



HAL
open science

Recherche de déterminants génétiques et moléculaires impliqués dans l'architecture racinaire et nodulaire des légumineuses et contribuant à une amélioration de la nutrition azotée

Virginie Quantin Bourion

► **To cite this version:**

Virginie Quantin Bourion. Recherche de déterminants génétiques et moléculaires impliqués dans l'architecture racinaire et nodulaire des légumineuses et contribuant à une amélioration de la nutrition azotée. Physiologie [q-bio.TO]. Université de Bourgogne, 2016. Français. NNT : 2016DIJOS042 . tel-01680215

HAL Id: tel-01680215

<https://theses.hal.science/tel-01680215>

Submitted on 10 Jan 2018

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

UNIVERSITE DE BOURGOGNE

Département Sciences Agronomiques et Ecologiques

THÈSE

Pour obtenir le grade de
Docteur de l'Université de Bourgogne

Discipline : **PHYSIOLOGIE**

par

Virginie BOURION

le 21 décembre 2016

**Recherche de déterminants génétiques et moléculaires impliqués dans
l'architecture racinaire et nodulaire des légumineuses et contribuant à
une amélioration de la nutrition azotée**

Directeur de thèse : Gérard DUC

Composition du jury :

| | | |
|------------------|--|--------------------|
| Florian FRUGIER | Directeur de Recherche, CNRS, Orsay | Rapporteur |
| Jacques LE GOUIS | Directeur de Recherche, INRA, Clermont-Ferrand | Rapporteur |
| Marc LEPETIT | Directeur de Recherche, INRA, Montpellier | Examineur |
| Christophe SALON | Directeur de Recherche, INRA, Dijon | Examineur |
| Daniel WIPF | Professeur, Université de Bourgogne, Dijon | Examineur |
| Gérard DUC | Directeur de Recherche, INRA, Dijon | Directeur de thèse |

REMERCIEMENTS

Je tiens tout d'abord à remercier Gérard Duc, pour avoir accepté d'être mon directeur de thèse et pour m'avoir accueillie dans « l'équipe racines » il y a quelques années déjà. Je remercie également ; Judith Burstin, responsable de l'équipe ECP, Christophe Salon, directeur du pôle GEAPSI, et Philippe Lemanceau, directeur de l'Unité Agroécologie pour m'avoir autorisée à accomplir ce travail de thèse tout en étant Ingénieur de recherche.

Je remercie vivement Florian Frugier et Jacques Le Gouis, rapporteurs de cette thèse, ainsi que Marc Lepetit, Christophe Salon et Daniel Wipf, examinateurs, pour avoir accepté de juger mon travail. Je remercie Christophe Salon et Marc Lepetit également pour avoir été membres de mon comité de thèse, ainsi que Marie-Laure Pilet-Nayel, et pour leurs conseils concernant l'analyse de mon travail.

Christophe et Marc, ainsi que Judith, Gérard, Sandrine Balzergue, Marie-Laure Martin-Magniette, Mathieu Siol et Nathalie Munier-Jolain ont été d'une précieuse aide lors de mes étapes d'analyse et d'interprétation des résultats des expérimentations ; je les en remercie. J'ai aussi beaucoup apprécié les discussions concernant la GWAS que j'ai eu avec Marie-Laure, Maria Manzanares et Aurore Desgroux dans le cadre du co-encadrement de sa thèse. Merci aussi à Marc, Judith, Gérard, Mathieu et à Brigitte Brunel, Antoine Lequerre, Frédéric Debellé, Julie Cullimore, Clare Gough, Marion Dalmais, Abdel Bendahame, Xavier Pinochet, Véronique Biarnès, Grégoire Aubert, Jonathan Kreplak, pour toutes les discussions passionnantes que nous avons eues lors de la construction du projet ANR, qui se base en partie sur des résultats obtenus pendant cette thèse.

Merci également à toutes les personnes qui m'ont dispensé des formations pendant ces années de thèse ; j'ai beaucoup apprécié les différentes formations que j'ai suivies à l'Université ou à l'INRA.

Aucun des travaux n'aurait pu être mené sans le soutien sans faille de « l'équipe racines », Henri de Larambergue, Chantal Martin et Véronique Aubert n'ont pas ménagé leur peine pour scanner et compté les racines et les nodosités sur des centaines

(milliers ?) de plantes : Merci à tous les autres membres de l'équipe ECP, Hervé et Céline en tête, qui ont participé aux opérations de phénotypage quand le volume de travail était trop important. Catherine et Marianne ont aussi été d'une grande aide pour le soutien qu'elles m'ont apporté concernant la fourniture des graines et des informations sur les accessions provenant de la collection de ressources génétiques. Le travail n'aurait pas pu se faire non plus sans l'aide de stagiaires et de mains d'œuvre occasionnelles, je les en remercie. Je garde un souvenir très fort des premiers phénotypages que nous avons réalisés à Epoisses, dans la salle du sous-sol, entre les caisses où étaient stockées des graines. Quel bonheur nous avons eu ensuite à notre arrivée à Dijon de disposer d'un vrai « labo » !

« L'équipe serre » a joué un rôle considérable dans la mise en place des expérimentations ; je remercie Estelle, Nouredine, Arnaud, Sébastien, Karine, Damien, Frank et Céline. Anne-Lyse m'a aussi apporté son aide en réalisant toutes les analyses des teneurs en N des échantillons.

Enfin, je voudrais remercier toutes les personnes de l'Unité qui m'ont encouragée, ces dernières semaines en particulier ; Dominique, Marianne, Damien, Chantal, Françoise, Karen, Céline, Brigitte, Greg, Jonathan, Corinne, les Catherine, Anne-Sophie, Marion, Géraldine, Hoai-Nam, Charlotte, Aurélie, Sarah... j'en oublie très certainement. Merci aussi à tous mes amis, et en particulier à tous les chanteurs.

Cela a été un grand bonheur (et aussi un grand stress...) d'accomplir ce travail. Cela n'aurait pas été possible sans le soutien de ceux qui me sont les plus chers : Pascal et nos trois filles, Carole, Florence et Mélodie. Et je n'oublie pas mes parents et ma belle-famille, tous prêts à nous apporter leur aide dès que nécessaire.

Pour finir, je voudrais dédier ce travail à Gisèle Laguerre, décédée en janvier 2013 ; juste quelques jours après mon inscription en thèse. Des années auparavant elle m'avait conseillée de faire une thèse. C'est avec elle qu'a été conçue l'expérimentation sur l'interaction entre pois et rhizobia et qui va se poursuivre dans le cadre du projet ANR ; je lui en suis extrêmement reconnaissante.

RÉSUMÉ

La culture de Légumineuses présente le double intérêt de permettre une production de graines à haute valeur nutritionnelle sans nécessité d'un apport d'engrais azoté. La nutrition azotée des légumineuses dépend en effet majoritairement de la fixation symbiotique de l'azote atmosphérique réalisée par des bactéries du sol, les rhizobia, au sein des nodosités, et dans une moindre mesure, de l'assimilation de l'azote minéral du sol par les racines.

Une meilleure compréhension a été acquise sur le contrôle génétique de la mise en place des racines et des nodosités et sur leur impact sur la nutrition azotée. Une grande variabilité génétique pour ces caractères a été mise en évidence, ainsi que l'existence de corrélations génétiques entre eux. Une approche de génétique quantitative a permis d'identifier des régions génomiques pouvant être impliquées dans leurs variations. Deux pistes d'amélioration de la nutrition azotée ont aussi été étudiées : l'amélioration de l'acquisition d'azote par les racines à partir d'une étude détaillée d'un mutant de développement racinaire, et l'amélioration de la symbiose via l'étude de la capacité des pois à favoriser les associations symbiotiques avec les rhizobia les plus performants.

Les résultats obtenus apportent des bases de réflexion concernant la conception d'un idéotype de nutrition azotée. Au-delà de la complémentarité indispensable entre les deux voies d'acquisition d'azote, il convient d'optimiser l'interaction entre les deux partenaires symbiotiques, mécanisme complexe mettant en jeu la formation et le fonctionnement des nodosités, en lien avec une signalétique et des interactions trophiques complexes entre partenaires et intra-plante.

ABSTRACT

Grain legume pulse crops are of great interest to allow a production of seeds high nutritional value without any contribution of nitrate fertilizer. The nitrogen nutrition of legumes depends indeed mainly on the fixation in nodules of atmospheric dinitrogen through the plant-rhizobium symbiosis, and to a lesser extent, absorption by roots of soil mineral nitrogen.

A better understanding has been obtained on the genetic control of the development of roots and nodules and on their impact on nitrogen nutrition. High genetic variability of these characters has been detected, and the existence of genetic correlations between them demonstrated. A quantitative genetic approach has identified several genomic regions that may be involved in their variations. The two different ways to improve nitrogen nutrition were also studied: the improvement of nitrogen acquisition by roots through a detailed study of a root architecture mutant, and the improvement of symbiosis via the study of the ability of peas to promote symbiotic associations with the most effective rhizobia.

The results provide interesting bases for the design of a pea nitrogen-nutrition 'ideotype'. Beyond the essential complementarity between the two pathways of nitrogen acquisition, it is necessary to optimize the interaction between the two symbiotic partners, which is a complex mechanism involving nodules formation and functioning in connection with complex signaling and trophic interactions between the partners and intra-plant.

TABLE DES MATIERES

| | |
|--|----|
| Introduction générale..... | 1 |
| Introduction bibliographique..... | 5 |
| 1. Généralités sur les symbioses rhizobia-légumineuses..... | 5 |
| Intérêt de la symbiose fixatrice d'azote..... | 5 |
| Les plantes hôtes | 7 |
| Les rhizobia..... | 8 |
| 2. Déterminants moléculaires et génétiques de la formation des nodosités | 10 |
| Généralités sur la formation des nodosités..... | 10 |
| Généralités sur le fonctionnement des nodosités | 11 |
| Bases moléculaires des étapes précoces de la nodulation | 13 |
| Régulation du nombre de nodosités | 20 |
| 3. Déterminants moléculaires et génétiques de la mise en place du système racinaire chez les dicotylédones | 21 |
| Formation des racines latérales chez les dicotylédones (modèle Arabidopsis)..... | 21 |
| Bases moléculaires de l'acquisition d'N et du développement des racines | 25 |
| 4. Ajustement entre les deux voies d'acquisition d'azote chez les légumineuses..... | 28 |
| Signalétique de la régulation entre les deux organogénèses racinaire et nodulaire | 29 |
| Ajustement de la nodulation au cours de la croissance du pois | 31 |
| 5. Diversité naturelle et évolution de la sélection du pois..... | 32 |
| La domestication du pois..... | 32 |
| Les principales évolutions de la sélection du pois | 33 |
| 6. L'amélioration de la nutrition azotée du pois : un levier pour augmenter et stabiliser son rendement ?..... | 35 |
| Les causes de la diminution des surfaces cultivées en pois | 35 |
| Les potentialités de l'amélioration de la nutrition azotée pour l'amélioration du rendement | 36 |
| 7. Objectifs et stratégie du travail de thèse..... | 38 |
| Chapitre I : Etude du déterminisme génétique de l'architecture racinaire nodulée et de la nutrition azotée du pois | 41 |
| 1. Introduction au chapitre I..... | 41 |
| 2. Publication n°1 | 43 |

| | |
|---|-----|
| Abstract | 44 |
| Introduction | 45 |
| Materials and methods | 47 |
| Results | 55 |
| Discussion | 63 |
| Conclusion..... | 68 |
| Acknowledgments | 68 |
| References | 69 |
| | |
| Chapitre II : Recherche de gènes candidats impliqués dans le développement racinaire et dans l'acquisition d'N..... | 73 |
| 1. Introduction au chapitre II..... | 73 |
| 2. Publication n°2 | 75 |
| Abstract | 76 |
| Introduction | 77 |
| Materials and methods | 79 |
| Results | 87 |
| Transcriptomic analysis..... | 91 |
| Discussion | 99 |
| Acknowledgments | 102 |
| References | 103 |
| | |
| Chapitre III : Variabilité génétique du choix entre partenaires symbiotiques pois et rhizobium..... | 109 |
| 1. Introduction au chapitre III | 109 |
| 2. Publication n°3 | 111 |
| Abstract | 112 |
| Introduction | 113 |
| Materials and methods | 115 |
| Results | 119 |
| Natural variability of pea nodulation and pea-Rlv partner choice | 123 |
| Correlation between partner choice and efficiency | 125 |
| Discussion | 127 |
| Conclusion..... | 133 |
| Acknowledgments | 134 |
| References | 135 |

| | |
|--|-----|
| Synthèse et perspectives | 141 |
| 1. Apport des résultats de la thèse en recherche..... | 141 |
| Mise au point d'une méthodologie de phénotypage..... | 142 |
| Mise en évidence d'une variabilité génétique | 143 |
| Mise en évidence de corrélations génétiques entre variables d'architecture racinaire nodulée et d'acquisition d'N | 144 |
| 2. Perspectives | 147 |
| Utiliser les nouveaux outils disponibles..... | 147 |
| Pour préciser l'architecture génétique des caractères..... | 148 |
| Pour valider de nouveaux gènes candidats..... | 150 |
| 3. Conclusion..... | 151 |
| Références bibliographiques | 153 |
| Annexes | 179 |
| Annexe 1 : Fichiers additionnels du chapitre I..... | 179 |
| Annexe 2 : Fichiers additionnels du chapitre II | 195 |
| Annexe 3: Fichiers additionnels du chapitre III | 204 |
| Annexe 4 : Liste des jeux de données utilisés au cours de la thèse..... | 221 |
| Annexe 5 : Poster décrivant la diversité de la collection de pois | 223 |

LISTE DES ABREVIATIONS

NB : abréviations utilisées plus d'une fois ou non définies dans le texte

| | |
|-----------|---|
| ALA | Alanine |
| AON | Autoregulation of Nodulation |
| ARG | Arginine |
| ANodB | Average Nodule Biomass |
| ASN | Asparagine |
| ASP | Aspartate |
| BC | Backcross |
| BegFlo | Beginning of Flowering date (°C.day) |
| BF | Beginning of Flowering stage |
| BGB | Belowground biomass = RootB + NodB |
| BGB:TB | Relative part of Belowground upon Total Biomass |
| BSF | Beginning of Seed Filling stage |
| BNF | Biological Nitrogen Fixation |
| CEP | C-TERMINALLY ENCODED PEPTIDE |
| CEPR | Récepteur CEP |
| CLE | CLAVATA3/EMBRYO SURROUNDING REGION |
| CK | Cytokinine |
| CYS | Cystéine |
| GLN | Glutamine |
| GLU | Glutamate |
| GRaSP | Genetics of Rhizobia Selection by Pea |
| GWAS | Genome Wide Association Study |
| INRA | Institut National de la Recherche Agronomique |
| LatApR | Total Root Elongation Rate (cm/°C.day) |
| LeafA | Leaf Area |
| LeafApR | Leaf Appearance Rate before BF (nb/°C.day) |
| LeafAR | Leaf Area Increase Rate before BF (cm ² /°C.day) |
| LeafStage | Number of nodes with leaves |
| LEU | Leucine |
| LRN | Number of first to third Lateral Roots |
| NBranch | Number of basal branches |
| NB:BGB | Nodule Biomass/Belowground Biomass |
| NDFFA | Percentage of N derived from Fixation |
| LG | Linkage Group |
| LOD score | Logarithm of Odds score |
| LRL | Mean Lateral Root Length = TRL - PRL / LRN |

| | |
|----------|---|
| LRR | Leucine-Rich Repeat |
| LSMeans | Least Square Means |
| LYS | Lysine |
| LysM | Lysine Motif |
| NF | Nod Factor |
| NLA | Efficiency of N conversion into Leaf Area |
| NLatRoot | Number of first order Lateral Roots |
| NNod | Number of Nodules |
| NodApR | Nodule Appearance Rate (nb/°C.day) |
| NodB | Nodule Biomass |
| NodE | Nodule Efficiency (g ShootQNfix/g nodule) |
| NodPAR | Nodule Projected Area Increase Rate (mm ² /°C.day) |
| NUR | N-Uptake Rate |
| PCA | Analyse en Composantes Principales |
| PRL | Length of the Primary Root (=TapRootL) |
| PRO | Proline |
| Q-RT-PCR | Quantitative Reverse Transcription Polymerase Chain Reaction |
| QTL | Quantitative Trait Locus/Loci |
| RCSP | Rhizobial Cell Surface Polysaccharide |
| RDW | Root Dry Weight |
| RIL | Recombinant Inbred Line |
| RLK | Receptor-Like Kinase |
| Rlv | <i>Rhizobium leguminosarum sv. viciae</i> |
| RootB | Root Biomass |
| RootE | Root Efficiency (g ShootQNabs/g root) |
| %RootN | Root N content (%) |
| RUE | Radiation Use Efficiency (g TotalB/MJ) |
| SDW | Shoot Dry Weight |
| SeedB | Seed Biomass |
| SeedN | Seed Number |
| SeedNC | Seed N content (%) |
| SeedQN | Seed N accumulation |
| SER | Serine |
| ShootB | Shoot Biomass |
| ShootL | Shoot Length |
| ShootNC | Shoot N content (%) (= %ShootN) |
| %ShootN | Shoot N content (%) (= ShootNC) |
| ShootQN | Shoot N accumulation |
| SLN | Specific Leaf Nitrogen (g ShootQN/m ² leaf) |
| SNP | Single Nucleotide Polymorphism |

| | |
|----------|--|
| SNU | Specific Nitrogen Uptake (g ShootQN/g belowground) |
| SPAD | Leaf Chlorophyll content estimated by SPAD measurement |
| SSR | Single Sequence Repeat |
| StrawB | Straw Biomass |
| StrawNC | Straw N content (%) |
| StrawQN | Straw N accumulation |
| subsp. | sub species |
| sv. | symbiovar |
| TapRootL | Tap Root Length |
| THR | Threonine |
| TotN | Total N accumulation = RDW x %RootN + SDW x %ShootN |
| TNodPA | Total Nodule Projected Area |
| TRL | Total Root Length (=TRootL) |
| TRootL | Total Root Length (=TRL) |
| TRootER | Total Root Elongation Rate (cm/°C.day) |
| TDW | Total Dry Weight = RDW + SDW |
| TSW | Thousand Seed Weight |
| UMR | Unité Mixte de Recherche |
| VAL | Valine |
| WT | Wild-type |

LISTE DES TABLEAUX

Tableau 1. Nombre de chromosomes et taille du génome des légumineuses modèles et des principales légumineuses d'intérêt agronomique. p 6

Table I.1. Pearson correlation coefficients between root and nodule structure traits and shoot nitrogen accumulation recorded on seven pea genotypes at four successive stages. p 54

Table I.2. Pearson correlation coefficients between shoot or seed nitrogen accumulation and nitrogen acquisition functioning or carbon accumulation traits recorded in RIL4 population in four experiments. p 58

Table II.1. Differentially accumulated transcripts between TR185 and wild type, annotated as related to N-acquisition pathway. p 94

Table II.2. Differentially accumulated transcripts between TR185 and wild type, annotated as related to cell wall modification. p 95

Table II.3. Differentially accumulated transcripts between TR185 and wild type, annotated as related to phenylpropanoid pathway. p 96

Table II.4. Differentially accumulated transcripts between TR185 and wild type, annotated as related to hormone metabolism and transport. p 98

LISTE DES FIGURES

- Fig. 1.** L'année internationale des légumineuses. p 2
- Fig. 2.** Relations taxonomiques entre les deux principaux clades subdivisant les légumineuses cultivées, avec les périodes de divergence. p 6
- Fig. 3.** Formation d'une nodosité racinaire de type indéterminé. p 12
- Fig. 4.** Structure des facteurs Nod. p 14
- Fig. 5.** Schéma conceptuel des bases moléculaires des étapes précoces de la nodulation. p 18
- Fig. 6.** Formation d'une racine latérale chez *Arabidopsis thaliana*. p 22
- Fig. 7.** Quatre modifications du système racinaire chez *Arabidopsis* en réponse à la disponibilité en N du milieu. p 24
- Fig. 8.** Représentation schématique du contrôle de l'allongement des racines latérales par NRT1.1 chez *Arabidopsis*. p 26
- Fig. 9.** Représentation schématique de la régulation de l'expression de NRT2.1 par N dans les racines d'*Arabidopsis*. p 26
- Fig. 10.** Représentation des voies signalétiques de régulation du nombre des nodosités et de racines latérales. p 30
- Fig. I.1.** Observation of pouch 15 days after seedling transfer into pouch (d5). p 50
- Fig. I.2.** Nodule number (a), nodule biomass (b), total nodule projected area (c), number of first order lateral roots (d), root biomass (e) and total root length (f) per plant, for seven pea genotypes, from the 4-leaf stage until the beginning of seed filling. p 52
- Fig. I.3.** Frequency distribution of RIL4 population for number of first order lateral roots (a, d), root biomass (b, e) and total root length (c, f) per plant, observed at the beginning of flowering in Pouches 2007 and Pots 2006 glasshouse experiments. p 56
- Fig. I.4.** Frequency distribution of RIL4 population for nodule number (a, d), nodule biomass (b, e), total nodule projected area (c, f) per plant, observed at the beginning of flowering in Pouches 2007 and Pots 2006 glasshouse experiments. p 56
- Fig. I.5.** Map position of QTL associated with nitrogen acquisition structure or functioning, carbon accumulation in aerial part or seed yield components, and nitrogen accumulation or content; in the pea population RIL4, in two glasshouse (07, 06) and two field experiments (06f, 04f). p 60
- Fig. II.1.** Highly-branched root architecture of the TR185 mutant irrespective of the nitrate supply. p 78

Fig. II.2. Dry weight and leaf area of wild-type (WT) and mutant (TR185) plants under high (10 mM, HN) or low (1 mM, LN) nitrate supply, from 7 to 28 days after germination. p 80

Fig. II.3. Grafting experiments. p 82

Fig. II.4. N concentration and efficiency of nitrogen accumulation into wild-type (WT) and mutant (TR185) plants under high (10 mM, HN) or low (1 mM, LN) nitrate supply, from 7 to 28 days after germination. p 86

Fig. II.5. Total amino acid content and contents of seven amino acids in wild-type (WT) and mutant (TR185) shoot. p 88

Fig. II.6. Total amino acid content and contents of seven amino acids in wild-type (WT) and mutant (TR185) root. p 90

Fig. II.7. Comparisons of mutant (TR185) and wild-type (WT) transcriptomes. p 92

Fig. III.1. Genetic structure of the pea collection using the DAPC method. p 118

Fig. III.2. *nodD* molecular phylogenetic analysis by Maximum Likelihood method of Rlv sequences available in GenBank. p 118

Fig. III.3. Relationship between shoot dry matter and nodule dry matter (a) or nodule number per plant (b), for 104 pea accessions multi-inoculated with a mixture of five Rlv strains. p 120

Fig. III.4. Strain frequencies in the nodules of 104 pea accessions multi-inoculated with a mixture of five Rlv strains (E1 experiment). p 120

Fig. III.5. Strain frequencies in the nodules of (a) the 104 pea accessions according to their membership to DAPC groups, (b) the 17 pea accessions belonging to D1 according to their membership to kluster groups. p 122

Fig. III.6. Shoot dry matter of 18 pea accessions with each of the five Rlv strains in mono-inoculation (E2 experiment). p 124

Fig. III.7. Relationship between nodulation index (relative to pea accession mean) in response to mono-inoculation with SF (E2 experiment) and SF competitiveness for nodulation evaluated in multi-inoculation (E1 experiment) for 18 pea accessions. p 126

Fig. III.8. Relationship between shoot dry matter index (relative to pea accession mean) in response to mono-inoculation (E2 experiment) and strain competitiveness for nodulation evaluated in multi-inoculation (E1 experiment) for 18 pea accessions. p 130

Fig. 11. Analyse en composantes principales des 104 accessions de pois avec 16 variables actives décrivant l'architecture racinaire nodulée, le développement aérien et l'acquisition d'N et 5 variables supplémentaires SA, SD, SE, SF et SK qui sont les proportions de chacune de ces souches dans les nodosités. p 146

LISTE DES ANNEXES

1. Annexe 1 : Fichiers additionnels du chapitre I

Supplemental Fig. I.1. Frequency distribution of RIL4 population for shoot dry matter per plant and percentage of nitrogen derived from fixation, at BF in two glasshouse experiments; Pouches in 2007 and Pots in 2006, and at maturity in two field experiments; 2006 and 2004. p 179

Supplemental Fig. I.2. LOD score curve on LGII, LGIII and LGVII for QTL associated with nitrogen acquisition structure or functioning, in two glasshouse experiments; Pouches in 2007 and Pots in 2006, and in a field experiment in 2004. p 180-182

Supplemental Table I.1. Effects of the different alleles of *Le* and *Af* on root and nodule traits evaluated at four developmental stages on seven pea genotypes. p 183

Supplemental Table I.2. Mean parental values, broad sense heritability (h^2), significance of genotype effect, Mean, Min and Max values for nitrogen acquisition structure traits recorded in RIL4 population in two glasshouse experiments (Pouches 2007, Pots 2006). p 184

Supplemental Table I.3. Pearson correlation coefficients between nitrogen acquisition structure traits recorded in RIL4 population in two glasshouse experiments. p 185

Supplemental Table I.4. Mean parental values, broad sense heritability (h^2) and significance of genotype effect for carbon accumulation, nitrogen acquisition functioning traits and developmental variables recorded in RIL4 population in two glasshouse and two field experiments. p 186

Supplemental Table I.5. Mean, Minimum and Maximum values of carbon accumulation, nitrogen acquisition functioning traits and developmental variables recorded in RIL4 population in two glasshouse and two field experiments. p 187

Supplemental Table I.6. Characteristics of the QTL for nitrogen acquisition structure and functioning traits detected in RIL4 population from both glasshouse and field experiments. p 188-189

Supplemental Table I.7. Characteristics of the QTL for carbon accumulation and seed traits detected in RIL4 population from both glasshouse and field experiments. p 190-191

Supplemental Table I.8. Characteristics of the QTL for nitrogen accumulation and content detected in RIL4 population from both glasshouse and field experiments. p 192

Supplemental Table I.9. Broad sense heritability (h^2), global R^2 , part of genetic variance explained by QTL(p) for nitrogen acquisition structure traits recorded in RIL4 population in two glasshouse experiments (Pouches 2007, Pots 2006). p 193

Supplemental Table I.10. Broad sense heritability (h^2), global R^2 , part of genetic variance explained by QTL (p) for carbon accumulation, nitrogen acquisition functioning traits and developmental variables recorded in RIL4 population in two glasshouse and two field experiments. p 194

2. Annexe 2 : Fichiers additionnels du chapitre II

Supplemental Fig. II.1. Q-RT-PCR validation of differentially accumulated transcripts initially identified by Affymetrix GeneChip analysis. p 195

Supplemental Fig. II.2. Relationship between shoot N concentration (%ShootN) and shoot dry weight (SDW). At each date, data are means from three biological replicates of six plants each \pm SE. p 196

Supplemental Fig. II.3. Content of ten amino acids in wild-type (WT) and mutant (TR185) shoots under high (10 mM. [HN]) or low (1 mM. [LN]) nitrate supply, at 14 and 21 days after germination. TRY, TYR, HIS were non-detectable, and therefore are not shown. At each date, data are expressed in $\mu\text{mol.g}^{-1}$ FW and are means from three biological replicates of six plants each \pm SE. Means followed by different letters are significantly different based on multiple comparisons (LSD test) at $p < 0.05$. p 197

Supplemental Fig. II.4. Content of eight amino acids in wild-type (WT) and mutant (TR185) roots under high (10 mM. [HN]) or low (1 mM. [LN]) nitrate supply, at 14 and 21 days after germination. TRY, TYR, GLY, CYS, MET were non-detectable, and therefore are not shown. At each date, data are expressed in $\mu\text{mol.g}^{-1}$ FW and are means from three biological replicates of six plants each \pm SE. Means followed by different letters are significantly different based on multiple comparisons (LSD test) at $p < 0.05$. p 198

Supplemental Fig. II.5. Root lignin content of the wild-type (WT) and the mutant (TR185) under high (10 mM. [HN]) or low (1 mM. [LN]) nitrate supply, 14 and 21 days after germination. p 199

Supplemental Table II.1. Genetic analyses of the highly-branched root mutant TR185. p 200

Supplemental Table II.2. Primers list used for Q-RT-PCR assays. p 201

Supplemental Table II.3. The 75 differentially accumulated transcripts responsive to N effect and common to those identified by Ruffel *et al.* (2008) as responsive to either local nitrate starvation (LNO₃vs-NO₃) or systemic signals (LNO₃vsSNO₃). p 202-203

3. Annexe 3 : Fichiers additionnels du chapitre III

Supplemental Table III.1. Pea collection. Passport and structure data. P 204-208

Supplemental Table III.2. Description of the *Rhizobium leguminosarum* symbiovar *viceae* strains. p 209

Supplemental Table III.3. Oligonucleotide primers used to identify specific strains. p 209

Supplemental Table III.4. Summary statistics of the ANOVA analyses performed on the phenotypic data collected in the E1 experiment. p 210

Supplemental Table III.5. Summary statistics of the ANCOVA analyses of the relationship between shoot and nodule dry weight in E1. p 210

Supplemental Table III.6. Median, Mean, Min and Max values of relative frequencies of the five Rlv strains within the DAPC or cluster groups. p 211

Supplemental Table III.7. Summary statistics of the ANOVA analyses performed on the phenotypic data collected in the E2 experiment. p 211

Supplemental Table III.8. Summary statistics of the linear regression analyses performed for the five strains between nodulation index evaluated in E2 and competitiveness evaluated in E1. p 212

Supplemental Table III.9. Summary statistics of the linear regression analyses performed for the five strains between shoot dry matter index evaluated in E2 and competitiveness evaluated in E1. p 212

Supplemental Fig. III.1. FastSTRUCTURE analysis. p 213

Supplemental Fig. III.2. Genetic structure of the pea collection using the fastSTRUCTURE analysis. p 214

Supplemental Fig. III.3. Relationship between shoot dry matter and nodule dry matter (a) or nodule number per plant (b), for the 17 pea accessions belonging to D1 according to their membership to kluster groups in the multi-inoculation experiment (E1). p 215

Supplemental Fig. III.4. Relationship between shoot dry matter and nodule dry matter (a) or nodule number per plant (b), for the 29 pea cultivars belonging to D2 or D3 according to their membership to kluster groups in the multi-inoculation experiment (E1). p 216

Supplemental Fig. III.5. Strain frequencies in the nodules of 104 pea accessions (E1 experiment). + indicates the mean frequency for each of the five strain. All outliers are identified by accession numbers. Colour indicates DAPC group affiliation. violet for D, green for D2 and brown for D3. p 217

Supplemental Fig. III.6. For 18 pea accessions multi-inoculated with a mixture of five Rlv strains (E1 experiment): (a) Strain frequencies in the nodules (b) Relationship between shoot dry matter and nodule dry matter. p 218

Supplemental Fig. III.7. Relationship between shoot dry matter in the mono-inoculation experiment (E2) and (a) shoot dry matter in multi-inoculation (E1 experiment) or (b) nodule number per plant in mono-inoculation (E2 experiment), for 18 pea accessions. p 219

Supplemental Fig. III.8. Nodule number per plant of 18 pea accessions with each of the five Rlv strains in mono-inoculation (E2 experiment). p 220

4. Annexe 4 : Liste des jeux de données utilisés au cours de la thèse

p 221-222

5. Annexe 5 : Poster décrivant la diversité de la collection de pois

p 223-226

INTRODUCTION GENERALE

INTRODUCTION GÉNÉRALE

Le contexte mondial nécessite un accroissement de la production agricole pour faire face à un accroissement de la population, tout en limitant l'apport d'intrants et en prenant en compte les contraintes de changement climatique. Face à ces enjeux, la culture de légumineuses présente le double intérêt de permettre une production de graines à haute valeur nutritionnelle, notamment riches en protéines, sans nécessité d'un apport d'engrais azoté. Pour la FAO (Organisation des Nations Unies pour l'alimentation et l'agriculture), l'année 2016 est d'ailleurs celle où les légumineuses à graines (pois, haricots, lentilles, pois chiches etc.) sont mises à l'honneur pour leur intérêt nutritionnel et leurs bienfaits agricoles (Fig. 1).

La nutrition azotée des légumineuses à graines dépend majoritairement de la fixation symbiotique de l'azote atmosphérique réalisée par des bactéries du sol, les rhizobia, au sein des nodosités racinaires et, dans une moindre mesure, de l'acquisition de l'azote minéral du sol par les racines. Cependant, la nutrition azotée des légumineuses peut être un facteur limitant de leur rendement. En effet, la fixation symbiotique d'azote est très sensible aux conditions environnementales (stress abiotiques, biotiques) et le faible développement des racines fréquemment observé chez les légumineuses peut limiter le prélèvement en eau et en éléments minéraux (dont les nitrates).

Compte-tenu de sa faible accessibilité, le système racinaire des plantes est particulièrement difficile à étudier. Chez les légumineuses, il est, de plus, le support de deux voies d'acquisition de l'azote et sa mise en place et son fonctionnement sont donc particulièrement complexes. En particulier, différentes études ont montré que les développements racinaire et nodulaire sont en compétition pour l'utilisation des ressources en C disponibles (Salon *et al.*, 2001; Voisin *et al.*, 2007). D'autres études ont montré que la structure génétique des populations de rhizobia nodulant le pois varie selon le génotype végétal et se traduit par une diversité fonctionnelle au niveau de la plante, avec des effets sur les développements racinaire et nodulaire (Bourion *et al.*, 2007; Laguerre *et al.*, 2007; Depret & Laguerre, 2008).

Cette thèse a été réalisée à l'UMR Agroécologie, Unité Mixte de Recherche (AgroSup Dijon, INRA, Université de Bourgogne Franche-Comté), et plus précisément au sein du pôle

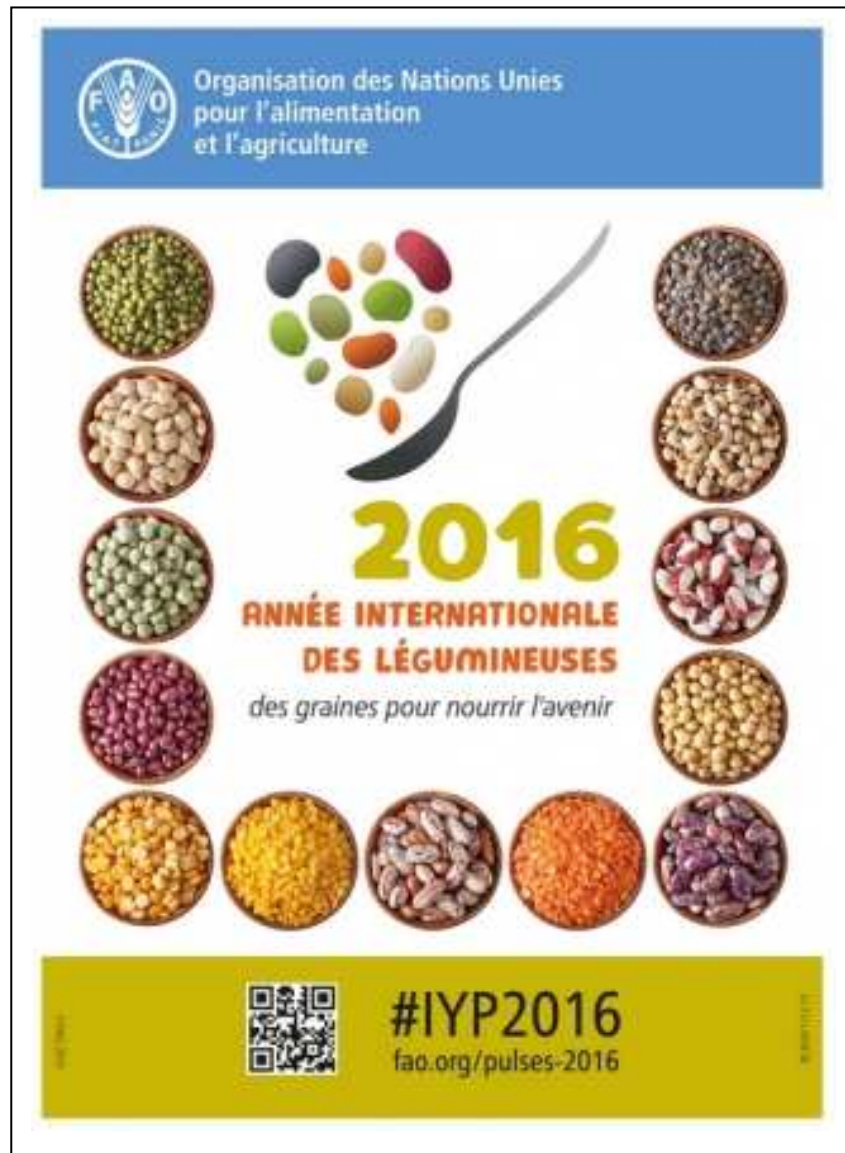


Fig. 1. L'année internationale des légumineuses vise à sensibiliser l'opinion publique aux avantages nutritionnels des légumineuses dans le cadre d'une production durable, en appui à la sécurité alimentaire et nutritionnelle de tous les pays.

GEAPSI (déterminismes Génétiques et Environnementaux de l'Adaptation des Plantes à des Systèmes de culture Innovants). Un des enjeux scientifiques de ce pôle est d'acquérir des connaissances sur les mécanismes génétiques et physiologiques à la base du prélèvement des ressources telluriques (N, S) par les légumineuses, ceci en interaction avec des partenaires biotiques (plantes adventices ou associées et microorganismes du sol) ; avec pour objectif finalisé l'identification d'idéotypes variétaux de plantes pouvant constituer des leviers pour la conception de systèmes de cultures plus économes en intrants. Les expérimentations réalisées dans le cadre de la thèse ont été réalisées dans le cadre de différents programmes (Projet européen GLIP, projet INRA AgroBI, projet ANR GENOPEA, projet BAP SYMBIOPEA) visant à enrichir les connaissances scientifiques et à promouvoir la culture des légumineuses.

L'objectif principal de cette thèse a été de rechercher des déterminants génétiques et moléculaires de la plante impliqués dans l'architecture racinaire et nodulaire du pois et d'évaluer leur impact sur la nutrition azotée de la plante en interaction avec des souches de rhizobium.

INTRODUCTION
BIBLIOGRAPHIQUE

INTRODUCTION BIBLIOGRAPHIQUE

1. Généralités sur les symbioses rhizobia-légumineuses

Intérêt de la symbiose fixatrice d'azote

L'azote (N) est un nutriment essentiel pour les plantes. C'est en effet un des éléments fondamentaux des composants cellulaires nécessaires à la vie, notamment pour la synthèse des acides nucléiques et des protéines indispensables à la reproduction et à la croissance. L'azote est très abondant dans l'atmosphère (79% de N₂), mais sa disponibilité dans le sol, sous forme de nitrates ou d'ammonium pouvant être prélevés par les racines des plantes, est souvent limitée. En conséquence, de nombreuses cultures nécessitent un apport de fertilisants azotés organiques ou de synthèse pour atteindre des rendements satisfaisants. Les besoins d'N sous forme d'engrais industriels représentent plus de 120 Mt par an (Galloway *et al.*, 2008; Fowler *et al.*, 2013). La production industrielle de ces engrais par le procédé Haber-Bosch consiste en une réduction à hautes température et pression du N₂ atmosphérique en ammonium, réaction appelée fixation azotée. La grande quantité d'énergie qu'elle nécessite est généralement d'origine fossile et cette consommation entraîne la libération d'une grande quantité de CO₂ dans l'atmosphère, estimée à 300 Mt par an (Jensen *et al.*, 2012). En outre, l'utilisation accrue des engrais azotés accroissent la formation d'azote réactif négatif pour l'environnement, sous forme de nitrate dans les sols non explorés par les racines ou les eaux de drainage et par émissions de gaz à effets de serre, ammoniac ou d'oxydes d'azote, dans l'air (Bouwman *et al.*, 2013; Fowler *et al.*, 2013).

Les légumineuses sont capables d'utiliser l'azote gazeux comme source principale d'azote, ceci grâce à une symbiose avec des bactéries du sol, collectivement appelés rhizobia. Ces bactéries naturellement existantes dans le sol convertissent le N₂ atmosphérique en ammonium assimilable par la plante ; on parle alors de fixation symbiotique de l'azote (en anglais, BNF, Biological Nitrogen Fixation). Les estimations de quantité d'N symbiotique fixé par les cultures de légumineuses sont de 60 Mt par an, auxquels s'ajoutent 60 Mt fixés par les forêts et prairies naturelles et 140 Mt par les océans (Herridge *et al.*, 2008; Fowler *et al.*, 2013). Plus précisément, la part fixée par les cultures de légumineuses à graines est estimée à 21 Mt, dont 3 Mt pour les légumineuses riches en protéines (pois chiche, haricot, pois, féverole, lentille pour les principales) et respectivement 16 Mt et 2 Mt pour les deux légumineuses à graines riches en huile, le soja et l'arachide (Herridge *et al.*, 2008).

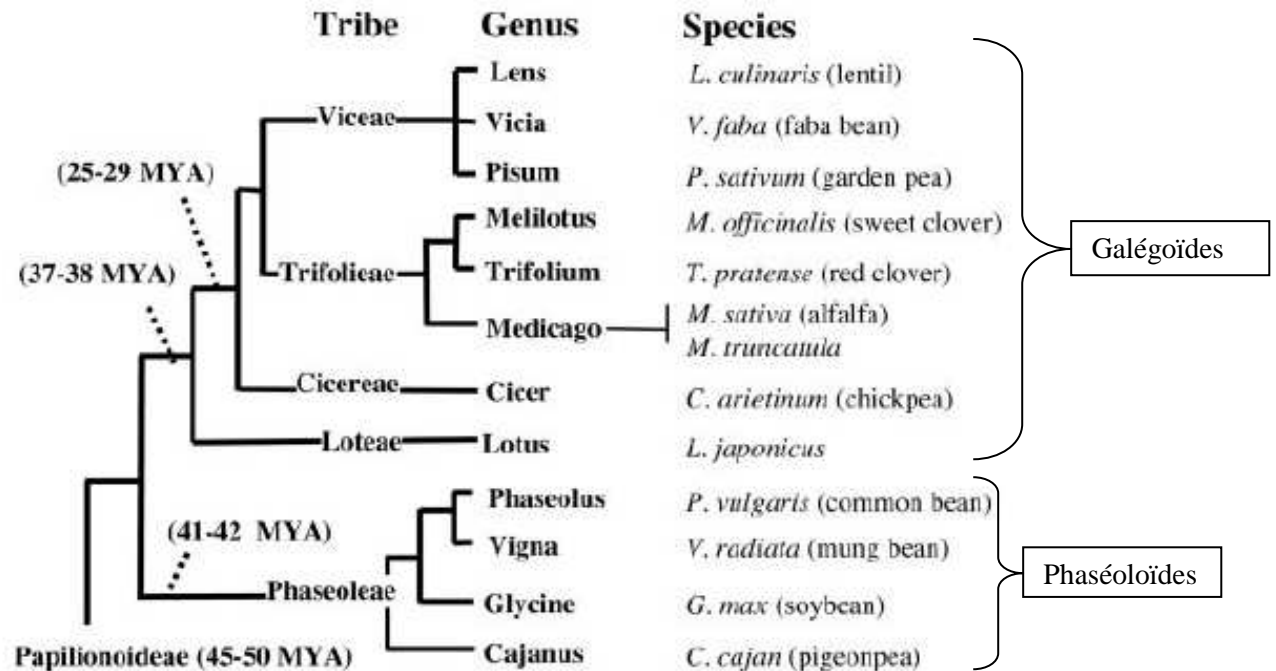


Fig. 2. Relations taxonomiques entre les deux principaux clades subdivisant les légumineuses cultivées, avec les périodes de divergence. MYA : Million years ago.
D'après Choi *et al.* 2004

| Tribe | Genus | Species | Chromosome No. | Genome Size | Self-Compatibility |
|------------|-----------|---|----------------|---------------------|--------------------|
| Trifolieae | Medicago | <i>M. truncatula</i> (barrel medic) | $2n = 2x = 16$ | <i>Mb/1C</i> 466 | Selfing |
| | | Alfalfa | $2n = 4x = 32$ | 1,715 | Outcrossing |
| | Trifolium | <i>Trifolium pratense</i> (red clover) | $2n = 2x = 14$ | 637 | Outcrossing |
| | | <i>Trifolium repens</i> (white clover) | $2n = 4x = 32$ | 956 | Outcrossing |
| Viceae | Melilotus | <i>Melilotus officinalis</i> (sweet clover) | $2n = 2x = 16$ | 1,103 | Outcrossing |
| | Pisum | Garden pea | $2n = 2x = 14$ | 4,337 | Selfing |
| | Vicia | <i>Vicia faba</i> (faba bean) | $2n = 2x = 12$ | 13,059 | Selfing |
| | Lens | Lentil | $2n = 2x = 14$ | 4,116 | Selfing |
| Cicereae | Cicer | Chickpea | $2n = 2x = 16$ | 931 | Selfing |
| Loteae | Lotus | <i>L. japonicus</i> | $2n = 2x = 16$ | 466 | Selfing |
| Phaseoleae | Phaseolus | Common bean | $2n = 2x = 22$ | 588 | Selfing |
| | | Mungbean | $2n = 2x = 22$ | 515 | Selfing |
| | Glycine | Soybean | $2n = 4x = 40$ | 1103 | Selfing |
| | Cajanus | <i>Cajanus cajan</i> (pigeon pea) | $2n = 2x = 22$ | 858 | Selfing |

^aData were from the Plant DNA C-values Database (<http://www.rbkew.org.uk/cval/homepage.html>).

Tableau 1. Nombre de chromosomes et taille du génome des légumineuses modèles et des principales légumineuses d'intérêt agronomique.
D'après Zhu *et al.* 2005

La quantité d’N fixée par les légumineuses fourragères se situe entre 12 à 25 Mt (Herridge *et al.*, 2008). Les légumineuses cultivées contribuent pour partie à l’excédent d’azote réactif, mais ne sont pas la cause fondamentale des déséquilibres et excès (Fowler *et al.*, 2013). L’azote fixé par fixation symbiotique est en effet directement incorporé, après une phase ammoniacale, dans la matière organique de la plante fixatrice (Cellier *et al.*, 2015). Des études ont montré que les cultures de légumineuses émettent près de trois fois moins de N₂O que les cultures de céréales (Jensen *et al.*, 2012). L’azote fixé constitue une source d’azote stable et peu volatil, donc peu susceptible de se disperser dans l’environnement et d’y créer des impacts négatifs, contrairement aux apports d’engrais appliqués sur les cultures. De plus, l’azote fixé profite non seulement à la légumineuse en place mais également aux plantes associées et aux cultures suivantes. En effet les reliquats après une culture de légumineuses contiennent 20 à 60 kg N/ha de plus qu’après une culture de céréales, ce qui permet de réduire d’autant les apports d’engrais azotés nécessaires à la culture suivante (Munier-Jolain & Carrouee, 2003). Enfin, au-delà de l’apport en azote et de la limitation des émissions des gaz à effet de serre, les légumineuses présentent d’autres atouts environnementaux. L’introduction des légumineuses dans les systèmes de culture dans les rotations ou en association crée en effet une diversification des cultures et permet, dans certains cas, de réduire la taille des populations de bioagresseurs et donc l’usage des produits phytosanitaires (Munier-Jolain & Carrouee, 2003).

Les plantes hôtes

Les plantes dites « hôtes » sont capables de former des organes symbiotiques appelés nodosités. A l’exception des *Parasponia*, toutes ces plantes capables de noduler appartiennent à la famille des légumineuses (*Fabaceae*) (Sprent *et al.*, 1987). Les *Fabaceae* sont des plantes dicotylédones et représentent une des trois plus importantes familles de plantes à fleur, comprenant près de 800 genres et 20000 espèces (Smykal *et al.*, 2015). Cette famille est constituée de trois sous-familles, les *Papilionoideae* et les *Mimosoideae*, auxquelles appartiennent la plupart des espèces capables d’établir une symbiose fixatrice d’azote, et les *Caesalpinioideae* dans laquelle peu d’espèces sont capables de noduler (Sprent, 2007). Les espèces de la sous-famille des *Mimosoideae* sont principalement des arbres et arbustes tropicaux ou subtropicaux, et incluent les genres *Acacia*, *Prosopis* et *Leucaena*. La sous-famille des *Papilionoideae* comprend la plupart des légumineuses cultivées, à graines ou fourragères. C’est la plus grande des trois sous-familles ; elle comprend 476 genres et près de 14000 espèces partageant un ancêtre commun autour de 50 MA (Lavin *et al.*, 2005). Quatre

clades subdivisent les légumineuses cultivées : les Phaséoloïdes, les Galégoïdes, les Génistoïdes et les Dalgergoïdes (Smykal *et al.*, 2015). Les Phaséoloïdes incluent les plantes dites « tropicales » comme le soja et le haricot (genres *Glycine*, *Phaseolus*) ; les Galégoïdes regroupent les plantes à culture en zone « tempérée » comme le pois, la lentille, la vesce, le pois chiche et la luzerne (genres *Pisum*, *Lens*, *Vicia*, *Cicer*, *Medicago*) (Fig. 2). Les genres *Pisum* et *Lens* appartiennent à la tribu des Fabeae (anciennement Viceae) qui comprend aussi trois autres genres: *Lathyrus*; *Vicia* (vesces, féverole) et le genre monotypique *Vavilovia formosa* (Mikic *et al.*, 2014b; Smykal *et al.*, 2015). La tribu des Fabeae est considérée comme l'un des groupes les plus jeunes de la famille des légumineuses avec un âge autour de 16 à 23 MA, au milieu du Miocène (Lavin *et al.*, 2005).

Parmi ces espèces, certaines ont été définies comme modèles pour la communauté scientifique s'intéressant en particulier aux mécanismes régissant la symbiose rhizobium-légumineuse. Les espèces *Medicago truncatula* et *Lotus japonicus* ont été les premières choisies, ceci en raison de la petite taille de leurs génomes (entre 450 et 500 Mb), de leur caractère autogame, du faible encombrement de leurs plantes et la courte durée de leur cycle de culture (Journet *et al.*, 2001; Stougaard, 2001) (Table 1). Il a été montré que ces espèces présentent des régions synténiques entre elles et avec les légumineuses d'intérêt agronomique (Choi *et al.*, 2004; Zhu *et al.*, 2005). Il était donc communément admis que le clonage des gènes d'intérêt chez les plantes cultivées serait possible une fois ces derniers identifiés chez les espèces modèles. *M. truncatula* présente aussi l'intérêt de pouvoir être transformé par *Agrobacterium rhizogenes* et *A. tumefaciens* (Chabaud *et al.*, 1996; Boisson-Dernier *et al.*, 2001). De grands projets nationaux et internationaux ont permis d'acquérir de nombreuses données génomiques sur ces espèces et les séquences de leurs génomes ont été publiées (Sato *et al.*, 2008; Young *et al.*, 2011). Les génomes de légumineuses d'intérêt agronomique ont aussi été séquencés ces dernières années : soja (*Glycine max*) (Schmutz *et al.*, 2010), pois cajan (*Cajanus cajan*) (Varshney *et al.*, 2012), pois chiche (*Cicer arietinum*) (Varshney *et al.*, 2013) et haricot (*Phaseolus vulgaris*) (Schmutz *et al.*, 2014). Des consortiums internationaux ont été lancés dans le but de générer la séquence complète de deux autres espèces cultivées ayant des génomes de taille supérieure d'environ 4,2 à 4,5 Gb : la lentille et le pois.

Les rhizobia

Les bactéries rhizobia, symbiotes à l'intérieur des nodosités, peuvent également vivre comme saprophytes dans le sol. L'association bénéfique entre les rhizobia et les légumineuses a été découverte à la fin du 19^{ème} siècle lorsque Beijerinck a obtenu la première culture bactérienne

pure à partir d'un nodule responsable de la fixation d'azote (Beijerinck, 1888). Cette bactérie a été ensuite nommée *Rhizobium leguminosarum*, le nom du genre dérivant des mots grecs, rhiza (racine) et bios (vie), et celui de l'espèce désignant l'hôte (Frank, 1889). Les espèces bactériennes symbiotiques découvertes par la suite ont ainsi été définies principalement sur la base de caractéristiques phénotypiques telles que la gamme d'hôtes, la morphologie et la vitesse de croissance des colonies sur des milieux sélectifs (Dangeard, 1926; Baldwin & Fred, 1929; Eckhardt *et al.*, 1931). De cette façon les bactéries à croissance rapide ont tout d'abord été différenciées en *R. leguminosarum*, *R. phaseoli*, *R. trifolii* et *R. meliloti*, et celles à croissance lente *R. japonicum* et *R. lupini*. A partir des années 80, la diversité de l'ADN a été étudiée chez ces bactéries, en particulier celle de l'ADNr 16S, permettant une autre classification en genres et espèces. L'utilisation de l'ADNr 16S était la technique la plus utilisée pour l'identification, la classification et la phylogénie de tous les organismes vivants et en particulier des bactéries (Woese, 1987). Les *R. japonicum* ont été reclassés comme faisant partie d'un nouveau genre *Bradyrhizobium* (du grec bradus = lent) (Jordan, 1982), et les rhizobia à croissance rapide subdivisés en plusieurs genres, parmi lesquels *Rhizobium*, *Mesorhizobium* et *Ensifer* (anciennement *Sinorhizobium*) (Jarvis *et al.*, 1997; Young, 2003). Les genres cités ci-dessus appartiennent à la classe des α -protobactéries, longtemps considérées comme seule classe contenant des bactéries nodulantes. Cependant, des β -protobactéries ont également été identifiées comme capables de noduler des légumineuses (Moulin *et al.*, 2001; Gyaneshwar *et al.*, 2011). Avec l'accroissement des études de diversité génétique, le nombre de genres et d'espèces reconnus parmi les rhizobia ne cesse de croître; actuellement les rhizobia sont considérés comme regroupant 98 espèces et 13 genres; (<http://www.rhizobia.co.nz/taxonomy/rhizobia>). Certaines de ces espèces ont été isolées à partir de racines de plantes autres que des légumineuses ou à partir de sols et identifiées comme non symbiotiques (Segovia *et al.*, 1991; Rivas *et al.*, 2004; Velázquez *et al.*, 2005). A noter aussi que des bactéries n'appartenant pas aux rhizobia ont parallèlement été identifiées comme capables de noduler des légumineuses, indiquant que la diversité des bactéries capables d'établir une fixation symbiotique n'est pas encore totalement connue; pour une revue voir (Peix *et al.*, 2015).

Enfin, la taxonomie basée sur l'ADNr 16S ne reflète pas les caractéristiques symbiotiques variables des rhizobia appartenant à une même espèce, en particulier de leur gamme de plantes hôtes. En conséquence, les études de taxonomie ont progressivement pris aussi en compte la diversité au niveau des gènes connus comme indispensables à la nodulation et à la fixation de l'azote (Laguerre *et al.*, 2001; Bailly *et al.*, 2007; Peix *et al.*,

2015). Ces gènes symbiotiques sont regroupés sur le mégaplasme nommé pSym chez les bactéries appartenant aux genres *Rhizobium* et *Sinorhizobium* (*Ensifer*) ; chez *Mesorhizobium* et *Bradyrhizobium*, ils sont situés sur le chromosome dans des îlots symbiotiques (Sullivan *et al.*, 1995; MacLean *et al.*, 2007). Cependant, des espèces différentes partagent les mêmes éléments symbiotiques et il a été montré que ces éléments sont transmissibles d'une espèce à l'autre (Sullivan *et al.*, 1995; Ding & Hynes, 2009). L'absence de correspondance simple entre diversité des gènes de symbiose et spécificité de nodulation a entraîné la préconisation d'une taxonomie dit polyphasique combinant informations phénotypiques et génétiques (Vandamme *et al.*, 1996). Le concept de « biovar » avait été introduit pour distinguer les rhizobia selon leur capacité à noduler spécifiquement une espèce ou une gamme réduite d'espèce de légumineuses ; il a été repris récemment sous le terme de « symbiovar » (Rogel *et al.*, 2011). L'étendue de gamme d'hôtes était évaluée à partir d'expérimentations lourdes et pas toujours standardisées (Ramírez-Bahena *et al.*, 2008). Les analyses de diversité sont de plus en plus nombreuses et associent un nombre croissant de gènes de symbiose, entraînant des modifications des contours des symbiovars (Ramírez-Bahena *et al.*, 2008; Kumar *et al.*, 2015).

2. Déterminants moléculaires et génétiques de la formation des nodosités

Différentes étapes sont nécessaires à la formation de nodosités fonctionnelles. Un dialogue moléculaire est indispensable entre les deux partenaires, notamment pour l'établissement des étapes précoces de la nodulation et la régulation du nombre de nodosités formées.

Généralités sur la formation des nodosités

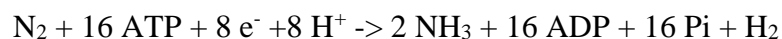
Selon les espèces de plante hôte, les nodosités se forment sur les tiges ou sur les racines. Chez la plupart des légumineuses à graines les nodosités sont racinaires. La formation des nodosités racinaires débute dans de rares cas par une pénétration des rhizobia dans les racines via des fissures formées lors de l'émergence des racines latérales. Le plus souvent, elle débute par une adhésion des bactéries à la surface des poils absorbants qui induit, au bout de 6 à 8 heures, leur courbure en « crosse de berger » (Fig. 3). Cette courbure piège les bactéries qui forment alors une micro-colonie. Les bactéries induisent une dégradation locale de la paroi cellulaire des poils absorbants ; celle-ci s'invagine et une nouvelle paroi est formée autour de cette invagination produisant une structure tubulaire appelée le cordon d'infection (Gage, 2004). Les cordons gagnent les cellules du cortex de la racine et se ramifient. Les cellules

bactériennes qu'ils renferment s'y multiplient. Pendant ce temps, les cellules du cortex interne situées sous le poil en cours d'infection se divisent pour former des primordia nodulaires. Les cordons infectieux pénètrent à l'intérieur de ces primordia et quelques jours après le début de l'infection libèrent les rhizobia dans des vésicules appelées symbiosomes où elles se différencieront en bactéroïdes capables de fixer l'azote. Une cellule infectée contient dans son cytoplasme le plus souvent un grand nombre de symbiosomes, pouvant aller jusqu'à plusieurs milliers (Udvardi & Day, 1997). La maturation se continue pour former une nodosité fixatrice.

La morphologie des nodosités matures dépend de la nature de leur méristème et est définie par l'espèce végétale (Sprent, 2007; Ferguson *et al.*, 2010). Chez les Phaséoloïdes, légumineuses « tropicales » auxquelles appartiennent le soja et la légumineuse modèle *Lotus japonicus*, les nodosités sont de type déterminé : leur méristème a une durée de vie limitée et elles sont sphériques. Les Galégoïdes, légumineuses « tempérées » auxquelles appartiennent le pois et la légumineuse modèle *Medicago truncatula*, se caractérisent par le maintien d'une zone méristématique pendant toute la vie des nodosités, dites de type indéterminé ; ceci leur confère une forme allongée et cylindrique. Une nodosité indéterminée mature est subdivisée en quatre zones, établies d'après la structure des bactéries qu'elles renferment (Vasse *et al.*, 1990). La zone I est constituée du méristème nodulaire et ne contient pas de bactéries. La zone II est la zone d'infection des cellules par les bactéries. La zone de transition comprend les cellules où les bactéries se différencient en bactéroïdes. La zone III contient les cellules contenant les bactéroïdes fixateurs, puis y succède la zone IV de sénescence du nodule.

Généralités sur le fonctionnement des nodosités

Au sein de la nodosité mature, les bactéroïdes fixent l'azote atmosphérique et produisent de l'ammonium. Cette réduction, catalysée par le complexe enzymatique de la nitrogénase est la suivante :



Deux types de gènes portés par les bactéroïdes sont indispensables à cette réaction ; ce sont les gènes *nif* et *fix*. Les gènes *nif* codent pour la biosynthèse de la nitrogénase. Les gènes *fix* sont quant à eux impliqués dans la régulation et le métabolisme de l'oxygène (Black *et al.*, 2012). La présence d'oxygène est indispensable aux bactéroïdes pour leur respiration et la synthèse d'ATP, mais un excès d'oxygène inactive irréversiblement la nitrogénase. Il existe des processus permettant de maintenir une pression faible en O₂ dans la zone centrale de la

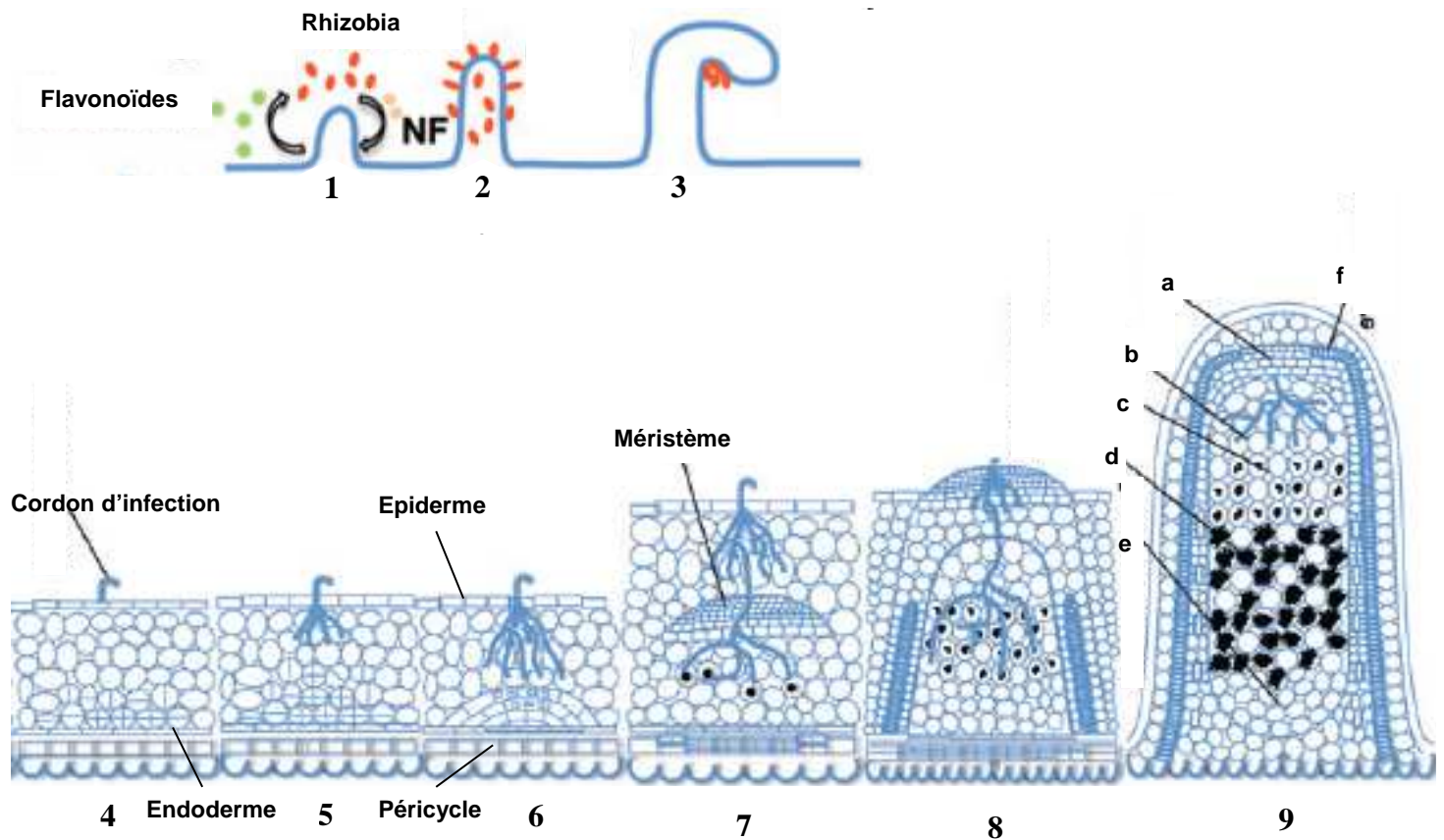


Fig. 3. Formation d'une nodosité racinaire de type indéterminé (1) Excrétion des flavonoïdes par la plante et des Facteurs Nod par les rhizobia (2) Chimiotactisme et attachement des rhizobia sur le poil absorbant (3) Formation de la crosse de Berger (4) Formation du cordon d'infection et divisions des cellules du cortex interne (5) Progression du cordon d'infection dans le cortex externe (6) Progression du cordon d'infection dans le cortex interne (7) Invasion des rhizobia dans les cellules du primodium nodulaire et formation du méristème (8) Maturation (9) Nodosité fixatrice d'azote avec (a) Zone I méristématique (b) Zone II d'infection (c) Interzone II-III (d) Zone III de fixation (e) Zone de sénescence (f) Vascularisation. D'après Ferguson *et al.* 2010

nodosité où se situent les bactéroïdes. Tout d'abord, le parenchyme nodulaire constitue une barrière de diffusion qui limite l'entrée de l'oxygène dans la nodosité ; la plante exerce un contrôle physiologique de sa perméabilité selon les conditions environnementales (en particulier disponibilité en O₂ et en nitrate dans la rhizosphère) (Denison *et al.*, 1992). Ensuite, les cellules infestées produisent la leghémoglobine, une enzyme à très haute affinité pour l'oxygène, qui transporte et tamponne la concentration d'oxygène indispensable à la respiration des bactéroïdes (Appleby, 1984; Ott *et al.*, 2005).

La nodosité est étroitement en liaison avec le système vasculaire de la plante hôte (Fig. 3), ce qui permet les échanges de nutriments et de signaux entre les deux partenaires. La plante fournit à la nodosité, via le phloème, l'énergie nécessaire tout d'abord à sa formation puis à son fonctionnement, ceci sous forme de photosynthétats et en particulier de saccharose (Udvardi & Day, 1997). Le saccharose est transformé, dans les cellules des nodosités, en acides dicarboxyliques C-4 (malate, fumarate, succinate) qui sont ensuite transportés dans les bactéroïdes. En retour, la majeure partie de l'ammonium produit diffuse passivement depuis les bactéroïdes dans le cytoplasme des cellules infectées pour y être assimilé (Prell & Poole, 2006). Chez les légumineuses « tempérées » et l'arachide, l'N est incorporé dans des acides aminés (majoritairement asparagine et glutamine) alors que les légumineuses « tropicales » synthétisent des uréides. Ces composés azotés sont transportés par le xylème pour être utilisés par la plante pour la synthèse des molécules azotées nécessaires à son métabolisme (acides aminés, protéines et acides nucléiques).

Bases moléculaires des étapes précoces de la nodulation

La mise en place de la nodulation implique une reconnaissance entre les partenaires symbiotiques. Une coordination de l'expression de nombreux gènes chez les deux partenaires, elle-même réglementée par l'échange de signaux moléculaires, est aussi nécessaire pour induire les importantes modifications racinaires relatives à la nodulation (Perret *et al.*, 2000; Oldroyd, 2013; Janczarek *et al.*, 2015).

Côté rhizobia

Les rhizobia contenus dans la rhizosphère sont attirés par chimiotactisme vers les racines des plantes hôtes, grâce aux différentes molécules exsudées ou excrétées par celles-ci (Gaworzewska & Carlile, 1982). Les bactéries se dirigent vers la source de ces molécules ; en arrivant contre les poils racinaires, elles s'y fixent et forment un bio-film (Downie, 2010). Les flavonoïdes sont les plus connus des premiers signaux émis par la plante (Redmond *et al.*, 1986). Ils activent la protéine NodD, facteur de transcription des gènes bactériens *nod*

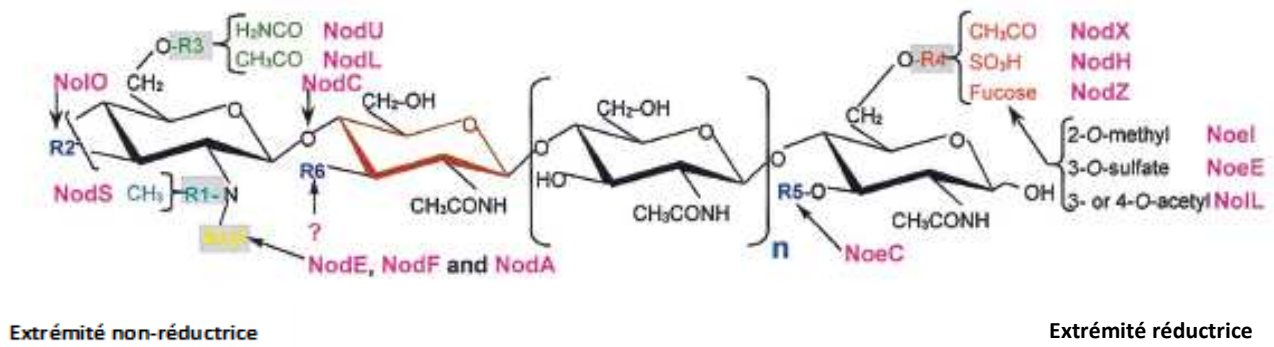


Fig. 4. Structure des facteurs Nod. Sont indiqués en rose les gènes impliqués (commun ou spécifiques) et les sites de décoration (R1 à R6). D'après Perret *et al.* 2000

(Mulligan & Long, 1985; Schultze & Kondorosi, 1998; Chen *et al.*, 2005). La protéine NodD se lie aux *nod boxes*, éléments de cis-régulation des opérons qui regroupent les gènes *nod*, et régule ainsi leur expression de manière coordonnée. Les protéines NodD sont présentes chez tous les rhizobia mais leurs propriétés symbiotiques varient d'une espèce à l'autre. La reconnaissance entre les protéines NodD d'une espèce de rhizobia et leur flavonoïde activateur est un des premiers mécanismes de spécificité dans la symbiose rhizobium-légumineuse (Spaink *et al.*, 1987; Denarie *et al.*, 1992; Maj *et al.*, 2010).

En plus des gènes régulateurs de transcription *nodD* codant pour les protéines NodD, deux autres classes fonctionnelles de gènes *nod* peuvent être identifiées : celle des gènes communs *nodABC* et celle des gènes de décoration spécifique à l'hôte (Debelle *et al.*, 2001). Les gènes *nodABC* sont responsables de la synthèse du squelette des facteurs Nod (en anglais, Nod factors ou NFs), molécules symbiotiques essentielles à la nodulation (Denarie *et al.*, 1992). Ce sont des lipo-chitooligosaccharides (LCOs), molécules composées d'un squelette de chitine comprenant trois à six résidus N-acetyl-glucosamine (GlcNAc) et dont l'extrémité non réductrice est N-acétylée avec un acide gras (Fig. 4). Le gène *nodC* code pour une N-acetylglucosamine transférase dont le rôle est de former le squelette de chitine ; *nodB* et *nodA* codent pour les enzymes qui interviennent successivement dans le processus de substitution à l'extrémité non réductrice (Perret *et al.*, 2000; Debelle *et al.*, 2001). Les gènes *nodABC* sont très conservés et présents chez les différentes espèces de rhizobia, à l'exception seulement de certains *Bradyrhizobium* (Giraud *et al.*, 2007). Les NFs produits diffèrent selon les espèces porteuses de ces gènes, avec des variations de longueur de l'oligomère de chitine et des substitutions portées par le sucre réducteur (notamment du degré de saturation de l'acide gras), indiquant une implication des gènes *nodABC* dans la spécificité d'hôte (Denarie *et al.*, 1996; D'Haese & Holsters, 2002). La diversité des gènes *nodABC* permet aussi de distinguer des biovars au sein d'une même espèce (Laguerre *et al.*, 2001; Bailly *et al.*, 2007).

Les gènes *nod* à décorations spécifiques ne sont présents que chez certaines espèces, certains symbiovars ou certaines souches parmi ces symbiovars. Ils induisent différents types de modification aux deux extrémités, non-réductrice et réductrice, du squelette de chitine. Chaque souche possède son propre cortège de gènes *nod* spécifiques qui lui permettent la production d'un cocktail de NFs (Long, 1996; Wais *et al.*, 2002). Les souches de *R. leguminosarum* sv. *viciae* (Rlv) se caractérisent par leur production d'un mélange de NFs pour lesquels les chaînes acyl (située à l'extrémité non réductrice du squelette de chitine) sont en C18:1 ou C18:4 (Spaink *et al.*, 1991; Ovtsyna *et al.*, 1999; Walker *et al.*, 2000). La substitution en C18:4 est spécifique des Rlv et est déterminé par le gène *nodE*, qui est un

déterminant majeur de leur gamme d'hôtes (Spaink *et al.*, 1991; Bloemberg *et al.*, 1995). Ce gène ainsi que le gène *nodO* sont d'avantage impliqués dans la croissance du cordon d'infection que dans la pénétration des Rlv dans les poils absorbants, ce qui suggère que la spécificité entre plante et bactérie se manifeste à différentes étapes de perception des NFs (Walker *et al.*, 2000). Un autre gène important est *NodL* ; il contrôle l'O-acétylation du sucre terminal non réducteur et influe également sur la nodulation spécifique à l'hôte (Spaink *et al.*, 1991). Les souches Rlv porteuses de *nodX* sont quant à elles les seules capables de noduler les pois de type « Cv Afghanistan » chez lesquels le gène *SYM2* est présent sous la forme allélique *sym2^A* (Lie, 1978; Young & Matthews, 1982; Davis *et al.*, 1988; Geurts *et al.*, 1997). Ce gène induit une O- acétylation du sucre réducteur (Firmin *et al.*, 1993; Ovtsyna *et al.*, 1999).

Des processus autres que ceux relatifs aux NFs sont nécessaires à la mise en place de la nodulation. Ainsi l'attachement des rhizobia est facilité par la liaison qui s'établit entre les lectines sécrétées par les plantes au niveau des poils absorbants et les polysaccharides de surface des rhizobia (en anglais, Rhizobial cell surface polysaccharides ou RCSPs) (Laus *et al.*, 2006). La composition et la structure des RCSPs varient selon les souches de rhizobia, suggérant leur implication dans la reconnaissance spécifique entre les partenaires (Robertsen *et al.*, 1981; Kawaharada *et al.*, 2015). Les fibrilles des rhizobia et des protéines de type adhésines/Ca²⁺dépendantes (ricadhésines) qu'elles produisent sont aussi impliquées dans cet attachement (Smit *et al.*, 1987). De façon plus générale, les rhizobia utilisent différents types de mécanismes de sécrétion de protéines pour adapter leur interaction avec les plantes hôtes, ceci de façon régulée par les flavonoïdes exsudés par la plante (Kobayashi *et al.*, 2004; Zehner *et al.*, 2008).

Côté légumineuses

Un très grand nombre d'études génétiques ont été réalisées chez les deux légumineuses modèles, *L. japonicus* et *M. truncatula*, qui ont permis de mettre en évidence les principaux gènes indispensables à la mise en place des premières étapes de la nodulation (perception des NFs et formation des cordons d'infection, transduction du signal et formation du primordium (Fig. 5). Certains gènes impliqués ont aussi été mis en évidence chez les espèces cultivées telles que le pois (*Pisum sativum*) et le soja (*Glycine max*).

Perception des facteurs Nod

Les récepteurs indispensables à la perception des NFs puis à la nodulation sont des LysM-RLKs. Ces receptor-like kinases (RLK) sont constitués d'un domaine kinase intracellulaire,

d'un domaine transmembranaire et d'un domaine extracellulaire comportant plusieurs motifs lysine (LysM) impliqués dans la perception des modifications des sucres réducteurs et non-réducteurs des NFs. Les LysM-RLKs ne sont connues que chez les plantes et sont codées par une famille multigénique (au moins 20 membres dans la plupart des légumineuses) ; pour une revue voir (Gough & Cullimore, 2011; Liang *et al.*, 2014). Deux gènes ont été identifiés comme indispensables à la nodulation chez plusieurs légumineuses. Le gène *MtNFP/LjNFR5/PsSYM10/GmNFR5* est indispensable à la perception des NFs (Walker *et al.*, 2000; Limpens *et al.*, 2003; Madsen *et al.*, 2003; Radutoiu *et al.*, 2003). L'autre gène, *MtLYK3/LjNFR1/PsSYM37/GmNFR1*, est impliqué plus spécifiquement dans la croissance des cordons d'infection (Geurts *et al.*, 1997; Limpens *et al.*, 2003; Zhukov *et al.*, 2008). Chez le pois, il a été montré que *PsSYM37* est situé dans une même région chromosomique que *PsSYM2*, ainsi que *PsK1* un autre gène codant pour une LysM-RLK (Zhukov *et al.*, 2008) mais la séquence de ces gènes n'a pas encore été clairement déterminée. Chez *L. japonicus*, EPR3, un autre membre de la famille LysM-RLKs, a été récemment identifié comme jouant un rôle dans la reconnaissance des rhizobia via la perception des RCSPs qu'elles produisent et ceci après induction par les NFs (Kawaharada *et al.*, 2015).

Enfin, un récepteur sérine/thréonine kinase de la famille des Leucine-Rich Repeat RLKs (LRR-RLKs) a aussi été identifié comme impliqué dans la perception des NFs. Il est codé par le gène *LjSYMRK/MtDMI2/PsSYM19/GmNORK* (Endre *et al.*, 2002; Stracke *et al.*, 2002). Récemment, il a été observé que LjSYMRK forme un complexe avec LjNFR5 (Antolín-Llovera *et al.*, 2014) et que MtDMI2 peut s'associer avec MtHMGR1, une enzyme de la voie de synthèse du mévalonate, capable elle-aussi d'induire des oscillations calciques (Venkateshwaran *et al.*, 2015).

Cascade signalétique de transduction du signal

La perception des facteurs Nod déclenche des modifications au niveau de l'épiderme des poils absorbants. Des canaux calciques sont tout d'abord activés dans la membrane plasmique provoquant un influx d'ions Ca^{2+} , suivie d'un efflux d'ions Cl^- et K^+ dans le milieu extracellulaire ; le gradient de calcium ainsi créé induirait la courbure du poil absorbant et faciliterait la formation du cordon d'infection (Esseling *et al.*, 2003). Cet influx de calcium est suivi par l'établissement d'oscillations calciques à proximité et à l'intérieur des noyaux des cellules épidermiques. Des nucléoporines ont été identifiées chez *L. japonicus* comme impliquées dans le transport du calcium à l'intérieur du noyau (Saito *et al.*, 2007). D'autres protéines situées dans la membrane nucléaire sont impliquées, en particulier celles codées par

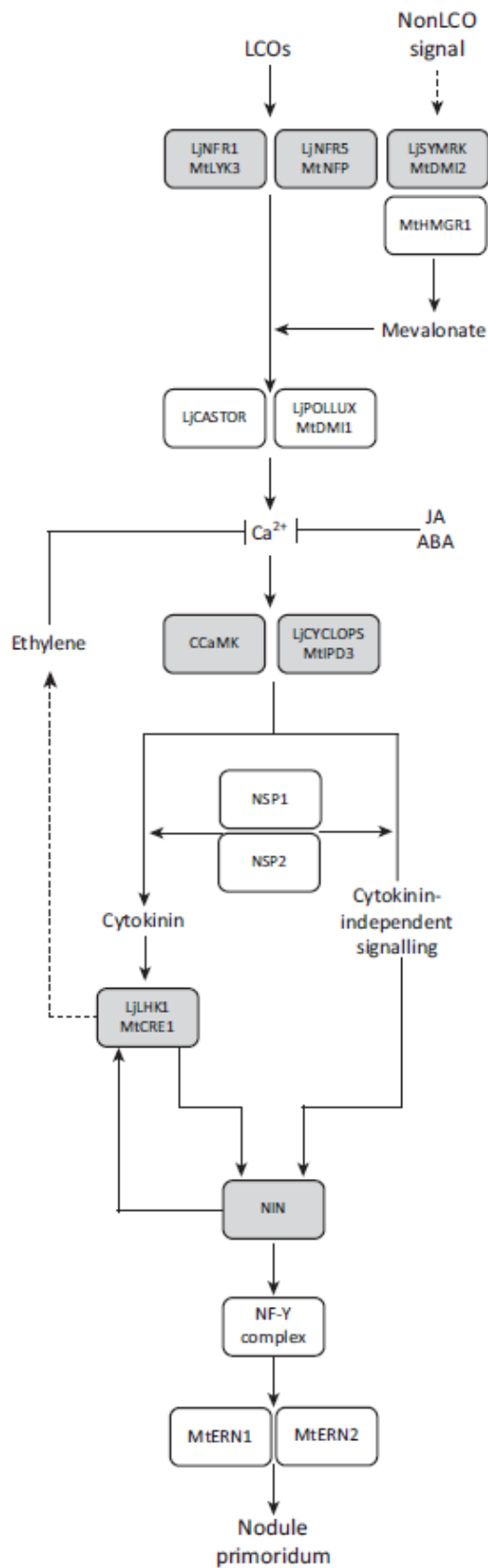


Fig. 5. Schéma conceptuel des bases moléculaires des étapes précoces de la nodulation. D’après Geurts *et al.* (2016)

MtDMI1/LjCASTOR et *LjPOLLUX* constituent des canaux permettent l'efflux de K^+ et la dépolarisation de la membrane nucléaire et du réticulum endoplasmique favorables à l'influx de Ca^{2+} (Ané *et al.*, 2004; Imaizumi-Anraku *et al.*, 2005; Riely *et al.*, 2007; Charpentier *et al.*, 2008). Des protéines responsables des oscillations calciques viennent d'être identifiées chez *M. truncatula* ; ces CNGC15 (Cyclic Nucleotide-Gated Channel) sont situées elles-aussi dans la membrane nucléaire et interagissent probablement avec DMI1 (Charpentier *et al.*, 2016).

Le signal induit par les oscillations calciques est perçu par une protéine kinase Ca^{2+} /Calmoduline dépendante (CCaMK) codée par *LjCCaMK/MtDMI3/PsSYM9* (Lévy *et al.*, 2004; Tirichine *et al.*, 2006). Cette protéine interagit avec et phosphoryle le facteur de transcription *LjCYCLOPS/MtIPD3* (Singh *et al.*, 2014). Une succession d'activation de facteurs de transcription, parmi lesquels NIN, NSP1, NSP2, ERN1 et ERN2, se produit ensuite ; pour une revue voir (Geurts *et al.*, 2016). Ces facteurs de transcription coordonnent l'expression dans l'épiderme de différents gènes codant pour des nodulines dont *ENOD11* (Andriankaja *et al.*, 2007).

Etapes précoces de la nodulation

La cascade signalétique induite par les facteurs Nod aboutit aussi à l'activation dans le cortex d'un récepteur de cytokinines (CKs) comprenant un domaine histidine kinase et codé par *MtCRE1/LjHK1* (Gonzalez-Rizzo *et al.*, 2006; Tirichine *et al.*, 2007). L'activation de ce récepteur est indispensable à l'induction des divisions cellulaires dans le cortex et la formation des primordia nodulaires (Frugier *et al.*, 2008; Plet *et al.*, 2011). Il a été montré que les activations de *MtCRE1* et de *MtNIN* dans le cortex sont corrélées de façon positive ; l'expression de l'un promouvant l'expression de l'autre (Vernié *et al.*, 2015). La forte accumulation de NIN dans le cortex induit l'activation des gènes du complexe *NF-Y* puis des gènes *MtERN1* et *MtERN2* nécessaires à la formation du nodule.

Rôle des phytohormones

Bien avant que leur rôle de signalisation via le gène *Cre1* soit démontré, les CKs ont été précocement pressenties comme jouant un rôle dans la nodulation. Leur rôle a tout d'abord été envisagé comme antagoniste à celui de l'auxine ; par une action directe sur l'accumulation d'auxine dans les cellules corticales et l'initiation des primordia nodulaires. Il avait été observé une accumulation de CKs dans les nodules de diverses légumineuses y compris le pois (Syōno & Torrey, 1976; Badenoch-Jones *et al.*, 1987) ; puis qu'une application de CKs sur les racines induisaient des divisions au niveau des cellules corticales et

l'expression de gènes codant pour les nodulines ENOD2 et ENOD40 (Hirsch *et al.*, 1997). Il a été montré récemment que l'accumulation des CKs dans les cellules du cortex des racines de *M. truncatula* suite à l'application de NFs est une étape clé pour l'induction de l'expression de la majeure partie des gènes nécessaires à la formation du nodule (van Zeijl *et al.*, 2015). Les CKs en tant que telles ne seraient pas impliquées dans les modifications du transport de l'auxine nécessaires à son accumulation dans les cellules corticales ; c'est l'accumulation de flavonoïdes induite par l'activation du gène *MtCRE1* qui en serait la cause (Ng *et al.*, 2015). L'accumulation des CKs serait quant à elle régulée négativement par l'éthylène (van Zeijl *et al.*, 2015). D'autres hormones comme l'acide abscissique (ABA) et les jasmonates (JA) ont un impact négatif sur la formation des nodules (Ferguson *et al.*, 2010) (Fig. 5).

Régulation du nombre de nodosités

La nodulation représente un coût en C élevé pour la plante, que ce soit pour la formation ou le fonctionnement des nodosités (Pate & Herridge, 1978; Voisin *et al.*, 2003a). En conséquence, la plante régule le nombre de nodosités qu'elle produit, ajustant ainsi son acquisition d'N via la fixation de N₂ à ses besoins en croissance et en fonction de la disponibilité en N sous forme minérale (ou organique) dans le milieu. Un contrôle local de la nodulation existe, par restriction du nombre d'infections et limitation de la formation du primordium nodulaire à une zone restreinte spatialement. Il existe différents modes de contrôle local de la nodulation. Chez les plantes à nodules indéterminés comme le pois et *M. truncatula*, il a été observé que les primordia se positionnent préférentiellement à proximité des vaisseaux de xylème et loin de ceux de phloème, ceci en lien avec un gradient d'éthylène (Heidstra *et al.*, 1997; Penmetsa & Cook, 1997). Récemment, il a été mis en évidence chez le soja un contrôle local par *GmNIC1*, gène codant pour un peptide CLE et dont l'expression est induite par une forte teneur en nitrate du milieu (Reid *et al.*, 2011).

La régulation du nombre de nodosités peut aussi se faire à distance ; cette régulation systémique a été nommée « autorégulation de la nodulation » (Autoregulation of nodulation, AON) (Caetano-Anolles & Gresshoff, 1991). Elle met en œuvre des signaux émis par les racines et reçus par les parties aériennes qui, en retour, envoient des signaux aux racines. Des mutants hypernodulants sont connus depuis longtemps chez différentes légumineuses dont le pois (Postma *et al.*, 1988; Sagan & Duc, 1996) ; la plupart ne perçoivent pas, au niveau de leur partie aérienne, un signal émis par leurs racines et sont donc impactés dans leur AON (Caetano-Anolles & Gresshoff, 1991). Il a été trouvé qu'ils présentent une mutation dans des gènes, *MtSUNN/LjHARI/GmNARK/PsSYM29*, codant pour des récepteurs LRR-RLKs.

(Leucine-Rich Receptor Like Kinase) ; (Krusell *et al.*, 2002; Nishimura *et al.*, 2002; Searle *et al.*, 2003; Schnabel *et al.*, 2005). Plus récemment, des peptides CLE (MtCLE12/13, LjRCLE-RS1/RS2, GmRIC1/2) émis par les racines et induits par les rhizobia ont été identifiés comme jouant ce rôle de molécule signal chez *M. truncatula*, *L. japonicus* et le soja (Mortier *et al.*, 2010; Reid *et al.*, 2011; Okamoto *et al.*, 2013).

Les teneurs élevées en N minéral inhibent la formation des nodosités (Carroll & Mathews, 1990; Barbulova *et al.*, 2007). Outre la réponse localisée, il existe aussi une réponse systémique, et les mutants hypernodulants sont connus depuis longtemps comme étant nitrate-tolérants (Carroll & Mathews, 1990; Sagan & Duc, 1996; Wopereis *et al.*, 2000; Schnabel *et al.*, 2005). Il a été montré récemment que le gène *LjHARI* est aussi impliqué dans cette régulation, comme récepteur du peptide CLE-RS2 émis par les racines soumises à une forte teneur en nitrate (Okamoto & Kawaguchi, 2015).

3. Déterminants moléculaires et génétiques de la mise en place du système racinaire chez les dicotylédones

Comparé à celui des céréales, le système racinaire des dicotylédones est peu développé. Chez les monocotylédones, plusieurs types de racines coexistent : les racines latérales qui se développent à partir de la racine primaire, les racines séminales et les racines adventives à partir du collet (Wachsman *et al.*, 2015). Chez les dicotylédones, le système racinaire est principalement pivotant, composé d'une racine principale (ou pivot) initiée au stade embryonnaire et de racines latérales qui apparaissent de manière acropète sur la racine principale puis sur les racines latérales d'ordre précédent. Plus occasionnellement des racines adventives peuvent aussi apparaître (Sorin *et al.*, 2005). La formation des racines latérales a été très étudiée chez *Arabidopsis thaliana* (*Arabidopsis*), modèle d'étude pour les plantes dicotylédones. Les études sur les racines des légumineuses sont beaucoup plus récentes.

Formation des racines latérales chez les dicotylédones (modèle *Arabidopsis*)

Depuis 20 ans, un très grand nombre d'études cellulaires et moléculaires de la formation des racines ont été réalisées sur *Arabidopsis* ; pour une revue voir (Peret *et al.*, 2009). C'est ce modèle que nous allons décrire ci-dessous.

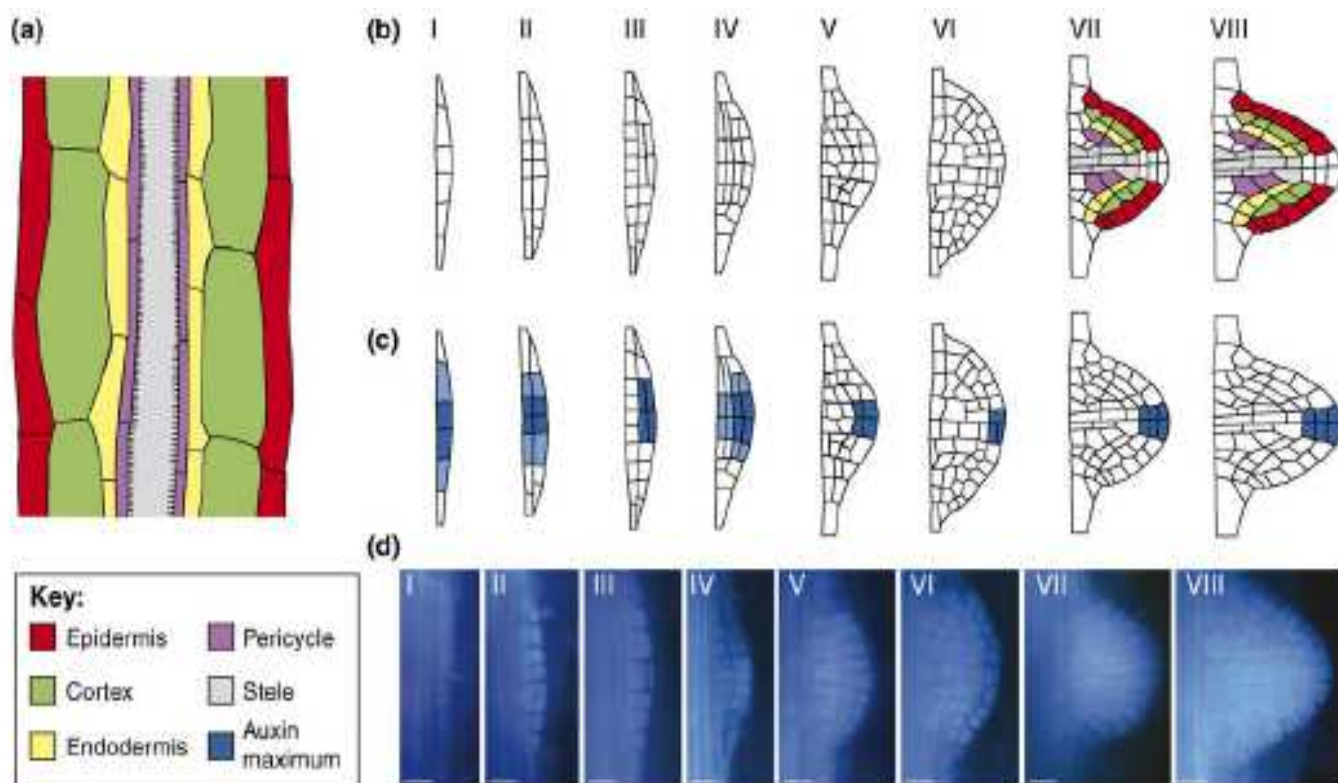


Fig. 6. Formation d'une racine latérale chez *Arabidopsis thaliana* (a) les différents tissus constitutifs d'une racine primaire (b) les 8 stades de développement du primordium racinaire formé à partir de cellules du péricycle de la racine primaire (c) les gradients de concentration en auxine (d) racines colorées par du bleu d'aniline ; barre d'échelle = 20µm. D'après Perret *et al.* (2009)

Contrairement aux ramifications aériennes, les racines latérales ne sont pas formées à partir de méristèmes axillaires préexistants dans l'embryon. Les primordia des racines sont initiés à partir de la dédifférenciation de quelques cellules du péricycle de la racine primaire, ceci à proximité de son apex (Fig. 6). Ces cellules fondatrices subissent alors des divisions anticlinales pour créer un primordia composé d'un maximum de dix petites cellules de longueur égale ; c'est le stade I de son développement (Malamy & Benfey, 1997). Une succession de divisions péricleinales et anticlinales d'un nombre limité de cellules aboutit à la formation progressive des différents tissus représentatifs d'une racine et confère une forme de dôme au méristème. Ce méristème traverse successivement les différents tissus de la racine primaire (endoderme, cortex, épiderme), ceci des stades IV à VI de son développement. Au stade VII, une nouvelle racine émerge de la racine primaire au stade VIII par expansion cellulaire.

Les phytohormones jouent un rôle prépondérant pour la formation des racines. Ce rôle a été abondamment décrit chez *Arabidopsis* et étudié plus récemment chez *M. truncatula*. Chez *Arabidopsis*, l'initiation des racines latérales se fait en réponse à un signal auxine, suite à l'expression du gène DR5 (De Smet *et al.*, 2007). L'accumulation d'auxine nécessaire aux premiers stades de formation des méristèmes est modulé notamment par les transporteurs AUX1, PINs et PGP/MDR et se fait grâce au transport acropétal via le phloème de l'auxine des méristèmes foliaires vers l'apex racinaire puis basipétal depuis la racine primaire (Casimiro *et al.*, 2001; Casson & Lindsey, 2003; Wu *et al.*, 2007). Lors de l'émergence de la nouvelle racine latérale, le primordium acquiert la capacité à produire de l'auxine mais néanmoins, l'afflux d'auxine venant des autres organes reste nécessaire à son allongement (Wu *et al.*, 2007). Le rôle négatif des CKs sur la formation des racines latérales a aussi été souvent souligné ; en particulier, les CKs interfèrent avec l'expression des gènes PINs, perturbant ainsi la formation du gradient d'auxine et l'initiation des racines (Laplaze *et al.*, 2007). Il a été observé chez *Arabidopsis* et *M. truncatula* que les flavonoïdes ont un impact négatif sur l'allongement des racines via une diminution du transport de l'auxine vers l'apex des racines (Peer *et al.*, 2004; Peer & Murphy, 2007; Laffont *et al.*, 2010). Enfin, il a été montré que les gibbérellines (GA), en stimulant la dégradation des protéines DELLAs, ont un effet antagoniste vis-à-vis des effets négatifs de l'éthylène sur l'allongement des racines (Achard *et al.*, 2003). L'auxine est quant à elle aussi connue pour favoriser l'allongement des racines en améliorant la destruction des protéines DELLAs par les GA (Ubeda-Tomas *et al.*, 2008).

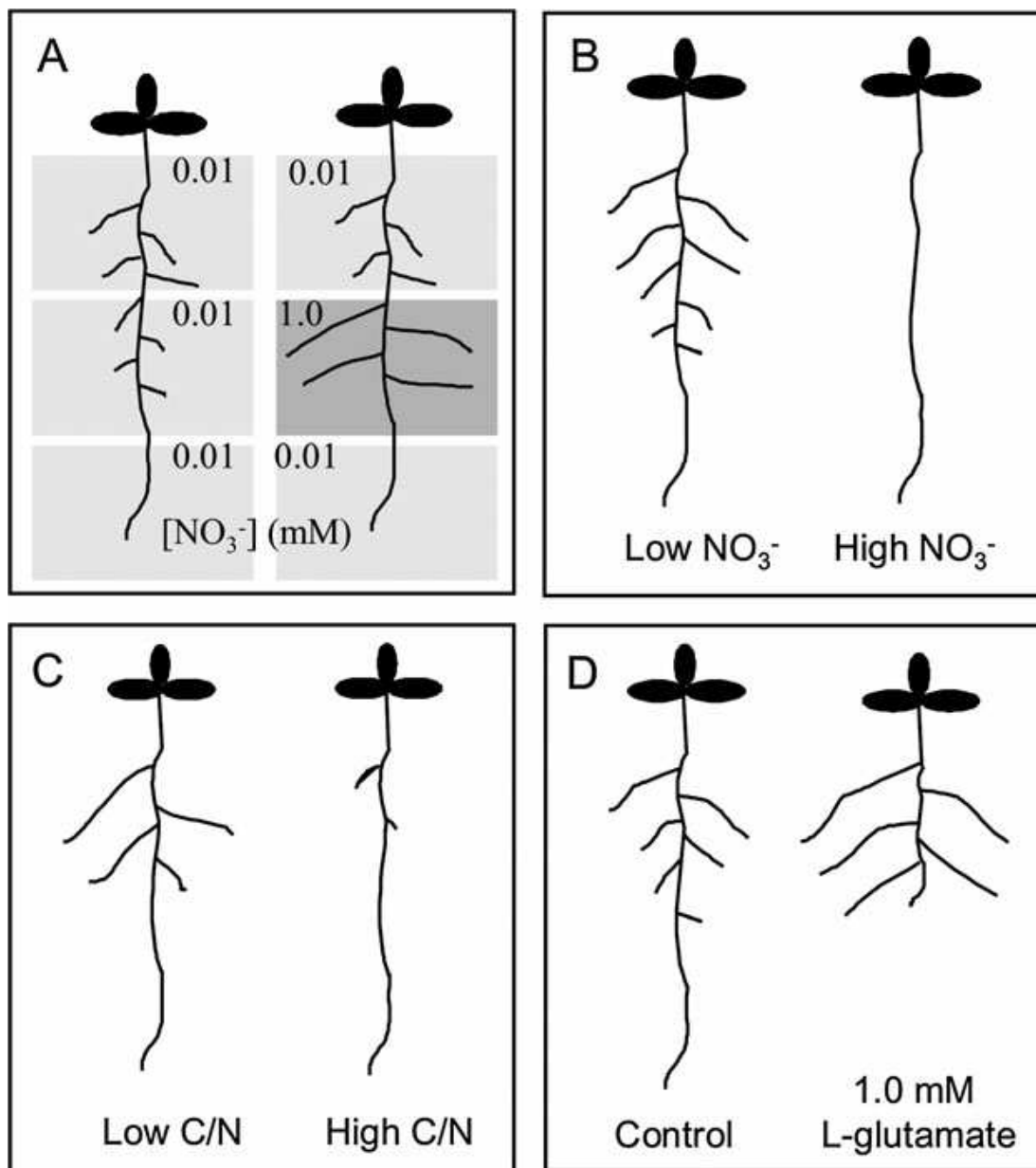


Fig. 7. Quatre modifications du système racinaire chez *Arabidopsis* en réponse à la disponibilité en N du milieu (A) Effet stimulateur de l’allongement des racines d’un apport localisé de NO_3^- (B) Inhibition de l’émergence des racines par un apport uniforme et très élevé en NO_3^- (C) Inhibition de l’initiation des racines par un faible rapport C/N (D) Effet d’un apport de glutamate : inhibiteur de l’allongement de la racine principale et stimulateur d’initiation et d’allongement des racines latérales. D’après Zhang *et al.* (2007)

Bases moléculaires de l'acquisition d'N et du développement des racines

Acquisition d'azote par les racines

Les plantes sont capables d'acquérir de l'N à partir des différentes formes présentes dans la rhizosphère ; principalement l'N sous forme minérale (ammonium, nitrate) présent quasiment universellement dans tous les sols mais aussi les composés organiques azotés solubles (urée, peptides, acides aminés) (Näsholm *et al.*, 2009). Comme pour la mise en place du système racinaire, l'étude des gènes impliqués dans le prélèvement des deux formes minérales a tout d'abord été très étudié chez *Arabidopsis*. Quelques gènes sont connus chez *M. truncatula* et chez *L. japonicus*.

Le nitrate et l'ammonium sont prélevés activement par les racines grâce à des transporteurs membranaires; pour une revue voir (Courty *et al.*, 2015; Kiba & Krapp, 2016). Les plantes acquièrent préférentiellement du nitrate comparativement à de l'ammonium qui en excès peut être toxique. Deux familles de transporteurs d'ammonium ont été identifiées chez *Arabidopsis* ; la famille AMT1 contrôle le transport et l'acquisition de l'ammonium et la famille AMT2 est impliquée dans des processus de régulation en fonction de la disponibilité en ammonium (Sohlenkamp *et al.*, 2002; Yuan *et al.*, 2007). Trois AMT1 et deux AMT2 ont été caractérisés chez *L. japonicus* (D'Apuzzo *et al.*, 2004; Rogato *et al.*, 2010). Les transporteurs de nitrate sont divisés en deux familles, NRT1/PTR/NFP et NRT2. Chez *Arabidopsis*, il a été identifié 53 membres de la famille NFP et 7 membres de la famille NRT2. Les membres de la famille NRT2 sont préférentiellement des transporteurs à haute affinité (high-affinity transporters, HATs) c'est-à-dire actifs lorsque la teneur en NO_3^- du milieu est faible (inférieure à 1 mM). Les transporteurs de la famille des NRT1/PTR/NFP sont préférentiellement des transporteurs à faible affinité (low-affinity transporters, LATs), actifs lorsque la teneur en NO_3^- du milieu est élevée (jusqu'à 50 mM), excepté NRT1.1 qui est à double affinité. Deux transporteurs NRT1 ont été identifiés chez *M. truncatula* : NIP/LATD et NRT1.3. Contre toute attente, MtLATD est un transporteur à haute affinité ; et il est surtout connu pour son rôle dans le développement racinaire et la nodulation (Yendrek *et al.*, 2010; Bagchi *et al.*, 2012). MtNRT1.3 n'aurait pas un rôle majeur comme transporteur de nitrate mais serait d'avantage impliqué dans la réponse de la plante à la limitation de la disponibilité en nitrate dans le milieu (Morère-Le Paven *et al.*, 2011).

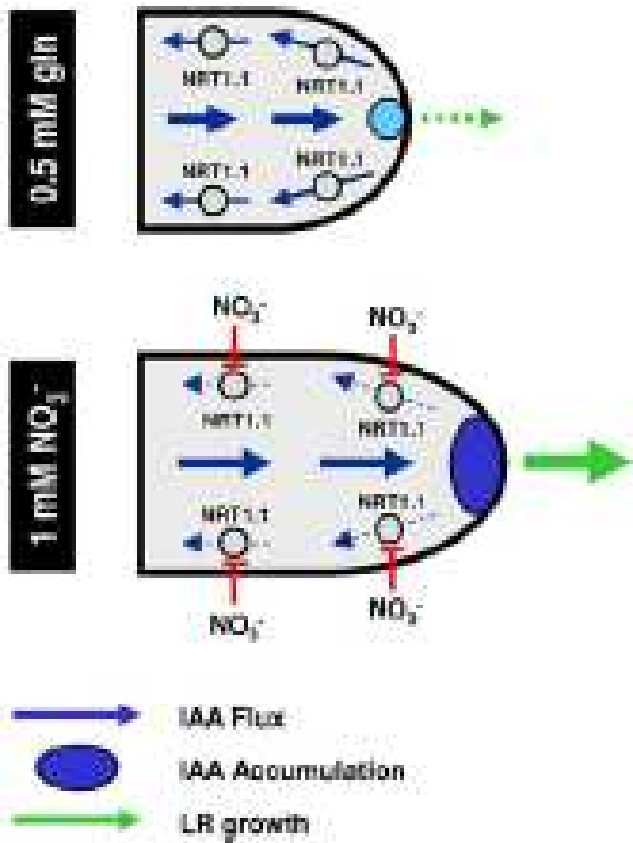


Fig. 8. Représentation schématique du contrôle de l’allongement des racines latérales par NRT1.1 chez *Arabidopsis* (A) En absence de NO_3^- , NRT1.1 active le transport basipétal de l’auxine ; l’allongement des racines est donc réduit car l’auxine ne s’accumule pas dans les apex (B) Un apport de 1 mM de NO_3^- réprime l’activation du transport basipétal de l’auxine par NRT1.1 ; l’auxine s’accumule dans les apex et l’allongement des racines est favorisé. D’après Krouk *et al.* (2010)

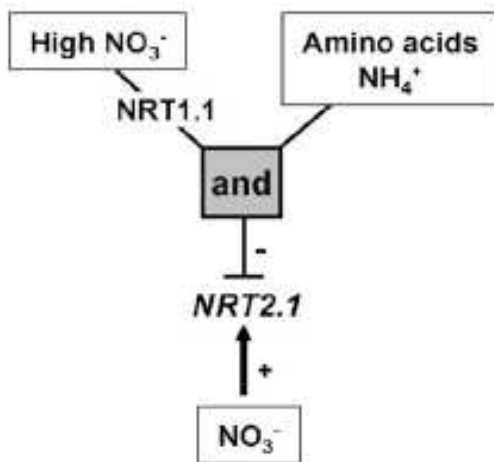


Fig. 9. Représentation schématique de la régulation de l’expression de NRT2.1 par N dans les racines d’*Arabidopsis*. En plus de l’induction par NO_3^- , l’expression de NRT2.1 est réprimée à la fois par les métabolites azotés présents dans le phloème et par une forte teneur en NO_3^- du milieu. D’après Krouk *et al.* (2006)

Développement des racines en interaction avec la ressource azotée minérale

Les racines ont la capacité de s'adapter de façon très importante à leur environnement local, ce qui leur permet de satisfaire au mieux les besoins de la plante, en particulier ceux en N (Li *et al.*, 2016). Des modifications de la croissance et du développement des racines ont été décrites chez *Arabidopsis* en réponse à des modifications de disponibilité de différentes formes d'N (Zhang *et al.*, 2007) (Fig. 7). Parmi ces réponses, la stimulation locale de l'allongement des racines par un apport de nitrate est la plus connue. Cette stimulation implique deux principales protéines, ANR1, un facteur de transcription MAD-box, et en amont, NRT1.1 impliqué comme nitrate-senseur dans la voie de signalisation, (Zhang & Forde, 1998; Remans *et al.*, 2006a). Il a été ensuite montré que NRT1.1 est aussi un transporteur d'auxine, permettant un fin ajustement de l'accumulation de l'auxine dans les apex des racines latérales et donc de leur allongement en fonction de la teneur en nitrate du milieu (Krouk *et al.*, 2010) (Fig. 8). L'allongement des racines est ainsi favorisé en présence d'un apport homogène et modéré de nitrate (<1mM). NRT1.1 a aussi un rôle antagoniste contre l'effet localement inhibiteur du glutamate sur la croissance de la racine principale (Walch-Liu & Forde, 2008). Un apport d'ammonium, quant à lui, provoque une augmentation de l'initiation de racines (Lima *et al.*, 2010).

Inversement, il a été observé qu'un très fort apport de nitrate (>>10mM) entraîne une inhibition de l'émergence des racines latérales (Zhang *et al.*, 1999; Remans *et al.*, 2006b) et de l'allongement de la racine primaire (Linkohr *et al.*, 2002). Cette inhibition mettrait en jeu une régulation systémique semblable à celle impliquant une répression du développement des racines par les produits d'assimilation de l'N (Zhang *et al.*, 1999; Walch-Liu *et al.*, 2006a; Gifford *et al.*, 2008; Gojon *et al.*, 2009). L'expression de gènes codant pour des transporteurs AMT1 et pour des transporteurs à haute-affinité NRT2 est réprimée lors d'un fort apport de nitrate ; c'est le cas en particulier du gène *AtNRT2.1* sous contrôle de *AtNRT1.1* (Krouk *et al.*, 2006; Loqué *et al.*, 2006; Remans *et al.*, 2006b) (Fig. 9). Les produits d'assimilation de l'N tels que la glutamine et l'asparagine pourraient être des signaux de satiété induisant une répression de l'émergence et de l'allongement des racines latérales (Gifford *et al.*, 2008; Ivanov *et al.*, 2012).

Une coordination très précise, via des molécules signal, de la modulation de l'architecture du système racinaire en réponse à la fourniture d'azote est indispensable. En effet, la plante investit du C et met en place des réponses adaptées au niveau de ses racines lui permettant une acquisition d'N suffisante pour soutenir la formation de l'appareil

photosynthétique. Le niveau d'initiation de racines latérales a ainsi été montré comme étant lié au rapport C/N (Malamy & Ryan, 2001) (Fig. 7). De même, la plupart des modifications de l'architecture racinaire en réponse à différents régimes de nutrition azotée peuvent être expliquées par des modifications des allocations de C au sein du système racinaire (Brun *et al.*, 2010).

Les hormones, en particulier l'auxine et les CKs, ont été précocement pressenties comme jouant un rôle comme molécules signal de l'état de nutrition azotée de la plante. Ainsi, l'accumulation de nitrate dans les feuilles (suite à un fort apport de nitrate) provoquerait une inhibition de la synthèse d'auxine dans les feuilles et donc de son transport vers les racines et de l'émergence des racines latérales (Forde, 2002; Walch-Liu *et al.*, 2006a). Les CKs ont été identifiés comme étant des signaux d'état d'insuffisance d'alimentation en N et il a été montré qu'un fort apport en nitrate induit une forte augmentation de la teneur en CKs des feuilles (Kiba *et al.*, 2011; Ruffel *et al.*, 2011). L'augmentation de CKs qui en résulte dans les racines y aurait un impact négatif sur le transport de l'auxine et inhiberait leur croissance. D'autres hormones comme l'éthylène et l'ABA sont aussi impliquées dans la réponse systémique à la disponibilité en N (Walch-Liu *et al.*, 2006a; Tian *et al.*, 2009; Ruffel *et al.*, 2011).

4. Ajustement entre les deux voies d'acquisition d'azote chez les légumineuses

Le système racinaire des légumineuses est nodulé : il est le support non seulement de l'acquisition de l'azote minéral du sol par les racines mais aussi de la fixation symbiotique de l'azote atmosphérique au sein des nodosités, et les proportions racines/nodosités sont variables selon l'état de la plante et de son milieu. Les développements racinaire et nodulaire sont en compétition pour l'utilisation des ressources disponibles en C, ce qui nécessite un ajustement très complexe entre ces développements pour satisfaire au mieux les besoins en N nécessaires à la croissance de la plante. Les molécules signal impliquées dans cette régulation font l'objet d'études chez les légumineuses modèles comme *M. truncatula*. Elles ne sont pas encore connues chez les plantes cultivées. Les légumineuses à graines quant à elles, et notamment le pois, ont fait l'objet de nombreuses études montrant un ajustement aux cours du cycle de la culture entre les deux voies d'acquisition d'N.

Signalétique de la régulation entre les deux organogènes racinaire et nodulaire

Les deux types d'organes sont initiés de façon post-embryonnaire et chez les légumineuses à nodosités à croissance indéterminée, à partir des mêmes tissus cellulaires ; péricycle, endoderme et cortex. Ainsi que nous l'avons vu dans les paragraphes précédents, la formation des nodosités et celle des racines latérales sont toutes les deux favorisées en conditions de faible teneur en nitrate du milieu et sont régulées par les mêmes phytohormones, le plus souvent de façon antagoniste. Différentes études menées chez *M. truncatula* ont confirmé cette double implication des hormones. Ainsi, l'implication du transport d'auxine pour la formation à la fois des racines et des nodosités a été démontrée : *MtLAX*, un gène codant pour un transporteur d'auxine, s'exprime dans les deux types de primordia, racinaire et nodulaire (de Billy *et al.*, 2001); de plus il a été observé que le nombre de nodosités et de racines formées est corrélé positivement à l'intensité du transport d'auxine via le phloème entre les parties aériennes et les racines (Jin *et al.*, 2012). Les études relatives au rôle de signalisation des CKs via le gène *MtCRE1* ont montré qu'elles sont indispensables à la formation des nodosités avec un impact négatif sur le développement des racines latérales (Gonzalez-Rizzo *et al.*, 2006; Frugier *et al.*, 2008; Laffont *et al.*, 2015).

En complément des phytohormones classiques, il a été montré récemment que des petits peptides sont des régulateurs potentiels de la nodulation et de la formation des racines. Il existe deux types principaux de petits peptides, les CLEs et les CEPs ; pour une revue voir (Djordjevic *et al.*, 2015; Okamoto *et al.*, 2016).

Les peptides CLE (CLAVATA3/EMBRYO SURROUNDING REGION) sont les plus étudiés de petits peptides. Ils sont constitués par 12 à 13 acides aminés. 32 peptides CLE ont été identifiés chez *Arabidopsis*. Ainsi que nous l'avons vu précédemment, les peptides *MtCLE12/13*, *LjRCLE-RS1/RS2* et *GmRIC1/2* ont été identifiés chez les légumineuses modèles et chez le soja, comme étant des molécules signal émises par les racines, induites essentiellement par l'inoculation par les rhizobies mais aussi par des fortes teneurs en nitrate, et impliquées dans la voie d'autorégulation du nombre de nodosités (Mortier *et al.*, 2010; Reid *et al.*, 2011; Okamoto *et al.*, 2013; Okamoto & Kawaguchi, 2015) (Fig. 10a). Ces signaux sont perçus dans les feuilles par les récepteurs LRR-RLKs codés par *MtSUNN/LjHARI/GmNARK/PsSYM29* (Krusell *et al.*, 2002; Nishimura *et al.*, 2002; Searle *et al.*, 2003; Schnabel *et al.*, 2005). Le signal synthétisé en retour au niveau des feuilles pourrait être constitué de CKs (Okamoto *et al.*, 2016).

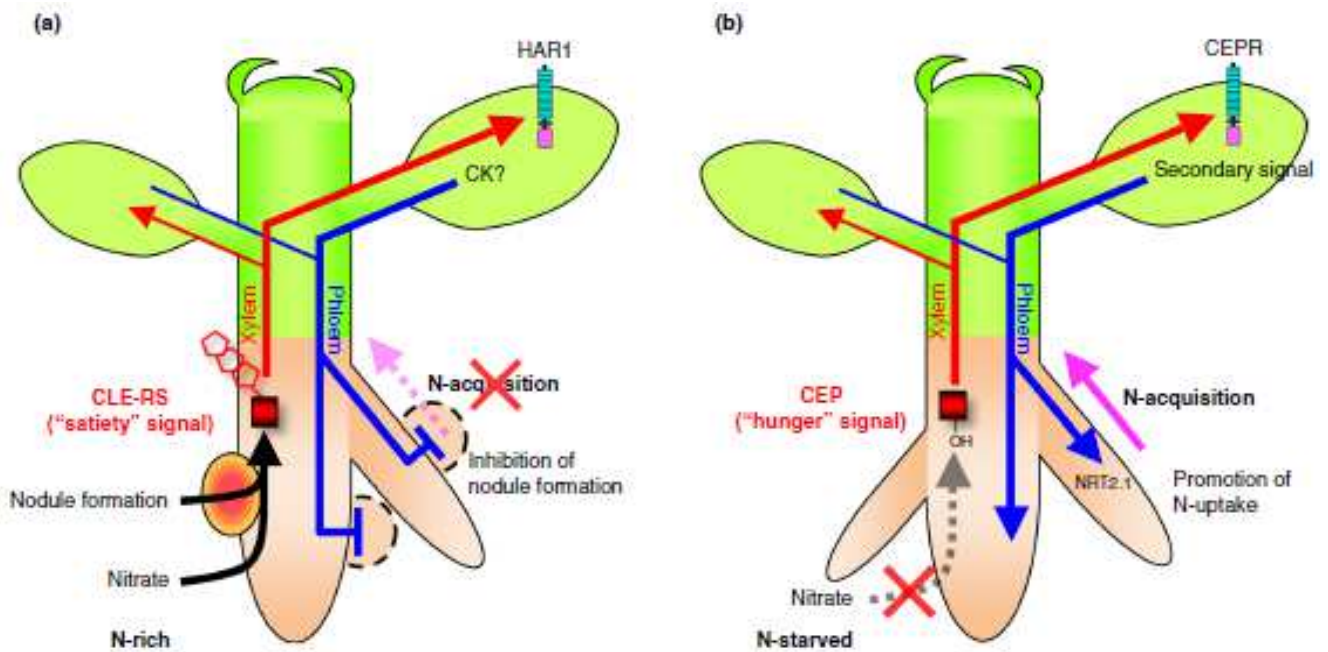


Fig. 10. Représentation des voies signalétiques de régulation du nombre des nodosités et de racines latérales (a) Chez *Lotus japonicus*, les peptides CLE-RS1/2 sont un signal de « satiété » transmis aux feuilles. CLE-RS1/2 sont induits par l'inoculation avec des rhizobia et CLE-RS2 est aussi induit par une forte teneur en nitrate du milieu. Le récepteur HAR1 reconnaît les peptides CLE-RS1/2 et envoie un signal, probablement sous forme de CKs, aux racines qui induit une inhibition de la nodulation (b) Chez *Arabidopsis*, les peptides CEPs sont un signal de « faim » en N transmis aux feuilles. Ils sont induits par une carence locale en nitrate. Le récepteur CEPR1 reconnaît les peptides CEPs et envoie un signal secondaire à des racines situées dans une zone non carencée pour y stimuler l'acquisition de nitrate. D'après Okamoto *et al.* (2016).

Les CEPs (C-TERMINALLY ENCODED PEPTIDES) sont des peptides constitués de 15 acides aminés. 11 gènes codant pour des CEPs sont connus chez *Arabidopsis* ; parmi lesquels sept sont sur-exprimés en conditions de carence en nitrate dans le milieu (Tabata *et al.*, 2014). Il est connu qu'il existe une réponse compensatoire à une carence locale en nitrate via une augmentation du prélèvement par les racines situées dans un milieu riche en nitrate et impliquant une régulation systémique (Ruffel *et al.*, 2011). Tabata *et al.* (2014) ont montré que les CEPs associés à des récepteurs CEP (CEPRs) de type LRR-RLK sont des éléments de cette régulation : les CEPs agissent comme des signaux ascendants de carence en nitrate, des racines vers les parties aériennes, où leur perception par *AtCEPR1* stimule l'expression des gènes transporteurs de nitrate probablement via la production d'un signal lui-même transmis aux racines (Fig. 10b). Chez *M. truncatula*, 11 gènes codants pour des CEPs ont aussi été identifiés ; il a été montré qu'une surexpression de l'un d'eux, *MtCEP1*, induit une inhibition de la formation des racines latérales et un accroissement du nombre de nodosités (Imin *et al.*, 2013). Parallèlement, *MtCRA2*, un homologue de *AtCEPR1*, a été identifié comme régulant positivement et de façon systémique la formation des nodosités, et négativement et localement celle des racines latérales (Huault *et al.*, 2014). *MtCRA2* est probablement un récepteur de *MtCEP1*, l'ensemble constituant une voie de régulation des développements racinaire et nodulaire différente de celle sous le contrôle de peptides CLE et de leur récepteur *MtSUNN* (Mohd-Radzman *et al.*, 2016).

Ajustement de la nodulation au cours de la croissance du pois

Des études écophysiologicals, menées en conditions de culture contrôlées ou au champ, ont mis en évidence que les deux voies d'acquisition d'N sont impliquées de façon différentielle au cours de la croissance du pois. Les études ont été menées principalement chez des variétés cultivées, mais aussi chez des mutants hypernodulants.

Que ce soit chez les variétés cultivées ou chez les mutants hypernodulants, l'activité fixatrice ne démarre pas immédiatement après la levée des plantules ; les premières nodosités sont généralement observées dans un délai de 10 à 15 jours (Voisin *et al.*, 2010). Pendant cette période précoce, la croissance de la plante repose uniquement sur les réserves contenues dans la graine (phase hétérotrophe) (Tricot *et al.*, 1997). En l'absence d'apport d'N minéral, une carence en N peut s'établir de façon temporaire pendant la période de formation des nodosités, entre le moment où les réserves de la semence sont épuisées et celui où les nodosités nouvellement formées deviennent fonctionnelles. En conditions agricoles, une telle carence est peu observée chez le pois, car compensée par les reliquats azotés présents dans les

sols (Voisin *et al.*, 2002a). Il a été observé néanmoins que la mise en place des nodosités est retardée si les reliquats azotés sont élevés ; l'acquisition de l'N se fait alors par assimilation racinaire des nitrates du sol. Cette assimilation racinaire diminue ensuite jusqu'à épuisement de l'N minéral du sol et l'inhibition de l'activité fixatrice est alors levée (Voisin *et al.*, 2002b). En l'absence d'N minéral, la majeure partie des nodosités est formée chez les cultivars lors de la première vague de nodulation. Les nodosités sont donc localisées majoritairement sur la partie supérieure du système racinaire ; leur densité est maximale à la base du pivot et sur les racines latérales situées à ce niveau, puis elle décroît rapidement à mesure que l'on s'éloigne de la base (Tricot *et al.*, 1997; Remmler *et al.*, 2014). Le nombre maximum de nodosités est atteint généralement à début floraison puis, en général, stagne jusqu'à la fin du franchissement du stade limite d'avortement des graines avant de chuter fortement en fin de cycle (Bourion *et al.*, 2007; Voisin *et al.*, 2010). Les nodosités formées deviennent sénescents, l'activité fixatrice décroît et l'acquisition d'N repose alors sur l'assimilation par les racines des nitrates du sol.

5. Diversité naturelle et évolution de la sélection du pois

Une très grande diversité phénotypique et génétique existe au sein du genre *Pisum*. Elle a fait l'objet d'études dès le milieu du 19^e siècle ; les travaux les plus connus étant ceux de Gregor Mendel (1822-1884) et de Nikolai Vavilov (1887-1943). Les processus de sélection végétale, menés pour répondre à l'évolution de la consommation et des modes de culture du pois, ont évolué au cours du temps. La connaissance de l'ensemble de cette diversité est indispensable à son utilisation dans les programmes de sélection végétale à venir.

La domestication du pois

Le pois est l'une des premières espèces végétales domestiquées dont les graines ainsi que celles d'autres légumineuses (lentille, pois chiche, fève) formaient avec les graines de céréales une composante alimentaire importante des premières civilisations du Moyen-Orient (Zohary, D. & Hopf, M., 1973; Mikic *et al.*, 2014a). Les premières traces de culture du pois ont été trouvées dans la région du Croissant Fertile s'étendant d'Israël à l'Irak en passant par le sud-est de la Turquie ; elles datent du début de Néolithique (7000-6000 avant J-C). La culture du pois s'est ensuite propagée vers l'ouest (Turquie, Grèce, Bulgarie) et vers l'est (Caucase, Iran, Afghanistan). L'Éthiopie est considérée comme étant un centre secondaire de diversification (Vershinin *et al.*, 2003; Jing *et al.*, 2010). À l'âge du bronze les pois étaient présents dans

toute l'Europe, de l'Est (Hongrie, Pologne, Russie) à l'Ouest (France, Suisse, Allemagne) (Zohary, D. & Hopf, M., 1973; Mikic *et al.*, 2014a). En parallèle, la culture de pois s'est déplacée vers l'Inde, où les premières références se trouvent vers 200 av. J.-C., et vers la Chine (Chimwamurombe & Khulbe, 2011; Smykal *et al.*, 2015).

Les premières études de classification, basées sur la morphologie des plantes et de leurs graines, leur zone géographique et sur les caryotypes, ont identifié cinq espèces parmi le genre *Pisum* : *P. sativum*, *P. humile*, *P. elatius*, *P. abyssinicum* et *P. fulvum* (Fourmont, 1956; Ben Ze'ev & Zohary, 1973). Actuellement, deux espèces seulement sont le plus généralement reconnues, *P. fulvum* et *P. sativum*, elle-même subdivisée en trois sous-espèces : *sativum*, *elatius* et *abyssinicum* (Smykal *et al.*, 2011). *P. fulvum*, dont la zone géographique est restreinte au Croissant Fertile, et *P. sativum* subsp. *elatius*, plus largement distribuée à travers le bassin méditerranéen, sont actuellement considérés comme étant les parents sauvages du pois cultivé *P. sativum* subsp. *sativum*. *P. sativum* subsp. *abyssinicum* dont la zone géographique se situe au Yémen et en Ethiopie est aussi un pois cultivé mais qui est considéré comme ayant dérivé indépendamment, jusqu'à constituer une troisième espèce (Vershinin *et al.*, 2003; Jing *et al.*, 2010).

Le pois est une espèce annuelle et préférentiellement autogame. Chez la plupart des pois cultivés, les fleurs sont cléistogames (l'autopollinisation a lieu avant l'ouverture de la fleur). Chez les pois sauvages ou fourragers la cléistogamie est moins stricte ; des Hyménoptères peuvent visiter les fleurs et transporter le pollen.

Les principales évolutions de la sélection du pois

Différents écrits datant de l'Antiquité puis du Moyen Age mentionnent que le pois était consommé en grains secs et avait un rôle déterminant dans la lutte contre les famines (Fourmont, 1956). Le pois mangetout, c'est-à-dire avec des gousses sans parchemin donc consommables, était déjà connu au XVIe siècle. C'est à cette époque que l'on commence à distinguer le pois des jardins et le pois des champs. Le pois commença à être consommé sous forme de grains verts retirés de la gousse au XVIIe siècle; il était très apprécié à la cour de Louis XIV et par la noblesse anglaise. Une des premières variétés dénommées connues est le pois « Michaux de Paris » ; elle aurait été créée en 1660. A partir de la fin du XVIIIe siècle sont créées beaucoup de nouvelles variétés en France ; telles que « Corne de bélier », « Merveille d'Etampes » ou « Serpette d'Auvergne » (Vilmorin-Andrieux, 1883). Les variétés devaient produire des grains verts et lisses et répondre aux exigences telles que productivité, facilité de récolte, précocité échelonnée pour un approvisionnement des marchés sur une

grande période de l'année. Les Anglais ont également beaucoup travaillé à l'amélioration du pois, notamment de sa précocité et de la taille des gousses et des grains ; les variétés les plus connues sont les pois Marrow ridés de Knight, « Champion d'Angleterre », « Plein le panier » et « Merveille de Kelvedon ». Les variétés françaises et anglaises pouvaient être naines ou à hautes tiges avec de nombreuses ramifications (pois à rames). Certaines des anciennes variétés françaises ou anglaises sont encore inscrites sur le catalogue des pois potagers français (<http://www.gnis.fr/index/action/page/id/257/title/Catalogues-francais>). Ce catalogue a été créé en 1952, année où toutes les variétés créées auparavant y ont été officiellement inscrites.

Entre les années 1950 et les années 1970, les industries de conserverie et de surgélation se sont beaucoup développées en France (Doré & Varoquaux, 2006). La production de pois potager a quintuplé en France ; elle est devenue ainsi le premier producteur en Europe de pois de conserve. Le pois appertisé représentait dans les années 1970 plus de 40% des conserves de légume. C'est dans ce contexte qu'ont été créées les premières variétés de pois potagers d'hiver dans un but d'allongement de la période de récolte des pois frais (Cousin, 1976).

En 1973, suite à l'embargo des Etats-Unis sur le soja, il est devenu nécessaire de cultiver en Europe des plantes riches en protéines destinées à l'alimentation du bétail. Un plan « protéines » accordant des subventions pour la production locale de cultures riches en protéines a été mise en place en Europe. Il a abouti à un intérêt accru pour le pois protéagineux, c'est-à-dire d'un pois cultivé en plein champ pour sa production de grains secs riches en protéines. Les entreprises semencières et les instituts publics en France, Suède, aux Pays-Bas, Danemark et Royaume-Uni ont investi des moyens sur la recherche de variétés adaptées et productives. En France, un catalogue des pois protéagineux a été créé en 1976. Les premières variétés de pois protéagineux ont été obtenues à partir de croisements entre des pois utilisés comme fourrages et des pois potagers ou de casserie. Les pois fourragers se caractérisent par leur haute taille et le plus souvent par la couleur anthocyanée de leurs fleurs. Il existe quelques variétés inscrites dans un catalogue (créé en 1961). Les pois de casserie sont des pois destinés à l'alimentation humaine, récoltés en sec et à forte teneur en protéines de leurs graines ; leur sélection spécifique a pris fin en 1977 (Doré & Varoquaux, 2006). Actuellement, les graines de pois protéagineux, avec une teneur moyenne en protéines autour de 24% de la matière sèche, sont essentiellement utilisées en alimentation animale (à destination surtout des monogastriques, porcs et volailles) mais connaissent de nouveaux débouchés à l'export en alimentation humaine.

Grâce aux progrès conjoints des techniques de culture et de la sélection végétale, les rendements moyens en pois secs ont progressés de 15 q/ha en moyenne en 1970 à 38 q/ha en 1982 et 45 q/ha en 1985 (Doré & Varoquaux, 2006; Schneider *et al.*, 2015). Une des premières pistes d'amélioration du rendement a été la création de variétés de pois d'hiver supposées apporter une productivité nettement supérieure à celle des pois de printemps ; ces variétés d'hiver ont été créées en utilisant des variétés-populations de pois fourragers collectées dans diverses régions de France ou d'Europe et repérées comme résistantes au froid (Cousin, 1976). Cependant toutes ces variétés, de printemps comme d'hiver, souffraient d'une faible résistance à la verse et donc de récoltes difficiles. Il a fallu attendre l'innovation apportée par la mutation *afila* transformant les folioles en vrilles pour assister à une augmentation des surfaces cultivées. « Solara », la première variété de type *afila* fut inscrite en 1984 ; c'est la version *afila* d'une des premières variétés de pois de printemps inscrite, « Finale » (Doré & Varoquaux, 2006). La France est devenue le premier producteur européen de pois protéagineux et un pic de production y a été atteint en 1993, avec plus de 700 000 ha semés.

6. L'amélioration de la nutrition azotée du pois : un levier pour augmenter et stabiliser son rendement ?

La France est aujourd'hui le sixième pays producteur de pois protéagineux dans le monde et est le premier en Europe (environ 500 Mt en 2013 ; <http://faostat.fao.org>). Néanmoins, les surfaces consacrées aux pois protéagineux ont très nettement baissé en France et en Europe qui ne produisent toujours que 50% de leurs besoins en protéines pour l'alimentation animale.

Les causes de la diminution des surfaces cultivées en pois

Un très net recul des surfaces cultivées en pois a été observé durant les années 2000 ; seulement 100 000 ha de pois ont été cultivés en France en 2008. La principale raison de ce déclin est le développement d'une maladie fongique racinaire due à *Aphanomyces euteiches*. S'y ajoutent un impact des politiques européennes, première réforme de la Politique Agricole Commune en 1992 puis Agenda 2000, défavorables au prix du protéagineux et en particulier du pois. En ont découlé une marginalisation de cette espèce, un plus faible investissement de la part des sélectionneurs et un déplacement de sa culture vers les terres les moins bonnes, les meilleures étant réservées aux cultures les plus rentables telles que le blé et le colza. Depuis 2010, de nouvelles politiques ont été mises en place pour relancer la culture des protéagineux ; avec en 2010 un nouveau plan européen « Protéines » et en 2014 le plan

« Protéines végétales pour la France » incluant des aides se situant entre 100 et 200 €/ha pour ces cultures ainsi que pour le soja. Par ailleurs, ces cultures capables de fixer l'azote atmosphérique sont aussi comptabilisées dans les surfaces d'intérêt écologique (SIE) et permettent de bénéficier d'un paiement vert. L'impact des politiques incitatives est certes positif mais ne perdure pas. Ainsi, les surfaces ont atteint 240 000 ha en 2010, chuté à 118 000 ha en 2013, et ont à nouveau progressé jusqu'à 183 000 en 2016 (Notes aux opérateurs, Terres Univia). Le principal frein à sa culture reste l'irrégularité de son rendement et de la teneur en azote de ses graines, auquel s'ajoutent le défaut de régularité d'approvisionnement pour les fabricants d'aliments pour le bétail et l'instabilité des débouchés vers les pays tiers.

Les potentialités de l'amélioration de la nutrition azotée pour l'amélioration du rendement

Une partie des baisses de rendement peut être attribuée à un déficit de nutrition azotée. En effet, une nette corrélation entre niveaux de nutrition azotée et de rendement a été observée en conditions de plein champ (Doré *et al.*, 1998; Voisin *et al.*, 2007).

Le levier de l'amélioration de la fixation symbiotique

La fixation symbiotique est très sensible aux conditions environnementales (stress abiotiques et biotiques) ; un déficit hydrique ou au contraire un excès d'eau, une alimentation carencée en P, K et S, un état structural du sol dégradé ou une infestation de sitones sont des facteurs limitants (Crozat *et al.*, 1994; Corre-Hellou & Crozat, 2005; Prudent *et al.*, 2016).

De plus, en conditions de plein champ, les plantes peuvent être nodulées par des rhizobia ayant une faible efficacité symbiotique (Triplett & Sadowsky, 1992). Les populations naturelles de rhizobia nodulant le pois varient selon les sols et les pratiques culturales (Fesenko *et al.*, 1995; Laguerre *et al.*, 2003; Depret *et al.*, 2004). Cette diversité bactérienne se traduit par une diversité fonctionnelle au niveau de la plante, avec des effets sur son développement aérien et sur celui de ses racines et nodosités, et sur l'efficacité d'acquisition d'N (Helz *et al.*, 1927; Fesenko *et al.*, 1995; Laguerre *et al.*, 2007). Les souches sont aussi connues pour être variables en compétitivité vis-à-vis de leur plante hôte (Amarger & Lobreau, 1982). Les rhizobia exercent un effet direct sur le processus de nodulation, notamment grâce à leur synthèse de facteurs Nod et sont ainsi plus ou moins compétitives. Certains rhizobia peuvent être compétitifs sans être bénéfiques à la plante ; ce sont « les tricheurs », symbiotes non mutualistes qui bénéficient de ses ressources sans rien lui fournir en échange (Sachs *et al.*, 2010). Face à ce problème encore mal quantifié en Europe et par

contre pris en compte au Canada par des inoculations systématiques, l'inoculation du pois par des souches efficaces peut être envisagée comme un moyen d'améliorer sa fixation symbiotique et donc son rendement (Bremer *et al.*, 1988; Fesenko *et al.*, 1995; McKenzie *et al.*, 2001; Depret, 2008). Cependant, les souches inoculées efficaces peuvent être moins compétitives que les rhizobia endogènes et dans ce cas l'inoculation inefficace pour augmenter le rendement (Meade *et al.*, 1985).

Les populations de rhizobia nodulant le pois varient également selon le génotype végétal, suggérant que la plante peut aussi exercer un contrôle sur ses partenaires symbiotiques (Bourion *et al.*, 2007; Depret & Laguerre, 2008). La nodulation induit un coût en C important pour la plante, généralement au détriment des parties aériennes et des racines. Il est communément admis que la plante a acquis au cours de l'évolution une préférence pour certains partenaires bactériens, soit par sélection des partenaires les plus bénéfiques (Simms & Taylor, 2002) ou à sanctionner les moins efficaces ou « tricheurs » (Oono *et al.*, 2011). La sélection végétale peut aussi avoir un impact. Ainsi, il a été observé que des variétés-population de luzerne, sélectionnées pour l'amélioration de leur fixation symbiotique, choisissent de préférence, parmi les souches indigènes, celles qui leur sont les plus favorables pour l'acquisition d'azote (Hardarson *et al.*, 1982). Néanmoins, la sélection végétale pratiquée ces dernières décennies, dans des systèmes de culture riches en nitrate peu favorables à la fixation symbiotique, peut avoir affecté la capacité de défense des plantes contre les symbiotes peu bénéfiques, comme suggéré par les travaux sur le soja (Kiers *et al.*, 2007). Ainsi, il devient indispensable de prendre en compte l'interaction avec les rhizobia dans les nouvelles démarches de sélection végétale, avec comme préliminaire la connaissance de la diversité génétique chez le pois pour le choix des rhizobia.

Le levier de l'augmentation du développement racinaire

L'acquisition de l'N minéral, complémentaire de la fixation symbiotique, peut être limitante, en particulier à partir du stade de remplissage des grains, si le système racinaire n'est pas assez développé. Les mutants hypernodulants de pois en représentent un cas extrême ; la formation des nodosités en nombre excessif engendre un coût en C très élevé limitant très fortement les croissances aérienne et racinaire et finalement la quantité totale d'N accumulée dans la plante (Salon *et al.*, 2001; Bourion *et al.*, 2007). L'étude de ces mutants a permis de progresser dans la connaissance de la régulation de la nodulation. L'intérêt en création variétale de ces mutants mériterait devrait être aussi testé en introgressant les allèles d'hypernodulation dans des génotypes à fort développement racinaire.

La variabilité du développement racinaire du pois a fait l'objet de différentes études au champ et en conditions contrôlées. Les études au champ ont montré que la profondeur d'enracinement des pois varie selon les génotypes de pois et les conditions environnementales, avec un effet fort du génotype (Thorup-Kristensen, 1998; Kraft & Boge, 2001; Vocanson *et al.*, 2006; Bourion *et al.*, 2007). Des expérimentations réalisées en conditions contrôlées ont permis d'étudier un plus grand nombre de génotypes de pois et ont mis en évidence une grande variabilité de longueur et biomasse de racines associées à une grande variabilité des biomasses aériennes (Ali-Khan & Snoad, 1977; McPhee, 2005). Ces expérimentations en conditions contrôlées ont été réalisées à des stades de développement très précoces (moins de 2 semaines). Elles avaient pour objectif principal l'étude des corrélations entre développement et croissance racinaire et tolérance à des maladies racinaires telles que celle induite par le pathogène *Aphanomyces euteiches*. La variabilité du système racinaire à des stades plus tardifs et les potentialités d'amélioration de l'acquisition d'N par une amélioration du développement racinaire restent à explorer. Enfin, jusqu'à présent, aucun gène n'a été identifié chez le pois comme étant associé à la variabilité de son développement racinaire.

7. Objectifs et stratégie du travail de thèse

Un idéotype de pois pour la nutrition azotée peut être considéré comme un génotype présentant une complémentarité optimale entre les deux voies d'acquisition d'N, avec une mise en place des nodosités qui ne se fasse pas au détriment du développement racinaire (Salon *et al.*, 2001). L'objectif de cette thèse a été d'acquérir une meilleure compréhension du contrôle génétique de la mise en place des racines et des nodosités et de leur impact sur la nutrition azotée de façon à explorer si un tel idéotype est concevable.

Pour répondre à cet objectif, deux questions de recherche ont été posées dans une première étape : i) Existe-il une variabilité génétique pour les caractères de mise en place des racines et des nodosités ? ii) Les variabilités associées à ces caractères sont-elles corrélées entre elles et aux caractères d'acquisition d'N de la plante via un contrôle génétique commun ? Deux pistes d'amélioration de la nutrition azotée ont ensuite été étudiées au cours de deux autres étapes; l'amélioration de leur développement racinaire à partir d'une étude détaillée d'un mutant de développement racinaire, puis l'amélioration de leur symbiose avec les rhizobia.

Deux types d'approche génétique ont été utilisés au cours de ces étapes. La première approche est quantitative et se base sur la recherche d'associations entre diversité de

l'architecture racinaire et nodulaire ou diversité de l'acquisition d'azote et polymorphisme moléculaire. La deuxième approche est transcriptomique et vise à mettre en évidence des différentiels d'expression de gènes corrélés à un phénotype racinaire et/ou d'acquisition d'azote. Ces deux types d'approches ont nécessité l'étude d'une grande variabilité de ressources génétiques, qui sont de différents types selon l'approche génétique utilisée ; une population de lignées recombinantes a été étudiée pour l'approche quantitative et un mutant de développement racinaire pour l'approche transcriptomique.

Enfin, la stratégie de recherche s'est caractérisée par une approche pluridisciplinaire du phénotype, comportant à la fois une description des structures (mise en place du système racinaire nodulé) et de leur fonctionnement (modèle écophysio-physiologique + métabolomique), tout en tenant compte du partenaire microbien (confrontation à diverses souches de *Rhizobium*).

Les résultats détaillés de chacune des trois étapes sont présentés et discutés dans trois chapitres successifs sous forme d'articles scientifiques en anglais. Les deux premiers articles ont été publiés dans des revues internationales à comité de lecture. Chacun de ces trois chapitres est complété d'une introduction spécifique. Le dernier chapitre du manuscrit présente une discussion sur les apports des résultats de la thèse, ainsi que des perspectives de recherche.

CHAPITRE I

CHAPITRE I : ETUDE DU DÉTERMINISME GÉNÉTIQUE DE L'ARCHITECTURE RACINAIRE NODULÉE ET DE LA NUTRITION AZOTÉE DU POIS

1. Introduction au chapitre I

Cette première étude a été réalisée pour déterminer si un idéotype de nutrition azotée, défini comme présentant une complémentarité optimale entre les deux voies d'acquisition d'azote, était concevable chez le pois. La variabilité génétique existante pour l'architecture racinaire nodulée et son impact sur l'acquisition d'azote ont été évaluées de façon conjointe sur une population de lignées recombinantes. Une recherche de Quantitative Trait Loci (QTL) a été réalisée pour chacune des variables mesurées afin d'étudier les contrôles génétiques de ces caractères et ainsi les possibilités de sélection sur ces caractères.

En préliminaire à cette étude, nous avons réalisé différentes expérimentations sur les 7 lignées parentes des populations de lignées recombinantes (RILs) qui avaient été créées dans l'Unité. Les lignées avaient été choisies essentiellement pour leur variabilité en teneur en protéines et en poids de graines (Burstin *et al.*, 2007); rien n'était connu sur leur développement racinaire et nodulaire. Les expérimentations ont été menées avec différentes conditions de nutrition azotée (dose d'N, souche de rhizobium à inoculer) avec différents types de substrat et contenant. Elles nous ont permis de : i) mettre au point des conditions et méthodologies de phénotypage de l'architecture racinaire et nodulaire applicables sur un grand nombre de lignées, et de préférence non destructives, ii) déterminer un ensemble de variables représentatives et mesurables sur un grand nombre de lignées de pois et iii) choisir, parmi les populations de RILs disponibles, celle créée à partir des deux lignées les plus contrastées pour la mise en place de leurs racines et nodosités et de leur acquisition d'N.

Deux expérimentations de phénotypage utilisant les méthodologies mises au point ont été réalisées en serre sur 153 des lignées recombinantes de la population choisie. Des variables intégratives clés d'efficacité d'acquisition de N et de C ont aussi été mesurées selon le modèle écophysique proposé par Moreau *et al.* (2007) et Voisin *et al.* (2007). Des données de deux expérimentations au champ sur les lignées de la RIL4 étaient aussi disponibles. Une recherche de QTL a été réalisée pour chacune des 57 variables mesurées sur

153 lignées recombinantes en utilisant la carte génétique comprenant 152 marqueurs qui était alors disponibles. Ont ainsi été détectés, 32 QTL relatifs aux racines et 26 QTL relatifs aux nodosités; avec des parts de variation phénotypique expliquée par chacun des QTL variant entre 9 et 49%. Parmi eux, 7 QTL relatifs aux racines et 11 des QTL relatifs aux nodosités ont été trouvés comme co-localisant sur le LGI à proximité du marqueur Af ; avec un effet négatif sur tous ces caractères de l'allèle *af* induisant la diminution de surface de feuilles par une transformation des folioles en vrilles, en accord avec l'hypothèse de l'impact de la disponibilité en C pour la mise en place des racines et des nodosités. Trois autres zones de co-localisation entre QTL racines et QTL nodosités ont été détectées ; les autres zones étant spécifiques, ce qui ouvre la possibilité de sélectionner sur les caractères de racines ou de nodosités de façon indépendante. La co-localisation entre la majeure partie des QTL relatifs à l'accumulation d'N dans les plantes ou dans les graines avec des QTL relatifs aux développements racinaire ou nodulaire est un autre résultat marquant de cette étude et ouvre des possibilités de sélection d'un idéotype de nutrition azotée.

L'ensemble de ces résultats a fait l'objet d'une publication dans la revue *Theoretical and Applied Genetics* (Bourion *et al.*, 2010).

2. Publication n°1

Genetic dissection of nitrogen nutrition in pea through a QTL approach of root, nodule, and shoot variability

Virginie Bourion*, Syed Masood Hasan Rizvi, Sarah Fournier, Henri de Larambergue, Fabien Galmiche, Pascal Marget, Gérard Duc, Judith Burstin
INRA, UMR102, Genetics and Ecophysiology of Grain Legumes, 17 rue de Sully, BP 86510, F-21065 Dijon Cedex, France

*Corresponding author: Virginie Bourion,
INRA, UMR Agroécologie, Pôle GEPASI
17 rue de Sully, BP 86510, F-21065 Dijon Cedex, France
Tel : 33 (3) 80 69 36 47
Fax : 33 (3) 80 69 32 63
Email: virginie.bourion@dijon.inra.fr

Les références citées sont situées à la fin de ce chapitre (p 67-70).

Les fichiers additionnels sont présentés en annexe 1.

Abstract

Pea (*Pisum sativum* L.) is the third most important grain legume worldwide, and the increasing demand for protein-rich raw material has led to a great interest in this crop as a protein source. Seed yield and protein content in crops are strongly determined by nitrogen (N) nutrition, which in legumes relies on two complementary pathways: absorption by roots of soil mineral nitrogen, and fixation in nodules of atmospheric dinitrogen through the plant-*Rhizobium* symbiosis. This study assessed the potential of naturally-occurring genetic variability of nodulated root structure and functioning traits to improve N nutrition in pea. Glasshouse and field experiments were performed on seven pea genotypes and on the 'Cameor' x 'Ballet' population of recombinant inbred lines selected on the basis of parental contrast for root and nodule traits. Significant variation was observed for most traits, which were obtained from non-destructive kinetic measurements of nodulated root and shoot in pouches, root and shoot image analysis, ¹⁵N quantification, or seed yield and protein content determination. A significant positive relationship was found between nodule establishment and root system growth, both among the seven genotypes and the population. Moreover, several Quantitative Trait Loci for root or nodule traits and seed N accumulation were mapped in similar locations, highlighting the possibility of breeding new pea cultivars with increased root system size, sustained nodule number, and improved N nutrition. The impact on both root or nodule traits and N nutrition of the genomic regions of the major developmental genes *Le* and *Af* was also underlined.

Keywords: *Pisum sativum* L., root and nodule development, pouches, root image analysis, shoot nitrogen accumulation, Quantitative Trait Loci.

Introduction

Pea (*Pisum sativum* L.) is the third most important grain legume worldwide, after soybean (*Glycine max* L.) and common bean (*Phaseolus vulgaris* L.) (FAOSTAT, 2007). The increasing demand for protein-rich raw material for animal feed or human nutrition has led to a greater interest in this crop as a protein source (Santalla *et al.*, 2001). Selection for high yield, high seed protein concentration and early maturity has been undertaken by pea breeders to develop cultivars with superior performance. However, seed yield and protein content are very variable, mostly because of biotic and environmental stresses. In all crops, nitrogen nutrition is one of the key processes involved in determining yield. In legumes, N acquisition relies on two complementary pathways: absorption of soil mineral nitrogen and fixation of atmospheric dinitrogen through the plant-*Rhizobium* symbiosis. N₂ fixation supplies the major part of plant nitrogen but is complemented by N absorption at critical stages, especially when N₂ fixation decreases during seed filling. Ecophysiological studies on legumes have established a relationship between respiration costs, which are mainly associated with energy demand for N₂ fixation in nodules, and C allocated to nodule and root structure (Voisin *et al.*, 2003a). Nitrogen flux through symbiotic activity has been modeled as a function of C flux towards nodulated roots, which is independent of nitrate availability but depends on nodule biomass. From the vegetative phase up to flowering, the developing nodules represent the largest carbon sink for the plant and N₂ fixation contributes to the largest part of N acquisition (Voisin *et al.*, 2003b). Conversely, at the end of growth cycle when filling seeds become the largest carbon sink, N-fixing activity decreases. At this stage, exogenous N supply relies on N assimilation by roots and should be limiting if the root system is too small (Bourion *et al.*, 2007).

We hypothesize that the genetic improvement of symbiotic efficiency and of nutrient acquisition by root systems can contribute to the improvement of legume crop performances and their stability. Due to the technical difficulty of studying the phenotype of root systems, the molecular determinants of root or nodule development are less understood than those for aerial plant parts. However, the molecular basis of the early nodule development has made significant progress since the discovery of plant responses to bacterial signals, known as Nod factors (Dénarié & Cullimore, 1993). Three genes controlling early steps of Nod factor signal transduction have been identified both in *Medicago truncatula* (Catoira *et al.*, 2000; Endre *et al.*, 2002; Ané *et al.*, 2004; Lévy *et al.*, 2004) and in pea (Endre *et al.*, 2002; Lévy *et al.*, 2004; Edwards *et al.*, 2007). Nodulation is then regulated through principally a major

systemic regulation pathway known as autoregulation of nodulation (AON), and mutants impaired in AON display a hypernodulating phenotype; for review see (Oka-Kira & Kawaguchi, 2006; Magori & Kawaguchi, 2009). In pea, the hypernodulation phenotype is under shoot control for *sym28* and *sym29* mutants (Sagan & Duc, 1996) but under root control for *nod3* mutant lines (Postma *et al.*, 1988). Most of these hypernodulating mutants have a reduced shoot and root growth (Salon *et al.*, 2001; Bourion *et al.*, 2007; Novak *et al.*, 2009), which may result from the competition for C between these different structures (Voisin *et al.*, 2007), and/or from complex meristematic response to hormonal signals (Krusell *et al.*, 2002).

A few experiments have investigated the spontaneous pea genetic variability of root development in young seedlings under controlled conditions (Ali-Khan & Snoad, 1977; McPhee, 2005) or in field experiments (Thorup-Kristensen, 1998; Kraft & Boge, 2001; Bourion *et al.*, 2007). Up to now, this variability in pea has not been associated with any plant genes. However, several regulators of root development have been identified in *Medicago truncatula*, and most of them monitor a fine and complex tuning of root versus nodule development. They include (i) genes involved notably in auxin, cytokinins and ABA transduction pathway: *MtLAX* gene is involved in local auxin transport and controls lateral root and nodule development (de Billy *et al.*, 2001; Mathesius, 2008), cytokinin signaling mediated by *MtCRE1* gene regulates nodule and lateral root organogenesis in an opposite manner (Gonzalez-Rizzo *et al.*, 2006; Frugier *et al.*, 2008), whereas ABA can rescue the root but not nodule meristem defect observed in *latd* mutants of *Mt* (Bright *et al.*, 2005; Liang *et al.*, 2007), (ii) genes involved in nitrate sensing and response: low mineral N condition is a prerequisite to allow nodule formation and function, whereas high levels of nitrate or ammonium inhibit nodule formation (Carroll & Mathews, 1990; Barbulova *et al.*, 2007) This inhibition of nodulation is primarily a localized response (Carroll & Mathews, 1990) but also includes AON, as exemplified by the nodule maintenance in hypernodulating mutants grown under high NO_3^- (Carroll & Mathews, 1990; Sagan & Duc, 1996; Wopereis *et al.*, 2000; Schnabel *et al.*, 2005). Recently, the *CLE* gene in *Lotus japonicus* was proposed to produce a root derived signal, which drives *HARI* mediated autoregulation and nitrate inhibition of nodulation (Okamoto *et al.*, 2009).

Quantitative trait loci (QTL) mapping has become a widespread approach to dissect the genetic determinism of many economically important complex traits in plant breeding. In pea, several genetic maps have been constructed using different types of markers, and genes of known function have been integrated into consensus maps (Weeden *et al.*, 1999; Aubert *et al.*, 2006). QTL for important traits in pea have been localized, including QTL for resistance

to root diseases (Pilet-Nayel *et al.*, 2002), QTL for frost tolerance (Lejeune-Hénaut *et al.*, 2008; Dumont *et al.*, 2009) or QTL of seed yield and protein content (Timmerman-Vaughan *et al.*, 1996; Tar'an *et al.*, 2004; Timmerman-Vaughan *et al.*, 2005; Burstin *et al.*, 2007). Most QTL for seed traits were shown to coincide with genes or QTL for aerial developmental traits, indicating either that the genomics regions associated with these QTL carry group of linked genes, or that single developmental genes underlying the QTL have pleiotropic effects on plant morphology, nitrogen source capacity and seed protein content and yield (Burstin *et al.*, 2007). Identifying the genetics determinants of nitrogen source capacity may unravel the molecular basis of the seed traits QTL. A few QTL for nitrogen source capacity were mapped in cereals, and showed overlaps between QTL of plant nitrogen use efficiency or root architecture and QTL for seed yield (Tuberosa *et al.*, 2002; Coque & Gallais, 2006; Laperche *et al.*, 2006).

In this study, we questioned the feasibility of improving nitrogen nutrition in legumes: is there a significant genetic variability for root and nodule development traits in pea ecotypes? Is there an antagonistic relationship between nodule and root developments? Can we identify root and/or nodule characteristics that are associated with seed nitrogen and biomass accumulation? To answer these questions, we characterized the genetic variability of the nodulated root compartment in pea and its relationship with nitrogen accumulation in the plant, in seven pea genotypes. Then, we identified QTL for these traits in a recombinant inbred line (RIL) population derived from the cross between two genotypes with contrasted nodulated root development.

Materials and methods

Plant material

Seven genotypes, parents of RIL populations ('Ballet', 'Cameor', China, VavD265, K586, 'Sommette', 'Terese') were assessed in glasshouse to evaluate root and nodule traits describing their nitrogen acquisition structure. These genotypes were described by (Baranger *et al.*, 2004), and their seed protein content and weight determined by Burstin *et al.* (2007). The population RIL4 comprised 207 recombinant inbred lines (RIL) deriving from a cross between 'Cameor' and 'Ballet'. 'Ballet' differs from 'Cameor' by lower seed protein content and by its semi-leafless type, due to the effect of *Af* gene.

Field trials

The population RIL4 was sown in two field experiments, on March 3d, 2004 (Exp04f; 180 F6:8 RIL) and on March 21st, 2006 (Exp06f; 153 F6:9 RIL deriving from the F8 RIL harvesting in 2004), at INRA-Dijon, Domaine d'Epoisses, Bretenière, France. At the sowing date, the ploughed layer (0-30 cm) of soil contained about 60 kg and 5 kg of N ha⁻¹ in 2004 and 2006, respectively. Three weeks after sowing, 1 kg N ha⁻¹ of ¹⁵N labelled ammonium nitrate was applied, providing 1% atom excess. P and K fertilisation was performed during the preceding autumn. Irrigation was provided at the beginning and end of flowering to avoid any drought stress. In these field trials, with two replicates in 2006 and one in 2004, each plot consisted in a 30-pea plant row grown on trellises and samples of ten pea plants per plot were harvested when seeds had ripened.

Glasshouse experiments

Four glasshouse experiments were carried out successively in February 2005 (Exp05a), December 2005 (Exp05b), February 2006 (Exp06) and February 2007 (Exp07). Plants were grown in controlled temperature (20°C/15°C) in a 16-h day-night cycle and under a mean photosynthetically active radiation (PAR) of 170 μmol photons m⁻² s⁻¹ guaranteed by high-pressure sodium lamps when daylight was declining. These experiments were performed in pots or in pouches, with surface sterilized seeds, in sterilized substrates inoculated with a cell suspension of a *Rhizobium leguminosarum* bv. *viciae* (*Rlv*) (ca. 10⁸ per seed; Smith & Wollum, 1989; Lira Junior *et al.*, 2005) and supplied with a low nitrate content (2.5 mM) nutrient solution for optimal root and nodule development. When grown in pots, plants were grown in 7-litre pots filled with a 1:1 (v/v) mixture of sterilized atapulgite and clay balls diameter). Inoculation was applied just after sowing. The sowing density was of 4 seeds per pot, but only the 3 most homogeneous seedlings over the 4 were kept after emergence.

When grown in pouches, 15 seeds by genotype were firstly left to germinate in plastic boxes filled with one litre of 4% (w/v) Kalys agar HP 696 gel, during four days, and then the 3 most homogeneous seedlings, with taproot length of about 3 cm long, were transferred into sterilized growth pouches and inoculated the day after. Each pouch consisted on a transparent plastic bag of 18 cm in width and 20 cm in length, containing a wick paper forming a trough in which the seed was placed (Fig. I.1). All pouches were covered with opaque paper. They received 50 ml of the low nitrate content nutrient solution, which was replaced twice a week. The solution had an initial pH of 6.5 (Novak *et al.*, 2002), and whatever the pouch, a final pH between 6.3 and 6.5 when renewed after 3 or 4 days in contact with the root.

Exp05a was set up to assess the genetic variability of the nodulated root development in 7 genotypes ('Ballet', 'Cameor', China, VavD265, K586, 'Sommette', 'Terese'). Seeds were sown in pots and inoculated with *Rlv* strain 1007 (Sagan & Duc, 1996; Laguerre *et al.*, 2007) in a three-block randomised design, with four pots per genotype in each block. Plants were harvested for measurements at 4 successive stages: the 4-leaf, 9-leaf, beginning of flowering (BF) and beginning of seed filling stages (BSF). In order to assess the effect of pots and pouches growing conditions as well as of the *Rlv* strain, Exp5b was performed with genotypes 'Ballet', 'Cameor', and VavD265 grown both in pots and pouches, and inoculated using either *Rlv* strain 1007 or P221 (Tricot *et al.*, 1997; Laguerre *et al.*, 2007). Plants were harvested for measurements at BF. The two following experiments were carried out in order to map QTL of nodulated root development in the mapping population RIL4. In Exp06, seeds of 153 F6:9 RIL and of their two parents were sown in a two-block randomized design, with one pot per RIL and six pots per parent in each block. In Exp07, after measurement of their tap root length (TRootLd0), seedlings of 117 F6:9 RIL and of their two parents were grown in sterilized growth pouches, in a three-block randomized design, with one pouch per line and two per parent in each block. Seeds or seedlings were inoculated with *Rlv* strain P221 and plants watered throughout the experiment, with a nutrient solution supplemented with 2.5 mM ¹⁵N labelled nitrate (1% ¹⁵N). Plants were harvested for measurements at BF.

Root measurements

When plants were harvested, their root system was carefully spread onto a transparent sheet to minimize root overlapping and scanned as digital images with an A3 color scanner (Epson; Tokyo, Japan). After image scanning, first order lateral roots and nodules were counted. Roots and nodules were then oven-dried at 80°C for 48 h, weighed separately, and their dry matter (RootB and NodB, respectively) and relative part of nodule upon belowground dry matter (NB:BGB) were determined. Total root length (TRootL) and nodule projected area (TNodPA) were further determined by image analysis using WinRHIZO[®] Software (Regent Instruments, Quebec, Canada). Growth pouches also allowed for non-destructive observation throughout the Exp07. Twice a week, from date 1 until date 7 corresponding respectively to two and 27 days after the seedlings transfer in pouches, number of first order lateral roots (NLatRoot) and number of nodules (NNod) were counted. The increase of first lateral root number was linear between date 1 and date 4 (twelve days after transfer), allowing the calculation of first order lateral root appearance rate (LatApR) within

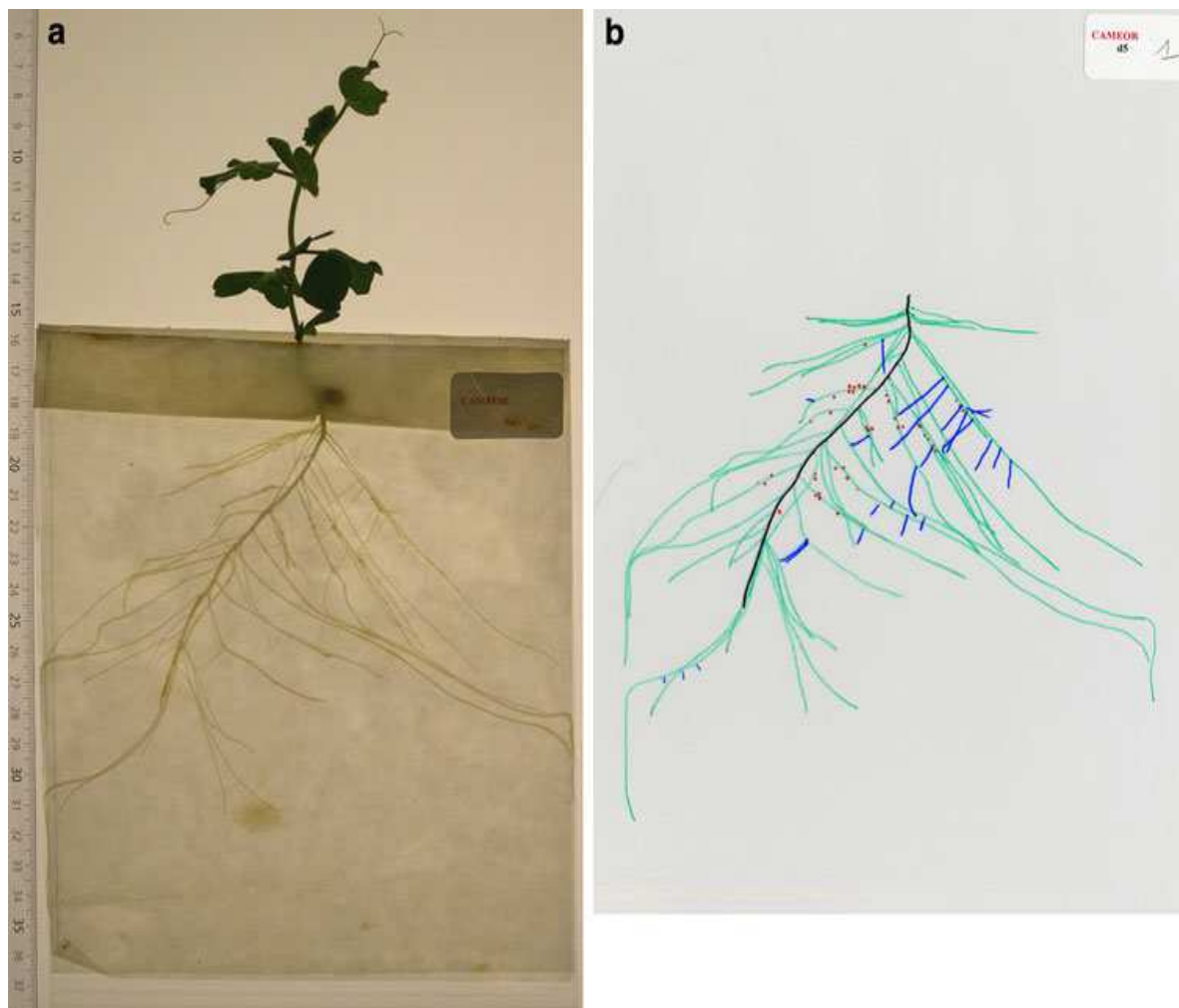


Fig. I.1. Observation of pouch 15 days after seedling transfer into pouch (d5) **(a)** the pouch consists on a transparent plastic bag of 18 cm in width and 20 cm in length, containing a wick paper forming a trough in which the seedling was placed, **(b)** roots and nodules are draw on a transparent sheet covering the pouch

this period. First nodules appeared only at date 4, and the nodule appearance rate (NodApR) was calculated within the linear increase period between date 4 and date 6 (20 days after transfer). Roots and nodules were also drawn on a transparent sheet covering the pouch, in different colours according to the root order, and red indicated nodules (Fig. I.1). After transparent sheets scanning, total root elongation rate (TRootER) and nodule projected area increase rate (TNodPAR) were calculated within the periods of linear increase. All these calculated traits were expressed as a function of cumulative degree°C.days from sowing, using a 0°C base temperature (Ney & Turc, 1993).

Aerial part measurements

The date of beginning of flowering (BegFlo), Shoot length (ShootL), and number of basal branches (NBranch) were measured at harvest in all experiments. In all the glasshouse experiments, the main stem leaf number was measured throughout the vegetative period, allowing the calculation of leaf appearance rate (leafApR). Shoots were harvested and oven-dried for dry matter measurement (ShootB) and calculation of the relative part of belowground upon total dry matter (BGB:TB). Shoot nitrogen content (ShootNC) was estimated according to the Dumas method for glasshouse Exp05a, or by mass spectrometry (SOCHROM) for glasshouse Exp06 and Exp07, and field Exp04f and Exp06f. Shoot nitrogen accumulation (ShootQN) was then calculated. For glasshouse Exp06 and Exp07, and field Exp04f and Exp06f, the part of nitrogen accumulation derived from symbiotic fixation (NDFA) was calculated using the isotope dilution technique (Duc *et al.*, 1988). In the glasshouse Exp06 and Exp07, specific nitrogen uptake (SNU) (Larigauderie *et al.*, 1994; Moreau *et al.*, 2007) was estimated as the amount of total shoot nitrogen uptake per total belowground dry matter; root efficiency and respectively nodule efficiency (RootE and NodeE) were calculated as the amount of shoot NO₃⁻ (respectively N₂) uptake per unit of root (respectively nodule) dry matter (Voisin *et al.*, 2007). In glasshouse Exp07, SPAD chlorophyll measurements (Minolta, Japan) were made on the 3 last expanded leaves of each harvested plant. Then, shoots were carefully spread onto a transparent sheet (Fig. I.1), the transparent sheets were scanned and analysed to determine total leaf area (LeafA), and thus estimate specific leaf nitrogen (SLN) (Sinclair & Horie, 1989) as total shoot nitrogen uptake per unit of leaf area. The leaf area was also estimated at successive dates, allowing the calculation of leaf area increase rate (LeafAR) and of radiation use efficiency (RUE) (Kiniry *et al.*, 1989; Sinclair & Horie, 1989).

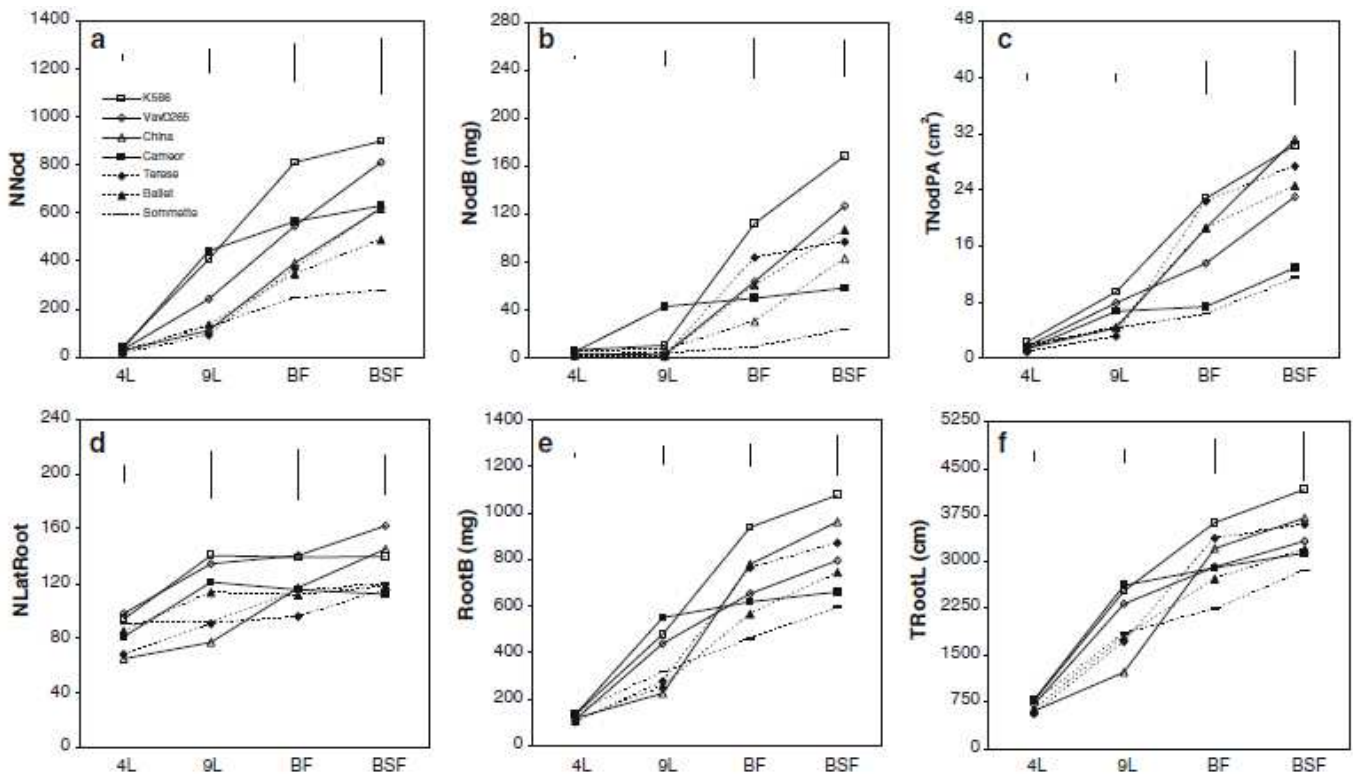


Fig. I.2. Nodule number (a), nodule biomass (b), total nodule projected area (c), number of first order lateral roots (d), root biomass (e) and total root length (f) per plant, for seven pea genotypes, from the 4-leaf stage until the beginning of seed filling. 4L, 9L, BF and BSF indicate the developmental stage and mean 4-leaf stage, 9-leaf stage, beginning of flowering and beginning of seed filling, respectively. Each point is the mean value of three replicates. Vertical bars represent LSD ($p < 0.05$). Open and solid symbols indicate *Le* and *le* genotypes, respectively. Full and dotted lines indicate *Af* and *af* genotypes, respectively

Finally, in field Exp04f and Exp06f, the number of seed (SeedN), the straw, seed and total shoot dry matter (StrawB, SeedB and ShootB) and thousand seed weight (TSW) were measured at maturity. Straw and seed nitrogen content (StrawNC, SeedNC) were estimated by near-infrared spectroscopy, allowing the calculation of straw and seed nitrogen accumulation (StrawQN, SeedQN) and of total shoot nitrogen content and accumulation (ShootNC, ShootQN).

RIL4 map construction

The 207 F6:8 RIL were genotyped using microsatellite and gene framework markers chosen to cover the pea genome from (Loridon *et al.*, 2005; Aubert *et al.*, 2006). The genetic map was built from using the *near*, *try*, *ripple* and *map* commands of MAPMAKER/EXP version 2.0. The RIL4 map comprises 152 markers and covers 1140 cM (Fig. I.5).

Statistical analyses

For each experiment, ANOVA were performed using the SAS GLM procedure (SAS Institute, 2000) to determine the significance level for genotype and block effects. The statistical model was: $Y_{ij} = \mu + g_i + b_j + e_{ij}$; where Y_{ij} is the value of the trait for genotype i in replicate j , μ the general mean, g_i the genotypic effect, b_j the replicate effect and e_{ij} the residual. Broad sense heritability (h^2) was then calculated as $h^2 = \sigma_G^2 / [\sigma_G^2 + \sigma_R^2/b]$, where σ_G^2 was the genotypic variance, σ_R^2 the error variance and b the number of blocks.

In glasshouse Exp05b, we tested the effect of the container, the *Rlv* strain, the genotype, as well as container x genotype and *rlv* strain x genotype effects. The analysis of variance did not show any significant *Rlv* strain effect or *Rlv* strain x genotype effect. Conversely, the effect of container (pot *versus* pouch) was significant for all traits, and the effect of container x genotype interaction was significant for all traits except NLatRoot, RootB, TRootL and NodB (data not shown).

In glasshouse Exp06 and Exp07, and field Exp04f and Exp06f, ANOVA were performed to test for year, RIL, and RIL x year interaction effects. For QTL analysis, adjusted genotype mean values were obtained for each experiment, using the *lsmeans* command of the SAS GLM procedure. For QTL analysis for traits measured at flowering in the two glasshouse experiments, corrected genotype mean values were the residuals of the following model: $Y_{ij} = \mu + \text{BegFlo}_{ij} + b_j + e_{ij}$; where BegFlo_{ij} is the beginning of flowering date for genotype i in replicate j .

Table I.1. Pearson correlation coefficients between root and nodule structure traits and shoot nitrogen accumulation recorded on seven pea genotypes at four successive stages

| Stage | Trait | TRootL | RootB | NNod | TNodPA | NodB | ShootQN |
|---------------------------|----------|--------|--------|--------|--------|--------|---------|
| 4-leaf | NLatRoot | 0.62 | 0.45 | 0.57 | 0.75* | 0.25 | 0.09 |
| | TRootL | | 0.87** | 0.77* | 0.51 | 0.62 | 0.66 |
| | RootB | | | 0.69 | 0.60 | 0.86** | 0.75* |
| | NNod | | | | 0.74 | 0.75* | 0.16 |
| | TNodPA | | | | | 0.70 | 0.05 |
| | NodB | | | | | | 0.42 |
| 9-leaf | NLatRoot | 0.92** | 0.79* | 0.77* | 0.89** | 0.27 | 0.60 |
| | TRootL | | 0.90** | 0.82* | 0.80* | 0.43 | 0.71 |
| | RootB | | | 0.95** | 0.80* | 0.72 | 0.92** |
| | NNod | | | | 0.83* | 0.77* | 0.88** |
| | TNodPA | | | | | 0.31 | 0.70 |
| | NodB | | | | | | 0.81* |
| Beginning of flowering | NLatRoot | 0.00 | 0.30 | 0.71 | -0.02 | 0.28 | -0.21 |
| | TRootL | | 0.81* | 0.59 | 0.70 | 0.82* | 0.72 |
| | RootB | | | 0.72 | 0.78* | 0.76* | 0.76* |
| | NNod | | | | 0.30 | 0.74 | 0.25 |
| | TNodPA | | | | | 0.73 | 0.96** |
| | NodB | | | | | | 0.72 |
| Beginning of seed filling | NLatRoot | 0.52 | 0.49 | 0.60 | 0.47 | 0.53 | -0.01 |
| | TRootL | | 0.70 | 0.78* | 0.48 | 0.46 | -0.04 |
| | RootB | | | 0.74 | 0.92** | 0.79* | 0.57 |
| | NNod | | | | 0.59 | 0.86** | 0.26 |
| | TNodPA | | | | | 0.75* | 0.79* |
| | NodB | | | | | | 0.59 |

*, **: significant correlation at the 0.05 and 0.01 probability level, respectively.

QTL were located using the composite interval mapping and iterative QTL mapping method (iQTLm) performed in MCQTL software (Jourjon *et al.*, 2005). Cofactors were selected by forward regression and QTL were searched, both using F tests. F thresholds were determined for all traits by 1 000 permutations test, for a global genome-wide type I risk of 10% for cofactor selection and of 5% for QTL detection. Mean F values over the traits of 11.3 (equivalent to LOD = 2.4) and of 12.9 (equivalent to LOD = 2.8) were used for cofactor selection and for QTL detection respectively. Allelic effect at each QTL and individual R², which represents the percentage of phenotypic variance explained by each QTL, were determined for all traits using MCQTL software. A global R² was also calculated for each trait by the multiple QTL model developed in iQTLm; its represents the percentage of phenotypic variance explained by all detected QTL. The global R² value was used to estimate p, the proportion of genotypic variance (σ^2_G) explained by all detected QTL, as $p = \text{global R}^2 / h^2$ (Charcosset & Gallais, 1996; Melchinger *et al.*, 2000). Pearson genetic correlation coefficients between traits were calculated using XLSTAT software (version 2006.4, <http://www.xlstat.com>).

Results

Variability of root and nodule structure among seven pea genotypes and its relation with nitrogen accumulation

Root and nodule traits describing the nitrogen acquisition structure were measured for seven pea genotypes at four successive stages. A significant genotype effect was observed whatever the stage, on the number of nodules (NNod), nodule biomass (NodB), total nodule projected area (TNodPA), number of lateral root (NlatRoot), total root length (TRootL) and root biomass (RootB). Genotypes showed contrasted kinetics of root and nodule development (Fig. I.2): in 'Cameor', NNod, NodB and RootB displayed a particularly fast increase at the beginning of the growth cycle, but rapidly reached their maximum at the 9-leaf stage; in 'Sommette', NNod, NodB, TNodPA and RootB were slow and remained limited until the end of the experiment; conversely, genotype K586 showed a fast and prolonged increase in NNod, TNodPA as well as in RootB and TRootL. The *Le* and *Af* genes, which respectively control the internode length and the semi-leafless trait, are segregating among the 7 genotypes (Fig. I.2). Both *Le* and *Af* genes had a significant effect on all root and nodule traits, at one stage or another, with higher values for *Le* or *Af* genotypes than for *le* or *af* ones (Supplemental Table

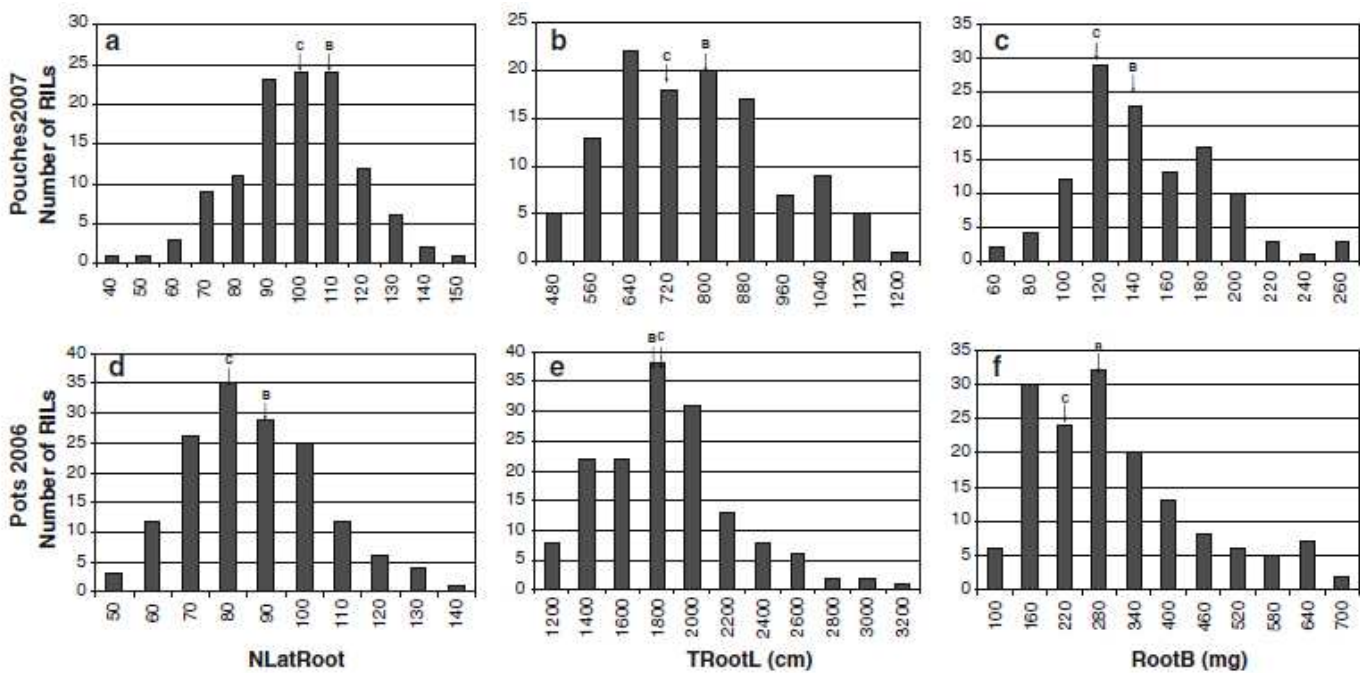


Fig. I.3. Frequency distribution of RIL4 population for number of first order lateral roots (**a, d**), root biomass (**b, e**) and total root length (**c, f**) per plant, observed at the beginning of flowering in Pouches 2007 and Pots 2006 glasshouse experiments. Arrows indicate the mean value of the parental lines: B, 'Ballet'; C, 'Cameor'

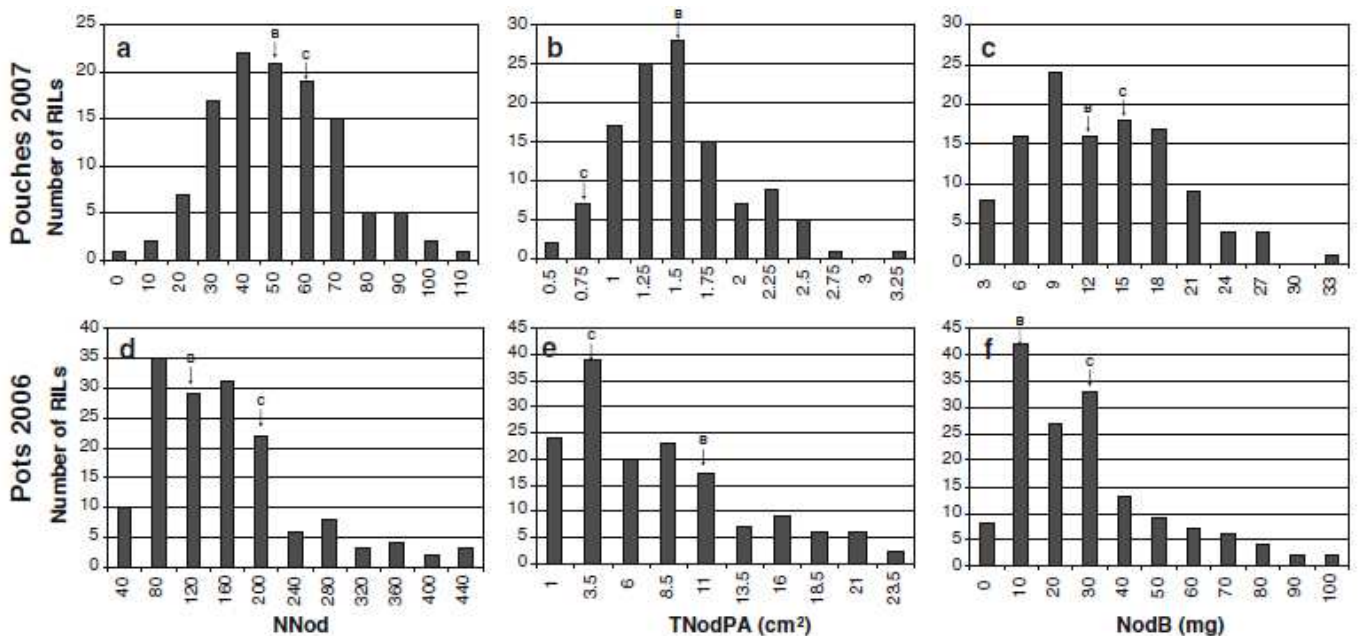


Fig. I.4. Frequency distribution of RIL4 population for nodule number (**a, d**), nodule biomass (**b, e**), total nodule projected area (**c, f**) per plant, observed at the beginning of flowering in Pouches 2007 and Pots 2006 glasshouse experiments. Arrows indicate the mean value of the parental lines: B, 'Ballet'; C, 'Cameor'

I.1). Among the *le* genotypes, ‘Cameor’ and ‘Ballet’ presented contrasted phenotypes both on rate of NNod appearance and on NodB, RootB and TRootL increase (Fig. I.2).

Shoot nitrogen accumulation (ShootQN) was significantly correlated with RootB from the 4-leaf stage until the beginning of flowering (BF), but neither with NLatRoot nor TRootL (Table I.1). RootB was highly correlated with TRootL and to NLatRoot only at the 9-leaf stage. ShootQN was highly correlated with NNod and NodB at the 9-leaf stage, and with TNodPA from BF to the beginning of seed filling (BSF). NodB was significantly correlated with NNod whatever the stage, and with TNodPA at BSF. Lastly, RootB and NodB were significantly correlated with each other at all stages except the 9-leaf stage, and NNod was significantly correlated with the TRoot at all stages except BF, and with NLatRoot only at the 9-leaf stage.

Variability of root and nodule structure in the ‘Cameor’ x ‘Ballet’ recombinant inbred population

Similar nitrogen acquisition structure traits were recorded in the ‘Cameor’ x ‘Ballet’ recombinant inbred line population (RIL4) during two glasshouse experiments: in 2006, plants were grown in pots and in 2007, plants were grown in pouches. Except for the number of lateral roots at BF, a highly significant effect of the genotype was detected for all root and nodule traits, included the number of lateral roots and the number of nodules as soon as their appearance date, which was respectively about two days (d1) and twelve days (d4) after the seedlings transfer in pouches (Supplemental Table I.2). Heritabilities were moderate to high, up to 0.88 and 0.77, for root and nodule traits, respectively. ‘Ballet’ had slightly higher values than ‘Cameor’ for most of the root traits, whereas nodule number and biomass were slightly lower for ‘Ballet’ than for ‘Cameor’. Compared with the parental values, transgressive segregants were observed for all traits in the two experiments (Fig. I.3 and I.4, Supplemental Fig. I.1). The effects of the experiment and of genotype x experiment interaction were also highly significant for all traits analysed ($P < 0.0001$) except NLatRoot. For all traits but NLatRoot, mean and range values differed markedly between the two experiments, with about 3-fold higher mean values in pots than in pouches. Nevertheless, TRootL, RootB, NNod, TNodPA and NodB measured in 2006 were highly significantly correlated with the same traits measured in 2007 (Supplemental Table I.3). Whatever the experiment, NodB was significantly correlated with both NNod and TNodPA, whereas TRootL and RootB were

Table I.2. Pearson correlation coefficients between shoot or seed nitrogen accumulation and nitrogen acquisition functioning or carbon accumulation traits recorded in RIL4 population in four experiments

| Trait | ShootQN-07 | ShootQN-06 | SeedQN-06f | SeedQN-04f |
|-------------|------------|------------|------------|------------|
| NLatRoot-07 | 0.33*** | 0.12 | 0.13 | 0.24* |
| NLatRoot-06 | 0.21* | 0.04 | 0.21* | 0.15 |
| TRootL-07 | 0.77*** | 0.70*** | 0.49*** | 0.32** |
| TRootL-06 | 0.59*** | 0.72*** | 0.40*** | 0.25* |
| RootB-07 | 0.73*** | 0.72*** | 0.41*** | 0.29** |
| RootB-06 | 0.65*** | 0.88*** | 0.43*** | 0.27** |
| NNod-07 | 0.47*** | 0.34*** | 0.13 | 0.24* |
| NNod-06 | 0.52*** | 0.75*** | 0.39*** | 0.27** |
| TNodPA-07 | 0.67*** | 0.62*** | 0.35*** | 0.19 |
| TNodPA-06 | 0.65*** | 0.91*** | 0.39*** | 0.31** |
| NodB-07 | 0.60*** | 0.38*** | 0.18 | 0.03 |
| NodB-06 | 0.50*** | 0.82*** | 0.40*** | 0.33*** |
| ShootB-07 | 0.83*** | 0.75*** | 0.35*** | 0.22* |
| ShootB-06 | 0.64*** | 0.97*** | 0.44*** | 0.30** |
| StrawB-06f | 0.32** | 0.43*** | 0.81*** | 0.40*** |
| StrawB-04f | 0.20* | 0.38*** | 0.35*** | 0.62*** |
| SeedB-06f | 0.31** | 0.47*** | 0.98*** | 0.44*** |
| SeedB-04f | 0.11 | 0.27** | 0.46*** | 0.98*** |
| NDFA-07 | 0.61*** | 0.40*** | 0.02 | -0.03 |
| NDFA-06 | 0.09 | 0.29** | 0.26** | 0.28** |
| NDFA-06f | 0.21* | 0.41*** | 0.64*** | 0.35*** |
| NDFA-04f | 0.18 | 0.38*** | 0.28** | 0.52*** |
| ShootNC-07 | -0.07 | -0.40*** | -0.21* | -0.20* |
| ShootNC-06 | -0.13 | -0.13 | -0.07 | -0.07 |
| StrawNC-06f | 0.09 | -0.10 | -0.40*** | -0.37*** |
| StrawNC-04f | 0.21* | 0.10 | -0.13 | -0.53*** |
| SeedNC-06f | -0.12 | -0.14 | 0.21* | 0.08 |
| SeedNC-04f | 0.13 | 0.18 | 0.07 | 0.36*** |
| RootE-07 | -0.21* | -0.42*** | -0.15 | -0.19 |
| RootE-06 | 0.05 | 0.07 | -0.12 | -0.15 |
| NodE-07 | -0.12 | -0.02 | -0.03 | -0.06 |
| NodE-06 | -0.08 | -0.15 | -0.27** | -0.14 |
| LeafA-07 | 0.79*** | 0.72*** | 0.42*** | 0.28** |
| LeafAR-07 | 0.48*** | 0.48*** | 0.37*** | 0.36*** |
| RUE-07 | -0.60*** | -0.57*** | -0.33** | -0.13 |
| SLN-07 | -0.15 | -0.41*** | -0.35*** | -0.28** |

*, **: significant correlation at the 0.05 and 0.01 probability level, respectively

highly correlated together ($r^2 > 0.6$), and both significantly correlated with all the nodule traits. Conversely, NLatRoot was neither highly correlated with RootB nor with nodule traits.

Variability of traits related to N acquisition efficiency, N and C accumulation in the plant, and plant development in the ‘Cameor’ x ‘Ballet’ recombinant inbred population

Different measurements related to C (ShootB, StrawB, SeedB, TSW) and N accumulation in aerial parts (ShootNC, ShootQN, StrawNC, StrawQN, SeedNC, SeedQN), and traits related to nitrogen acquisition efficiency (NDFA, SNU, RootE, NodeE) were determined. Some developmental traits (ShootL, Nbranch, BegFlo, LeafApR) were also recorded.

The effect of the genotype was significant for most traits, with generally higher broad-sense heritabilities (h^2) in glasshouse experiments than in field experiments (Supplemental Table I.4). In all experiments, ‘Ballet’ had higher BegFlo, LeafApR than ‘Cameor’. Concerning traits related to C accumulation, ‘Ballet’ had higher ShootB, StrawB, TSW, RUE and ShootL than ‘Cameor’, and lower SeedN, LeafA and LeafAR (Supplemental Table I.4). Concerning N acquisition efficiency and accumulation, ‘Cameor’ had higher NDFA, SPAD, ShootNC, SeedNC and SeedQN, and lower StrawQN and NodeE.

The effects of year and genotype x year interaction were highly significant for most traits. Higher mean values were observed in pots than in pouches, with about a 4-fold increase of ShootB or ShootQN, and a 2-fold increase of SNU, RootE and NodeE (Supplemental Table I.5). In field experiments, higher StrawB and SeedB were observed in 2004 than in 2006, associated with higher StrawQN and SeedQN in spite of lower SeedNC. NDFA was noticeably lower in 2004 (Supplemental Table I.5), which is consistent with higher soil nitrate content at sowing (Sagan *et al.*, 1993b; Voisin *et al.*, 2002a). ShootQN and SeedQN were highly significantly correlated with RootB and TRootL measured at BF in pot or pouch experiment, and only in some cases with NLatRoot (Table I.2). ShootQN and SeedQN were also significantly correlated with all the nodule traits and NDFA, and with ShootB, StrawB or SeedB. Conversely, ShootQN and SeedQN were hardly ever correlated with ShootNC, SeedNC, RootE and NodeE. Lastly, ShootQN and SeedQN were significantly negatively correlated with SLN and RUE, whereas they were significantly positively correlated with leafA and LeafAR.

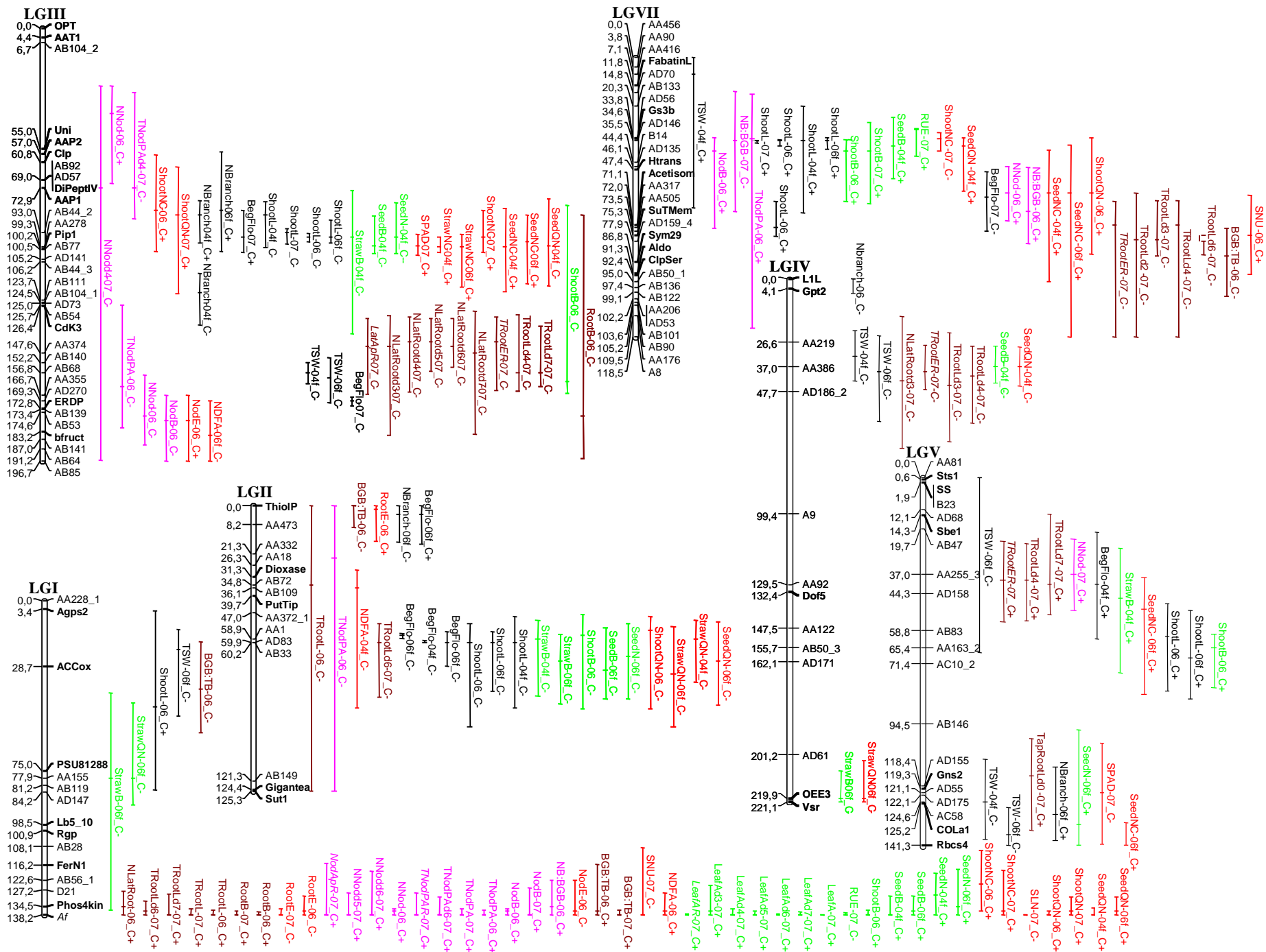


Fig. I.5. Map position of QTL associated with nitrogen acquisition structure or functioning, carbon accumulation in aerial part or seed yield components, and nitrogen accumulation or content; in the pea population RIL4, in two glasshouse (07, 06) and two field experiments (06f, 04f). For each QTL, the name of the trait, the number of the experiment, and the sign of the additive effect of the parental ‘Cameor’ allele are indicated

Mapping QTL for root and nodule structure

A total of 32 QTL was detected for root traits on six linkage groups (LG): 8 were related to number of lateral roots, 21 to root length, and 3 to root dry matter (Supplemental Table I.6). Concerning the nodule traits, a total of 26 QTL were detected on five linkage groups; 9 were related to nodule number, 8 to nodule area, 4 to nodule dry matter, and 3 to the relative part of the nodule dry matter. 5 QTL for the relative part of belowground upon total dry matter (BGB:TB) were also detected. Seven of the 32 root QTL and 11 of the 26 nodule QTL were detected in region of LGI close to the *Af* gene (LGI-*Af*). All showed a positive additive effect of the 'Cameor' allele with parts of the phenotypic variation (R^2) explained by the QTL ranging from 9 to 49%.

The other QTL for root or nodule traits were located on fourteen other genomic regions, with R^2 ranging from 10 to 20%. Some clusters were observed (Fig. I.5): nodule and root traits QTL were detected in LGII close to the marker Dioxase, with a negative additive effect of 'Cameor'; nodule traits QTL were found in LGIII near the marker AAP1; six of the eight QTL for lateral root number and three QTL for root length were detected in the same region of LGIII, near the marker AA374, with a negative additive effect of 'Cameor'; several QTL for nodule traits were found at LGIII near marker AB139, with a negative effect of 'Cameor' allele; root traits QTL were found on LGIV near marker AA386, with a negative effect of 'Cameor' allele; root and nodule traits QTL were found on LGV near AD158, with a positive effect of 'Cameor' allele; on LGVII, three QTL for nodule traits were found near Gs3b, and two other QTL for nodule traits were found between Htrans and Acetisom, with a positive effect of 'Cameor' allele, whereas five different QTL for root length with a negative effect of 'Cameor' allele were detected between AD159_4 and Sym29.

Co-location of QTL for root and nodule structure with QTL for N acquisition efficiency, N and C accumulation in plant, and plant development

Ten QTL for N efficiency traits were detected (Supplemental Table I.6): 7 QTL related to the N acquisition efficiency (SNU, RootE, Node), and 3 QTL for the percentage of N derived from fixation (NDFA). All of them mapped to genomic regions involved in root or nodule variation (Fig. I.5). Five of them were detected in the genomic region LGI-*Af*, and their R^2 ranged from 9 to 21%, with a positive additive effect of the 'Cameor' allele for NDFA, and negative additive effect for all other N acquisition efficiency traits. Co-locations of QTL of

Nefficiency with root and nodule traits were also observed in 4 other genomic regions, with negative effect of the ‘Cameor’ allele for all the NDFA QTL and positive effect for all the efficiency QTL. Each of these QTL accounted for 8 to 12% of the phenotypic variation.

A total of 26 QTL related to C accumulation in aerial parts (LeafA, LeafAR, ShootB, StrawB, SeedB), 15 QTL for seed yield components (SeedN, TSW) and 3 QTL for RUE were detected (Supplemental Table I.7). Sixteen QTL for N accumulation in the plant (ShootQN, StrawQN, SeedQN, SLN) and 15 QTL for N content (ShootNC, StrawNC, SeedNC) and SPAD were detected (Supplemental Table I.8). Twenty of these QTL were detected in the LGI-Af region (Fig. I.5), with positive additive effect of the ‘Cameor’ allele for all traits, except RUE. QTL for RUE and for ShootB-06 accounted for respectively 76% and 42% of the phenotypic variation of these traits measured at BF. For StrawB, SeedB and SeedN, which were measured at harvest, R^2 ranged from 10 to 17%. Co-locations of N and C accumulation with root and/or nodule QTL were also observed on LGII-AB33, LGIII-AAP1, LGIV-AA386, LGV-AD158, LGVII-Gs3b and LGVII-Htrans (Fig. I.5). A close inspection of LOG curves revealed a secondary peak for root and nodule traits near LGIII-AB44_2 (100 cM), which co-located with shoot and seed N accumulation traits (Supplemental Fig. I.2). Interestingly, LGIII-AA44_2 was the only region in which QTL for SeedQN co-located with QTL for SeedNC, and they displayed antagonistic effects. Otherwise, whatever the genomic region, QTL for SeedQN always co-located with SeedB, with never antagonistic effects.

Most of the QTL related to aerial part or to seed N and biomass accumulation were consistent across experiments, and many correspond to genomic regions that we also identified in the pea mapping population RIL1 (Burstin *et al.*, 2007). Among them, LGI-Af was contributing in both RIL4 and RIL1 to almost all the traits evaluated. Three other genomic regions in RIL4 involved in C and N accumulation in aerial parts and seeds (LGII-AB33 for ShootQN and flowering; LGIII-AB44_2 for StrawB, StrawNC, SeedN and flowering; LGVII-Gs3b for ShootB, ShootNC and SeedB) also correspond to genomic regions in RIL1. Moreover, four regions involved in the variation of TSW and/or SeedNC were located in similar regions in RIL1 and RIL4 (LGIII-AA374, LGIV-AA386, LGV-AD158, LGV-Rbcs4). Moreover, the root length QTL near LGVII-AD159_4 may correspond to the region of marker Amy associated with root biomass reported by Weeden and Moffet (2002).

Discussion

This study was carried out to identify the genetic determinants of the nitrogen nutrition in pea, which could be involved in the determinism of seed yield and protein content. Some studies identified major gene effects for root biomass in pea (Weeden & Moffet, 2002; Kof *et al.*, 2006), and some searched for genomic regions involved in nodule number or biomass on common bean and soybean (Nodari *et al.*, 1993; Souza *et al.*, 2000; Nicolas *et al.*, 2006). To our knowledge, our study is the first integrated approach of the genetic basis of nitrogen nutrition in legumes investigating not only QTL involved in nodulated root structure and functioning variability, but also those related with C accumulation variability, which is known to be closely linked to nitrogen acquisition capacity (Voisin *et al.*, 2003a). We used a set of different methods, ranging from root washing at specific stages, kinetic measurements of roots and nodules in pouches, to ^{15}N quantification experiments in the field, in order to approach the different facets of the nodulated root development as related to N nutrition. Our experiments in artificial conditions used *Rhizobium leguminosarum* bv. *viciae* (*Rlv*) strains previously demonstrated to be efficient on a large range of pea genotypes (Laguerre *et al.*, 2007), and our field experiments were conducted on a soil previously shown to contain *Rlv* populations efficient on a majority of pea accessions in our collection. Our study in pouches allowed an establishment of nodules on all plant studied, confirming that the conditions of high inoculation level and 50 ml of nutrient solution with pH at 6.5 were suitable for nodulation, in agreement with various experiments on legumes (Smith & Wollum, 1989; Novak *et al.*, 2002; Lira Junior *et al.*, 2005). Both pouch and pot experiments in artificial conditions provided a range of measurements of good quality, as revealed by the high heritabilities obtained for root and nodule traits (Supplemental Tables I.4 and I.9), in comparison with those obtained in a field experiment within a soybean RIL population (Kuang *et al.*, 2005). Our glasshouse experiments allowed detection of QTL for most of root and nodule traits, explaining up to 68% of the genetic variance observed for these traits (Supplemental Table I.9). High heritabilities were also obtained for C and N accumulation traits in such conditions, and QTL were detected, explaining similar part of genetic variance existing within the RIL population than those detected in field conditions (Supplemental Table I.10).

Towards the improvement of N nutrition through root and nodule structure traits

As for other legumes, N nutrition in pea relies both on atmospheric N₂ fixation by nodules and on soil mineral N uptake by roots, and it is considered a major limiting factor of plant growth (Voisin *et al.*, 2007). In pea, N-fixing activity increases during the vegetative phase up to flowering, concomitantly with the development of nodules which are then the largest carbon sink for the plant (Voisin *et al.*, 2003b). Then, both symbiotic fixation and mineral N root absorption decline at the end of the growth cycle when filling seeds become the largest carbon sink (Jeuffroy & Warembourg, 1991; Voisin *et al.*, 2003a). One strategy for enhancing exogenous nitrogen supply late during the growth cycle could be to select pea lines with a prolonged period of nodule development, which would maintain their symbiotic N₂ fixation activity during seed filling. Another complementary strategy is to increase root development before the beginning of seed filling, in order to enhance exogenous nitrogen supply at later stages (Bourion *et al.*, 2007). This would also contribute through deeper roots to water stress tolerance, and probably interact with root rot disease tolerance.

In this study, we assessed the potential of naturally-occurring genetic variability for root and nodule traits to improve nitrogen nutrition in pea. A significant variability was observed for both root (RootB, TRootL, NLatRoot) and nodule traits (NodB, NNod, TNodPA), among 7 contrasted pea genotypes and among a pea RIL population. Nodule traits exhibited ten-fold variations or more (Fig. I.2), depending on the stage at which the nodulated root system was observed. Root traits, with a two-fold average variation, were less variable (Fig. I.2). Then, we assessed the relationship among desirable traits, to identify possible antagonistic relationship between root and nodule development, and between root or nodule and shoot development. A complex interaction between hormonal and trophic factors determines the root-nodule-shoot development. Indeed, hypernodulating mutants were considered potential candidates for enhancing N₂ fixation through an increase in nodule number (Carroll & Mathews, 1990). However, various studies have indicated that hypernodulating mutants did not accumulate more nitrogen than the wild line (Sagan *et al.*, 1993b; Bourion *et al.*, 2007), and often displayed depressed shoot and root growth, probably due to high C costs for nodulation and N₂ fixation (Voisin *et al.*, 2007). Gonzalez-Rizzo *et al.* (2006) also demonstrated that the cytokinin receptor MtCRE1 regulates nodule and lateral root organogenesis in an opposite manner. In the present study, root and nodule traits did not show antagonistic relationship both among the 7 genotypes and the RIL4 population (Table

I.1; Supplemental Table I.3). Conversely, nodule traits were highly positively correlated with TRootL and RootB, and to a lesser extent with NLatRoot. Consistently, the 4 common QTL controlling root and nodule traits showed additive allele effects of the same sign (LGI-Af, LGII-Dioxase, LGIII-AB139, LGV-AD158; Fig. I.5). On the other hand, some QTL regions were specific of roots traits (LGIII-AA374, LGIV-AA386). This makes it possible to select simultaneously or separately for root and nodule traits.

We further identified that NodB was determined both by NNod (3 QTL in common; LGI-Af, LGIII-AB139, LGVII-Htrans; Supplemental Fig. I.2) and by TNodPA (3 QTL in common; LGI-Af, LGIII-AB139, LGVII-Gs3b), which reflects nodule number and nodule size, respectively. Conversely, RootB was hardly ever correlated to NLatRoot (Table I.1; Supplemental Table I.3) and highly correlated to TRootL (with 2 QTL in common; LGI-Af, LGIII-AA374). TRootL was correlated both with TRootER (4 QTL in common; LGIII, LGIV, LGV, LGVII) and little with LatApR (1 QTL in common; LGIII-AA374). Interestingly, QTL for TRootER were co-located either with NNod (LGV-AD158) or with NLatRoot (LGIII-AA374, LGIV-AA386). As nodules as well as lateral root primordia form on elongating parts of the root (Tricot *et al.*, 1997), this result suggests that some genomic regions may control either nodule or root initiation, but not both.

Root and nodule structures contribute to N acquisition and accumulation in the plant

All QTL for NDFA, which described N acquisition by fixation, corresponded to QTL controlling both root and nodule traits (LGI-Af, LGII-Dioxase, LGIII-AB139; Fig. I.5, Supplemental Fig. I.2). They displayed additive effects of same sign, indicating that an increase of NDFA is tightly linked to an increase of NodB and RootB. This result seemed different from what was observed in pea hypernodulating mutants (Sagan *et al.* 1993; Salon *et al.* 2001), for which increased NodB was associated with decreased RootB and NDFA (Bourion *et al.*, 2007). However, all these results suggest that the N accumulation through fixation relies on a good development of both roots and nodules. Interestingly, QTL for NDFA were not always co-located with QTL for SeedQN, which is in agreement with previous observations of no significant effect of N symbiotic fixation level in seed N accumulation (Sagan *et al.* 1993; Voisin *et al.* 2002).

In most cases, shoot and seed N accumulation were correlated with root and nodule traits. As such, among the seven pea accessions, ShootQN was significantly correlated with

RootB at early stages (from 4-leaf stage to BF), NNod at the 9-leaf stage, and TNodPA at later stages (from BF to BSF); Table I.1). Among the RIL4 genotypes, ShootQN and SeedQN were significantly correlated with RootB, TRootL, NNod, and TNodPA and NodB (Table I.2). Consistently, common QTL displaying effects of the same sign were found between root and/or nodule traits and ShootQN, SeedQN or SeedNC (LGI-*Af*, LGII-AB33, LGIV-AA386, LGV-AA158, LGVII-Htrans; Fig. I.5). These results may suggest that genes controlling nitrogen nutrition structure traits are significant determinants of shoot and/or seed N accumulation. Conversely, these results may equally suggest that ShootQN accumulation, which is assumed to control the elaboration of leaf area and thus the C supply (Laperche *et al.*, 2006; Moreau *et al.*, 2009), promotes root and nodule establishment and growth.

In other cases, shoot or seed N accumulation did not appear to be directly correlated with root or nodule traits. As such, the genomic regions near LGIII-AB44_2 and near LGVII-Gs3b displayed QTL with opposite effects for ShootNC or SeedNC on one hand, and SeedB, SeedN and SeedQN on the other hand, but no strong effect QTL for root and nodule traits. As these genomic regions appeared to also control ShootL and BegFlo, they may correspond to a QTL controlling N partitioning between seeds and aerial parts through plant development. Lastly, the QTL clusters for root elongation rate, root length at early stages, and thousand seed weight that were found at LGIII-AA374 and LGIV-AA386 may illustrate the link between seed cotyledon reserves and root growth during the heterotrophic phase. Indeed, Tricot *et al.* (1997) observed a rough decline of root elongation rate and of roots number, between the 4- and 6-leaf stages in conjunction with the exhaustion of seed reserves. Other experiments have confirmed the impact of seed size on root elongation rate, root length or root dry matter during the early growth of the plant (Thorup-Kristensen, 1998; McPhee, 2005). Thus, the QTL controlling thousand seed weight in these regions may have a pleiotropic effect on the root elongation rate. However, a higher root elongation rate and root length could conversely enhance seed storage compound accumulation at the end of the plant life cycle by a sustained water and nutrient supply, and hence increase thousand seed weight.

Root and nodule traits are impacted by major developmental genes such as *Le* and *Af*

In pea, *Le*, which encodes gibberellin 3 β -hydroxylase, controls inter-node length whereas *Af* controls the switch between leaflets and tendrils. In a previous work, we showed that in pea, the genomic regions encompassing the developmental genes *Le* and *Af* have pleiotropic

effects on plant morphology, source capacity, and seed protein content and yield (Burstin *et al.*, 2007). Consistently, Weeden and Moffet (2002) showed a significant association between *Le* and root biomass, in 42 RIL derived from a cross between a *Pisum elatius* line and a pea cultivar, and Kof *et al.* (2006) observed a significant effect of *Af* on root biomass. The results obtained herein confirm and specify these findings. In 7 pea accessions, we found a significant effect of the genes *Le* and *Af* on root and nodule development, at different stages of plant development (Supplemental Table I.1). The effect of *Le* on root and nodule traits increased from the earlier stage (at 4-leaf stage, only nodule traits showed a significant effect of *Le*) towards the latest stage analysed (at BSF, all root and nodule traits showed a significant effect of *Le*). Conversely, the effect of *Af* on root and nodule traits decreased from the earlier stage (at 4-leaf stage, all traits showed a significant effect of *Af*) towards the latest stages analysed (at BSF, only NLatRoot and NNod showed a significant effect). In the RIL4 population, *Le* is not segregating, and neither QTL for seed N content and yield nor QTL for root and nodule development were detected in the corresponding genomic region, at the bottom end of LGIII. *Af* is segregating in the RIL4 population. A major QTL cluster was identified in the LGI-*Af* region, where *Af* is the best candidate gene for having pleiotropic effects on leaf area, nitrogen acquisition structure traits, and seed or shoot N accumulation traits. This region controlled 16% of the variation of leaf area eight days after germination when one leaf was fully-expanded, and accounted for more than 60% twenty days after germination when five leaves were fully expanded. It also controlled 75% of the variation of RUE, indicating a reduction of C accumulation in *af* genotypes. This could be the cause of the reduction of root and nodule growth. Indeed, QTL for root and nodule dry matter and for the relative part of nodule upon belowground dry matter were consistently detected at beginning of flowering, with a negative effect of the *af* allele for all these traits including the relative part of nodule upon belowground dry matter. This may indicate a greater impact of low carbon availability on nodules than on roots, which, according to Voisin *et al.* (2003b) represent the largest carbon sink for the plant during the vegetative stage up to flowering. Consistently, no QTL for TRootER was detected at the *Af* locus, whereas QTL for NodApR and TNodPAR were. This reinforces the hypothesis of a trophic control of this locus on nodule appearance and growth rather than on root elongation. The potentiality of this region for N nutrition improvement depends on the nature of the gene involved: if the gene *Af* is responsible for the N nutrition variation, then the usefulness of this locus will be limited since the *afila* trait is extremely desirable for lodging resistance. If the gene responsible for the

variation is a gene close to *Af*, then the linkage may be broken. This question could be checked when the *Af* gene will be identified.

Conclusion

This study showed the usefulness of experiment in pouches combined with image analysis for investigating the nodulated root development and growth in a large number of plants. This methodology provided consistent results with those acquired in pots and facilitated the selection of contrasted accessions for root and nodule features. This information can be used as a valuable baseline for breeding programs. Using this methodology, we investigated the variability and relationship of nitrogen acquisition structure together and with C and N accumulation in the plant. We have found a significant positive relationship between nodule establishment and root system growth, which should allow building a pea nitrogen-nutrition 'ideotype', with increased root system size and no decreased nodule number. We also specified the significant contribution of N acquisition structure to seed N content and yield. Our results point to regions of interest for root and nodule development. Because QTL associations described herein may be caused either by pleiotropic effects of one gene or by linkage between different genes, these regions will need to be refined through fine-mapping and/or the use of association genetics, and through the comparison with another large-seed legume such as soybean or with the model species *Medicago truncatula*.

Acknowledgments

We thank, for their help in the experimental work, Hervé Houtin, Céline Rond, Nicolas Jeannin, Abdelkrim Souiah and Chantal Martin of the UMR102, and Nouredine El Mjiyad of the Experimental Unit (UE115, Dijon). We also thank Laurence Moreau (INRA, UMR320, Gif-sur-Yvette) for useful help in statistical analysis and Olivier Delfosse (INRA, UMR614, Reims) for ¹⁵N analyses. Gisèle Laguerre (INRA, UMR113, Montpellier) and Céline Faivre (INRA, UMR1229, Dijon) are acknowledged for their guidance in the use of growth pouches. This work was supported by the French programme Genoplante (GOP PeaC and GOP PeaC2), and by the European Union (Grain Legumes Integrated Project, a Framework Programme 6 project, grant no. FOOD-CT-2004-506223). We are grateful to the anonymous referees for their constructive criticisms.

References

- Ali-Khan ST, Snoad B. 1977. Root and shoot development in peas. *Annals of Applied Biology* **85**(1): 131-136.
- Ané JM, Kiss GB, Riely BK, Penmetsa RV, Oldroyd GED, Ayax C, Lévy J, Debelle F, Baek JM, Kalo P, Rosenberg C, Roe BA, Long SR, Dénarié J, Cook DR. 2004. *Medicago truncatula* *DMII* required for bacterial and fungal symbioses in legumes. *Science* 303(5662): 1364-1367.
- Aubert G, Morin J, Jacquin F, Loridon K, Quillet MC, Petit A, Rameau C, Lejeune-Hénaut I, Huguet T, Burstin J. 2006. Functional mapping in pea, as an aid to the candidate gene selection and for investigating synteny with the model legume *Medicago truncatula*. *Theoretical and Applied Genetics* **112**(6): 1024-1041.
- Baranger A, Aubert G, Arnau G, Laine AL, Deniot G, Potier J, Weinachter C, Lejeune-Henaut I, Lallemand J, Burstin J. 2004. Genetic diversity within *Pisum sativum* using protein- and PCR-based markers. *Theoretical and Applied Genetics* **108**(7): 1309-1321.
- Barbulova A, Rogato A, D'Apuzzo E, Omrane S, Chiurazzi M. 2007. Differential effects of combined N sources on early steps of the nod factor-dependent transduction pathway in *Lotus japonicus*. *Molecular Plant-Microbe Interactions* **20**(8): 994-1003.
- Bourion V, Laguerre G, Depret G, Voisin AS, Salon C, Duc G. 2007. Genetic variability in nodulation and root growth affects nitrogen fixation and accumulation in pea. *Annals of Botany* **100**(3): 589-598.
- Bright LJ, Liang Y, Mitchell DM, Harris JM. 2005. The *LATD* gene of *Medicago truncatula* is required for both nodule and root development. *Molecular Plant-Microbe Interactions* **18**(6): 521-532.
- Burstin J, Marget P, Huart M, Moessner A, Mangin B, Duchene C, Desprez B, Munier-Jolain N, Duc G. 2007. Developmental genes have pleiotropic effects on plant morphology and source capacity, eventually impacting on seed protein content and productivity in pea. *Plant Physiology* **144**(2): 768-781.
- Caroll BJ, Mathews A 1990. Nitrate inhibition of nodulation in legumes. In: Gresshoff PM ed. *Molecular biology of symbiotic nitrogen fixation*. Boca Raton: CRC Press, 159-180.
- Catoira R, Galera C, de Billy F, Penmetsa RV, Journet EP, Maillet F, Rosenberg C, Cook D, Gough C, Dénarié J. 2000. Four genes of *Medicago truncatula* controlling components of a nod factor transduction pathway. *Plant Cell* **12**(9): 1647-1665.
- Charcosset A, Gallais A. 1996. Estimation of the contribution of quantitative trait loci (QTL) to the variance of a quantitative trait by means of genetic markers. *Theoretical and Applied Genetics* **93**(8): 1193-1201.
- Coque M, Gallais A. 2006. Genomic regions involved in response to grain yield selection at high and low nitrogen fertilization in maize. *Theoretical and Applied Genetics* **112**(7): 1205-1220.
- de Billy F, Grosjean C, May S, Bennett M, Cullimore JV. 2001. Expression studies on *AUX1*-like genes in *Medicago truncatula* suggest that auxin is required at two steps in early nodule development. *Molecular Plant-Microbe Interactions* **14**(3): 267-277.
- Dénarié J, Cullimore J. 1993. Lipo-Oligosaccharide Nodulation Factors - a Minireview New Class of Signaling Molecules Mediating Recognition and Morphogenesis. *Cell* **74**(6): 951-954.
- Duc G, Mariotti A, Amarger N. 1988. Measurements of genetic variability for symbiotic dinitrogen fixation in field-grown fababean (*Vicia faba* L.) using a low-level ^{15}N -tracer technique. *Plant and Soil* **106**(2): 269-276.
- Dumont E, Fontaine V, Vuylsteker C, Sellier H, Bodèle S, Voedts N, Devaux R, Frise M, Avia K, Hilbert J-L, Bahrman N, Hanocq E, Lejeune-Hénaut I, Delbreil B. 2009. Association of sugar content QTL and PQL with physiological traits relevant to frost damage resistance in pea under field and controlled conditions. *Theoretical and Applied Genetics* **118**(8): 1561-1571.
- Edwards A, Heckmann AB, Yousafzai F, Duc G, Downie JA. 2007. Structural implications of mutations in the pea *SYM8* symbiosis gene, the *DMII* ortholog, encoding a predicted ion channel. *Molecular Plant-Microbe Interactions* **20**(10): 1183-1191.

- Endre G, Kereszt A, Kevei Z, Mihacea S, Kalo P, Kiss GB. 2002.** A receptor kinase gene regulating symbiotic nodule development. *Nature* **417**(6892): 962-966.
- FAOSTAT 2007.** Food and Agriculture Organization website. <http://faostat.fao.org>.
- Frugier F, Kosuta S, Murray JD, Crespi M, Szczyglowski K. 2008.** Cytokinin: secret agent of symbiosis. *Trends in Plant Science* **13**(3): 115-120.
- Gonzalez-Rizzo S, Crespi M, Frugier F. 2006.** The *Medicago truncatula* CRE1 cytokinin receptor regulates lateral root development and early symbiotic interaction with *Sinorhizobium meliloti*. *Plant Cell* **18**(10): 2680-2693.
- Jeuffroy M-H, Warembourg FR. 1991.** Carbon transfer and partitioning between vegetative and reproductive organs in *Pisum sativum* L.. *Plant Physiology* **97**(1): 440-448.
- Jourjon MF, Jasson S, Marcel J, Ngom B, Mangin B. 2005.** MCQTL: multi-allelic QTL mapping in multi-cross design. *Bioinformatics* **21**(1): 128-130.
- Kiniry JR, Jones CA, Otoole JC, Blanchet R, Cabelguenne M, Spanel DA. 1989.** Radiation-Use Efficiency in Biomass Accumulation Prior to Grain-Filling for 5 Grain-Crop Species. *Field Crops Research* **20**(1): 51-64.
- Kof EM, Vinogradova IA, Oorzhak AS, Kalibernaya ZV. 2006.** The rates of shoot and root growth in intact plants of pea mutants in leaf morphology. *Russian Journal of Plant Physiology* **53**(1): 116-125.
- Kraft JM, Boge W. 2001.** Root characteristics in pea in relation to compaction and fusarium root rot. *Plant Disease* **85**(9): 936-940.
- Krusell L, Madsen LH, Sato S, Aubert G, Genua A, Szczyglowski K, Duc G, Kaneko T, Tabata S, de Bruijn F, Pajuelo E, Sandal N, Stougaard J. 2002.** Shoot control of root development and nodulation is mediated by a receptor-like kinase. *Nature* **420**(6914): 422-426.
- Kuang R-B, Liao H, Yan X-L, Dong Y-S. 2005.** Phosphorus and Nitrogen Interactions in Field-Grown Soybean as Related to Genetic Attributes of Root Morphological and Nodular Traits. *Journal of Integrative Plant Biology* **47**(5): 549-559.
- Laguerre G, Depret G, Bourion V, Duc G. 2007.** *Rhizobium leguminosarum* bv. viciae genotypes interact with pea plants in developmental responses of nodules, roots and shoots. *New Phytologist* **176**(3): 680-690.
- Laperche A, Devienne-Barret F, Maury O, Le Gouis J, Ney B. 2006.** A simplified conceptual model of carbon/nitrogen functioning for QTL analysis of winter wheat adaptation to nitrogen deficiency. *Theoretical and Applied Genetics* **113**(6): 1131-1146.
- Larigauderie A, Reynolds JF, Strain BR. 1994.** Root response to CO₂ enrichment and nitrogen supply in loblolly-pine. *Plant and Soil* **165**(1): 21-32.
- Lejeune-Hénaut I, Hanocq E, Béthencourt L, Fontaine V, Delbreil B, Morin J, Petit A, Devaux R, Boilleau M, Stempniak J-J, Thomas M, Lainé A-L, Foucher F, Baranger A, Burstin J, Rameau C, Giauffret C. 2008.** The flowering locus Hr colocalizes with a major QTL affecting winter frost tolerance in *Pisum sativum* L. *Theoretical and Applied Genetics* **116**(8): 1105-1116.
- Lévy J, Bres C, Geurts R, Chalhoub B, Kulikova O, Duc G, Journet EP, Ané JM, Lauber E, Bisseling T, Dénarié J, Rosenberg C, Debelle F. 2004.** A putative Ca²⁺ and calmodulin-dependent protein kinase required for bacterial and fungal symbioses. *Science* **303**(5662): 1361-1364.
- Liang Y, Mitchell DM, Harris JM. 2007.** Abscisic acid rescues the root meristem defects of the *Medicago truncatula* latd mutant. *Developmental Biology* **304**(1): 297-307.
- Lira Junior MdA, Lima AST, Arruda JRF, Smith DL. 2005.** Effect of root temperature on nodule development of bean, lentil and pea. *Soil Biology and Biochemistry* **37**(2): 235-239.
- Loridon K, McPhee K, Morin J, Dubreuil P, Pilet-Nayel ML. 2005.** Microsatellite marker polymorphism and mapping in pea (*Pisum sativum* L.). *Theor Appl Genet.* **111**.
- Magori S, Kawaguchi M. 2009.** Long-distance control of nodulation: Molecules and models. *Molecules and Cells* **27**(2): 129-134.
- Mathesius U. 2008.** Auxin: at the root of nodule development? *Functional Plant Biology* **35**(8): 651-668.

- McPhee K. 2005.** Variation for seedling root architecture in the core collection of pea germplasm. *Crop Science* **45**(5): 1758-1763.
- Melchinger A, Chandra S, Utz H, eds. 2000.** New ways in quantitative genetics: results of QTL analyses revisited by cross validation and bootstrapping. Les Colloques. Paris (France).
- Moreau D, Salon C, Munier-Jolain N. 2007.** A model-based framework for the phenotypic characterization of the flowering of *Medicago truncatula*. *Plant Cell and Environment* **30**(2): 213-224.
- Moreau D, Schneider C, Huguet T, Salon C, Munier-Jolain N. 2009.** Can differences of nitrogen nutrition level among *Medicago truncatula* genotypes be assessed non-destructively? Probing with a recombinant inbred lines population: Probing with a recombinant inbred lines population. *Plant Signaling & Behavior* **4**(1): 30-32.
- Ney B, Turc O. 1993.** Heat-unit-based description of the reproductive development of pea. *Crop Science* **33**(3): 510-514.
- Nicolas MF, Hungria M, Arias CAA. 2006.** Identification of quantitative trait loci controlling nodulation and shoot mass in progenies from two Brazilian soybean cultivars. *Field Crops Research* **95**(2-3): 355-366.
- Nodari RO, Tsai SM, Guzmán P, Gilbertson RL, Gepts P. 1993.** Toward an integrated linkage map of common bean. III. Mapping genetic factors controlling host-bacteria interactions. *Genetics* **134**(1): 341-350.
- Novak K, Biedermannova E, Vondrys J. 2009.** Symbiotic and growth performance of supernodulating forage pea lines. *Crop Science* **49**(4): 1227-1234.
- Novak K, Chovanec P, Skrdleta V, Kropacova M, Lisa L, Nemcova M. 2002.** Effect of exogenous flavonoids on nodulation of pea (*Pisum sativum* L.). *Journal of Experimental Botany* **53**(375): 1735-1745.
- Oka-Kira E, Kawaguchi M. 2006.** Long-distance signaling to control root nodule number. *Current Opinion in Plant Biology* **9**(5): 496-502.
- Okamoto S, Ohnishi E, Sato S, Takahashi H, Nakazono M, Tabata S, Kawaguchi M. 2009.** Nod Factor/Nitrate-Induced *CLE* Genes that Drive HARA1-Mediated Systemic Regulation of Nodulation. *Plant and Cell Physiology* **50**(1): 67-77.
- Pilet-Nayel ML, Muehlbauer FJ, McGee RJ, Kraft JM, Baranger A, Coyne CJ. 2002.** Quantitative trait loci for partial resistance to *Aphanomyces* root rot in pea. *Theoretical and Applied Genetics* **106**(1): 28-39.
- Postma JG, Jacobsen E, Feenstra WJ. 1988.** Three pea mutants with an altered nodulation studied by genetic analysis and grafting. *Journal of Plant Physiology* **132**(4): 424-430.
- Sagan M, Duc G. 1996.** *Sym28* and *Sym29*, two new genes involved in regulation of nodulation in pea (*Pisum sativum* L.). *Symbiosis* **20**(3): 229-245.
- Sagan M, Ney B, Duc G. 1993.** Plant symbiotic mutants as a tool to analyse nitrogen nutrition and yield relationship in field-growth peas (*Pisum sativum* L.). *Plant and Soil* **153**(1): 33-45.
- Salon C, Munier-Jolain NG, Duc G, Voisin AS, Grandgirard D, Larmure A, Emery RJN, Ney B. 2001.** Grain legume seed filling in relation to nitrogen acquisition: A review and prospects with particular reference to pea. *Agronomie* **21**(6-7): 539-552.
- Santalla M, Amurrio JM, De Ron AM. 2001.** Symbiotic Interactions between *Rhizobium leguminosarum* Strains and Elite Cultivars of *Pisum sativum* L. *Journal of Agronomy and Crop Science* **187**(1): 59-68.
- Schnabel E, Journet EP, de Carvalho-Niebel F, Duc G, Frugoli J. 2005.** The *Medicago truncatula* *SUNN* gene encodes a *CLVI*-like leucine-rich repeat receptor kinase that regulates nodule number and root length. *Plant Molecular Biology* **58**(6): 809-822.
- Sinclair TR, Horie T. 1989.** Leaf Nitrogen, Photosynthesis, and Crop Radiation Use Efficiency - a Review. *Crop Science* **29**(1): 90-98.
- Smith GB, Wollum AG. 1989.** Nodulation of *Glycine max* by six *Bradyrhizobium japonicum* strains with different competitive abilities. *Applied and Environmental Microbiology* **55**(8): 1957-1962.

- Souza AA, Boscariol RL, Moon DH, Camargo LE, Tsai SM. 2000.** Effects of *Phaseolus vulgaris* QTL in controlling host-bacteria interactions under two levels of nitrogen fertilization. *Genetics and Molecular Biology* **23**(1): 155-161.
- Tar'an B, Warkentin T, Somers DJ, Miranda D, Vandenberg A, Blade S, Bing D. 2004.** Identification of quantitative trait loci for grain yield, seed protein concentration and maturity in field pea (*Pisum sativum* L.). *Euphytica* **136**(3): 297-306.
- Thorup-Kristensen K. 1998.** Root growth of green pea (*Pisum sativum* L.) genotypes. *Crop Sci* **38**.
- Timmerman-Vaughan GM, McCallum JA, Frew TJ, Weeden NF, Russell AC. 1996.** Linkage mapping of quantitative trait loci controlling seed weight in pea (*Pisum sativum* L.). *Theoretical and Applied Genetics* **93**(3): 431-439.
- Timmerman-Vaughan GM, Mills A, Whitfield C, Frew T, Butler R, Murray S, Lakeman M, McCallum J, Russell A, Wilson D. 2005. Linkage mapping of QTL for seed yield, yield components, and developmental traits in pea. *Crop Science* **45**(4): 1336-1344.
- Tricot F, Crozat Y, Pellerin S. 1997.** Root system growth and nodule establishment on pea (*Pisum sativum* L.). *Journal of Experimental Botany* **48**(11): 1935-1941.
- Tuberosa R, Sanguinetti MC, Landi P, Giuliani MM, Salvi S, Conti S. 2002.** Identification of QTLs for root characteristics in maize grown in hydroponics and analysis of their overlap with QTLs for grain yield in the field at two water regimes. *Plant Mol Biol* **48**.
- Voisin AS, Bourion V, Duc G, Salon C. 2007.** Using an ecophysiological analysis to dissect genetic variability and to propose an ideotype for nitrogen nutrition in pea. *Annals of Botany* **100**(7): 1525-1536.
- Voisin AS, Salon C, Jeudy C, Warembourg FR. 2003a.** Symbiotic N₂ fixation activity in relation to C economy of *Pisum sativum* L. as a function of plant phenology. *Journal of Experimental Botany* **54**(393): 2733-2744.
- Voisin AS, Salon C, Jeudy C, Warembourg FR. 2003b.** Root and nodule growth in *Pisum sativum* L. in relation to photosynthesis: Analysis using ¹³C-labelling. *Annals of Botany* **92**(4): 557-563.
- Voisin AS, Salon C, Munier-Jolain NG, Ney B. 2002.** Effect of mineral nitrogen on nitrogen nutrition and biomass partitioning between the shoot and roots of pea (*Pisum sativum* L.). *Plant and Soil* **242**(2): 251-262.
- Weeden NF, Moffet M. 2002.** Identification of genes affecting root mass and root/shoot ratio in a JI 1794 x 'Slow' RIL population. *Pisum Genetics* **34**: 28-31.
- Weeden NF, Tonguc M, Boone WE. 1999.** Mapping coding sequences in pea by PCR. *Pisum Genet* **31**: 30-32.
- Wopereis J, Pajuelo E, Dazzo FB, Jiang QY, Gresshoff PM, de Bruijn FJ, Stougaard J, Szczyglowski K. 2000.** Short root mutant of *Lotus japonicus* with a dramatically altered symbiotic phenotype. *Plant Journal* **23**(1): 97-114.

CHAPITRE II

CHAPITRE II : RECHERCHE DE GENES CANDIDATS

IMPLIQUES DANS LE DEVELOPPEMENT RACINAIRE ET

DANS L'ACQUISITION D'N

1. Introduction au chapitre II

Le levier de l'amélioration de l'acquisition d'N chez les légumineuses via une amélioration de leur développement racinaire a été abordé par l'étude d'un mutant d'architecture racinaire chez *Medicago truncatula*, espèce modèle pour laquelle des données génomiques étaient disponibles. Ce mutant, TR185, du fait de son phénotype racinaire hyper-ramifié, était supposé avoir un haut potentiel d'acquisition d'N minéral. L'objectif de l'étude a été i) de déterminer le potentiel d'acquisition d'N chez ce mutant, ii) de mettre en évidence des différentiels d'expression de gènes entre ce mutant et le sauvage pouvant être corrélés au phénotype racinaire et à l'acquisition d'N de ce mutant.

Il a été observé que TR185 conserve son architecture racinaire ramifiée quelle que soit la teneur en nitrate du milieu et contre toute attente, présente des caractéristiques de nutrition N sous-optimale : allocation préférentielle de la biomasse vers les racines, faible teneur en N des parties aériennes, et faible efficacité d'acquisition d'N. L'expérimentation a été menée en conditions non symbiotiques : aucune inoculation n'a été réalisée et la culture effectuée en chambre climatisée en respectant des mesures strictes de désinfection a empêché toute nodulation. La culture a aussi été réalisée en hydroponie, de façon à avoir un accès facilité aux racines. Un nouveau protocole d'expérimentation a donc été mis au point, inspiré de celui utilisé par Ruffel *et al.* (2008). Les descriptions de l'architecture racinaire et des efficacités d'acquisition d'N et de C ont été faites de façon similaire à celle réalisées précédemment sur les lignées de pois. L'analyse transcriptomique réalisée à partir de prélèvements de racines a mis en évidence 484 différentiellement exprimés entre TR185 et le sauvage (parmi les 61 278 transcrits disponibles sur les puces Affymetrix) ; un script d'analyse statistique adapté à cette expérimentation à deux facteurs (génotype de plante et teneur en azote du milieu) a été écrit en collaboration avec M-L Martin-Magniette (INRA URGV, Evry).

L'analyse des voies métaboliques impliquées conjointe avec des analyses de teneur en acides aminés des parties aériennes et des racines, ainsi que la réalisation de greffes ont

permis de tester les hypothèses permettant d'expliquer la nutrition sous-optimale observée chez TR185 en dépit de son nombre élevé de racines. L'hypothèse d'une perturbation de sa perception de la disponibilité en nitrate a ainsi été rejetée au profit de celle d'une hyper-ramification racinaire induite par un signal systémique de carence en N.

L'ensemble de ces résultats a fait l'objet d'une publication dans la revue *Journal of Experimental Botany* (Bourion *et al.*, 2014).

2. Publication n°2

Unexpectedly low nitrogen acquisition and absence of root architecture adaptation to nitrate supply in a *Medicago truncatula* highly-branched root mutant

Virginie Bourion^{1*}, Chantal Martin¹, Henri de Larambergue¹, Françoise Jacquin¹, Grégoire Aubert¹, Marie-Laure Martin-Magniette²³⁴⁵⁶, Sandrine Balzergue⁴⁵⁶, Geoffroy Lescure⁷, Sylvie Citerne⁷, Marc Lepetit⁸, Nathalie Munier-Jolain¹, Christophe Salon¹, Gérard Duc¹

¹INRA, UMR1347 Agroécologie, BP 86510, F-21065 Dijon, France

²INRA, UMR518 MIA, F-75231 Paris, France.

³AgroParisTech, UMR MIA, F-75231 Paris, France.

⁴INRA, UMR1165 URGV, F-91057 Evry, France.

⁵UEVE, UMR URGV, F-91057 Evry, France.

⁶CNRS, ERL8196 UMR URGV, F-91057 Evry, France.

⁷ Institut Jean-Pierre Bourgin, UMR1318 INRA/AgroParisTech, F-78026 Versailles, France

⁸USC1342 INRA, UMR113 IRD-CIRAD-SupAgro-UM2, Symbioses Tropicales et Méditerranéennes, Campus de Baillarguet, TA A-82/J, F-34398 Montpellier Cedex 5, France

*Corresponding author: Virginie Bourion,

INRA, UMR1347 Agroécologie, Pôle GEAPSI, BP 86510, F-21065 Dijon, France

Tel : 33 (3) 80 69 36 47

Fax : 33 (3) 80 69 32 63

Email: virginie.bourion@dijon.inra.fr

Les références citées sont situées à la fin de ce chapitre (p 101-105).

Les fichiers additionnels sont présentés en annexe 2.

Abstract

To complement N₂ fixation through symbiosis, legumes can efficiently acquire soil mineral nitrogen (N) through adapted root architecture. However, root architecture adaptation to mineral N availability has been little studied in legumes. Therefore, we investigated the effect of nitrate availability on root architecture in *Medicago truncatula* and assessed the N-uptake potential of a new highly-branched root mutant. The effects of varying nitrate supply on both root architecture and N-uptake were characterized in the mutant and in the wild type. Surprisingly, the root architecture of the mutant was not modified by nitrate supply variation. Moreover, despite its highly-branched root architecture, TR185 has a permanently N-starved phenotype. A transcriptome analysis was performed to identify genes differentially expressed between the two genotypes. This analysis revealed differential responses related to the nitrate acquisition pathway and confirmed that N-starvation occurred in TR185. Changes in amino acids content and in expression of genes involved in the phenylpropanoid pathway were associated with differences in root architecture between the mutant and the wild type.

Keywords: highly-branched root mutant, *Medicago truncatula*, root architecture, nitrogen limitation, nitrogen acquisition, amino acids, phenylpropanoid

Short statement: Physiological and developmental analyses provide evidence that the highly-branched root architecture of the mutant results from systemic regulation by its nitrogen status, possibly involving glutamine or asparagine signals.

Introduction

Nitrogen (N) is one of the most limiting resources for plant growth. Legumes have natural ability to use, as main N source, atmospheric N₂ via symbiosis in nodules with Rhizobiaceae spp. However, N nutrition can still limit yield and seed quality in legumes, especially under abiotic or biotic stress conditions. In that conditions, the fixation of N₂ is impacted and cannot totally fulfil N demand (Salon *et al.*, 2001), and the poorly developed root systems of N₂ fixing legumes are unable to explore a large soil volume (Bourion *et al.*, 2007). In this context, the genetic improvement of root system development is a target for increasing legume yield performance.

Up to now, the molecular determinants of root development in legumes have been little characterised. The naturally occurring genetic variability of root development in legumes has been investigated (Kraft & Boge, 2001; McPhee, 2005; Bourion *et al.*, 2010), but few genes involved in root development have been characterized (Yendrek *et al.*, 2010; Jin *et al.*, 2012). A complex tuning of root versus nodule development seems to operate in legumes, as mutants impaired in the autoregulation of nodulation display shorter root length or enhanced lateral root (LR) number (Wopereis *et al.*, 2000; Krusell *et al.*, 2002; Schnabel *et al.*, 2005; Schnabel *et al.*, 2011; Jin *et al.*, 2012). Hormones have been shown to be involved in their common molecular pathways; particularly auxin (de Billy *et al.*, 2001; Jin *et al.*, 2012), cytokinin (Gonzalez-Rizzo *et al.*, 2006; Frugier *et al.*, 2008; Plet *et al.*, 2011), and abscisic acid (Bright *et al.*, 2005; Liang *et al.*, 2007; Yendrek *et al.*, 2010).

The paramount importance of hormones in the regulation of root growth and development has been thoroughly investigated in *Arabidopsis* (Peret *et al.*, 2009). Auxin delivery, which promotes LR initiation, is regulated by the auxin influx carrier AUX1 and auxin efflux transporters PINs and PGP/MDR (Muday & DeLong, 2001; Marchant *et al.*, 2002). Auxin transport remains necessary for root elongation (Wu *et al.*, 2007). Interacting effects of auxin and cytokinin disrupt LR initiation by interfering with PINs genes expression and the associated auxin-gradient formation (Laplaze *et al.*, 2007). Cytokinin has been shown to reduce the root elongation rate through an ethylene-induced production (Benkova & Hejatko, 2009; Ruzicka *et al.*, 2009), whereas gibberellin antagonizes the negative effects of ethylene on root growth (Fu & Harberd, 2003; Ubeda-Tomas *et al.*, 2008).

In addition, root growth and development are known in *Arabidopsis* to be modulated by external NO₃⁻ availability. The localized stimulatory effect of external nitrate on LR

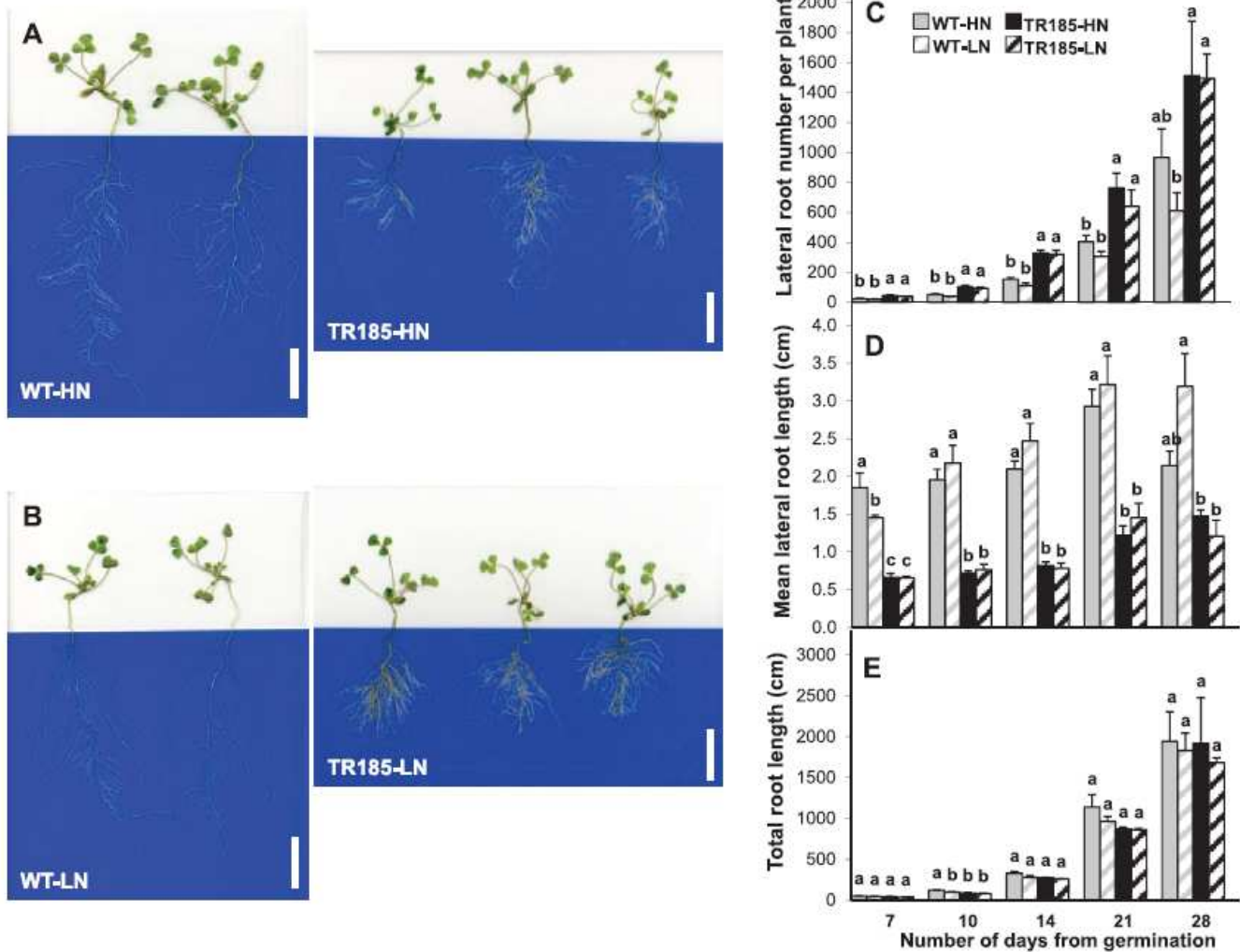


Fig. II.1. Highly-branched root architecture of the TR185 mutant irrespective of the nitrate supply. (A and B) Representative examples of wild-type (WT) and TR185 plants grown for 14 days in a growth chamber on hydroponic culture tanks, filled with a nutrient solution with high (A; 10 mM, HN) or with low (B; 1 mM, LN) nitrate supply. Bars, 5 cm. (C-E) Quantification of root architecture from 7 to 28 days after germination: lateral root number per plant (C), lateral root length (D), and total root length per plant (E; TRL). Data are means±SE from three biological replicates of six plants each. Different letters above the columns indicate significant difference based on multiple comparisons ($p < 0.05$, LSD test). The decrease in lateral root length at 28 days after germination in WT plants grown in HN has no biological significance.

elongation has been shown to involve both ANR1 and NRT1.1, which act together as a NO_3^- sensor promoting auxin transport (Zhang & Forde, 1998; Remans *et al.*, 2006a; Krouk *et al.*, 2010; Gojon *et al.*, 2011). Evidences of roles for cytokinin and abscisic acid in the root architectural response to nitrate have been presented (Walch-Liu *et al.*, 2006a; Kiba *et al.*, 2011; Ruffel *et al.*, 2011). A systemic regulation of the root architecture by the plant N status has been described (Zhang & Forde, 2000; Remans *et al.*, 2006b), involving a feedback repression of root development by products of N assimilation (Walch-Liu *et al.*, 2006b; Gifford *et al.*, 2008). The modulation of the root system architecture in response to N supply is also known to depend on the plant carbon (C) allocation within the root system (Brun *et al.*, 2010), and LR initiation level has been shown to be related to the C:N ratio (Zhang & Forde, 2000; Malamy & Ryan, 2001; Malamy, 2005). Transcriptomic analyses have confirmed that many genes involved in N assimilation or C primary metabolism are responsive to variation of nitrate supply (Wang *et al.*, 2003; Scheible *et al.*, 2004; Bi *et al.*, 2007). Transcriptomic studies of legumes subjected to variation in nitrate supply are consistent with those obtained in *Arabidopsis* (Ruffel *et al.*, 2008; Omrane *et al.*, 2009).

In this study, we describe a new highly-branched root *Medicago truncatula* and showed its unexpectedly low nitrogen acquisition and absence of root architecture adaptation to nitrate supply.

Materials and methods

Plant material

Medicago truncatula cv. Jemalong J5 was used as the wild-type reference (WT) and for backcrosses of the TR185 mutant. The mutant TR185 was selected after γ -ray mutagenesis on J5 (Sagan *et al.*, 1995), and displayed a phenotype with highly-branched roots and few small nodules (Salon *et al.*, 2009). The mutation was stable over four generations of selfing. Genetic analyses revealed that the highly-branched root architecture of TR185 is determined by a single recessive mutation (Supplemental Table II.1).

Plant growth conditions

Scarified seeds of both genotypes were surface sterilized for 7 min with a 3% sodium hypochlorite solution and rinsed seven times with sterile water (Garcia *et al.*, 2006). Seeds were then placed on sterilized plastic boxes filled with one litre of 4% (w/v) Kalys agar HP 696 gel. Boxes were left in the dark for 4 days of cold-treatment at 4 °C followed by 4 days of

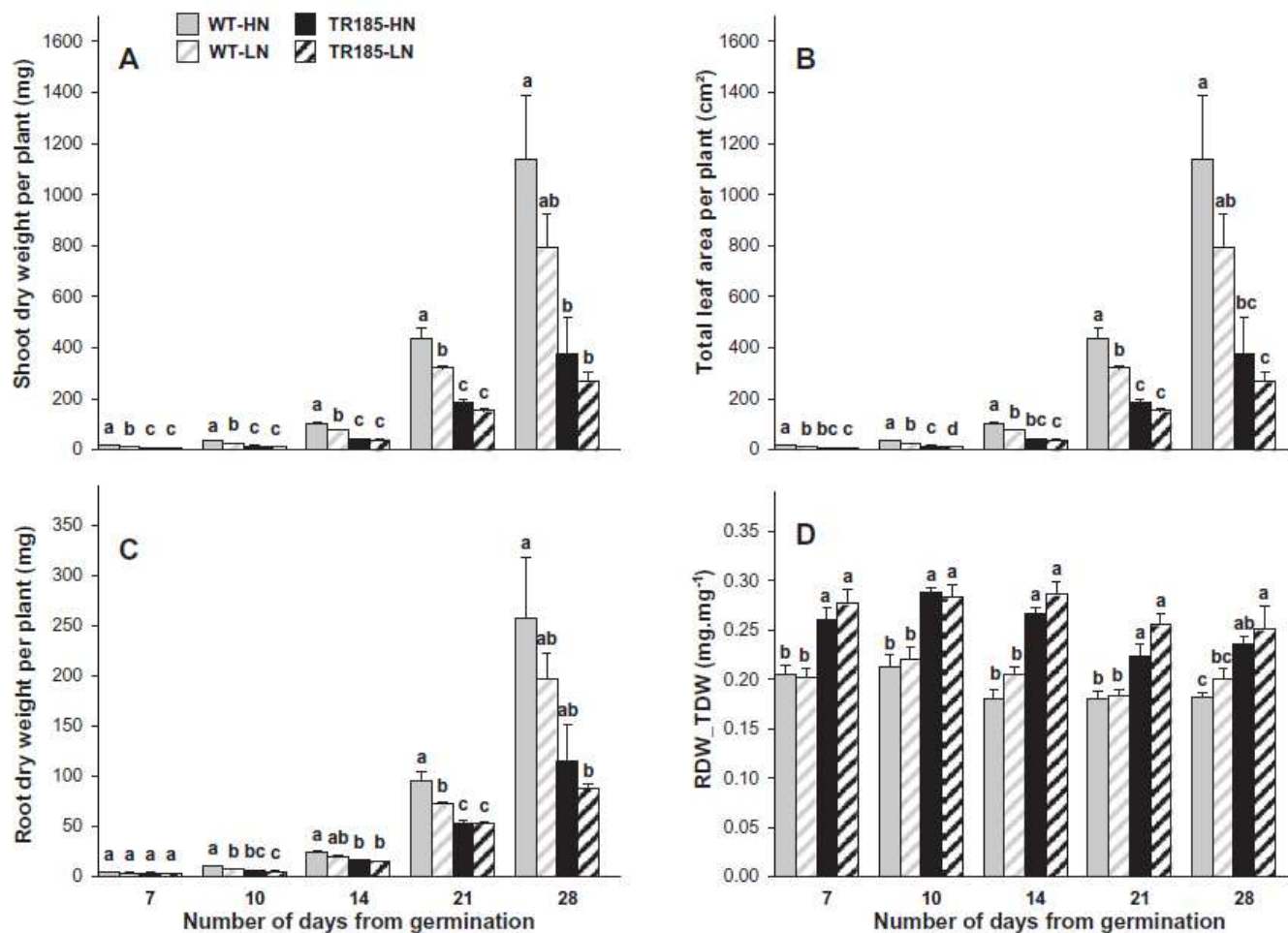


Fig. II.2. Dry weight and leaf area of wild-type (WT) and mutant (TR185) plants under high (10 mM, HN) or low (1 mM, LN) nitrate supply, from 7 to 28 days after germination. (A) Shoot dry weight, (B) Total leaf Area and (C) Root dry weight per plant. (D) Root-to-total dry weight ratio. Data are means±SE from three biological replicates of six plants each. Different letters above the columns indicate significant difference based on multiple comparisons ($p < 0.05$, LSD test)

germination at 20°C. Germinated seeds were transferred to hydroponic culture tanks filled with an aerated nutrient solution (Barker *et al.*, 2006). The basal nutrient solution (Ruffel *et al.*, 2008) was supplemented with 1 mM KNO₃ (LN; Low Nitrate) or 10 mM KNO₃ (HN; High Nitrate) as N source. *Rhizobium* inoculation was performed neither in LN nor in HN condition. The two nitrate levels were determined on the basis of previous studies of nitrogen nutrition on *M. truncatula* (Moreau *et al.*, 2008): for non-nodulating plants, the optimal N nutrition was achieved with 10 mM nitrate supply, whereas the N nutrition index represented only 35% of the optimum at 0.625 to 1.25 mM nitrate supply. Both nutrient solutions have an initial pH of 6.6 and were renewed every week. Measurements in the hydroponic culture tanks before renewing the solution indicated a slight increase of the pH to 7.2 after 4 weeks of experiment, irrespective of the N supply level. Plants were grown in a growth chamber under the controlled conditions of 24°C/19°C in a 16-h day-night cycle, a mean photosynthetically active radiation (PAR) of 200 μmol photons/m²/s, and 70% hygrometry. Each tank contained 6 WT and 6 TR185 plants. On one shelf of the growth chamber, nutrient solution in the tanks was supplemented by 1mM of KNO₃ (LN); on the other shelf, the concentration of KNO₃ in the solution was 10mM (HN). Three successive experiments in the growth chamber were performed on the two different genotypes. Each experiment constituted a biological replicate. In each experiment, plants were collected at five successive dates from 7 to 28 days after the transfer of germinated seeds into the tanks.

Plant measurements, ecophysiological modelling, and grafting

At each of the five dates, six plants of each genotype were collected both in one LN and one HN tank. Length of the primary root (PRL) was measured. The first to third order lateral roots were counted, allowing the calculation of total lateral root number (LRN). No nodule was found in any root observed. Then, the shoot and root systems were carefully spread separately onto transparent sheets and scanned as digital images with an A3 color scanner (Epson; Tokyo, Japan). Total leaf area (LeafA), total root length (TRL) and total root surface area per plant were further determined by image analysis using WinRHIZO[®] Software (Regent Instruments, Quebec, Canada). Mean lateral root length (LRL) was then calculated as: TRL-PRL / LRN. Roots and shoots were oven-dried separately at 80°C for 48 h for shoot, root and total dry weight determination (SDW, RDW and TDW). Shoot and root N concentrations of ground dried tissues (%ShootN, %RootN) were estimated following the Dumas' method, and the total N accumulation in the plant (TotN) calculated. Then, three integrative variables,

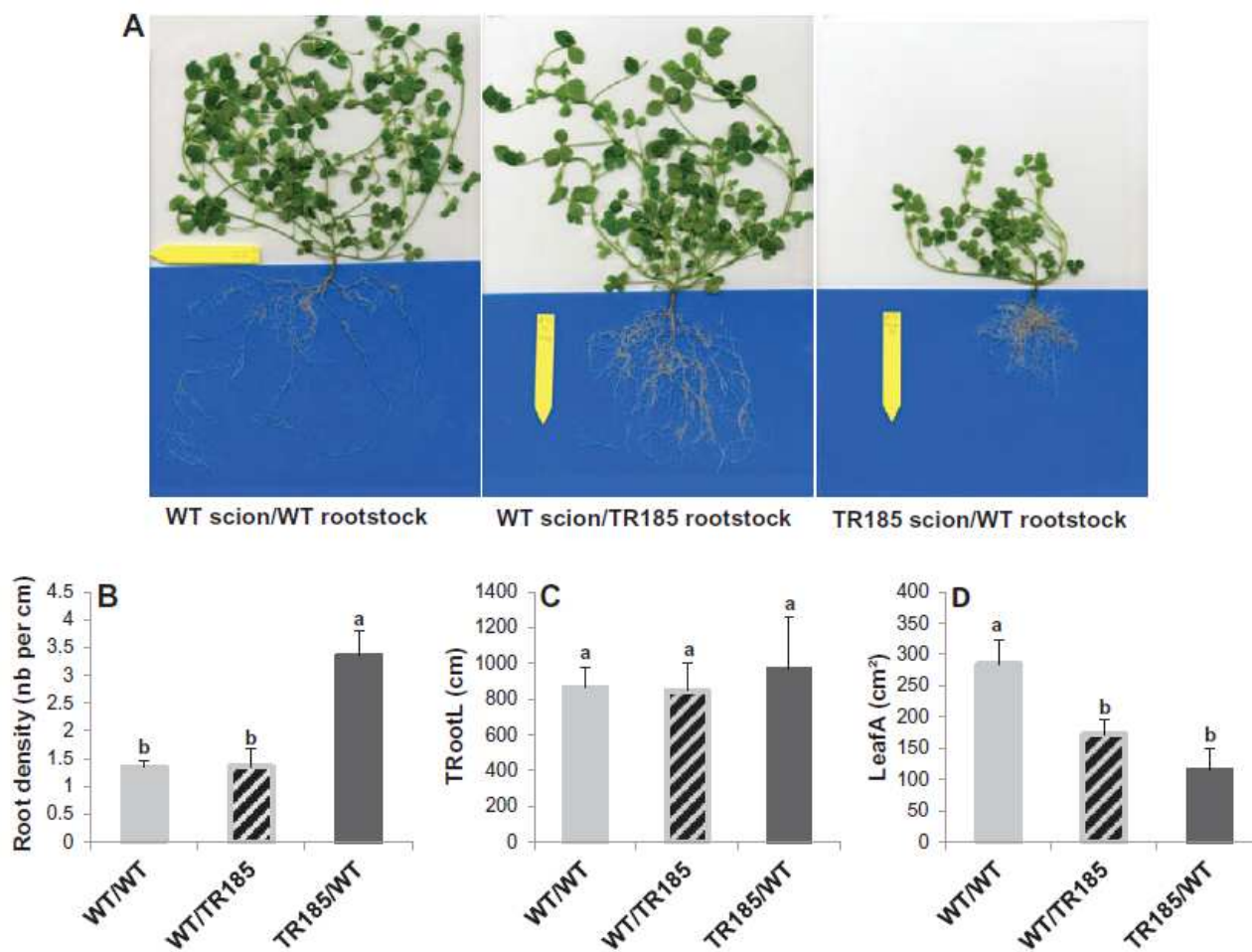


Fig. II.3. Grafting experiments. (A) Representative 10-week old plants, (B) Number of lateral roots per length of primary root (Root density), (C) Total root length (TRootL) and (D) Total Leaf Area (LeafA) of 10-week old plants. Data are means \pm SE from three plants.

characterizing the relationship between the four state variables LeafA, TDW, RDW, and TotN, were calculated (Moreau *et al.*, 2012). They represent efficiencies of C or N acquisition. The LeafA is considered as the C source, which is distributed to roots according to root-to-total dry weight ratio (RDW_TDW). The RDW or more precisely the root surface area pilots the N entrance onto the plant, according to N-uptake rate (NUR). The TotN accumulated into the plant allows the elaboration of the LeafA, according to efficiency of N conversion into leaf area (NLA).

Grafting was performed as described in the “cuttings and grafts” chapter of the Medicago handbook (<http://www.noble.org/medicagohandbook/>). Grafts were initially generated *in vitro*, and after three weeks were potted in an attapulgate:clay balls mixture (1:1) in the greenhouse for an additional seven weeks. Three plants per combination were then carefully spread onto transparent sheets and scanned as digital images, and LeafA and TRL determined by image analysis as described previously. The number of first lateral roots per length of primary root was also determined for each plant.

For graft experiment and each sampling date, means and SE values were calculated for all variables and ANOVA were performed using XLSTAT software (version 2010.6.03, <http://www.xlstat.com>). Means were classified using the least significant difference (LSD) range test at the 0.05 probability level.

Metabolic analyses

Amino acids content

The levels of the 20 standard amino acids synthesized by plants were measured in TR185 and WT plants. 100 mg of lyophilized powder were weighed and extracted in a three-step ethanol–water procedure, as described by (Loudet *et al.*, 2003). Using the method described by (Ikram *et al.*, 2012), ninhydrin colour reagent was added to the extract and absorbance read at 570 nm on a spectrophotometer. This result was used to calculate the amino acid content in $\mu\text{mol.g}^{-1}$ FW.

Lignin content

Lignin content in roots was determined using the Acetyl bromide method adapted from (Fukushima & Hatfield, 2001). To prepare the root cell wall (CW), 100 mg of the dried root

grinded samples were extracted sequentially with stirring with water, ethanol and acetone. An acetyl bromide/acetic acid solution (1/3, v/v) was added to about 5 mg of the CW dried extract obtained. Lignins were solubilized whereas polysaccharides were hydrolysed. After the reaction, the excess of acetyl bromide and polybromide ions were destroyed by adding water and hydroxylamine chlorhydrate. Lignin content was calculated from absorbance readings at 280 nm, and expressed as mg.g^{-1} of root DW.

Transcriptomic analyses

RNA extraction and Affymetrix geneChip

Total RNA was extracted from roots using the Plant RNeasy Mini Kit with on-column DNase digestion (Qiagen). All RNA samples were checked for their integrity on the Agilent 2100 Bioanalyzer according to the Agilent Technologies (Waldbronn, Germany) specifications. For microarray analyses, two micrograms of total RNA were transcribed as described in (Rey *et al.*, 2013). The labelled cDNA produced was used to hybridize Affymetrix GeneChip® Medicago genome arrays at INRA-URGV (Evry, France). The raw CEL files were imported in R software for data analysis. All raw and normalized data are available through the CATdb database ((Gagnot *et al.*, 2008); project “AFFY_root_dvt_Nitrogen_Medicago”), and from the Gene Expression Omnibus (GEO) repository at the National Center for Biotechnology Information (NCBI) ((Barrett *et al.*, 2007), accession number GSE18318).

Statistical Analysis of Microarray Data

The data were normalized with the GC RMA algorithm (Irizarry *et al.*, 2003), available in the Bioconductor package (Gentleman & Carey, 2002). We performed a two-way ANOVA on the normalized expression signals, which was modeled as follows: $Y_{ijk} = \mu + G_i + N_j + GN_{ij} + e_{ijk}$, where Y is the normalized expression signal of a transcript for genotype i at nitrate supply j in replicate k, μ the global mean, G_i the genotypic effect, N_j the nitrate effect, GN_{ij} the genotype x nitrate interaction effect, and e_{ijk} are normally distributed zero-mean random errors. Due to the limited number of observations, the degree of freedom was too weak to perform tests based on the specific residual variance of each transcript. Thus, a global residual variance was calculated after the removal of the transcripts displaying extreme variation. Three contrasts were considered to classify genes as either responsive to the genotype effect independently of the nitrate supply (G), or responsive to the nitrate supply across both genotypes (N), or not

responsive to the nitrate supply in the same way in both genotypes (G x N interaction). For each contrast, the test statistic was calculated from the global variance, and the P values were adjusted by the Bonferroni method, which controls the Family Wise Error Rate (FWER) (Ge *et al.*, 2003). For a given contrast, a gene is declared differentially expressed if its adjusted P value is lower than 0.05. A functional classification of the differentially expressed genes was visualized using MapMan version 3.5.0 (<http://mapman.gabipd.org/web/guest/mapman>; (Thimm *et al.*, 2004; Tellstrom *et al.*, 2007)).

Q-RT-PCR

A set of 18 genes identified as differentially expressed in roots was chosen for validation of Affymetrix genome arrays by Q-RT-PCR (Supplemental Fig. II.1). Primer sequences are available in Supplemental Table II.2. For each sample, 1 µg of total RNA was treated with RQ1 DNase (Promega) and reverse transcription was carried out using the IScript cDNA synthesis Kit (BIO-RAD). Q-RT-PCR reactions were performed on a LC480 apparatus (Roche) using the GoTaq qPCR Mastermix (Promega). Three technical replicates were performed for each one of the three independent biological replicates. Relative expression levels were calculated according to the relative standard curve method (Δ CT) using Elongation factor 1 (EF1) and Ubiquitin genes as reference genes.

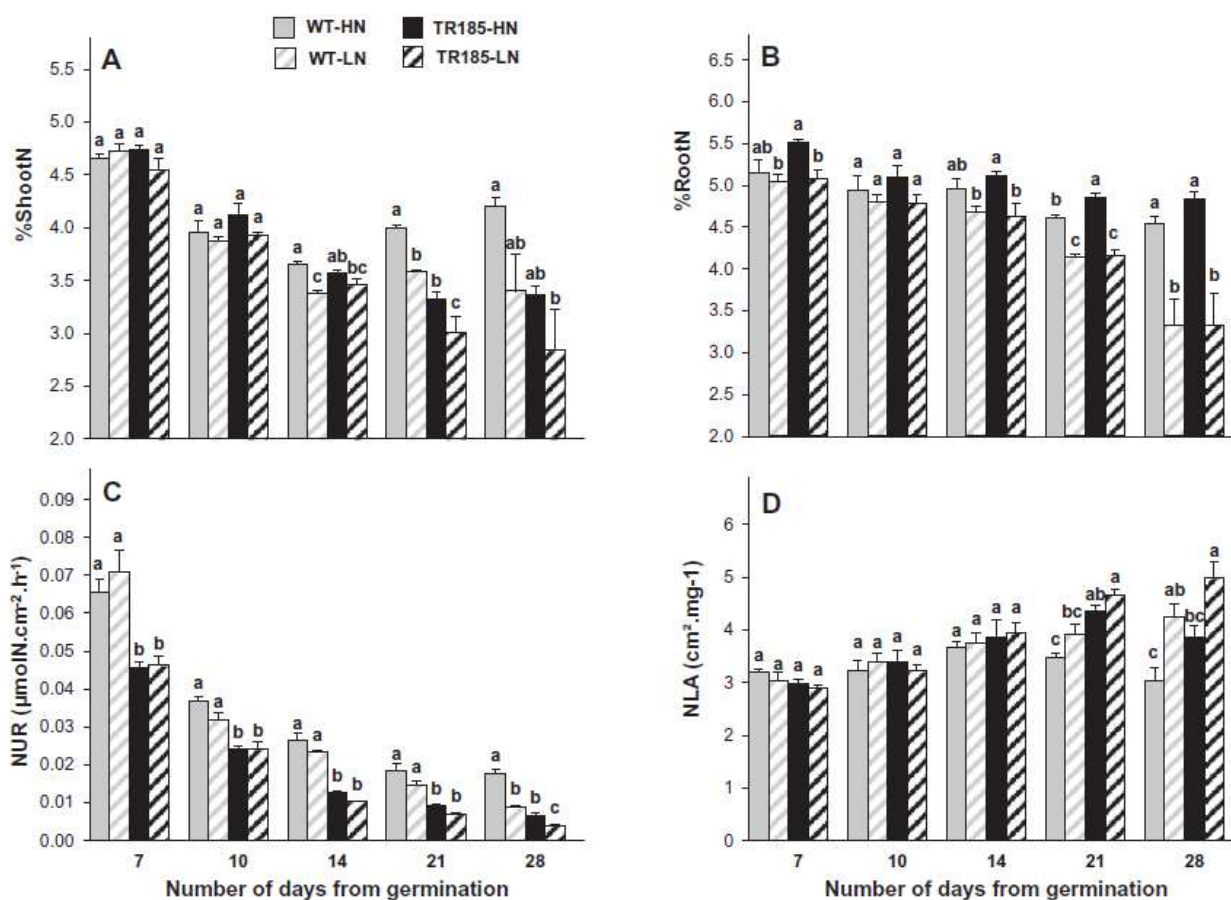


Fig. II.4. N concentration and efficiency of nitrogen accumulation into wild-type (WT) and mutant (TR185) plants under high (10 mM, HN) or low (1 mM, LN) nitrate supply, from 7 to 28 days after germination. (A and B) Shoot N concentration (A; %ShootN) and Root N concentration (B; %RootN) estimated following the Dumas method. (C and D) N-uptake rate (C; NUR) and efficiency of N conversion into leaf area (D; NLA). Data are means±SE from three biological replicates of six plants each. Different letters above the columns indicate significant difference based on multiple comparisons ($p < 0.05$, LSD test)

Results

Root architecture and N-uptake

The highly-branched root architecture of TR185 is associated with depressed growth irrespective of the nitrate supply, and is shoot determined

The root architecture of TR185 was highly branched under both high and low nitrate supply (Fig. II.1). This resulted in a significantly higher lateral root number (LRN) and significantly lower mean lateral root length (LRL) when compared with WT, throughout the growth period and irrespective of the nitrate supply (Fig. II.1C and D). Despite this, TR185 had a similar total root length (TRL) as WT (Fig. II.1E). In the WT plants, LRN decreased slightly with decreasing nitrate supply (Fig. II.1C). An effect of decreased nitrate supply was also observed on the LRL of the WT, with a transient significant decrease at the seven-day stage followed by an increase at the later stages (Fig. II.1D).

TR185 displayed significantly lower shoot and root dry weights (SDW; RDW) and leaf area (LeafA) than WT, as early as the seven- or ten-day stage (Fig. II.2A-C). These differences were associated throughout the growth period with a higher root-to-total dry weight ratio in TR185 compared with WT (Fig. II.2D). Both RDW and SDW of the WT were reduced with LN supply, whereas no significant decrease was observed in TR185.

To determine whether the highly-branched root architecture of TR185 was shoot- or root-determined, we performed grafting experiments (Fig. II.3). Analysis of roots in the different grafting combinations revealed that the root architecture phenotype was graft transmissible from shoots (Fig. II.3A-C). The shoot leaf area was also most reduced in the combination with TR185 as scion (Fig. II.3D).

Compared with WT, TR185 has reduced %shoot N and N-uptake efficiency

Up to the 14-day stage, shoot N concentration (%ShootN) decreased continuously for both genotypes without significant difference between them (Fig. II.4A). From the 21-day stage onwards, %ShootN became significantly lower in TR185 than in WT, and the depressing effect of LN was significant for both genotypes. TR185 has lower %Shoot N than WT even when normalized to the SDW measures (Fig. II.S2). Few differences in root N concentration (%RootN) were significant between the two genotypes (Fig. II.4B). For both genotypes, %RootN decreased throughout the study period, and especially under LN supply.

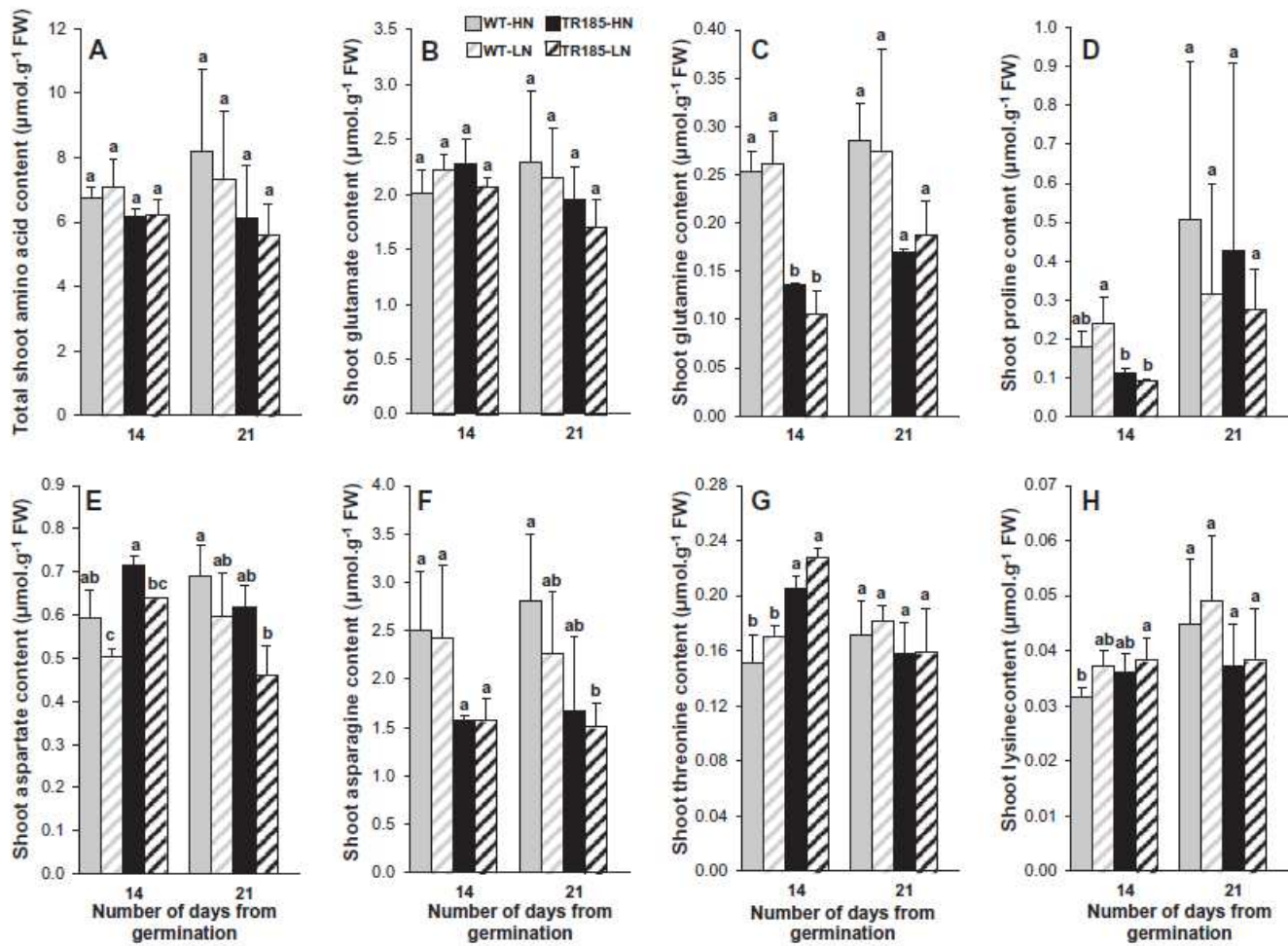


Fig. II.5. Total amino acid content (A) and contents of seven amino acids in wild-type (WT) and mutant (TR185) shoot: Glutamate (B), Glutamine (C), Proline (D), Aspartate (E), Asparagine (F), Threonine (G), Lysine (H)

The N-uptake rate (NUR) was significantly lower in TR185 than in WT, as soon as the seven-day stage and under both nitrate conditions (Fig. II.4C). For both genotypes, NUR decreased throughout the growth period and, at the 28-day stage, was significantly lower under LN supply. Concerning the amount of leaf area produced per N acquired by the roots (NLA), significantly higher values were observed for TR185 from the 21-day stage onwards (Fig. II.4D). From that stage, both genotypes had higher NLA values under LN than for HN supply.

TR185 has lower ASN and GLN contents than WT

The levels of the 20 standard amino acids synthesized by plants were measured at 14-day and 21-day stages. No differences in total free amino acid or glutamate (GLU) content in shoots were observed between TR185 and the WT or between LN and HN supply at the 14-day stage, whereas differences none significant but similar to that observed in %ShootN appeared at the 21-day stage (Fig. II.5A,B). Six other amino acids did not show any significant variations at the two stages considered (Supplemental Fig. II.3B-E, H-J). In contrast, significant differences between the two genotypes in the contents of eight amino acids were observed as soon as the 14-day stage, with lower values for glutamine (GLN), proline (PRO), asparagine (ASN) and alanine (ALA) (Fig. II.5C,D,F; Supplemental Fig. II.3A), and higher values for threonine (THR), lysine (LYS), valine (VAL) and serine (SER), in TR185 compared with WT (Fig. II.5G,H; Supplemental Fig. II.3B,F). For three amino acids, aspartate (ASP), SER and cysteine (CYS), a significant effect of N supply was observed (Fig. II.5E; Supplemental Fig. II.3F-G).

A lower total free amino acid content was observed in the roots of TR185 when compared with WT roots (Fig. II.6A). However, no significant differences were observed between TR185 and the WT for their root levels of GLU and ASP, and for both genotypes, the level of these two amino acids was lower under LN than under HN supply (Fig. II.6B, E). By contrast, TR185 had a lower level of GLN and ASN, and no significant effect of N supply was observed for these two amino acids (Fig. II.6C, F). A genotype effect was also observed for PRO, THR and LYS, at least at the 14-day stage (Fig. II.6D, G, H). Arginine (ARG), histidine (HIS), isoleucine (ILE), SER, phenylalanine (PHE) and all the derivatives of pyruvate (ALA, VAL, LEU) did not show any significant variations (Supplemental Fig. II.4).

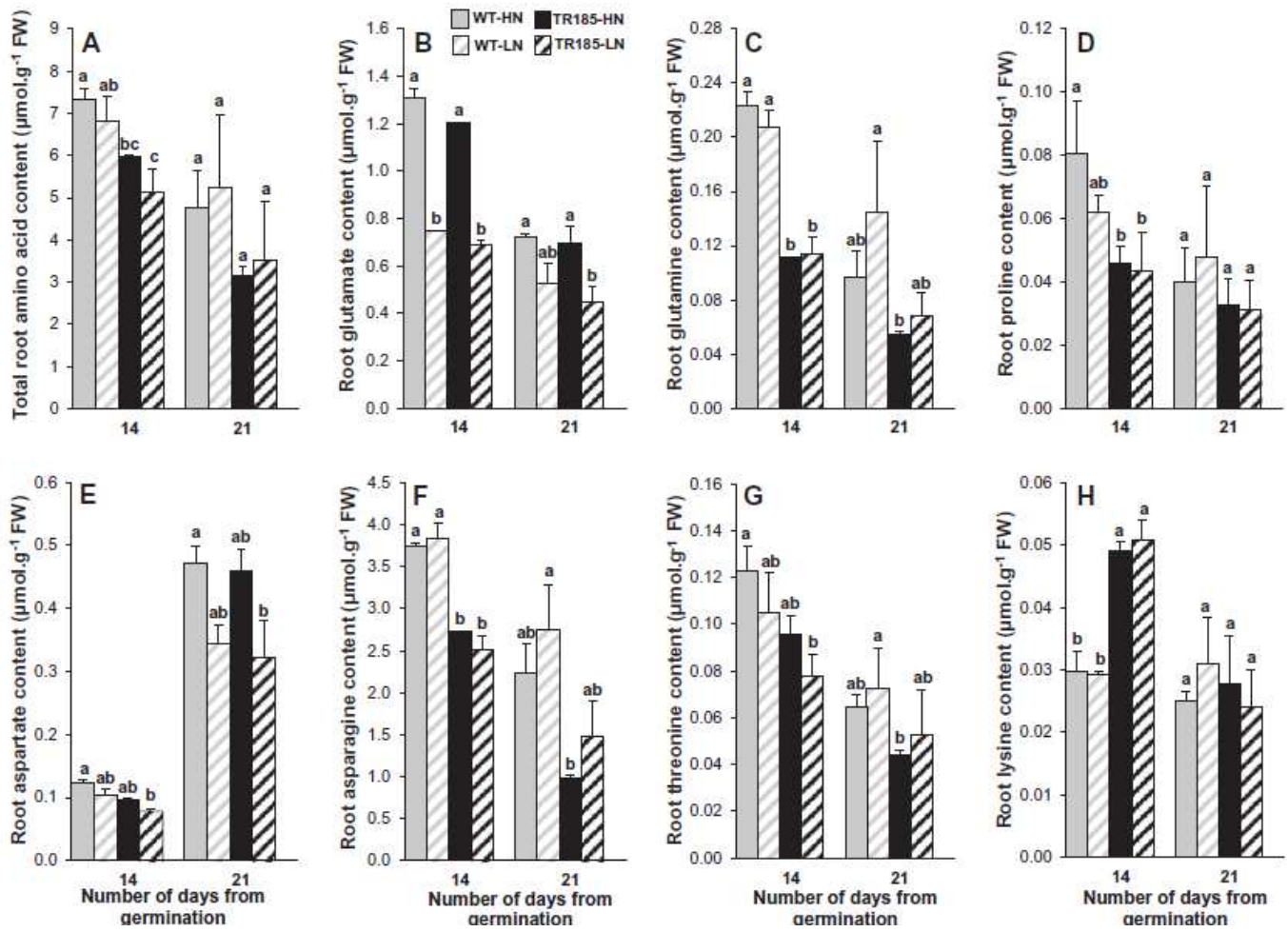


Fig. II.6. Total amino acid content (A) and contents of seven amino acids in wild-type (WT) and mutant (TR185) root: Glutamate (B), Glutamine (C), Proline (D), Aspartate (E), Asparagine (F), Threonine (G), Lysine (H)

Transcriptomic analysis

Most of the genes were differentially expressed between TR185 and WT

A global gene expression profiling of root cells using a microarray analysis was conducted on both the WT and TR185, under high and low nitrate concentrations. The transcriptomic analysis was performed on the 10-day stage, at which the two genotypes were significantly different for most of the traits related to the root architecture or plant growth.

Significant hybridization in at least one root sample was found on 26754 probe sets among the 61278 tested (Filter based on signal values > 4). 586 of these transcripts were differentially expressed; among them, 475 were differentially expressed in response to genotype effect (G) independently of nitrate supply, 156 in response to nitrate effect (N) across both genotypes, and 20 in response to G x N effect (Fig. II.7A). Several of these genes were responsive to either two or three effects in common. 77 of the 168 transcripts responding to N or GxN effects were previously identified by Ruffel *et al.* (2008) to be regulated in wild-type roots in response to either local nitrate starvation (65 transcripts) or to systemic signals related to the plant N status (20 transcripts), with 8 in response to both signals (Supplemental Table II.3). Fifty-six of the common transcripts previously found to be up-regulated in response to local nitrate starvation by Ruffel *et al.* (2008) were significantly up-regulated in LN compared with HN. Altogether, these results confirmed that our LN treatment resulted in N-limitation.

Using the MapMan software, an overall comparison of the main metabolic pathways highlights differential gene expression between TR185 and WT in N acquisition and amino acid synthesis, cell wall and lipid metabolism, phenylpropanoid and flavonoid biosynthetic pathways (Fig. II.7B).

Most of the genes involved in N acquisition and assimilation were up-regulated in TR185 when compared with WT

Among the annotated transcripts differentially expressed in roots between TR185 and WT, 23 are involved in the N acquisition and assimilation pathway (Table II.1). One of the most up-regulated transcripts in TR185 encodes a putative nitrate transporter of the NRT2 family (Table II.1). A transcript encoding a putative NRT1 Nitrate transporter and two transcripts

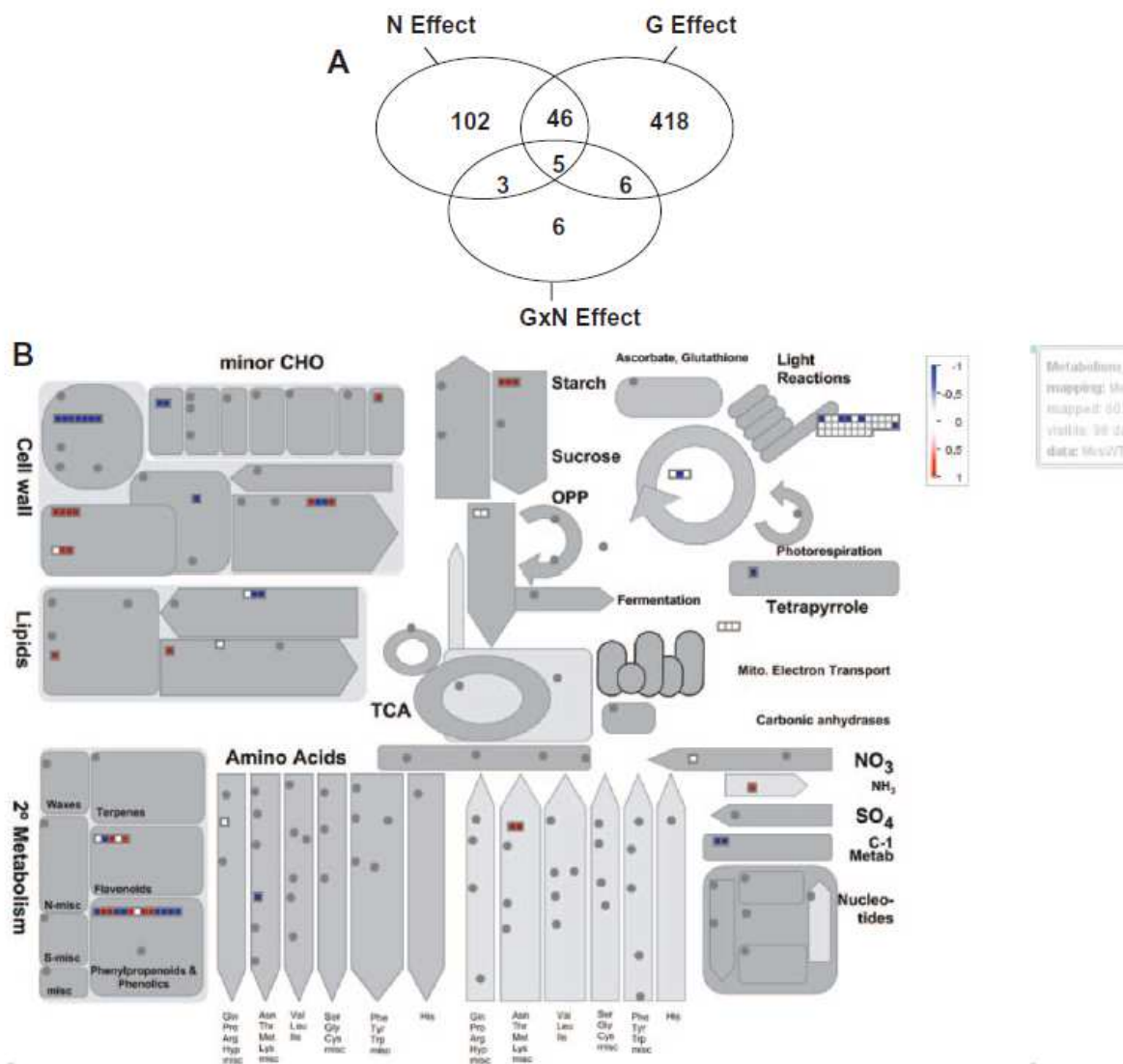


Fig. II.7. Comparisons of mutant (TR185) and wild-type (WT) transcriptomes. (A) Venn diagram of transcripts identified as differentially expressed in roots in response to genotype effect (G), to nitrate supply across both genotypes (N) or to genotype effect in a different way according to the nitrate supply (GxN interaction). (B) Overall picture of the gene expression changes in roots between TR185 and WT in the main metabolic pathways. This MapMan representation is based on annotations of *Medicago_AFFY_09 M. truncatula*. Only transcripts significantly differentially expressed are shown. Differential values are expressed in a log₂ scale. Transcripts differentially expressed by more than the threshold value of 1 are shown in colour; red for up-regulated and blue for down-regulated in TR185; in both cases with a colour scale representing the intensity of up- or down-regulation. Absent transcripts are shown in grey

encoding putative ammonium transporters of AMT1 and AMT2 family were also more highly expressed in TR185. Concerning the ammonium assimilation, a transcript encoding a glutamate synthase was up-regulated in TR185 under HN supply but down-regulated under LN supply. A transcript encoding a Pyrroline-5-carboxylate synthetase, which is involved in conversion of GLU to PRO, was down-regulated, in TR185 compared with WT, under HN supply and up-regulated under LN supply. A transcript encoding a glutamate dehydrogenase was more highly expressed in TR185 than in the WT irrespective of the nitrate supply. Two transcripts encoding an L-asparagine amidohydrolase involved in ASN degradation were up-regulated in TR185 compared with WT, whereas the expression of a transcript encoding a Dihidropicolinate synthase involved in LYS synthesis was down-regulated.

A differential expression between TR185 and WT was also observed for genes involved in the synthesis of organic acids which are required for N assimilation. Transcripts involved in starch degradation were up-regulated in TR185 compared with WT, irrespective of the N supply for those encoding beta-amylase and starch phosphorylase, and under HN supply only for one encoding a Phosphoenolpyruvate carboxylase kinase. Differential expressions of transcripts encoding sugar transporters were observed also between TR185 and WT.

Genes involved in cell wall and lipid metabolism were differentially expressed between TR185 and WT

Twenty-four of the annotated transcripts differentially expressed between TR185 and WT were found to be involved in cell wall-modification or lipid metabolism (Fig. II.7B; Table II.2). Most of the transcripts encoding cell-wall modifying enzymes, like expansins, pectinesterases, and polygalacturonases were up-regulated in TR185. Other notable changes in TR185 concerned differential expression of transcripts encoding cell wall polysaccharide synthases, with down-regulation of a cellulose synthase and up-regulation of a callose synthase. Lastly, most of the transcripts encoding AGP cell wall or lipid binding proteins were down regulated in TR185. Concerning the lipid metabolism, the main changes in expression between TR185 and WT were the down-regulation of transcripts related to fatty acid elongation and lipid synthesis, and in the up-regulation of a transcript encoding a lipase.

Table II.1. Differentially accumulated transcripts between TR185 and wild type, annotated as related to N-acquisition pathway

| Annotation | MHN | MLN | WTHN | WTLN | Effect |
|---|------------|------------|-------------|-------------|-------------------|
| NRT2 Nitrate transporter | 5.13 | 4.15 | 2.76 | 3.19 | <u>G</u> |
| NRT1 Nitrate transporter | 7.10 | 6.97 | 6.19 | 5.81 | <u>G</u> |
| AMT1 transporter | 7.78 | 8.54 | 6.75 | 7.43 | <u>G</u> |
| AMT2 transporter | 7.60 | 7.11 | 5.89 | 5.21 | <u>G</u> |
| Ferredoxin-dependent glutamate synthase | 4.53 | 3.60 | 3.58 | 4.55 | GxN |
| Pyrroline-5-carboxylate synthetase | 8.80 | 9.58 | 9.83 | 8.66 | GxN |
| Glutamate deshydrogenase | 11.81 | 12.07 | 11.04 | 10.90 | <u>G</u> |
| Putative L-asparagine amidohydrolase | 14.05 | 13.66 | 12.57 | 12.76 | <u>G</u> |
| Putative L-asparagine amidohydrolase | 12.92 | 12.80 | 11.40 | 11.72 | <u>G</u> |
| Dihydrodipicolinate synthase | 3.27 | 4.48 | 6.19 | 6.77 | G |
| Proline transporter | 9.30 | 9.68 | 10.30 | 10.63 | G |
| Lysine Histidine Transporter | 8.03 | 8.52 | 6.77 | 6.99 | <u>G</u> |
| Amino acid transporter | 5.82 | 6.28 | 4.74 | 5.43 | <u>G</u> |
| NRT1 Peptide transporter | 9.21 | 9.63 | 8.22 | 8.62 | <u>G</u> |
| Proton-dependent oligopeptide transporter | 5.15 | 4.42 | 3.92 | 3.50 | <u>G</u> |
| beta-amylase | 7.24 | 7.39 | 6.25 | 6.30 | <u>G</u> |
| beta-amylase | 6.87 | 7.13 | 5.82 | 5.73 | <u>G</u> |
| Starch phosphorylase | 5.15 | 5.07 | 3.41 | 3.97 | <u>G</u> |
| Phosphoenolpyruvate carboxylase kinase | 7.45 | 6.57 | 5.94 | 7.53 | N* GxN |
| Malate transporter | 10.85 | 11.33 | 9.95 | 10.32 | <u>G</u> |
| Sugar transporter | 5.47 | 4.89 | 4.10 | 5.24 | <u>G</u> * |
| Glucose transporter | 7.65 | 8.58 | 8.97 | 9.37 | G |
| leghemoglobin MtLb1 | 6.51 | 6.64 | 6.04 | 4.37 | <u>G</u> |

Average Affymetrix GeneChip normalized expression values across three biological replicates for the mutant TR185 (M) and the wild type (WT), in high and low N conditions. G, N and GxN indicate transcripts responsive to genotype, nitrate and genotype x nitrate effect, respectively (adjusted P -values<0.05). G* indicates a transcript responsive to G effect only under HN condition. N* indicates a transcript responsive to N effect only for the wild type. **G** and G indicate up-regulation and down-regulation, respectively, in TR185 compared with the wild type

Table II.2. Differentially accumulated transcripts between TR185 and wild type, annotated as related to cell wall modification

| Target Identifier | Annotation | MHN | MLN | WTHN | WTLN | Effect |
|-----------------------------|--|-------|-------|-------|-------|----------|
| cell wall modifying enzymes | | | | | | |
| Mtr.9830.1.s1_at | Expansin | 8.23 | 7.88 | 6.86 | 7.37 | <u>G</u> |
| Mtr.20107.1.s1_at | Expansin-related protein precursor | 8.04 | 7.73 | 9.31 | 8.87 | G |
| Mtr.22752.1.s1_s_at | Expansin | 9.64 | 9.93 | 8.82 | 8.74 | <u>G</u> |
| Mtr.4467.1.s1_at | Pectinesterase | 11.16 | 10.88 | 10.15 | 9.08 | <u>G</u> |
| Mtr.274.1.s1_at | Pectinesterase | 7.54 | 7.79 | 6.78 | 5.86 | <u>G</u> |
| Mtr.7581.1.s1_s_at | Pectinesterase | 9.13 | 8.88 | 8.36 | 7.33 | <u>G</u> |
| Mtr.41480.1.s1_at | Polygalacturonase | 6.65 | 7.55 | 5.86 | 6.33 | <u>G</u> |
| Mtr.4713.1.s1_at | Lyase | 5.39 | 5.93 | 4.75 | 4.51 | <u>G</u> |
| Mtr.39445.1.s1_at | Polygalacturonase | 6.93 | 8.04 | 8.13 | 8.79 | G, N |
| Mtr.43680.1.s1_at | Dehydration-induced protein | 7.07 | 8.07 | 8.41 | 8.78 | G |
| cell wall polysaccharides | | | | | | |
| Mtr.28768.1.s1_at | Cellulose synthase | 6.46 | 6.83 | 7.56 | 8.00 | G |
| Mtr.17447.1.s1_at | Callose synthase | 5.47 | 5.42 | 3.69 | 4.81 | <u>G</u> |
| cell wall proteins AGPs | | | | | | |
| Mtr.18563.1.s1_at | fasciclin-like arabinogalactan-protein | 6.88 | 7.55 | 7.98 | 8.56 | G |
| Mtr.51607.1.s1_at | fasciclin-like arabinogalactan-protein | 10.26 | 11.14 | 11.47 | 11.95 | G |
| Mtr.50900.1.s1_at | fasciclin-like arabinogalactan-protein | 8.82 | 9.53 | 9.79 | 10.46 | G |
| Mtr.10992.1.s1_at | fasciclin-like arabinogalactan-protein | 10.13 | 10.82 | 11.17 | 11.77 | G |
| Mtr.18380.1.s1_at | fasciclin-like arabinogalactan-protein | 9.05 | 9.69 | 10.05 | 10.76 | G |
| Mtr.50897.1.s1_at | fasciclin-like arabinogalactan-protein | 6.81 | 7.51 | 7.95 | 8.60 | G |
| Mtr.13136.1.s1_at | fasciclin-like arabinogalactan-protein | 9.09 | 9.82 | 10.19 | 10.75 | G |
| Mtr.32740.1.S1_at | Lipid binding protein | 10.45 | 9.68 | 8.99 | 9.00 | <u>G</u> |
| Mtr.37476.1.S1_at | Lipid binding protein | 8.35 | 8.84 | 9.73 | 9.39 | G |
| Lipid metabolism | | | | | | |
| Mtr.12519.1.s1_at | beta-ketoacyl-CoA synthase | 4.88 | 5.11 | 5.82 | 6.17 | G |
| Mtr.41116.1.s1_at | Acyl carrier protein | 3.59 | 3.66 | 5.08 | 4.68 | G |
| Mtr.12518.1.s1_at | lipase | 11.47 | 11.86 | 10.55 | 10.74 | <u>G</u> |

Average Affymetrix GeneChip normalized expression values across three biological replicates for the mutant TR185 (M) and the wild type (WT), in high and low N conditions. G and N indicate transcripts responsive to genotype and nitrate effect, respectively (adjusted P -values<0.05). G and G indicate up-regulation and down-regulation, respectively, in TR185 compared with the wild type

Table II.3. Differentially accumulated transcripts between TR185 and wild type, annotated as related to phenylpropanoid pathway

| Annotation | MHN ^o | MLN | WTHN | WTLN | Effect |
|--|------------------|-------|-------|-------|----------|
| Phenylalanine ammonia-lyase | 10.39 | 11.19 | 9.46 | 10.18 | <u>G</u> |
| 4-coumarate-CoA ligase | 6.64 | 6.68 | 7.54 | 7.71 | G |
| Transferase family protein (HCT) | 5.94 | 7.29 | 7.58 | 7.86 | G |
| Caffeoyl-CoA 3-O-methyltransferase | 5.45 | 5.80 | 6.22 | 6.95 | G |
| Caffeoyl-CoA 3-O-methyltransferase | 4.26 | 4.52 | 5.36 | 5.39 | G |
| Isoflavone-O- methyltransferase | 9.49 | 9.80 | 10.44 | 10.79 | G |
| O-methyltransferase | 3.21 | 3.42 | 3.97 | 4.75 | G |
| Chalcone synthase | 5.01 | 4.81 | 4.15 | 3.22 | <u>G</u> |
| UDP-glucose flavonol 3-O-glucosyltransferase | 7.81 | 7.03 | 6.09 | 5.46 | <u>G</u> |
| UDP-glucose flavonol 3-O-glucosyltransferase | 6.46 | 6.06 | 5.32 | 5.25 | <u>G</u> |
| UDP-glucose flavonol 3-O-glucosyltransferase | 6.15 | 5.29 | 4.89 | 3.97 | <u>G</u> |
| UDP-glucosyltransferase | 6.82 | 6.33 | 4.94 | 5.39 | <u>G</u> |
| UDP-glucosyltransferase | 8.45 | 7.81 | 7.37 | 6.82 | <u>G</u> |
| Transferase | 7.77 | 7.10 | 6.61 | 6.35 | <u>G</u> |
| Transferase | 6.94 | 6.37 | 5.83 | 5.23 | <u>G</u> |
| Transferase | 8.30 | 8.92 | 7.19 | 7.58 | <u>G</u> |
| Flavonol synthase/flavanone 3-hydroxylase | 11.62 | 11.40 | 10.88 | 10.21 | <u>G</u> |
| Flavonoid biosynthetic process DMR6 | 5.54 | 5.20 | 4.17 | 4.75 | <u>G</u> |
| Dihydroflavonol 4-reductase | 7.99 | 8.72 | 8.97 | 7.72 | GxN |
| Anthocyanin 5-aromatic acyltransferase | 4.90 | 5.37 | 6.31 | 6.03 | G |
| Anthocyaninless2 transcription factor | 7.83 | 6.97 | 6.29 | 5.95 | <u>G</u> |
| Anthocyaninless2 transcription factor | 6.41 | 5.59 | 4.65 | 4.34 | <u>G</u> |
| Protein Transparent Testa 12 | 2.87 | 3.62 | 3.34 | 5.03 | G, N |

Average Affymetrix GeneChip normalized expression values across three biological replicates for the mutant TR185 (M) and the wild type (WT), in high and low N conditions. G and N indicate transcripts responsive to genotype and nitrate effect, respectively (adjusted P -values<0.05). G and G indicate up-regulation and down-regulation, respectively, in TR185 compared with the wild type

Most of the genes involved in phenylpropanoid pathway were up-regulated in TR185 when compared with WT

Twenty-three of the annotated transcripts differentially expressed between TR185 and WT were found to be involved in the phenylpropanoid and flavonoid pathways (Fig. II.7B; Table II.3). A large number of transcripts involved in lignin synthesis were repressed in TR185 compared with WT, including those encoding HCT and Caffeoyl-CoA 3-O-methyltransferase. Conversely, a transcript encoding a chalcone synthase, which is a crucial flavonoid biosynthesis enzyme, and numerous transcripts involved in the flavonol glycosides synthesis were up-regulated in TR185 compared with WT, independently of the nitrate supply. Various transcripts involved in the anthocyanin pathway were also differentially expressed between TR185 and WT; with up-regulation of the transcription factor ANL2 and down-regulation of an Anthocyan-5-aromatic acyltransferase and a gene similar to *tt12*, both irrespective of the nitrate supply.

Genes involved in hormone metabolism and transport were differentially expressed between TR185 and WT

Twenty-three of the annotated transcripts differentially expressed between TR185 and WT were found to be involved in hormone metabolism or transport (Table II.4). The transcripts encoding auxin-induced or -binding proteins, among them an Indole-3-acetic acid amido synthetase and an auxin efflux transporter similar to AtPin5, were mostly down-regulated in TR185 compared with WT. A transcript encoding a homeobox transcription factor similar to the TF IFL of AtPIN1 was differentially expressed in response to G x N effect, with a lower expression in TR185 in response to LN only. Differential expression was also observed for various transcripts involved in the metabolism of cytokinin, with, in TR185 compared with WT, up-expression of a transcript involved in its degradation and down-expression of two transcripts possibly involved in its signalling. An up-regulation of a gene encoding an ethylene-responsive transcription factor and a down-regulation of transcripts involved in Gibberellin synthesis or signalling were also observed in TR185. Lastly, two main genes involved in Jasmonate metabolism were also differentially regulated between TR185 and WT, with down-regulation of transcripts encoding the lipoxygenase AtLOX1, and up-regulation of transcripts encoding the lipoxygenase AtLOX.

Table II.4. Differentially accumulated transcripts between TR185 and wild type, annotated as related to hormone metabolism and transport

| Annotation | MHN | MLN | WTHN | WTLN | Effect |
|---|-------|-------|-------|-------|----------|
| Indole-3-acetic acid amido synthetase | 6.63 | 5.73 | 5.19 | 5.07 | <u>G</u> |
| Indole-3-acetic acid-amido synthetase | 2.57 | 2.57 | 3.62 | 3.42 | G |
| Auxin-induced protein 5NG4 | 4.41 | 4.76 | 5.43 | 6.51 | G |
| Auxin-binding protein ABP19b precursor | 8.04 | 8.60 | 9.26 | 9.49 | G |
| Auxin:hydrogen symporter similar to AtPin5 | 2.08 | 2.16 | 3.04 | 3.39 | G |
| Transcription factor similar to IFL | 4.89 | 4.11 | 3.55 | 4.68 | GxN |
| Transcription factor similar to AtHB2 | 5.79 | 4.88 | 4.58 | 3.96 | <u>G</u> |
| Cytokinin dehydrogenase | 6.01 | 6.91 | 5.25 | 5.62 | <u>G</u> |
| Transcription factor similar to APRR2 | 4.20 | 4.16 | 5.07 | 5.90 | G |
| Oxygen transporter activity | 6.63 | 8.34 | 8.39 | 9.12 | G, N |
| Ethylene-responsive transcription factor | 7.53 | 6.65 | 6.08 | 5.89 | <u>G</u> |
| Gibberellin 20-oxidase | 6.73 | 7.25 | 7.87 | 8.01 | G |
| Gibberellin 20 oxidase 1-B | 6.50 | 6.78 | 7.65 | 7.78 | G |
| Gibberellin-regulated family protein | 6.11 | 6.25 | 7.95 | 7.61 | G |
| Scarecrow transcription factor family protein | 3.24 | 3.77 | 4.28 | 4.76 | G |
| Lipoxygenase similar to AtLOX1 | 11.50 | 11.92 | 12.68 | 12.67 | G |
| Lipoxygenase similar to AtLOX1 | 10.33 | 11.03 | 11.60 | 11.84 | G |
| Lipoxygenase similar to AtLOX1 | 4.61 | 5.03 | 6.19 | 5.81 | G |
| Lipoxygenase similar to AtLOX1 | 4.69 | 5.47 | 6.07 | 5.77 | G |
| Lipoxygenase similar to AtLOX1 | 4.54 | 5.73 | 6.45 | 6.53 | G |
| Lipoxygenase similar to AtLOX1 | 2.88 | 2.99 | 3.20 | 4.36 | G |
| Lipoxygenase similar to AtLOX5 | 4.64 | 5.70 | 4.17 | 4.10 | <u>G</u> |
| Lipoxygenase similar to AtLOX5 | 5.60 | 4.73 | 4.34 | 3.97 | <u>G</u> |

Average Affymetrix GeneChip normalized expression values across three biological replicates for the mutant TR185 (M) and the wild type (WT), in high and low N conditions. G and N indicate transcripts responsive to genotype and nitrate effect, respectively (adjusted P -values<0.05). G and G indicate up-regulation and down-regulation, respectively, in TR185 compared with the wild type

Discussion

The size and architecture of the root system determine the surface area of exchange between roots and the soil medium, and both are known to adapt in response to fluctuations of nutrient availability. Among the key nutrients, NO_3^- is well known to markedly affect root system architecture. We report here a new highly branched *M. truncatula* mutant, TR185, which lacks the capacity to adapt root architecture to nitrate supply and shows an unexpectedly low nitrogen acquisition. TR185 was selected among various γ -ray mutants because of its highly-branched root phenotype and expected enhanced nitrogen acquisition; its numerous young roots which have not yet developed strong lignin barriers were predicted to exploit more efficiently the soil for uptake of both water and nutrients (Steudle & Peterson, 1998; Naseer *et al.*, 2012). However, our study demonstrated that TR185 displayed N-limited responses; under both LN and HN supply, TR185 was depressed in shoot and root dry weight, and had a preferential dry weight allocation to roots at the expense of shoots when compared with the WT. The sub-optimal N nutrition of TR185 became evident as the growth cycle progressed, as from the 21-day stage, TR185 had lower %ShootN than the WT at both nitrate conditions. Furthermore, its low N-uptake rate and high amount of leaf area produced per N acquired were both typical for plants under very low N status (Larigauderie *et al.*, 1994; Moreau *et al.*, 2012). In *Arabidopsis*, root N uptake and architecture are both known to be regulated by external N supply and internal N demand. Based on these well-known responses in *Arabidopsis*, we investigated whether TR185 is impaired in either local acquisition/perception of nitrate availability or in systemic regulation by nitrogen status of the whole plant.

Molecular studies in *Arabidopsis* have highlighted that nitrate per se is a signal leading to an up-regulation of N transporters and thus of N acquisition of plants which have been previously N-starved (Lejay *et al.*, 1999; Wang *et al.*, 2003; Scheible *et al.*, 2004; Bi *et al.*, 2007). The localized stimulatory effect of external nitrate on lateral root (LR) elongation and/or emergence has also been thoroughly investigated (Zhang & Forde, 1998; Remans *et al.*, 2006a; Krouk *et al.*, 2010; Gojon *et al.*, 2011). Nitrate has been demonstrated to be itself the signal for the stimulation of LR emergence (thus LR number) and elongation. This stimulation has been associated with an enhanced auxin accumulation in apex of root primordia in newly emerged LRs, and has been shown to involve both ANR1 and NRT1.1 genes. In our study, microarray analysis revealed an up-regulation of various transcripts belonging to NO_3^- or NH_4^+ transporters families in TR185 when compared with WT. Such up-

regulation occurred irrespective of the N supply and could thus indicate a permanent local perception of high nitrate availability in the mutant. However, this up-regulation did not increase either root amino acid content or %RootN in TR185 compared with WT. Furthermore, no effect of nitrate supply on LR elongation or number was observed in TR185. Moreover, its highly-branched root architecture is not representative of plants impaired in local perception of nitrate; its reduced responsiveness differed from that observed in *nrt1.1* mutants or *ANRI*-repressed lines of Arabidopsis, in which LR number was never higher than in the wild type even under high nitrate availability. Taken together, these molecular and developmental responses of TR185 to N availability indicate that its highly-branched root system architecture is not mainly induced by an impaired local perception of nitrate availability.

Besides the local stimulatory effect of nitrate, a feedback repression is known to be exerted by high N status of the whole plant which down-regulates high-affinity N transporters, whereas N-starvation results in the opposite response. Specific members of the NRT2 and AMT1 families in Arabidopsis and MtNRT2 genes in *M. truncatula* are known to be involved in this response (Loqué *et al.*, 2006; Yuan *et al.*, 2007; Ruffel *et al.*, 2008; Okamoto *et al.*, 2009; Girin *et al.*, 2010). A systemic repression of LR development by the high N status of the plant has also been described in Arabidopsis. A nitrate-dependent signalling pathway controlling LR elongation has been described, in which nitrate supply above 10 mM blocks the elongation of LR post-emergence in response to a high shoot nitrate accumulation (Zhang *et al.*, 1999; Zhang & Forde, 2000; Remans *et al.*, 2006b). More recently, an additional signalling pathway has been pointed out, in which LR emergence is controlled by N assimilation products. According to Gifford *et al.* (2008), GLN is the predominant signal regulating repression of LR emergence, but an inhibition of root growth by ASN was also shown by Ivanov *et al.* (2012) and its possible role as an N-satiety signal suggested. In our study, molecular and developmental analyses converge to indicate that the mutant could perceive a permanent N-starvation signal, which induced modification of root N acquisition and architecture in it when compared with WT. Indeed, the up-regulation of root N transporters in TR185 compared with WT could be characteristic of the plant N-starvation status in the mutant. Importantly, the decreased GLN and ASN root content in TR185 compared with WT could explain its highly-branched root architecture in agreement with Gifford *et al.* (2008) and Ivanov *et al.* (2012). Grafting experiments revealed that the highly-branched root phenotype in TR185 was transmissible from shoots and not from roots

(Supplemental Fig. II.5), thus reinforcing the hypothesis of phloem transport of a signal in the mutant. As the GLN and ASN shoot contents were also lower in TR185 than in the WT, the signal could be the low GLN/ASN phloem content itself. That hypothesis does not exclude a possible higher degradation in roots of these two major N storage forms, as suggested by the observed differential expression of transcripts involved in ammonium assimilation. As such, the lower root content of GLN and ASN could be related to the up-regulation of a glutamate synthase and a putative L-asparagine amidohydrolase respectively in TR185 when compared with the WT.

Additional analyses of expression of genes involved in cell wall modification, phenylpropanoid pathway and hormone transport confirmed that TR185 plants were under N-starvation, and provided further explanation of their root architecture. Most of the genes involved in cell wall degradation were up-regulated in the TR185 compared with WT, whereas those involved in cell wall synthesis were down-regulated. Such modifications have been previously observed for *L. japonicus* in N-starvation conditions (Omrane *et al.*, 2009). Most of the transcripts encoding AGP cell wall proteins were down regulated in TR185, in agreement with the reduced root elongation observed in Arabidopsis AGP-defective mutants (van Hengel & Roberts, 2002; Shi *et al.*, 2003; Seifert & Roberts, 2007). Widespread differential expression of transcripts for the phenylpropanoid pathway was also observed between the two genotypes. The phenylpropanoid pathway serves as a rich source of metabolites in plants, being required for the biosynthesis of both lignin and many other important compounds such as the flavonoids (Fraser & Chapple, 2011). A large number of genes involved in lignin synthesis were repressed in TR185, whereas transcripts encoding a chalcone synthase or involved in flavonol glycoside synthesis were up-regulated. The slightly lower root lignin content observed in TR185 compared with WT was in agreement with these results (Fig. II. S6). The higher expression in TR185 of genes involved in flavonoid synthesis support the hypothesis that the mutant was under permanent N-starved conditions, in agreement with previous observations in *L. japonicus* roots (Omrane *et al.*, 2009). Flavonoid accumulation is known to decrease polar auxin transport, inducing a deregulation of LR elongation and thus short root architecture (Peer *et al.*, 2004; Peer & Murphy, 2007; Laffont *et al.*, 2010). Interestingly, most of the transcripts encoding auxin-induced or -binding proteins or related to the auxin efflux transporters PIN were down-regulated in TR185 when compared with WT, suggesting a decreased root auxin accumulation or transport. Differentially expressed genes between TR185 and WT related to other hormones could also

explain TR185 root architecture; in particular the up-regulation of a transcript involved in cytokinin degradation, and thus in enhanced LR initiation (Laplaze *et al.*, 2007), and the down-regulation of transcripts involved in GA synthesis, which depressed root elongation (Beemster & Baskin, 2000; Achard *et al.*, 2003; Benkova & Hejatko, 2009).

In conclusion, the mutant TR185 displayed highly-branched root architecture and impaired N acquisition, both irrespective of the nitrate supply. Physiological and developmental analyses of its responses to N supply suggested that the root architecture of TR185 results from a systemic regulation by the plant nitrogen status, possibly involving GLN or ASN signals. Altered expression of genes of the phenylpropanoid pathway could also explain its root architecture. Further studies are needed both to determine in which gene the mutation occurred and fully understand the TR185 phenotype under conditions when it is relying exclusively on symbiotic N fixation for its N acquisition. Such results will identify the genes and physiological mechanisms that regulate legume root architecture and activity as a function of plant N status, and give new targets for legume breeding.

Acknowledgments

The authors thank Estelle Carteret, Arnaud Bartet and Sébastien Brenot of the Experimental Unit for their technical support for the experiment in growth chamber. Anne-Lise Santoni of the UMR Agroécologie is acknowledged for the nitrogen concentration analyses, and Catherine Bonnefoy for her help in the grafting experiment. This work was supported by INRA (AgroBI program) and the Burgundy Council (PARI-Agrale6 program). The authors thank Richard Thompson for his critical reading of the manuscript and the anonymous referees for their constructive criticisms.

References

- Achard P, Vriezen WH, Van Der Straeten D, Harberd NP. 2003. Ethylene regulates Arabidopsis development via the modulation of DELLA protein growth repressor function. *Plant Cell* **15**, 2816-2825.
- Barrett T, Troup DB, Wilhite SE, Ledoux P, Rudnev D, Evangelista C, Kim IF, Soboleva A, Tomashevsky M, Edgar R. 2007. NCBI GEO: mining tens of millions of expression profiles-database and tools update. *Nucleic Acids Res* **35**, D760-765.
- Beemster GTS, Baskin TI. 2000. STUNTED PLANT 1 mediates effects of cytokinin, but not of auxin, on cell division and expansion in the root of Arabidopsis. *Plant Physiology* **124**, 1718-1727.
- Benkova E, Hejatko J. 2009. Hormone interactions at the root apical meristem. *Plant Molecular Biology* **69**, 383-396.
- Bi YM, Wang RL, Zhu T, Rothstein SJ. 2007. Global transcription profiling reveals differential responses to chronic nitrogen stress and putative nitrogen regulatory components in Arabidopsis. *Bmc Genomics* **8**.
- Bourion V, Laguerre G, Depret G, Voisin AS, Salon C, Duc G. 2007. Genetic variability in nodulation and root growth affects nitrogen fixation and accumulation in pea. *Annals of Botany* **100**, 589-598.
- Bourion V, Rizvi SMH, Fournier S, de Larambergue H, Galmiche F, Marget P, Duc G, Burstin J. 2010. Genetic dissection of nitrogen nutrition in pea through a QTL approach of root, nodule, and shoot variability. *Theoretical and Applied Genetics* **121**, 71-86.
- Bright LJ, Liang Y, Mitchell DM, Harris JM. 2005. The *LATD* gene of *Medicago truncatula* is required for both nodule and root development. *Molecular Plant-Microbe Interactions* **18**, 521-532.
- Brun F, Richard-Molard C, Pagès L, Chelle M, Ney B. 2010. To what extent may changes in the root system architecture of *Arabidopsis thaliana* grown under contrasted homogenous nitrogen regimes be explained by changes in carbon supply? A modelling approach. *Journal of Experimental Botany* **61**, 2157-2169.
- de Billy F, Grosjean C, May S, Bennett M, Cullimore JV. 2001. Expression studies on *AUX1*-like genes in *Medicago truncatula* suggest that auxin is required at two steps in early nodule development. *Molecular Plant-Microbe Interactions* **14**, 267-277.
- Fraser CM, Chapple C. 2011. The phenylpropanoid pathway in Arabidopsis. *The Arabidopsis book / American Society of Plant Biologists* **9**, e0152-e0152.
- Frugier F, Kosuta S, Murray JD, Crespi M, Szczyglowski K. 2008. Cytokinin: secret agent of symbiosis. *Trends in Plant Science* **13**, 115-120.
- Fu XD, Harberd NP. 2003. Auxin promotes Arabidopsis root growth by modulating gibberellin response. *Nature* **421**, 740-743.
- Fukushima RS, Hatfield RD. 2001. Extraction and isolation of lignin for utilization as a standard to determine lignin concentration using the acetyl bromide spectrophotometric method. *Journal of Agricultural and Food Chemistry* **49**, 3133-3139.
- Gagnot S, Tamby JP, Martin-Magniette ML, Bitton F, Taconnat L, Balzergue S, Aubourg S, Renou JP, Lecharny A, Brunaud V. 2008. CATdb: a public access to Arabidopsis transcriptome data from the URGV-CATMA platform. *Nucleic Acids Research* **36**, D986-D990.
- Garcia J, Barker DG, Journet EP. 2006. Seed storage and germination. In: Mathesius U, Journet EP, Sumner LW, eds. *The Medicago truncatula handbook*.
- Ge YC, Dudoit S, Speed TP. 2003. Resampling-based multiple testing for microarray data analysis. *Test* **12**, 1-77.
- Gentleman R, Carey V. 2002. Bioconductor. *R News* **2**, 11-16.
- Gifford ML, Dean A, Gutierrez RA, Coruzzi GM, Birnbaum KD. 2008. Cell-specific nitrogen responses mediate developmental plasticity. *Proceedings of the National Academy of Sciences of the United States of America* **105**, 803-808.

- Girin T, El-Kafafi ES, Widiez T, Erban A, Hubberten HM, Kopka J, Hoefgen R, Gojon A, Lepetit M. 2010.** Identification of Arabidopsis mutants impaired in the systemic regulation of root nitrate uptake by the nitrogen status of the plant. *Plant Physiology* **153**, 1250-1260.
- Gojon A, Krouk G, Perrine-Walker F, Laugier E. 2011.** Nitrate transceptor(s) in plants. *Journal of Experimental Botany* **62**, 2299-2308.
- Gonzalez-Rizzo S, Crespi M, Frugier F. 2006.** The *Medicago truncatula* CRE1 cytokinin receptor regulates lateral root development and early symbiotic interaction with *Sinorhizobium meliloti*. *Plant Cell* **18**, 2680-2693.
- Ikram S, Bedu M, Daniel-Vedele F, Chaillou S, Chardon F. 2012.** Natural variation of Arabidopsis response to nitrogen availability. *Journal of Experimental Botany* **63**, 91-105.
- Irizarry RA, Hobbs B, Collin F, Beazer-Barclay YD, Antonellis KJ, Scherf U, Speed TP. 2003.** Exploration, normalization, and summaries of high density oligonucleotide array probe level data. *Biostatistics* **4**, 249-264.
- Ivanov A, Kameka A, Pajak A, Bruneau L, Beyaert R, Hernandez-Sebastia C, Marsolais F. 2012.** Arabidopsis mutants lacking asparaginases develop normally but exhibit enhanced root inhibition by exogenous asparagine. *Amino Acids* **42**, 2307-2318.
- Jin J, Watt M, Mathesius U. 2012.** The autoregulation gene *SUNN* mediates changes in root organ formation in response to nitrogen through alteration of shoot-to-root auxin transport. *Plant Physiology* **159**, 489-500.
- Kiba T, Kudo T, Kojima M, Sakakibara H. 2011.** Hormonal control of nitrogen acquisition: roles of auxin, abscisic acid, and cytokinin. *Journal of Experimental Botany* **62**, 1399-1409.
- Kraft JM, Boge W. 2001.** Root characteristics in pea in relation to compaction and fusarium root rot. *Plant Disease* **85**, 936-940.
- Krouk G, Lacombe B, Bielach A, Perrine-Walker F, Malinska K, Mounier E, Hoyerova K, Tillard P, Leon S, Ljung K, Zazimalova E, Benkova E, Nacry P, Gojon A. 2010.** Nitrate-Regulated Auxin Transport by NRT1.1 Defines a Mechanism for Nutrient Sensing in Plants. *Developmental Cell* **18**, 927-937.
- Krusell L, Madsen LH, Sato S, Aubert G, Genua A, Szczyglowski K, Duc G, Kaneko T, Tabata S, de Bruijn F, Pajuelo E, Sandal N, Stougaard J. 2002.** Shoot control of root development and nodulation is mediated by a receptor-like kinase. *Nature* **420**, 422-426.
- Laffont C, Blanchet S, Lapierre C, Brocard L, Ratet P, Crespi M, Mathesius U, Frugier F. 2010.** The Compact Root Architecture1 gene regulates lignification, flavonoid production, and polar auxin transport in *Medicago truncatula*. *Plant Physiology* **153**, 1597-1607.
- Laplaze L, Benkova E, Casimiro I, Maes L, Vanneste S, Swarup R, Weijers D, Calvo V, Parizot B, Herrera-Rodriguez MB, Offringa R, Graham N, Doumas P, Friml J, Bogusz D, Beeckman T, Bennett M. 2007.** Cytokinins act directly on lateral root founder cells to inhibit root initiation. *Plant Cell* **19**, 3889-3900.
- Larigauderie A, Reynolds JF, Strain BR. 1994.** Root response to CO₂ enrichment and nitrogen supply in loblolly-pine. *Plant and Soil* **165**, 21-32.
- Lejay L, Tillard P, Lepetit M, Olive FD, Filleur S, Daniel-Vedele F, Gojon A. 1999.** Molecular and functional regulation of two NO₃⁻ uptake systems by N- and C-status of Arabidopsis plants. *Plant Journal* **18**, 509-519.
- Liang Y, Mitchell DM, Harris JM. 2007.** Abscisic acid rescues the root meristem defects of the *Medicago truncatula latd* mutant. *Developmental Biology* **304**, 297-307.
- Loque D, Yuan L, Kojima S, Gojon A, Wirth J, Gazzarrini S, Ishiyama K, Takahashi H, von Wiren N. 2006.** Additive contribution of AMT1;1 and AMT1;3 to high-affinity ammonium uptake across the plasma membrane of nitrogen-deficient Arabidopsis roots. *Plant Journal* **48**, 522-534.
- Loudet O, Chaillou S, Krapp A, Daniel-Vedele F. 2003.** Quantitative trait loci analysis of water and anion contents in interaction with nitrogen availability in *Arabidopsis thaliana*. *Genetics* **163**, 711-722.
- Malamy JE. 2005.** Intrinsic and environmental response pathways that regulate root system architecture. *Plant Cell and Environment* **28**, 67-77.

- Malamy JE, Ryan KS. 2001.** Environmental regulation of lateral root initiation in Arabidopsis. *Plant Physiology* **127**, 899-909.
- Marchant A, Bhalerao R, Casimiro I, Eklof J, Casero PJ, Bennett M, Sandberg G. 2002.** AUX1 promotes lateral root formation by facilitating indole-3-acetic acid distribution between sink and source tissues in the Arabidopsis seedling. *Plant Cell* **14**, 589-597.
- McPhee K. 2005.** Variation for seedling root architecture in the core collection of pea germplasm. *Crop Science* **45**, 1758-1763.
- Moreau D, Burstin J, Aubert G, Huguet T, Ben C, Prospero JM, Salon C, Munier-Jolain N. 2012.** Using a physiological framework for improving the detection of quantitative trait loci related to nitrogen nutrition in *Medicago truncatula*. *Theoretical and Applied Genetics* **124**, 755-768.
- Moreau D, Voisin AS, Salon C, Munier-Jolain N. 2008.** The model symbiotic association between *Medicago truncatula* cv. Jemalong and *Rhizobium meliloti* strain 2011 leads to N-stressed plants when symbiotic N₂ fixation is the main N source for plant growth. *Journal of Experimental Botany* **59**, 3509-3522.
- Muday GK, DeLong A. 2001.** Polar auxin transport: controlling where and how much. *Trends in Plant Science* **6**, 535-542.
- Naseer S, Lee Y, Lapierre C, Franke R, Nawrath C, Geldner N. 2012.** Casparian strip diffusion barrier in Arabidopsis is made of a lignin polymer without suberin. *Proceedings of the National Academy of Sciences of the United States of America* **109**, 10101-10106.
- Okamoto S, Ohnishi E, Sato S, Takahashi H, Nakazono M, Tabata S, Kawaguchi M. 2009.** Nod Factor/Nitrate-Induced *CLE* Genes that Drive HAR1-Mediated Systemic Regulation of Nodulation. *Plant and Cell Physiology* **50**, 67-77.
- Omrane S, Ferrarini A, D'Apuzzo E, Rogato A, Delledonne M, Chiurazzi M. 2009.** Symbiotic competence in *Lotus japonicus* is affected by plant nitrogen status: transcriptomic identification of genes affected by a new signalling pathway. *New Phytologist* **183**, 380-394.
- Peer WA, Bandyopadhyay A, Blakeslee JJ, Makam SI, Chen RJ, Masson PH, Murphy AS. 2004.** Variation in expression and protein localization of the PIN family of auxin efflux facilitator proteins in flavonoid mutants with altered auxin transport in *Arabidopsis thaliana*. *Plant Cell* **16**, 1898-1911.
- Peer WA, Murphy AS. 2007.** Flavonoids and auxin transport: modulators or regulators? *Trends in Plant Science* **12**, 556-563.
- Peret B, De Rybel B, Casimiro I, Benkova E, Swarup R, Laplaze L, Beeckman T, Bennett MJ. 2009.** Arabidopsis lateral root development: an emerging story. *Trends in Plant Science* **14**, 399-408.
- Plet J, Wasson A, Ariel F, Le Signor C, Baker D, Mathesius U, Crespi M, Frugier F. 2011.** MtCRE1-dependent cytokinin signaling integrates bacterial and plant cues to coordinate symbiotic nodule organogenesis in *Medicago truncatula*. *Plant Journal* **65**, 622-633.
- Remans T, Nacry P, Pervent M, Filleur S, Diatloff E, Mounier E, Tillard P, Forde BG, Gojon A. 2006a.** The Arabidopsis NRT1.1 transporter participates in the signaling pathway triggering root colonization of nitrate-rich patches. *Proceedings of the National Academy of Sciences of the United States of America* **103**, 19206-19211.
- Remans T, Nacry P, Pervent M, Girin T, Tillard P, Lepetit M, Gojon A. 2006b.** A central role for the nitrate transporter NRT2.1 in the integrated morphological and physiological responses of the root system to nitrogen limitation in Arabidopsis. *Plant Physiology* **140**, 909-921.
- Rey T, Nars A, Bonhomme M, Bottin A, Huguet S, Balzergue S, Jardinaud MF, Bono JJ, Cullimore J, Dumas B, Gough C, Jacquet C. 2013.** NFP, a LysM protein controlling Nod factor perception, also intervenes in *Medicago truncatula* resistance to pathogens. *New Phytologist* **198**, 875-886.
- Ruffel S, Freixes S, Balzergue S, Tillard P, Jeudy C, Martin-Magniette ML, van der Merwe MJ, Kakar K, Gouzy J, Fernie AR, Udvardi M, Salon C, Gojon A, Lepetit M. 2008.** Systemic signaling of the plant nitrogen status triggers specific transcriptome responses depending on the nitrogen source in *Medicago truncatula*. *Plant Physiology* **146**, 2020-2035.

- Ruffel S, Krouk G, Ristova D, Shasha D, Birnbaum KD, Coruzzi GM. 2011.** Nitrogen economics of root foraging: Transitive closure of the nitrate-cytokinin relay and distinct systemic signaling for N supply vs. demand. *Proceedings of the National Academy of Sciences of the United States of America* **108**, 18524-18529.
- Ruzicka K, Simaskova M, Duclercq J, Petrasek J, Zazimalova E, Simon S, Friml J, Van Montagu MCE, Benkova E. 2009.** Cytokinin regulates root meristem activity via modulation of the polar auxin transport. *Proceedings of the National Academy of Sciences of the United States of America* **106**, 4284-4289.
- Sagan M, Morandi D, Tarengi E, Duc G. 1995.** Selection of nodulation and mycorrhizal mutants in the model plant *Medicago truncatula* (Gaertn.) after gamma-ray mutagenesis. *Plant Science* **111**, 63-71.
- Salon C, Lepetit M, Gamas P, Jeudy C, Moreau S, Moreau D, Voisin AS, Duc G, Bourion V, Munier-Jolain N. 2009.** Analysis and modeling of the integrative response of *Medicago truncatula* to nitrogen constraints. *Comptes Rendus Biologies* **332**, 1022-1033.
- Salon C, Munier-Jolain NG, Duc G, Voisin AS, Grandgirard D, Larmure A, Emery RJN, Ney B. 2001.** Grain legume seed filling in relation to nitrogen acquisition: A review and prospects with particular reference to pea. *Agronomie* **21**, 539-552.
- Scheible WR, Morcuende R, Czechowski T, Fritz C, Osuna D, Palacios-Rojas N, Schindelasch D, Thimm O, Udvardi MK, Stitt M. 2004.** Genome-wide reprogramming of primary and secondary metabolism, protein synthesis, cellular growth processes, and the regulatory infrastructure of Arabidopsis in response to nitrogen. *Plant Physiology* **136**, 2483-2499.
- Schnabel E, Journet EP, de Carvalho-Niebel F, Duc G, Frugoli J. 2005.** The *Medicago truncatula* *SUNN* gene encodes a *CLV1*-like leucine-rich repeat receptor kinase that regulates nodule number and root length. *Plant Molecular Biology* **58**, 809-822.
- Schnabel EL, Kassaw TK, Smith LS, Marsh JF, Oldroyd GE, Long SR, Frugoli JA. 2011.** The *ROOT DETERMINED NODULATION1* gene regulates nodule number in roots of *Medicago truncatula* and defines a highly conserved, uncharacterized plant gene family. *Plant Physiology* **157**, 328-340.
- Seifert GJ, Roberts K. 2007.** The biology of arabinogalactan proteins. *Annual Review of Plant Biology*, Vol. 58. Palo Alto: Annual Reviews, 137-161.
- Shi HZ, Kim Y, Guo Y, Stevenson B, Zhu JK. 2003.** The Arabidopsis *SOS5* locus encodes a putative cell surface adhesion protein and is required for normal cell expansion. *Plant Cell* **15**, 19-32.
- Steudle E, Peterson CA. 1998.** How does water get through roots? *Journal of Experimental Botany* **49**, 775-788.
- Tellstrom V, Usadel B, Thimm O, Stitt M, Kuster H, Niehaus K. 2007.** The lipopolysaccharide of *Sinorhizobium meliloti* suppresses defense-associated gene expression in cell cultures of the host plant *Medicago truncatula*. *Plant Physiology* **143**, 825-837.
- Thimm O, Blasing O, Gibon Y, Nagel A, Meyer S, Kruger P, Selbig J, Muller LA, Rhee SY, Stitt M. 2004.** MAPMAN: a user-driven tool to display genomics data sets onto diagrams of metabolic pathways and other biological processes. *Plant Journal* **37**, 914-939.
- Ubeda-Tomas S, Swarup R, Coates J, Swarup K, Laplaze L, Beemster GTS, Hedden P, Bhalerao R, Bennett MJ. 2008.** Root growth in Arabidopsis requires gibberellin/DELLA signalling in the endodermis. *Nature Cell Biology* **10**, 625-628.
- van Hengel AJ, Roberts K. 2002.** Fucosylated arabinogalactan-proteins are required for full root cell elongation in arabidopsis. *Plant Journal* **32**, 105-113.
- Walch-Liu P, Ivanov, II, Filleur S, Gan YB, Remans T, Forde BG. 2006a.** Nitrogen regulation of root branching. *Annals of Botany* **97**, 875-881.
- Walch-Liu P, Liu LH, Remans T, Tester M, Forde BG. 2006b.** Evidence that L-glutamate can act as an exogenous signal to modulate root growth and branching in Arabidopsis thaliana. *Plant and Cell Physiology* **47**, 1045-1057.
- Wang RC, Okamoto M, Xing XJ, Crawford NM. 2003.** Microarray analysis of the nitrate response in Arabidopsis roots and shoots reveals over 1,000 rapidly responding genes and new linkages

- to glucose, trehalose-6-phosphate, iron, and sulfate metabolism. *Plant Physiology* **132**, 556-567.
- Wopereis J, Pajuelo E, Dazzo FB, Jiang QY, Gresshoff PM, de Bruijn FJ, Stougaard J, Szczyglowski K. 2000.** Short root mutant of *Lotus japonicus* with a dramatically altered symbiotic phenotype. *Plant Journal* **23**, 97-114.
- Wu G, Lewis DR, Spalding EP. 2007.** Mutations in Arabidopsis multidrug resistance-like ABC transporters separate the roles of acropetal and basipetal auxin transport in lateral root development. *Plant Cell* **19**, 1826-1837.
- Yendrek CR, Lee YC, Morris V, Liang Y, Pislariu CI, Burkart G, Meckfessel MH, Salehin M, Kessler H, Wessler H, Lloyd M, Lutton H, Teillet A, Sherrier DJ, Journet EP, Harris JM, Dickstein R. 2010.** A putative transporter is essential for integrating nutrient and hormone signaling with lateral root growth and nodule development in *Medicago truncatula*. *Plant Journal* **62**, 100-112.
- Yuan LX, Loque D, Ye FH, Frommer WB, von Wiren N. 2007.** Nitrogen-dependent posttranscriptional regulation of the ammonium transporter AtAMT1;1. *Plant Physiology* **143**, 732-744.
- Zhang HM, Forde BG. 1998.** An Arabidopsis MADS box gene that controls nutrient-induced changes in root architecture. *Science* **279**, 407-409.
- Zhang HM, Forde BG. 2000.** Regulation of Arabidopsis root development by nitrate availability. *Journal of Experimental Botany* **51**, 51-59.
- Zhang HM, Jennings A, Barlow PW, Forde BG. 1999.** Dual pathways for regulation of root branching by nitrate. *Proceedings of the National Academy of Sciences of the United States of America* **96**, 6529-6534.

CHAPITRE III

CHAPITRE III : VARIABILITÉ GÉNÉTIQUE DU CHOIX ENTRE PARTENAIRES SYMBIOTIQUES POIS ET RHIZOBIUM

1. Introduction au chapitre III

Le levier de l'amélioration de l'acquisition d'N chez le pois via une amélioration de sa symbiose avec les bactéries de type *Rhizobium leguminosarum* sv. *viciae* (Rlv) a été abordé par l'étude de la diversité des choix entre ces deux partenaires symbiotiques et de la variabilité des associations entre pois et Rlv pour leur capacité à former des nodosités et leur efficacité à fixer l'N atmosphérique. Hormis le cas de spécificité d'hôte mis en évidence à la fin des années 1970 entre des pois principalement originaires d'Afghanistan et des souches originaires du Moyen-Orient (Lie, 1978; Young & Matthews, 1982), très peu de choses étaient connues concernant la diversité des choix entre partenaires. Les objectifs de cette étude relatifs à l'étude de cette diversité étaient de vérifier i) si la plante peut exercer un contrôle sur ses partenaires symbiotiques; ii) si ce choix peut être basé sur l'efficacité d'acquisition d'N obtenue avec les Rlv choisies ; iii) si ces choix ont pu être modifiés lors de la domestication de l'espèce associée à sa migration géographique ou lors des processus de création variétale plus récents.

Cette étude de diversité a été réalisée sur une collection de 104 accessions de pois inoculée par un mélange de cinq souches de Rlv. La collection des accessions de pois a été constituée comme comprenant principalement la « core » collection créée dans l'Unité, à laquelle nous avons ajouté différentes accessions connues pour leur spécificité d'hôte ou pour la variabilité, mise en évidence précédemment, de leur développement racinaire ou nodulaire. Le « core » collection avait été définie comme représentative de la diversité au sein du genre *Pisum* évaluée sur la base de l'étude de 28 marqueurs microsattellites et de connaissance sur les origines géographiques des accessions. Néanmoins, un certain nombre de données « passeport » relatives aux statuts et usages de ces accessions étaient manquantes ; nous avons complété ces données à partir d'un travail bibliographique ou d'enquête auprès de sélectionneurs. Les 5 souches de Rlv ont été choisies par notre collègue microbiologiste, Gisèle Laguerre, pour leur diversité de type de gène *nodD* et, quand elles avaient été évaluées (en comparaison

avec une souche de référence et sur un faible nombre de géotypes de pois), pour leur diversité d'efficacité ou de compétitivité.

Des mesures, identiques à celles utilisées dans les deux chapitres précédents et décrivant les structures d'acquisition de l'N, leur fonctionnement et la croissance des plantes, ont été réalisées sur l'ensemble de la collection de pois inoculée par le mélange des 5 souches de Rlv. Une large gamme de variation de biomasse aérienne a été observée entre les différentes accessions, en corrélation hautement significative et positive avec la biomasse des nodosités. Cette corrélation a été observée quelle que soit l'origine géographique ou la diversité d'usage des géotypes de pois, révélant une faible variabilité génétique pour la relation qui relie croissance de la plante et sa demande en N. Une forte variabilité pour le nombre de nodosités a aussi été observée entre les accessions de pois, mais sa corrélation avec la biomasse aérienne était moins significative que celle obtenue pour la biomasse des nodosités.

En complément, les compositions des populations de Rlv associées à chacune des accessions de pois ont été déterminées à Montpellier au LSTM sur un échantillon représentatif de 60 nodules par accession. Il a ainsi été mis en évidence que la composition des populations de Rlv associées varie selon le géotype de pois et plus globalement entre les groupes de structuration génétique de la collection de pois. Ainsi une plus grande diversité de choix a été observée chez les accessions sauvages ou appartenant aux premières espèces domestiquées en comparaison avec les cultivars plus récents. Ces différences pourraient témoigner de changements dans le choix du partenaire symbiotique lors de la domestication et de la sélection. Les expérimentations complémentaires réalisées en mono-inoculation sur un sous-ensemble de 18 accessions de pois ont montré que i) l'efficacité de la fixation de l'azote n'est néanmoins pas un déterminant majeur du choix par les géotypes de pois de leur partenaire symbiotique; ii) la compétitivité en mono-inoculation d'une souche n'est pas un bon indicateur de sa capacité à noduler un pois lorsqu'elle est en mélange avec d'autres souches. Ces travaux ont donc montré l'importance de la diversité du choix entre partenaires symbiotiques et l'existence d'un contrôle génétique de ce caractère; sa prise en compte dans les programmes de sélection à venir nécessitera une meilleure connaissance de ses déterminants génétiques.

Ce travail est présenté sous forme d'un article prévu pour soumission à la revue *New phytologist*.

2. Publication n°3

Genetic diversity of pea-Rhizobium leguminosarum partner choice

Virginie Bourion^{1*}, Karine Heulin-Gotty², Henri de Larambergue¹, Pierre Tisseyre²,
Véronique Aubert¹, Marjorie Pervent², Marianne Chabert-Martinello¹, Denis Vile³, Mathieu
Sio¹, Gérard Duc¹, Brigitte Brunel², Judith Burstin¹, Marc Lepetit^{2*}

¹Agroécologie, AgroSup Dijon, INRA, Univ. Bourgogne Franche-Comté, F-21000 Dijon,
France

²Symbioses Tropicales et Méditerranéennes, INRA, UMR1342 INRA-IRD-CIRAD-SupAgro-
UM2, Campus de Baillarguet, TA-A82/J, F-34398 Montpellier, France

³Ecophysiologie des Plantes sous Stress Environnementaux, INRA, UMR759 INRA-
SupAgro, 3 place Viala, 34070 Montpellier

This contribution is dedicated to the memory of Dr. Gisèle Laguerre, who initiated this work
and through her activities raised interest in rhizobial resources as the foundation for improved
legume agriculture.

*Corresponding authors:

Virginie Bourion,

INRA, UMR1347 Agroécologie, Pôle GEAPSI, 17 rue Sully, BP 86510, F-21065 Dijon
cedex, France

Tel.: 33 (3) 80 69 36 47

Email: virginie.bourion@dijon.inra.fr

Marc Lepetit

INRA, UMR1342 IRD-INRA-CIRAD-SupAgro-UM2, Symbioses Tropicales et
Méditerranéennes, Campus de Baillarguet, TA-A82/J, F-34398 Montpellier Cedex 5, France

Tel.: 33 (4) 67 59 38 62

Email: lepetit@supagro.inra.fr

Les références citées sont situées à la fin de ce chapitre (p 133-137).

Les fichiers additionnels sont présentés en annexe 3.

Abstract

Research conducted, including the rationale

In the field, peas are nodulated by indigenous *Rhizobium leguminosarum* sv. *viciae* (Rlv) diverse strains varying in their competitiveness for nodulation and efficiency of nitrogen fixation. Even several cases of pea-Rlv specificity were evidenced in the past, the genetic diversity for pea-Rlv partner choice is not fully known.

Methods

The variability of pea-Rlv partner choice was investigated within a 104-pea collection representative of the variability encountered in the genus *Pisum*, co-inoculated with a mixture of five diverse Rlv strains. Additional single-inoculated experiments estimated the competitiveness for nodulation and nitrogen fixation efficiency conferred by each Rlv strain to 18 contrasted pea accessions.

Key results

Differences in Rlv choice were observed between the different pea genetic groups identified, revealing changes in partner choice during domestication and breeding selection. Differences in competitiveness for nodulation in multi-inoculation were found between the five strains without correlation with competitiveness for nodulation or nitrogen fixation efficiency obtained in mono-inoculation.

Main conclusion, including key points of discussion

The results show that nitrogen fixation efficiency is not a major determinant of pea-Rlv partner choice and that mono-inoculation experiments fail to determine the strain ability to compete with other strains in a mixture. A successful inoculant must not only provide enhanced symbiotic nitrogen fixation but be competitive for nodulation in the presence of a large population of indigenous rhizobia.

Keywords: *Pisum sativum*, *Rhizobium leguminosarum* sbv. *viciae*, pea-rhizobium symbiosis, genetic diversity, competitiveness for nodulation, nitrogen fixation efficiency, partner choice, breeding selection

Introduction

Legumes have a key role as a sustainable source of protein in both human and animal diets. Thanks to their ability to establish a beneficial symbiotic interaction with the nitrogen-fixing bacteria called rhizobia, legumes do not require nitrogen fertiliser which is a major source of greenhouse gases and energy consumption (Jensen & Hauggaard-Nielsen, 2003; Galloway *et al.*, 2008). The biological nitrogen fixation (BNF) obtained from grain legume crops (pulses and oilseed legumes) represents a quarter of the annual N applied to arable lands as chemical fertilizers (Herridge *et al.*, 2008). Grain legume crops are also a high valuable source of protein (115 million t of protein per year in the world) (Duc *et al.*, 2015). Despite these benefits, grain legumes are under-cultivated in many agricultural systems, as they suffer from low productivity and yield instability, in comparison to cereals, whose productivity is governed by the use of low cost fertilizers. With a surface less than 2% in grain legumes, Europe has to import about 70% of its plant proteins. Therefore, improving the productivity and stability of grain legumes are major objectives to extend their cultivation.

Pea (*Pisum sativum* L.) is one of the first domesticated crops, together with other legumes and cereals which formed important dietary component of early civilizations in Middle East and Mediterranean (Zohary, Daniel & Hopf, Maria, 1973; Cousin, 1997). Archaeological evidence dates the appearance of pea in farming villages back to the early Neolithic phase (7000 to 6000 B.C.) in the area of the Fertile Crescent through Israel, south-eastern Turkey and Iraq (Zohary, Daniel & Hopf, Maria, 1973). Pea genetic diversity later spreads to western Turkey, Greece and Bulgaria, and eastwards to the Caucasus, Iran and Afghanistan (Zohary, Daniel & Hopf, Maria, 1973; Smykal *et al.*, 2011). Finally, cultivated peas were found at the Bronze Age all over Europe (Fourmont, 1956; Zohary, Daniel & Hopf, Maria, 1973; Cousin, 1997). In parallel, pea cultivation moved eastward to India, where the earliest references are found in c. 200 B.C., and to China (Chimwamurombe & Khulbe, 2011; Smykal *et al.*, 2015). The wild relatives of pea contain both the species *P. fulvum* found in the Fertile Crescent and the *P. sativum* subsp. *elatius* which is distributed widely across the Mediterranean basin from Spain to the Middle East. Cultivated pea is dominated by *P. sativum* subsp. *sativum* whereas the less abundant *P. sativum* subsp. *abyssinicum* from Yemen and Ethiopia is considered as an independently derived cultivated type (Vershinin *et al.*, 2003; Jing *et al.*, 2010; Smykal *et al.*, 2011).

Pea is nodulated by *Rhizobium leguminosarum* symbiovar *viciae* (Rlv) bacteria. The term 'symbiovar' (previously "biovar") refers to the ability of rhizobia to establish symbiosis

with specific legumes (Rogel *et al.*, 2011). Within the Rlv biovar, variation in sequences of symbiotic genes has been described, suggesting variation in their nodulation abilities and partner choice (Laguerre *et al.*, 2001; Kumar *et al.*, 2015; Peix *et al.*, 2015). Indeed, although Rlv has long been regarded as able to nodulate all the species of the legume tribe Viciaeae, differences in symbiotic host range have been described between Rlv strains (Laguerre *et al.*, 2003; Mutch & Young, 2004). Variation of Rlv host specificity within the *Pisum* genus is one of the earliest reported cases of host-controlled nodulation restriction. Some cultivated peas from Afghanistan were identified as resistant to nodulation by European Rlv strains and as requiring specific Rlv strains found in Israel, Turkey or Afghanistan, whereas European pea cultivars were nodulated by both types of strains (Lie, 1978; Young & Matthews, 1982). The genes *SYM2* in the plant and *nodX* in the rhizobium confer this specificity (Holl, 1975; Davis *et al.*, 1988). Several examples of specificity for particular strains have also been obtained in pea after mutagenesis (Sagan *et al.*, 1993a; LaRue *et al.*, 1996). More generally, variations in *nod* genes which lead to variations in the structure and level of production of the symbiotic signals Nod factors, together with variations in the legume genes encoding lysine motif receptor like kinases (LysM-RLKs) which are required for Nod factors perception, are associated to the specificity of legume-rhizobia association (Spaink *et al.*, 1991; Denarie *et al.*, 1992; Walker *et al.*, 2000; D'Haese & Holsters, 2002). In addition to the Nod factor-mediated signalling, other mechanisms – among them those involving the rhizobial cell surface polysaccharides and secreted proteins – required to initiate legume infection (Masson-Boivin *et al.*, 2009; Downie, 2010). To our knowledge, these mechanisms have not yet been investigated in the pea-rlv symbiosis.

In the complex environment of the soil, legume roots are exposed to heterogeneous rhizobial populations, with strains varying in symbiotic performance (Denison, 2000; Laguerre *et al.*, 2003; Sachs *et al.*, 2009; Rahi *et al.*, 2012). The nodulation process has a high metabolic cost for both rhizobial and plant partners (Phillips, 1980; Schulze *et al.*, 1999; Trainer & Charles, 2006; Voisin *et al.*, 2007). The co-evolution between legumes and symbiotic bacteria is generally assumed to be highly dependent on both the photosynthetic performance of the plant and nitrogen fixation efficiency of the symbiont. Legumes can monitor and respond to the nitrogen-fixing performance of symbiotic bacteria, promoting the high- and excluding or punishing the low-efficient strains; for reviews see (Simms & Taylor, 2002; Oono *et al.*, 2011). However, poorly-fixing rhizobial strains can also gain advantage over beneficial strains and offers no growth benefit to their plant host (Sachs *et al.*, 2010). Domesticated crops such as pea and faba bean crops tend to have fewer compatible symbionts

than their wild relatives (Mutch & Young, 2004). In soybean, a less ability was observed for modern as compared to older cultivars to establish beneficial associations with rhizobia, which may be explained by decrease in genetic diversity and breeding in high-N soils (Kiers *et al.*, 2007). Such a constraint can led to low yield in soils where the beneficial strains are unavailable for modern cultivars. There is a general agreement concerning the interest of rhizobial inoculation for improving BNF and thus pea yield (Bremer *et al.*, 1988; Fesenko *et al.*, 1995; McKenzie *et al.*, 2001). However, even when pea seeds are inoculated with efficient Rlv strains these can be outcompeted by naturally occurring rhizobia (Meade *et al.*, 1985), indicating that competitiveness for nodulation could not be related to efficiency.

The genetic basis of pea-Rlv partner choice is far to be fully understood, particularly when roots are exposed to a mixture of compatible Rlv strains, which is the case in field conditions. This study investigates the variability in partner choice in pea-Rlv symbiosis. A pea core-collection representative of the genetic and biogeographic diversity encountered within the genus *Pisum* was inoculated with a mixture of five diverse compatible Rlv strains. Differences in Rlv choice according to the pea diversity and selection history are evidenced. Relationship between nodulation competitiveness and nitrogen fixation efficiency is evaluated. Consequences for breeding and inoculation strategies to improve symbiotic traits in pea crop are discussed.

Materials and methods

Biological material

104 *Pisum* accessions were selected among the reference pea collection available at INRA Dijon (<http://www.thelegumeportal.net/>) according to their known genetic or origin diversity and to their variability of agronomic traits such as their cultivation status, end use and type of sowing (Burstin *et al.*, 2015). This collection of 104 accessions includes 12 wild or semi-wild genotypes, 36 landraces, 12 inbred lines or germplasm, and 44 cultivars (Supplemental Table III.1). Accessions are originating from 36 countries including representatives of the centres of pea genetic diversity and initial domestication (Middle East, Abyssinia, Afghanistan) and of its extension throughout the world in Africa, Asia, Europe and America. Among the wild, semi-wild or landraces genotypes, two are *Pisum fulvum* accessions and nine accessions are identified as belonging to the subspecies *abyssinicum*, *elatius* or *humile*. The 44 cultivars consisted a representative panel of the selection performed towards nowadays.

The five Rlv strains selected have been identified as nodulating pea in previous experiments (Supplemental Table III.2) and have diverse geographical origins. They included

the reference strain 3841 (SK) originated from England (Brewin *et al.*, 1980), two strains originated from France (SA and SD), a strain collected in Algeria (SE) and the TOM strain originated from Turkey and well-known to be required by some Afghan peas (SF) (Lie, 1978; Brewin *et al.*, 1980; Young *et al.*, 1982).

Pea collection genetic structure analyses

The 104 pea accessions were genotyped in 2015 using the GenoPea 13.2 K SNP Array (Tayeh *et al.*, 2015). A filtering was performed to exclude highly heterozygous SNPs -which are unexpected given the high selfing rate in pea- and SNPs with minor allele frequency lower than 0.02. Following these steps, a set of 11,218 markers were used for population structure analyses. The genetic structure of the sample was investigated using two methods: (1) a model-based Bayesian clustering assignment algorithm implemented in the software fastSTRUCTURE (Raj *et al.*, 2014) and (2) a discriminant analysis of principal components – DAPC – a multivariate method which employs PCA to reduce the number of correlated variables (SNP markers) to be analyzed using a discriminant analysis implemented in the R package Adegenet (Jombart *et al.*, 2010). The fastSTRUCTURE analysis was run for a number of clusters (K) ranging from 1 to 20 with 5 replicates per K value and using the ‘simple prior’ option (Supplemental Fig. III.1). To evaluate the repeatability of runs and check for the absence of true multimodality the program CLUMPP v.1.1.2 was run using the Greedy algorithm (Jakobsson & Rosenberg, 2007). The putative number of optimal of clusters was assessed from the likelihood profile and admixture plots were performed using a custom python script. The second method, DAPC, was run without prior knowledge of groups. The optimal number of clusters was thus assessed through sequential K-means and model selection using Bayesian Information Criterion. The number of principal components was determined to be 2 through maximization of the α -score measuring the difference between the proportion of successful reassignment of the analysis and values obtained using random groups.

Phenotyping

In all experiments, plants were grown in greenhouse in controlled temperature (21°C/16°C) in a 16-h day-night cycle and under a mean photosynthetically active radiation (PAR) of 250 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ guaranteed by high-pressure sodium lamps when daylight was declining. Prior to sowing, the seeds of the *P. fulvum* and some *P. sativum* subsp. *elatius* or *humile* accessions were scarified, and seeds of all the accessions sterilized in 10% bleach for 10

minutes and five-time rinsed in sterile water. The 2-L sterilized pots were filled with a 1:1 (v/v) mixture of sterilized atapulgitite and clay balls (2–6 mm diameter) and were top-watered (reverse osmosis water) from one day until eight days after sowing.

In a first experiment (E1), the 104 pea accessions of the pea collection were assessed for their ability to form nodules and for their partner choice among the five selected Rlv strains, using a three-block design, with two pots per accession in each block and three seeds sown per pot. At sowing, each seed was inoculated with 1 mL ($\sim 10^8$ cfu) of rhizobium inoculum, comprised of an equal proportion of the five Rlv strains. Eight days after sowing, two of the three plantlets were kept per pot and supplied through sterilized drippers with a low nitrate content (0.625 mM) nutrient solution in order to prevent from strong N limitation without impairing nodulation as already observed in *Medicago truncatula* (Moreau *et al.*, 2008) or in pea with a slightly higher nitrate content (Bourion *et al.*, 2010). Four weeks after sowing, all the 1,248 plants kept were harvested. For each accession, all nodules formed on roots were counted on one plant of one pot of each block and their total dry weight determined. On the two plants on the pot, the aerial part oven-dried and weighted. Rhizobia were isolated for each accession from a sample of 60 nodules collected haphazardly on the plants of the other pot of the block. After nodule surface sterilization, each nodule was crushed and undifferentiated bacteria present inside cultivated on YM agar plates. Bacterial identities of individual nodules were determined either by assaying antibiotic resistance on YM medium or by PCR amplification using specific primers (Tables S2 & S3). Very few mixed nodules were found (<1%; data not shown). Frequencies of nodules containing each strain were then calculated for each pea genotype, allowing estimation of the competitiveness of the strains.

In a second experiment (E2), the nodulation ability and efficiency of each of the five Rlv strains involved in E1 was assessed in a subset of 18 pea accessions selected among the 104. The 18 accessions were mono-inoculated with each of the five Rlv strains. Each mono-inoculation test was performed in one assigned bank not adjacent to the others, in order to prevent contamination. For each strain, a four-block randomised design was used with one pot per accession in each block and four seeds sown per pot. As a control, all the 18 pea accessions were grown in one another bank without any inoculation (NI). At sowing, each seed was inoculated with a 1 mL cell suspension of one of the five Rlv strains ($\sim 10^7$ cfu). Eight days after sowing, two of the four plantlets per pot were then harvested and the absence of nodules confirmed in the NI bank. The remaining plants were supplied with a nutrient

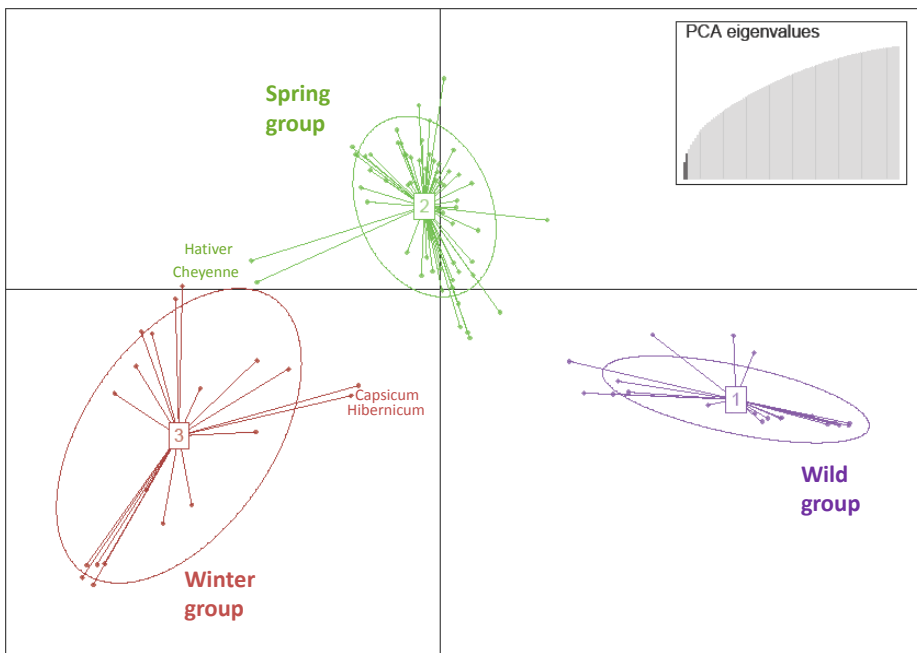


Fig. III.1. Genetic structure of the pea collection using the DAPC method

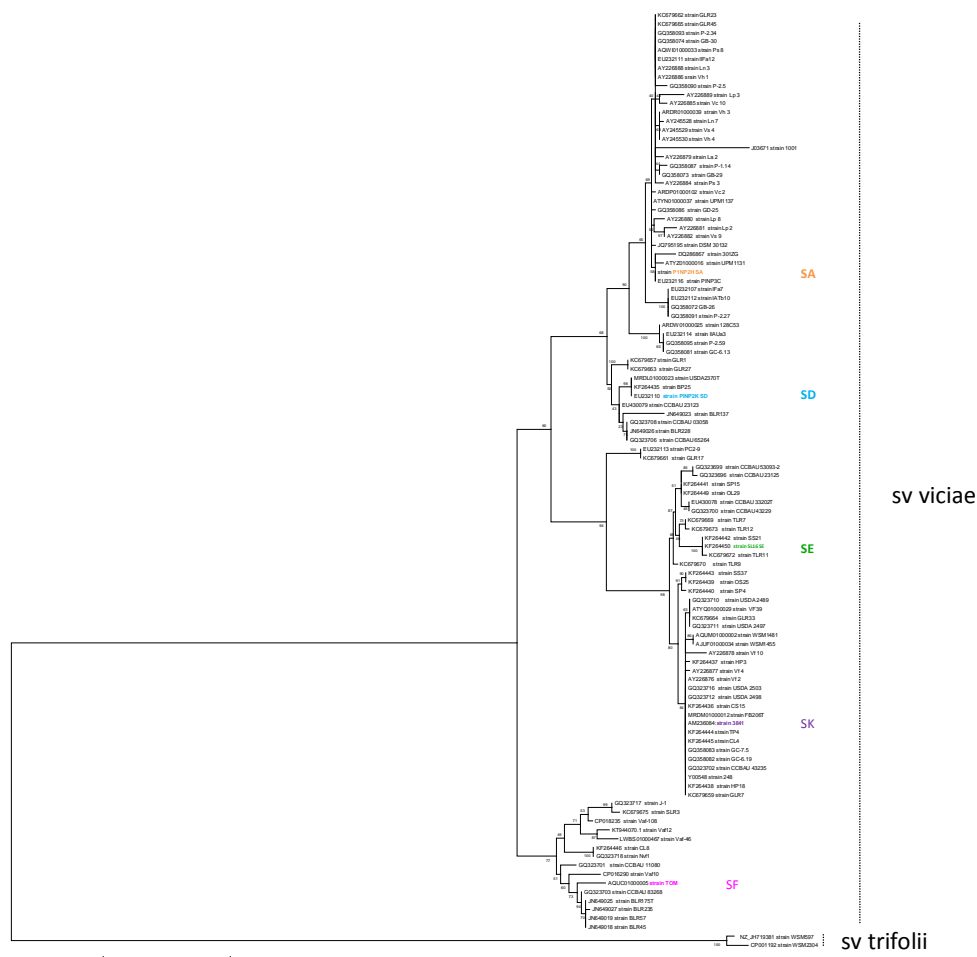


Fig. III.2. *nodD* molecular phylogenetic analysis by Maximum Likelihood method of R1v sequences available in GenBank. The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model using MEGA7 software (Kumar *et al.*, 2016)

solution without any N. Five weeks after sowing, 695 plants were harvested. For each pea accession, all the nodules formed on two plants were counted, and a sample of 16 nodules was collected over the four blocks. Nodule identities were checked using the same methods as in E1. The very few cross-contaminated plants were discarded. The aerial dry weight of the 689 remaining plants was determined. In such conditions of no N supply, symbiotic N acquisition was the main limiting factor for plant growth and the shoot biomass determined for each pea-Rlv association representative of its nitrogen fixation efficiency (Laguerre *et al.*, 2012). A shoot dry matter index was calculated for each pea accession x Rlv strain association as the shoot dry matter of the pea accession inoculated with the strain divided by the pea accession mean (Heath & Tiffin, 2009). A nodulation index was similarly calculated as the nodule number of a pea accession inoculated with a strain divided by the pea accession mean.

Statistical analysis of phenotypic data

Each phenotypic variable was analysed by a two-way ANOVA using a linear model (R function `lm`) including a genotype and a block effects. Normality of the residuals of the model, as well as independence and homogeneity of their variance were checked. Regression analysis was performed between shoot biomass and nodule biomass or number. Analysis of covariance (ANCOVA) was then performed to test if either the intercept or the slope of the relationship between these variables was depending on the level of each of the categorical variables describing the collection.

Results

Genetic structure of the pea collection

Two complementary methods were used to assess the population genetic structure of the collection of 104 *Pisum* accessions. The DAPC analysis uncovered three genetic groups (Fig. III.1). D1 was named 'Wild group' as it comprised nine of the 12 wild or semi-wild accessions plus 12 landraces (Supplemental Table III.1). The 'Spring group' D2 comprised 64 accessions among them all the accessions of known spring sowing type. The 'Winter group' (D3) comprised 18 genotypes among which 16 were of winter type. The two accessions of unknown sowing type were both sampled at an altitude of more than 2000 metres (JI1844 and JI1431, JIC *Pisum* Collection database, <https://www.seedstor.ac.uk>), confirming that the D3 group was characterized by cold tolerance. Only four accessions displayed ambiguous position between D2 and D3: two winter peas ('Hativer' and 'Cheyenne'), plus '*Pisum sativum*-*Hibernicum* JI1846' and 'Capsicum' respectively from Egypt and Azerbaidjan.

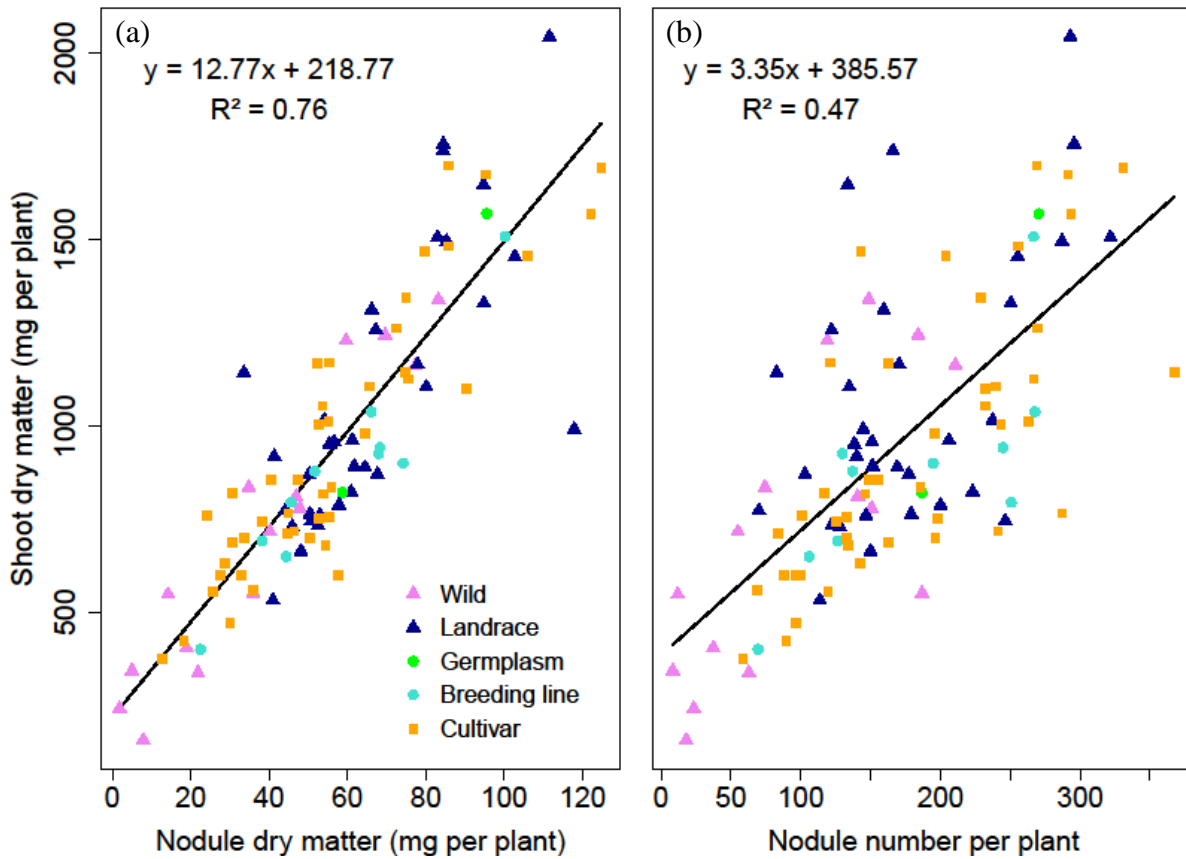


Fig. III.3. Relationship between shoot dry matter and nodule dry matter (a) or nodule number per plant (b), for 104 pea accessions multi-inoculated with a mixture of five Rlv strains (E1 experiment)

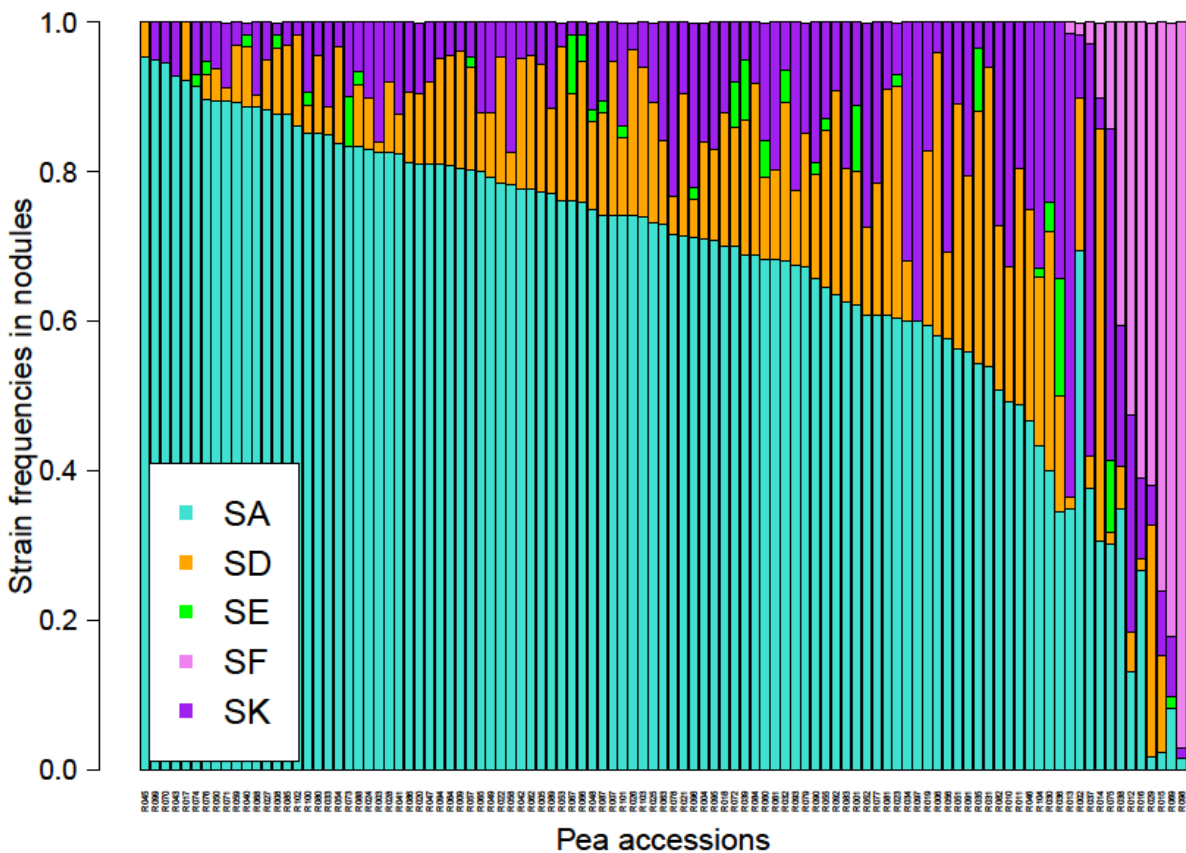


Fig. III.4. Strain frequencies in the nodules of 104 pea accessions multi-inoculated with a mixture of five Rlv strains (E1 experiment)

The fastSTRUCTURE analysis identified ten different clusters which interestingly were found to subdivide the three DAPC groups (Supplemental Fig. III.2).

Thanks to a careful examination of the 70 accessions with membership probabilities higher than 80%, cluster assignments were found to correlate with species affiliation or geographic origin and breeding history (Supplemental Table III.1). Within D1, K01 was composed of the two *P. sativum* subsp. *abyssinicum* and of five wild accessions originating from the Middle East: the two *P. fulvum*, two *P. sativum* subsp. *elatius* and JI1794 identified as belonging to subsp. either *humile* (LaRue & Weeden, 1992; Ellis *et al.*, 1998) or *elatius* (Weeden *et al.*, 2002; Bogdanova *et al.*, 2014). K02 included three of the four accessions collected in Afghanistan, the second *P. sativum* subsp. *humile* and all the three accessions from Nepal or India. K03 consisted of three *P. sativum* from Abyssinia (Ethiopia, Sudan) or Lybia. Within the D2 ‘Spring group’, K04 was the ‘North-eastern European garden pea group’ as it gathered almost all the old landraces from Baltic States, Ukraine and Russia, plus the old garden pea ‘Torsdag’ released in Sweden in 1925 and three garden or fodder cultivars from Germany including ‘Hohenheimer Pink Flowered’. K05, the ‘French garden pea group’, included five old cultivars created in France, especially the mangetout ‘Corne de Bélier’ and the garden pea ‘Serpette d’Auvergne’ which were first mentioned respectively in 1818 and 1829 (Fourmont, 1956). K06 gathered most of the English garden peas of the collection, among them two of the oldest, ‘Téléphone à rames’ (1878) and ‘Petit provençal’ (1910), and also breeding lines created in the United States at the end of the 20th century. K07 included two Dutch accessions created in the 1950s and a *P. sativum* subsp. *elatius* (JI1703) whom origin is unknown. K08 gathered most of the French spring dry pea cultivars of the collection including Baccara which was the most cultivated during the 1990s (Cousin, 1997). Within the D3 group, K09 gathered winter European fodder peas and two winter cultivars created in the United States at the end of the 1970’s, whereas K10 gathered all the French winter dry peas of the collection including ‘Frisson’ (1979).

Genetic diversity of the five Rlv strains

The five strains have various geographical origins: SA and SD are from France, SE is the reference strain 3841 from England, SF is from Algeria, and SF is the TOM strain from Turkey known as required for Afghan peas. The genetic diversity of the strains was ascertained by phylogeny analyses made on the basis of symbiotic *nodD* markers and showed that these five Rlv strains are representative of a large diversity among the symbiovar *viciae* (Fig. III.2).

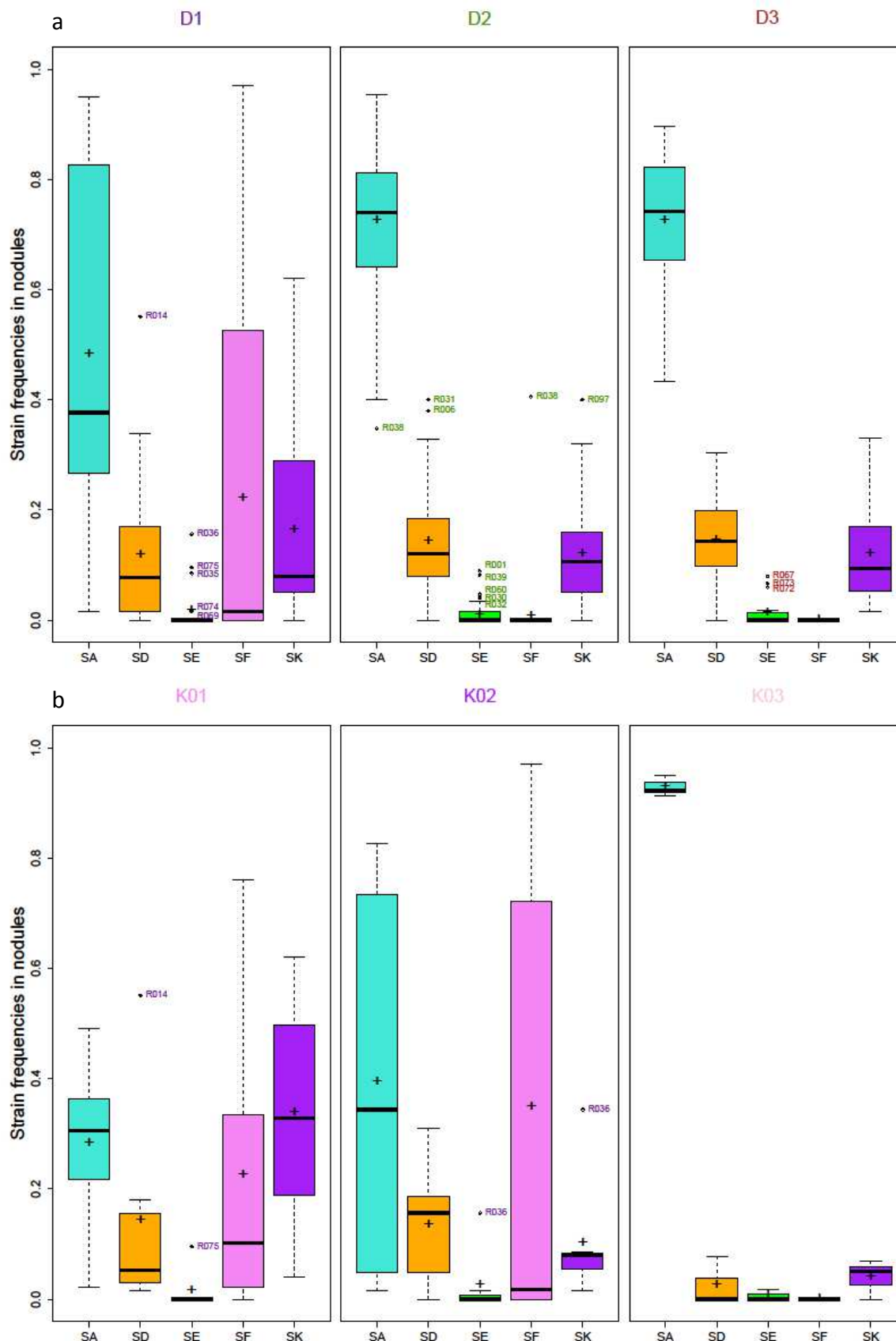


Fig. III.5. Strain frequencies in the nodules of (a) the 104 pea accessions according to their membership to DAPC groups (E1 experiment), (b) the 17 pea accessions belonging to D1 according to their membership to cluster groups (E1 experiment). + indicates the mean frequency for each of the five strain. All outliers are identified by accession numbers. Colour indicates DAPC group affiliation: violet for D, green for D2 and brown for D3

Natural variability of pea nodulation and pea-Rlv partner choice

Significant variations in nodule biomass and number were observed between the 104 pea accessions when co-inoculated with an equal mixture of the five Rlv strains (E1 experiment; Supplemental Table III.4). Both nodule biomass and number were found to be positively and significantly correlated with shoot biomass (Fig. III.3). In both relationships there were no significant differences according to the status of the pea accessions i.e. between wild, landraces, germplasm, breeding lines or cultivars (Supplemental Table III.5). However, the lowest biomasses or nodule number were observed mainly in wild accessions or for few cultivars, whereas the highest ones were obtained by landraces or other cultivars. Within the ‘Wild group’ D1, biomasses and nodule number were lower in the five wild accessions clustered in K01 (*P. fulvum* or *P. sativum* sbsp. *humile* or *elatius*) than in the two *P. s. abyssinicum* and all the members of K02 or K03 (Supplemental Fig. III.3). Similarly, fodder and dry winter pea cultivars (K09 and K10) and spring dry pea cultivars (K08) had lower biomasses and nodule number than the garden peas created in North-eastern Europe and France (K04, K05, K07) (Supplemental Fig. III.4).

A large variability of the relative frequencies of the five Rlv strains was observed in the nodules of the 104 pea accessions (Fig. III.4). With a mean frequency value of 67% among all the 104 accessions, SA was the more competitive strain, far ahead from SD (14%), SK (13%), SF (5%) and SE (2%) (Supplemental Fig. III.5). However, variations between pea accessions were found around these mean values and differences were observed according to their membership to DAPC groups (Fig. III.5a; Supplemental Table III.6). The relative frequency of SA was much more diverse in D1 than in D2 or D3 in which it was always higher than 40%. Conversely, SF was detected in none of the members of D2 or D3 except R038 (“Capsicum”) but in members of D1 with a frequency up to 97%. The maximum SK relative frequency was lower in D2 and D3 than in D1. We also observed differences between the three clusters which subdivided the D1 group (Fig. III.5b; Supplemental Table III.6). In K01, very few accessions had a strain preference (i.e. frequency higher than 80%). In K02, the relative frequencies of SA and SF were highly variable, with a strain preference either for SA or SF. In K03 all the accessions had a SA relative frequency in nodules higher than 91% and none was nodulated by SF.

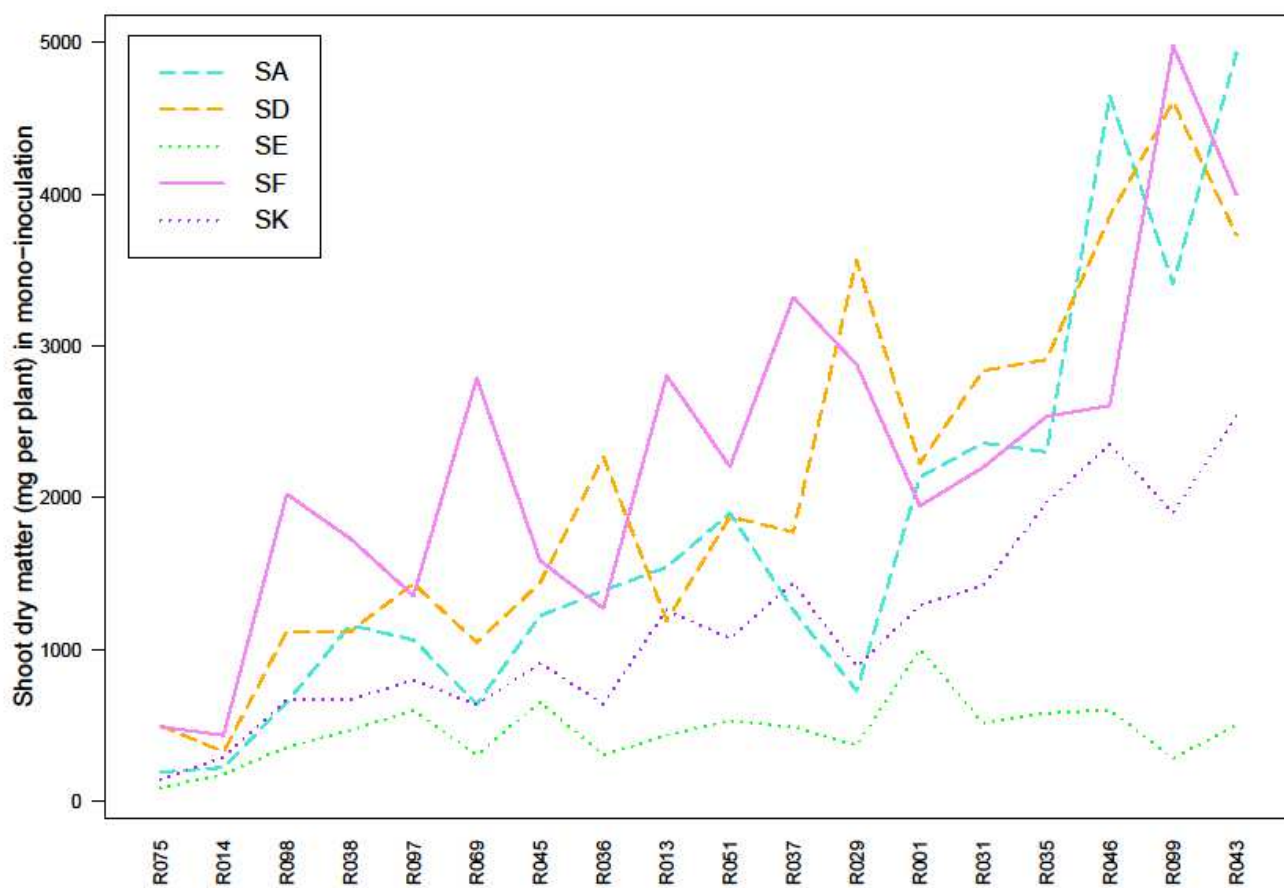


Fig. III.6. Shoot dry matter of 18 pea accessions with each of the five Rlv strains in mono-inoculation (E2 experiment)

Correlation between partner choice and efficiency

To investigate whether competitiveness for nodulation was related with nitrogen fixation efficiency, 18 pea accessions were inoculated separately with each of the five Rlv strains (E2 experiment). The 18 accessions were selected from the E1 experiment as displaying contrasted partner choice (Supplemental Fig. III.6). They belonged either to the D1 or the D2 group and were representative of the shoot and nodule biomass variations encountered within the pea collection.

Both pea genotype and inoculated strain were found to have a significant effect on the shoot biomass of the 90 pea-Rlv associations and the interaction between the two factors was significant (Fig. III.6; Supplemental Table III.7). An overall trend of variation of shoot biomass was observed between the 18 pea accessions and comparison with the data obtained in E1 suggested that this variation was related to their growth potential. Indeed the mean values of shoot biomass obtained for the 18 pea accessions over the five Rlv strains in E2 were significantly correlated to those obtained in E1 ($r^2 = 0.51$; $p=0.000531$; Supplemental Fig. III.7a). Overall differences in the shoot biomass produced were also observed according to the Rlv strain associated: SF, SD and in a less extend SA displayed higher efficiencies than SK in nearly all the pea accessions and SE in all cases (Fig. III.6). However, the most efficient strain varied according to the pea accession. SF was the most efficient for four pea accessions belonging to D1 – two members of K01 (the two *P. s. abyssinicum*: R013, R037) and two members of K02 (R069 from Israël, R098 from Afghanistan) - and in a less extend for two accessions of D2 (R038, R051) (Fig. III.6). SD was the most efficient for three accessions belonging to D1 (K02: R029, R036; NA: R035) and one from D2 (R031), and SA for the two tested accessions belonging to K04 (R046, R043) (Fig. III.6). The increase in the mean shoot biomass within the 18 pea accessions over the five strains was also correlated to the increase in their mean nodule number ($r^2=0.62$; $p=6.541e-05$; Supplemental Fig. III.7b), in agreement with the positive and significant relationship observed between shoot and nodule biomass in E1. However, high differences in the nodulation of pea accessions according to the Rlv strain inoculated were observed (Supplemental Fig. III.8). As for the shoot biomass, both pea and strain had a significant effect, and the interaction between the two factors was significant (Supplemental Table III.7). In most cases SE, SK or SD produced the highest numbers of nodules, and in nearly all cases SA the lowest (Supplemental Fig. III.8). Only six pea accessions had more nodules with SF than with at least the strains SA, SD and SK.

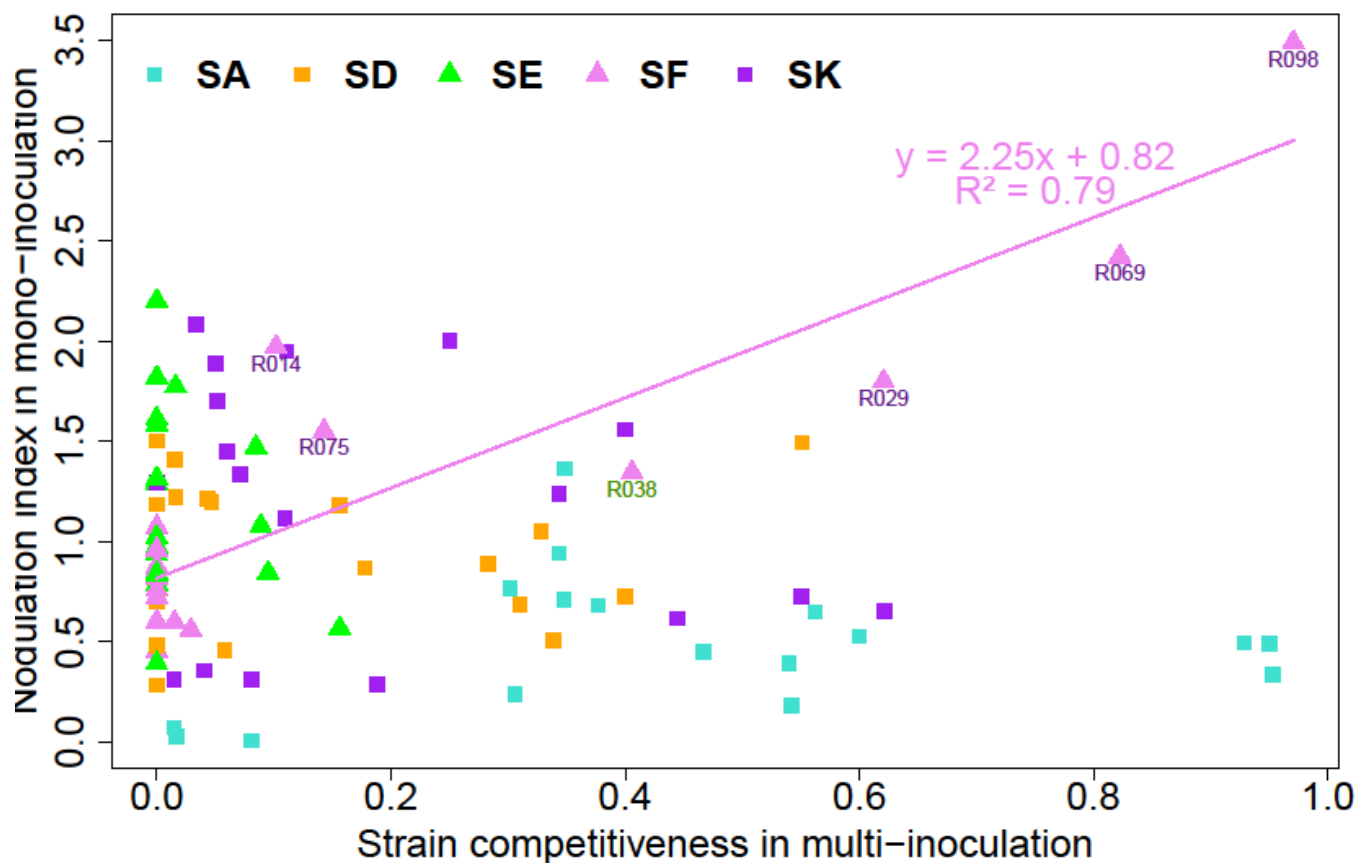


Fig. III.7. Relationship between nodulation index (relative to pea accession mean) in response to mono-inoculation with SF (E2 experiment) and SF competitiveness for nodulation evaluated in multi-inoculation (E1 experiment) for 18 pea accessions

To reduce the impact of the growth potential differences between the pea accessions and thus more specifically determine the effect of the strain, both shoot dry matter and nodulation indexes were calculated as the shoot dry matter (number of nodules) of the pea accession inoculated with the strain divided by the pea accession mean. For all the four strains SA, SD, SE and SK, the nodulation index evaluated in the E2 experiment was found to be not correlated to the competitiveness for nodulation evaluated in E1 (Supplemental Table III.8). Conversely, we observed for SF a significant correlation between its nodulation index obtained in mono-inoculation and its competitiveness for nodulation in multi-inoculation ($r^2=0.79$; $p=5.512e-07$; Supplemental Table III.8; Fig. III.7). The two accessions (R098, R069) with higher than 80% of nodulation by SF in multi-inoculation were the most successfully nodulated by SF in the E2 experiment. At the opposite, the SF nodulation index was low as compared with the other strains for all the accessions with less than 10% of nodulation by SF when inoculated in mixture; most of them belonged to the DAPC group D2 (Fig. III.7). Between these two extreme positions, were the two *P. s. abyssinicum* (R014, R075) and the two accessions, R038 from Caucasus and R029 from Afghanistan, for which the SF nodulation ability in mono-inoculation was between 1.5 and 2. The competitiveness for nodulation was also found to be poorly related to the shoot biomass index (Supplemental Table III.9; Fig. III.8). No correlation at all was found for the three strains SD, SE and SK. Significant but highly dispersed correlations were only observed for the two strains SF ($r^2=0.47$; $p=0.001$) and SA ($r^2=0.41$; $p=0.002$). The pea accessions were distributed along the regression line according to their level of resistance to European strains, the most resistant (R098, R029, R069) having the highest shoot biomass indexes and the partially resistant (R038, R014, R075) obtaining intermediate values. Consistently, when inoculated by SA, the most resistant peas had the lowest shoot biomass indexes.

Discussion

To our knowledge, this is the first time that the genetic diversity for pea-Rlv partner choice is investigated within a pea collection representative of the variability encountered in the genus *Pisum* co-inoculated with a mixture of diverse *Rhizobium leguminosarum* ssp. *viciae* (Rlv) strains. Little is known about the mechanisms underlying the competitiveness of different Rlv strains for pea nodulation and the various abilities of different pea genotypes to choose among diverse compatible Rlv strains. Moreover, whether competitiveness for nodulation of a given

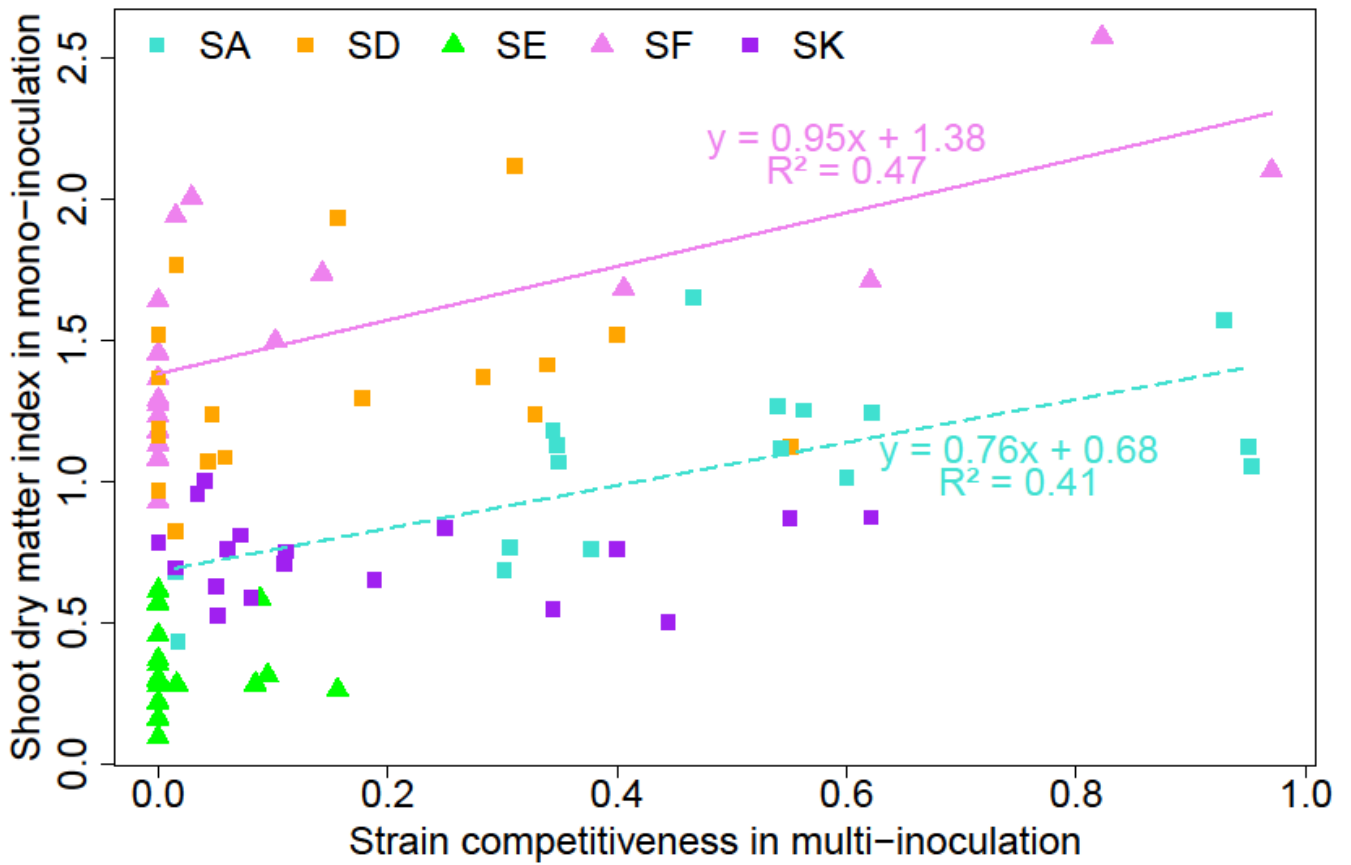


Fig. III.8. Relationship between shoot dry matter index (relative to pea accession mean) in response to mono-inoculation (E2 experiment) and strain competitiveness for nodulation evaluated in multi-inoculation (E1 experiment) for 18 pea accessions

pea-Rlv symbiotic association could be related to nitrogen fixation efficiency has been few investigated.

Natural variability of biomass and pea nodulation

Differences in shoot biomass were found between the pea accessions according to their SNP-based cluster affiliation. As cluster assignments were found to correlate with species affiliation, geographic origin and year of cultivar registration, these results give an overview of the modifications which had occurred during domestication and breeding history. Within the D1 group, the landraces of *P. sativum* or *P. s.* subsp. *abyssinicum* were found to have higher size than the *P. fulvum* accessions and most of the other wild peas. The *P. s.* subsp. *abyssinicum* clustered with the *P. fulvum* and some *P. s.* subsp. *elatius* or *humile*, which supports the model of independent domestications for *P. sativum* subsp. *abyssinicum* and *P. sativum* and the origin of *P. s.* subsp. *abyssinicum* as a hybrid between *P. fulvum* and a subset of *P. s.* subsp. *elatius* (Vershinin *et al.*, 2003; Jing *et al.*, 2010). Within the ‘Spring’ D2 group, the oldest known cultivars – created at the end of the 18th century - are garden peas. Garden peas have been selected until the end of the 60’s mainly in the Netherlands, France and Great Britain (Vilmorin-Andrieux, 1883; Fourmont, 1956), which is consistent with their gathering into distinct clusters. We found that Dutch and French peas presented similar characteristics of high biomass than fodder or garden peas from North-eastern Europe, whereas English peas were smaller and thus are likely another source of diversity in Europe as indicated by Fourmont (1956). In response to the soya exports embargo from US, European breeding moved during the 70’s towards field dry peas for animal feeding. Crosses were made between the former garden peas with high seed protein content and European fodder peas, with the aim of increasing seed yield by reducing the plant biomass or by fall-sowing (Acikgoz, 1982; Cousin, 1997). The low biomasses we observed within the cluster of spring dry peas and the ‘Winter’ D3 group are consistent with this breeding evolution and use of fodder peas as a new source of diversity.

Significant positive correlations were observed between shoot biomass and nodule biomass or number, independently of species affiliation and breeding history. A close adjustment between symbiotic organs development and plant growth has been already observed in a smaller genetic diversity within pea or *M. truncatula* (Bourion *et al.*, 2010; Voisin *et al.*, 2010; Laguerre *et al.*, 2012). Pea and *M. truncatula* forms indeterminate nodules, following two sequential processes: nodule formation and mature nodule expansion. Both processes require an adjustment between the satisfaction of whole plant N demand and

the allocation of photosynthates needed for this function. The nodule formation determines the nodule number and is the result of early plant x bacteria interaction. It only occurs under situations of N limitation and is repressed by high N supply (Jeudy *et al.*, 2010; Voisin *et al.*, 2010). The nodule expansion is also strongly submitted to a systemic N-signaling regulation and locally highly reduced in fix- nodules (Laguerre *et al.*, 2012). In our study supplied with a low nitrate content, we observed a closer adjustment between shoot and nodule biomasses than between shoot biomass and nodule number, highlighting that the peas fine-tuned their symbiotic capacity to their N demand and C allocation potential by modulating the expansion of their nodules. Their nodule numbers more likely resulted from early plant x rhizobia interaction and partner choice.

Competitiveness for nodulation in single- and multi-inoculum environment are not correlated

Both experiments of mono-inoculation and multi-inoculation give complementary information and new insights into the natural variability of pea-Rlv partner choice. Our results obtained in mono-inoculation experiments confirmed that SF (TOM) is required for nodulation of pea accessions originated mainly from Afghanistan or in few cases in Middle East or Ethiopia (Lie, 1978; Winarno & Lie, 1979; Young *et al.*, 1982; Young & Matthews, 1982). We also found in agreement with these authors that some other peas from Afghanistan are only partially resistant or even susceptible to European Rlv strains and we enlarged the existence of resistant or partially resistant wild/landrace accessions to other countries (Caucasus). In complement to the former studies of SF/TOM, we found a high correlation between its nodulation ability in mono-inoculation as compared with the other strains and its nodulation competitiveness in multi-inoculation. Indeed, two pea accessions of our pea collection, R098 from Afghanistan and R069 from Israel (the *Pisum humile* JI241 described by Young & Matthews, 1982, as resistant to nodulation by European strains) were successfully nodulated by SF, formed no or only few nodules when mono-inoculated with SA, SD or SK and were also the two accessions with higher than 80% of nodulation by SF in multi-inoculation. Of the two other afghan peas tested in mono-inoculation, R029 had a partial resistant phenotype with less nodules with the European strains than with SF and showed an intermediate competitiveness of SF in multi-inoculation, whereas R035 had a clearer susceptible phenotype. Interestingly, and not already mentioned in the literature, the two *P. sativum* originated from Caucasus were found to be partially nodulated by SF in multi-inoculation, and the one tested in the mono-inoculation experiment confirmed its partial

resistance to European strains. All the other accessions tested were found to be nodulated by SF in the mono-inoculation experiment but not at all in the multi-inoculation experiment. The ability of SF/TOM, in a single-inoculum environment, to nodulate a wide diversity of peas is in agreement with former observations (Lie, 1981; Young *et al.*, 1982; Fobert *et al.*, 1991). The inhibiting effect of European strains has been well documented on few peas including ‘cv Afghanistan’ and induces no or a reduced number of TOM nodules (Winarno & Lie, 1979; Hogg *et al.*, 2002). Our findings extend the knowledge of this inhibiting effect, as to our knowledge, no literature mentioned a total blocking effect of European strains on nodulation by TOM of all the peas susceptible to European strains.

With the exception of the correlation between the resistance of nodulation by European strains and the SF competitiveness in multi-inoculation, no correlation was observed between the ability of a strain to form nodules in mono-inoculation and its competitiveness for nodulation in multi-inoculation. SA was the most competitive strain in multi-inoculation but, for nearly all the accessions tested, formed the lowest number of nodules in mono-inoculation as compared with the other strains. More variations in nodule number were observed between the pea accessions when mono-inoculated with SD, SE or SK as compared with SA, but without any correlation with the level of competitiveness of those strains in multi-inoculation. Taken together, these results indicate that the nodulation ability of a strain evaluated in mono-inoculation is not a good predictor of its competitiveness for nodulation in the presence of other strains in a mixed-inoculum and, a fortiori, in the complex rhizobial environment of fields.

Partner choice varies according to pea genetic and biogeographic diversity

Some relation between pea genetic diversity and choice among the five Rlv strains was observed in our study. As shown before (Lie, 1978; Lie *et al.*, 1987), cultivated peas showed less variation with regard to their symbiotic partner choice than wild peas. Indeed, in the multi-inoculation experiment, the two genetic groups D2 and D3 presented a less diversity in Rlv choice than the ‘Wild group’ D1. The D2 and D3 groups included all the cultivars, breeding lines and landraces from Europe or America and some landraces from Africa or Asia. D1 comprised landraces or wild accessions, and most of them was originating from the centers of pea genetic diversity and initial domestication. This ‘Wild group’ was subdivided into three genetic clusters, which could be distinguished from one another according to the species/subspecies or origin and the partner choice pattern of their belonging accessions. The K01 cluster, the most diverse in partner choice, was composed of all the *P. fulvum* and *P.*

sativum subsp. *abyssinicum* of the collection plus some *P. sativum* wild accessions representative of the Middle East genetic diversity centre. The K02 cluster was representative of the further extend of peas to Afghanistan and India and comprised the landrace peas highly specific to nodulation by TOM. At the opposite, all the accessions of the K03 group were *P. sativum* originating from Abyssinia or Lybia and had an SA frequency higher than 90%. This observation of higher preference for a specific strain among the *P. sativum* originating from this region has not already been mentioned in the literature. All these observations provide evidence that the very first domestication processes and spread of peas from the Fertile Crescent may have resulted in a higher specificity for some Rlv. Conversely, as D2 and D3 had similar choice among the five Rlv strains in our experiment, there is little evidence of modification of partner choice subsequent to the spread of peas to Europe, and this whatever the modification of end use from forage or garden to dry peas and the different breeding strategies followed from the end of the 18th century until nowadays.

Thanks to genetic analyses of peas resistant to nodulation by European strains, the first symbiotic pea genes *SYM1* and *SYM2* were identified (Lie, 1971; Holl, 1975). The *sym2^A* allele induces arrested nodulation on ‘cv Afghanistan’ pea inoculated with European strains (Geurts *et al.*, 1997). These strains lack the host specificity gene *nodX* carried by TOM (Davis *et al.*, 1988) and evidence has been provided that their inhibition of the nodulation by TOM of ‘cv Afghanistan’ results from an overproduction of Nod factors (Hogg *et al.*, 2002). Another *nod* genes such as *nodE*, *nodO* and *nodL* have been identified as modifying the variety and level of Nod factor production and determining host specificity variation (Spaink *et al.*, 1991; Walker *et al.*, 2000). In pea, only one another gene affecting nodulation has been identified among the natural diversity (*SYM22* in JI1794=R012) and the use of mutagens has allowed the identification of other genes (LaRue & Weeden, 1992; Borisov *et al.*, 2007). Among them, *SYM10* and *SYM37* were shown to encode LysM-RLKs and to be required for nodulation and Nod factor perception (Madsen *et al.*, 2003; Zhukov *et al.*, 2008). *SYM37* maps at the same position than *SYM2*, and it is likely that this region contains different repertoires of LysM-RLK encoding genes (Zhukov *et al.*, 2008). Recent evidence from model legumes has shown that some LysM-RLK encoding genes other than *PsSYM10* and *PsSYM37* orthologues are also likely to be involved in strain specificity through Nod factor or rhizobia cell surface recognition (Kawaharada *et al.*, 2015; Malkov *et al.*, 2016). Our study revealed an unknown natural variability for partner choice among wild peas which could be useful for further studies of the genetic basis of pea-Rlv nodulation.

Nitrogen fixation efficiency is not a major determinant of partner choice

We found very little evidence that competitiveness for nodulation of a given pea-Rlv symbiotic association is related to its nitrogen fixation efficiency. First of all, in the single-inoculum environment, the SE strain displayed a high ability to form nodules but in all cases had the lowest efficiency. Moreover, the relationship between strain competitiveness in multi-inoculation and relative shoot biomass of mono-inoculated plants was only significant for two strains, SA and SF, and is likely mainly related to differences between accessions in resistance for nodulation by SA and specificity for SF. The weak relationship between competitiveness for nodulation and nitrogen fixation efficiency is in agreement with previous observations made in other plant-rhizobia associations with indeterminate nodules, in which Fix^- mutants of *R. meliloti* were found to not significantly differ in nodulation competitiveness of alfalfa from their Fix^+ parent strains (Amarger, 1981). More recently we showed in *M. truncatula/Sinorhizobium* that plant N status does not impact partner choice for nodule formation but results in preferential expansion of nodules formed with the most efficient strains (Laguerre *et al.*, 2012). Nodulation competitiveness and symbiotic efficiency traits are likely under distinct selection pressures. Competitiveness is most probably determined during early plant-bacterial interactions and/or the bacterial colonization of the symbiotic organ, whereas efficiency of symbiotic nitrogen fixation requires later additional interactions with the plant. Nutritional interactions result in a strong allocation of metabolites by the plant to the bacteroids and the induction of nitrogen fixation in bacteria.

Conclusion

This work confirms the importance of considering legumes as exposed to several symbiotic compatible rhizobia rather than to a single strain (Friesen & Heath, 2013; Kiers *et al.*, 2013). Mono-inoculation experiments are useful to evaluate the ability of a strain to form efficient symbiosis with a given host, but they fail to determine the ability of this strain to compete with other strains in a mixture, which is the common situation in the field. Abiotic soil conditions and biotic composition of the rhizosphere may also modulate the fitness of free bacteria, their abilities to infect plant root and their competitiveness toward other microorganisms (Philippot *et al.*, 2013). Integrating this complexity is a major challenge for future studies on pea-Rlv partner choice.

This work also documents that competitiveness for nodulation is under the control of both pea and Rlv genetic factors and confirms that a successful inoculant must not only provide enhanced symbiotic nitrogen fixation but be competitive for nodulation in the

presence of a large population of indigenous rhizobia (Triplett & Sadowsky, 1992). Further experiments are planned to decipher the genetic architecture of the partner choice trait and identify specific loci underlying this phenotype by performing Genome Wide Association Studies. They will involve larger pea and Rlv collections and increased genomic resources and high-throughput phenotyping abilities than those presented in this study. Such knowledge of the key genetic factors involved will allow to develop new pea varieties and successful Rlv inoculants and enhance the agronomic potential of the pea crop.

Acknowledgments

The authors thank Karine Palavioux, Franck Zenk, Nouredine El Mjyyad and Damien Gironde (UMR1347, Dijon) for their technical support in the greenhouse experiments; Hervé Houtin, Anthony Klein, Chantal Martin, Jean-Bernard Magnin-Robert, and Céline Rond (UMR1347, Dijon) for their help in plant phenotyping, and Catherine Delaître for her providing the seeds. They also thank Jean-Michel Retailleau, Peter Spencer-Phillips, Marie-Laure Pilet-Nayel and Dominique Millot (UMR1347, Dijon), for providing information about the pea accessions; and Fabrice Dessaint (UMR1347, Dijon) for its help concerning the statistical analyses performed. This work was supported by the French ANR GENOPEA and the “INRA-BAP projet scientifique” SYMBIOPEA programs.

References

- Acikgoz E. 1982.** Cold tolerance and its association with seedling morphology and chemical composition in annual forage legumes. I. Field peas (*Pisum sativum* ssp. *arvense* L. Poir.) *Zeitschrift Fur Pflanzenzuchtung-Journal of Plant Breeding* **88**(2): 118-126.
- Amarger N. 1981.** Competition for nodule formation between effective and ineffective strains of *Rhizobium meliloti*. *Soil Biology & Biochemistry* **13**(6): 475-480.
- Bogdanova VS, Kosterin OE, Yadrikhinskiy AK. 2014.** Wild peas vary in their cross-compatibility with cultivated pea (*Pisum sativum* subsp. *sativum* L.) depending on alleles of a nuclear-cytoplasmic incompatibility locus. *Theoretical and Applied Genetics* **127**(5): 1163-1172.
- Borisov AY, Danilova TN, Koroleva TA, Kuznetsova EV, Madsen L, Mofett M, Naumkina TS, Nemankin TA, Ovchinnikova ES, Pavlova ZB, Petrova NE, Pinaev AG, Radutoiu S, Rozov SM, Rychagova TS, Shtark OY, Solovov, II, Stougaard J, Tikhonovich IA, Topunov AF, Tsyganov VE, Vasil'chikov AG, Voroshilova VA, Weeden NF, Zhernakov AI, Zhukov VA. 2007.** Regulatory genes of garden pea (*Pisum sativum* L.) controlling the development of nitrogen-fixing nodules and arbuscular mycorrhiza: A review of basic and applied aspects. *Applied Biochemistry and Microbiology* **43**(3): 237-243.
- Bourion V, Rizvi SMH, Fournier S, de Larambergue H, Galmiche F, Marget P, Duc G, Burstin J. 2010.** Genetic dissection of nitrogen nutrition in pea through a QTL approach of root, nodule, and shoot variability. *Theoretical and Applied Genetics* **121**(1): 71-86.
- Bremer E, Rennie RJ, Rennie DA. 1988.** Dinitrogen fixation of lentil, field pea and fababean under drylands conditions. *Canadian Journal of Soil Science* **68**(3): 553-562.
- Brewin N, Beringer J, Johnston A. 1980.** Plasmid-mediated transfer of host-range specificity between two strains of *Rhizobium leguminosarum*. *Microbiology* **120**(2): 413-420.
- Burstin J, Salloignon P, Chabert-Martinello M, Magnin-Robert JB, Siol M, Jacquin F, Chauveau A, Pont C, Aubert G, Delaitre C, Truntzer C, Duc G. 2015.** Genetic diversity and trait genomic prediction in a pea diversity panel. *Bmc Genomics* **16**.
- Chimwamurombe P, Khulbe R. 2011.** 1 History, origin and evolution. *Biology and Breeding of Food Legumes*: 1-18.
- Cousin R. 1997.** Peas (*Pisum sativum* L). *Field Crops Research* **53**(1-3): 111-130.
- D'Haeze W, Holsters M. 2002.** Nod factor structures, responses, and perception during initiation of nodule development. *Glycobiology* **12**(6): 79R-105R.
- Davis EO, Evans IJ, Johnston AWB. 1988.** Identification of nodX, a gene that allows *Rhizobium leguminosarum* bv *viciae* strain TOM to nodulate Afghanistan peas. *Molecular & General Genetics* **212**(3): 531-535.
- Denarie J, Debelle F, Rosenberg C. 1992.** Signaling and host range variation in nodulation. *Annual Review of Microbiology* **46**: 497-531.
- Denison RF. 2000.** Legume sanctions and the evolution of symbiotic cooperation by rhizobia. *American Naturalist* **156**(6): 567-576.
- Downie JA. 2010.** The roles of extracellular proteins, polysaccharides and signals in the interactions of rhizobia with legume roots. *Fems Microbiology Reviews* **34**(2): 150-170.
- Duc G, Agrama H, Bao SY, Berger J, Bourion V, De Ron AM, Gowda CLL, Mikic A, Millot D, Singh KB, Tullu A, Vandenberg A, Patto MCV, Warkentin TD, Zong XX. 2015.** Breeding Annual Grain Legumes for Sustainable Agriculture: New

- Methods to Approach Complex Traits and Target New Cultivar Ideotypes. *Critical Reviews in Plant Sciences* **34**(1-3): 381-411.
- Ellis THN, Poyser SJ, Knox MR, Vershinin AV, Ambrose MJ. 1998.** Polymorphism of insertion sites of Ty1-copia class retrotransposons and its use for linkage and diversity analysis in pea. *Molecular and General Genetics* **260**(1): 9-19.
- Fesenko AN, Provorov NA, Orlova IF, Orlov VP, Simarov BV. 1995.** Selection of *Rhizobium leguminosarum* bv. *viciae* strains for inoculation of *Pisum sativum* L. cultivars - Analysis of symbiotic efficiency and nodulation competitiveness. *Plant and Soil* **172**(2): 189-198.
- Fobert P, Roy N, Nash J, Iyer V. 1991.** Procedure for obtaining efficient root nodulation of a pea cultivar by a desired *Rhizobium* strain and preempting nodulation by other strains. *Applied and Environmental Microbiology* **57**(6): 1590-1594.
- Fourmont R. 1956.** Les variétés de pois (*Pisum sativum* L.) cultivées en France. *INRA*: 1-253.
- Friesen ML, Heath KD. 2013.** One hundred years of solitude: integrating single-strain inoculations with community perspectives in the legume-rhizobium symbiosis. *New Phytologist* **198**(1): 7-9.
- Galloway JN, Townsend AR, Erisman JW, Bekunda M, Cai Z, Freney JR, Martinelli LA, Seitzinger SP, Sutton MA. 2008.** Transformation of the Nitrogen Cycle: Recent Trends, Questions, and Potential Solutions. *Science* **320**(5878): 889-892.
- Geurts R, Heidstra R, Hadri AE, Downie JA, Franssen H, vanKammen A, Bisseling T. 1997.** Sym2 of pea is involved in a nodulation factor-perception mechanism that controls the infection process in the epidermis. *Plant Physiology* **115**(2): 351-359.
- Heath KD, Tiffin P. 2009.** Stabilizing mechanisms in a legume-rhizobium mutualism. *Evolution* **63**(3): 652-662.
- Herridge DF, Peoples MB, Boddey RM. 2008.** Global inputs of biological nitrogen fixation in agricultural systems. *Plant and Soil* **311**(1-2): 1-18.
- Hogg B, Davies AE, Wilson KE, Bisseling T, Downie JA. 2002.** Competitive nodulation blocking of cv. Afghanistan pea is related to high levels of nodulation factors made by some strains of *Rhizobium leguminosarum* bv. *viciae*. *Molecular Plant-Microbe Interactions* **15**(1): 60-68.
- Holl FB. 1975.** Host plant control of the inheritance of dinitrogen fixation in the *Pisum-Rhizobium* symbiosis. *Euphytica* **24**(3): 767-770.
- Jakobsson M, Rosenberg NA. 2007.** CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics* **23**(14): 1801-1806.
- Jensen ES, Hauggaard-Nielsen H. 2003.** How can increased use of biological N₂ fixation in agriculture benefit the environment? *Plant and Soil* **252**(1): 177-186.
- Jeady C, Ruffel S, Freixes S, Tillard P, Santoni AL, Morel S, Journet EP, Duc G, Gojon A, Lepetit M, Salon C. 2010.** Adaptation of *Medicago truncatula* to nitrogen limitation is modulated via local and systemic nodule developmental responses. *New Phytol* **185**.
- Jing R, Vershinin A, Grzebyta J, Shaw P, Smykal P, Marshall D, Ambrose MJ, Ellis THN, Flavell AJ. 2010.** The genetic diversity and evolution of field pea (*Pisum*) studied by high throughput retrotransposon based insertion polymorphism (RBIP) marker analysis. *Bmc Evolutionary Biology* **10**(1): 44.
- Jombart T, Devillard S, Balloux F. 2010.** Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. *Bmc Genetics* **11**.
- Kawaharada Y, Kelly S, Nielsen MW, Hjuler CT, Gysel K, Muszynski A, Carlson RW, Thygesen MB, Sandal N, Asmussen MH, Vinther M, Andersen SU, Krusell L, Thirup S, Jensen KJ, Ronson CW, Blaise M, Radutoiu S, Stougaard J. 2015.**

- Receptor-mediated exopolysaccharide perception controls bacterial infection. *Nature* **523**(7560): 308-315.
- Kiers ET, Hutton MG, Denison RF. 2007.** Human selection and the relaxation of legume defences against ineffective rhizobia. *Proceedings of the Royal Society B-Biological Sciences* **274**(1629): 3119-3126.
- Kiers ET, Ratcliff WC, Denison RF. 2013.** Single-strain inoculation may create spurious correlations between legume fitness and rhizobial fitness. *New Phytologist* **198**(1): 4-6.
- Kumar N, Lad G, Giuntini E, Kaye ME, Udomwong P, Shamsani NJ, Young JPW, Bailly X. 2015.** Bacterial genospecies that are not ecologically coherent: population genomics of *Rhizobium leguminosarum*. *Open Biology* **5**(1).
- Kumar S, Stecher G, Tamura K. 2016.** MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Molecular Biology and Evolution* **33**(7): 1870-1874.
- Laguerre G, Heulin-Gotty K, Brunel B, Klonowska A, Le Quéré A, Tillard P, Prin Y, Cleyet-Marel J-C, Lepetit M. 2012.** Local and systemic N signaling are involved in *Medicago truncatula* preference for the most efficient *Sinorhizobium* symbiotic partners. *New Phytologist* **195**(2): 437-449.
- Laguerre G, Louvrier P, Allard MR, Amarger N. 2003.** Compatibility of rhizobial genotypes within natural populations of *Rhizobium leguminosarum* biovar *viciae* for nodulation of host legumes. *Applied and Environmental Microbiology* **69**(4): 2276-2283.
- Laguerre G, Nour SM, Macheret V, Sanjuan J, Drouin P, Amarger N. 2001.** Classification of rhizobia based on *nodC* and *nifH* gene analysis reveals a close phylogenetic relationship among *Phaseolus vulgaris* symbionts. *Microbiology* **147**(4): 981-993.
- LaRue T, Weeden N. 1992.** The symbiosis genes of pea. *Pisum Genet* **24**: 5-12.
- Lie T. 1971.** Temperature-dependent root-nodule formation in pea cv. Iran. *Plant and Soil*: 751-752.
- Lie TA. 1978.** Symbiotic Specialization in Pea Plants - Requirement of Specific Rhizobium Strains for Peas from Afghanistan. *Annals of Applied Biology* **88**(3): 462-465.
- Lie TA, Goktan D, Engin M, Pijnenborg J, Anlarsal E. 1987.** Coevolution of the Legume-Rhizobium Association. *Plant and Soil* **100**(1-3): 171-181.
- Madsen EB, Madsen LH, Radutoiu S, Olbryt M, Rakwalska M, Szczyglowski K, Sato S, Kaneko T, Tabata S, Sandal N, Stougaard J. 2003.** A receptor kinase gene of the LysM type is involved in legume perception of rhizobial signals. *Nature* **425**(6958): 637-640.
- Malkov N, Fliegmann J, Rosenberg C, Gascioli V, Timmers AC, Nurisso A, Cullimore J, Bono J-J. 2016.** Molecular Basis of Lipo-chitoooligosaccharide Recognition by the Lysin Motif Receptor-like Kinase LYR3 in Legumes. *Biochemical Journal*: BCJ20160073.
- Masson-Boivin C, Giraud E, Perret X, Batut J. 2009.** Establishing nitrogen-fixing symbiosis with legumes: how many rhizobium recipes? *Trends in Microbiology* **17**(10): 458-466.
- McKenzie RH, Middleton AB, Solberg ED, DeMulder J, Flore N, Clayton GW, Bremer E. 2001.** Response of pea to rhizobia inoculation and starter nitrogen in Alberta. *Canadian Journal of Plant Science* **81**(4): 637-643.
- Meade J, Higgins P, Ogara F. 1985.** Studies on the inoculation and competitiveness of a *Rhizobium leguminosarum* strain in soils containing indigenous rhizobia. *Applied and Environmental Microbiology* **49**(4): 899-903.
- Moreau D, Voisin AS, Salon C, Munier-Jolain N. 2008.** The model symbiotic association between *Medicago truncatula* cv. Jemalong and *Rhizobium meliloti* strain 2011 leads

- to N-stressed plants when symbiotic N₂ fixation is the main N source for plant growth. *Journal of Experimental Botany* **59**(13): 3509-3522.
- Mutch LA, Young JPW. 2004.** Diversity and specificity of *Rhizobium leguminosarum* biovar *viciae* on wild and cultivated legumes. *Molecular Ecology* **13**(8): 2435-2444.
- Oono R, Anderson CG, Denison RF. 2011.** Failure to fix nitrogen by non-reproductive symbiotic rhizobia triggers host sanctions that reduce fitness of their reproductive clonemates. *Proceedings of the Royal Society B-Biological Sciences* **278**(1718): 2698-2703.
- Peix A, Ramirez-Bahena MH, Velazquez E, Bedmar EJ. 2015.** Bacterial Associations with Legumes. *Critical Reviews in Plant Sciences* **34**(1-3): 17-42.
- Philippot L, Raaijmakers JM, Lemanceau P, van der Putten WH. 2013.** Going back to the roots: the microbial ecology of the rhizosphere. *Nat Rev Micro* **11**(11): 789-799.
- Phillips DA. 1980.** Efficiency of symbiotic nitrogen fixation in legumes. *Annual review of plant physiology* **31**(1): 29-49.
- Rahi P, Kapoor R, Young JPW, Gulati A. 2012.** A genetic discontinuity in root-nodulating bacteria of cultivated pea in the Indian trans-Himalayas. *Molecular Ecology* **21**(1): 145-159.
- Raj A, Stephens M, Pritchard JK. 2014.** fastSTRUCTURE: Variational Inference of Population Structure in Large SNP Data Sets. *Genetics* **197**(2): 573-U207.
- Rogel MA, Ormeno-Orrillo E, Romero EM. 2011.** Symbiovars in rhizobia reflect bacterial adaptation to legumes. *Systematic and Applied Microbiology* **34**(2): 96-104.
- Sachs JL, Ehinger MO, Simms EL. 2010.** Origins of cheating and loss of symbiosis in wild *Bradyrhizobium*. *Journal of Evolutionary Biology* **23**(5): 1075-1089.
- Sachs JL, Kembel SW, Lau AH, Simms EL. 2009.** In Situ Phylogenetic Structure and Diversity of Wild *Bradyrhizobium* Communities. *Applied and Environmental Microbiology* **75**(14): 4727-4735.
- Sagan M, Messenger A, Duc G. 1993a.** Specificity of the *Rhizobium*-Legume Symbiosis Obtained after Mutagenesis in Pea (*Pisum-Sativum* L). *New Phytologist* **125**(4): 757-761.
- Schulze J, Adgo E, Merbach W. 1999.** Carbon Costs Associated with N₂ Fixation in *Vicia faba* L and *Pisum sativum* L. over a 14-Day Period. *Plant Biology* **1**(6): 625-631.
- Simms EL, Taylor DL. 2002.** Partner choice in nitrogen-fixation mutualisms of legumes and rhizobia. *Integrative and Comparative Biology* **42**(2): 369-380.
- Smykal P, Coyne CJ, Ambrose MJ, Maxted N, Schaefer H, Blair MW, Berger J, Greene SL, Nelson MN, Besharat N, Vymyslicky T, Toker C, Saxena RK, Roorkiwal M, Pandey MK, Hu JG, Li YH, Wang LX, Guo Y, Qiu LJ, Redden RJ, Varshney RK. 2015.** Legume Crops Phylogeny and Genetic Diversity for Science and Breeding. *Critical Reviews in Plant Sciences* **34**(1-3): 43-104.
- Smykal P, Kenicer G, Flavell AJ, Corander J, Kosterin O, Redden RJ, Ford R, Coyne CJ, Maxted N, Ambrose MJ, Ellis NTH. 2011.** Phylogeny, phylogeography and genetic diversity of the *Pisum* genus. *Plant Genetic Resources-Characterization and Utilization* **9**(1): 4-18.
- Spaink HP, Sheeley DM, Vanbrussel AAN, Glushka J, York WS, Tak T, Geiger O, Kennedy EP, Reinhold VN, Lugtenberg BJJ. 1991.** A novel highly unsaturated fatty-acid moiety of lipo-oligosaccharide signals determines host specificity of *Rhizobium*. *Nature* **354**(6349): 125-130.
- Tayeh N, Aluome C, Falque M, Jacquin F, Klein A, Chauveau A, Bérard A, Houtin H, Rond C, Kreplak J. 2015.** Development of two major resources for pea genomics: the GenoPea 13.2 K SNP Array and a high-density, high-resolution consensus genetic map. *The plant journal* **84**(6): 1257-1273.

- Trainer MA, Charles TC. 2006.** The role of PHB metabolism in the symbiosis of rhizobia with legumes. *Applied Microbiology and Biotechnology* **71**(4): 377-386.
- Triplett EW, Sadowsky MJ. 1992.** Genetics of competition for nodulation of legumes. *Annual Review of Microbiology* **46**: 399-428.
- Vershinin AV, Allnutt TR, Knox MR, Ambrose MJ, Ellis THN. 2003.** Transposable elements reveal the impact of introgression, rather than transposition, in *Pisum* diversity, evolution, and domestication. *Molecular Biology and Evolution* **20**(12): 2067-2075.
- Vilmorin-Andrieux. 1883.** Les Plantes potagères. Chapitre : Pois. *Vilmorin-Andrieux & Cie*: 423-474.
- Voisin AS, Bourion V, Duc G, Salon C. 2007.** Using an ecophysiological analysis to dissect genetic variability and to propose an ideotype for nitrogen nutrition in pea. *Annals of Botany* **100**(7): 1525-1536.
- Voisin AS, Munier-Jolain NG, Salon C. 2010.** The nodulation process is tightly adjusted to plant growth. An analysis using environmentally and genetically induced variation of nodule number and biomass in pea. *Plant and Soil* **337**(1-2): 399-412.
- Walker SA, Viprey V, Downie JA. 2000.** Dissection of nodulation signaling using pea mutants defective for calcium spiking induced by Nod factors and chitin oligomers. *Proceedings of the National Academy of Sciences of the United States of America* **97**(24): 13413-13418.
- Weeden N, Brauner S, Przyborowski J. 2002.** Genetic analysis of pod dehiscence in pea (*Pisum sativum* L.). *Cellular and Molecular Biology Letters* **7**(2B): 657-664.
- Winarno R, Lie TA. 1979.** Competition between Rhizobium strains in nodule formation: Interaction between nodulating and non-nodulating strains. *Plant and Soil* **51**(1): 135-142.
- Young JPW, Johnston AWB, Brewin NJ. 1982.** A Search for Peas (*Pisum-Sativum-L*) Showing Strain Specificity for Symbiotic Rhizobium-Leguminosarum. *Heredity* **48**(APR): 197-201.
- Young JPW, Matthews P. 1982.** A Distinct Class of Peas (*Pisum-Sativum-L*) from Afghanistan That Show Strain Specificity for Symbiotic Rhizobium. *Heredity* **48**(APR): 203-210.
- Zhukov V, Radutoiu S, Madsen LH, Rychagova T, Ovchinnikova E, Borisov A, Tikhonovich I, Stougaard J. 2008.** The Pea Sym37 Receptor Kinase Gene Controls Infection-Thread Initiation and Nodule Development. *Molecular Plant-Microbe Interactions* **21**(12): 1600-1608.
- Zohary D, Hopf M. 1973.** Domestication of Pulses in the Old World Legumes were companions of wheat and barley when agriculture began in the Near East. *Science* **182**(4115): 887-894.

SYNTHESE ET PERSPECTIVES

SYNTHESE ET PERSPECTIVES

Les légumineuses sont des cultures de choix pour répondre à la nécessité d'une agriculture combinant production de qualité et durabilité : elles produisent des graines à haute valeur nutritionnelle, notamment riches en protéines ; ceci sans nécessité d'un apport d'engrais azoté grâce à la symbiose qu'elles établissent avec des bactéries fixatrices de l'azote de l'air. Cependant, les irrégularités du rendement et de teneur en protéines que connaît la culture de pois limitent son extension. Elles sont, en partie, liées à des problèmes de nutrition azotée. En effet, la fixation symbiotique d'azote est très sensible aux conditions environnementales (stress abiotiques, biotiques) et le faible développement des racines fréquemment observé chez les légumineuses peut affecter le prélèvement en eau et en éléments minéraux (dont les nitrates). Pour créer des variétés de pois possédant une meilleure nutrition azotée, une complémentarité est donc à rechercher entre les deux voies d'acquisition d'N, avec en particulier une mise en place des nodosités qui ne se fasse pas au détriment du développement racinaire.

L'objectif de cette thèse a été d'acquérir une meilleure compréhension du contrôle génétique de la mise en place des racines et des nodosités et de leur impact sur la nutrition azotée de façon à explorer si un tel idéotype est concevable.

1. Apport des résultats de la thèse en recherche

Des approches très diverses ont été utilisées au cours de cette thèse. Une approche de génétique quantitative a tout d'abord été utilisée, basée sur la recherche d'associations entre diversité de l'architecture racinaire et nodulaire ou de l'acquisition d'azote et polymorphisme moléculaire (QTL). Elle a permis d'identifier des zones du génome pouvant être impliquées. La deuxième approche, transcriptomique, a mis en évidence des différentiels d'expression de gènes corrélés à un phénotype racinaire et/ou d'acquisition d'azote. Enfin, une troisième approche nous a permis d'examiner la question de la capacité des génotypes de pois à favoriser les associations symbiotiques avec les souches de rhizobium les plus performantes pour l'acquisition d'azote et l'impact de la sélection végétale sur cette capacité.

Différents jeux de données, acquis en amont ou au cours de la thèse, ont été utilisés pour les trois différentes approches (Annexe 4). La quasi-totalité de ces données a été valorisée dans les articles qui constituent cette thèse ; un petit nombre n'a été valorisé jusqu'à présent que sous forme de posters ou dans le rapport final du projet ANR GENOPEA.

Certaines d'entre-elles ont également constitué des données de base pour l'établissement de nouveaux projets.

Mise au point d'une méthodologie de phénotypage

En dépit de leur diversité, les approches menées ont toutes eu pour dénominateur commun : la description de la variabilité des structures d'acquisition de l'N (racines + nodosités) et de leur fonctionnement.

Des méthodes de phénotypage racinaire et nodulaire ont donc été mises au point, de façon à être i) applicables sur un grand nombre de géotypes ; ii) effectuées à des stades représentatifs des capacités d'acquisition d'N des plantes. Outre la définition des systèmes et conditions de culture, elles ont nécessité la mise au point de techniques d'acquisition d'images (techniques de lavage et d'étalement des racines, type d'image, résolution) et d'analyse de ces images (utilisation du logiciel WinRHIZO[®], Regent Instruments, Quebec, Canada). Des caractères représentatifs de la mise en place du système racinaire nodulé ont ainsi été déterminés pour toutes les expérimentations, par analyse d'images (longueur et surface des racines, surface des nodosités) ou par comptage direct (nombre des racines et des nodosités). En complément, une technique d'étalement ou acquisition et d'analyse d'image des parties aériennes a été mise au point, permettant d'estimer les surfaces foliaires et la variabilité des couleurs des feuilles comme indicateur de leur teneur en chlorophylle, sans nécessiter l'utilisation d'un planimètre ou d'un SPAD.

Ces méthodologies ont depuis été citées et utilisées pour décrire de la variabilité de nodulation, de fixation symbiotique ou de surface foliaire dans des populations de lignées recombinantes de légumineuses, *L. japonicus*, haricot, soja et pois (Tominaga *et al.*, 2012; Ramaekers *et al.*, 2013; Hwang *et al.*, 2014; Klein *et al.*, 2014). La méthodologie de description du système racinaire et de la partie aérienne a été appliquée à une collection de 266 accessions de pois dans le cadre d'une thèse visant à déterminer le rôle de l'architecture racinaire dans le contrôle génétique de la diminution des symptômes de pourriture racinaire dus à *Aphanomyces euteiches* chez le pois (Desgroux, 2016). 33, 38 et 7 QTL relatifs respectivement aux racines, aux parties aériennes et plante entière ont ainsi été détectés dans cette étude basée une analyse de génétique d'association (Genome Wide Association Study, GWAS).

Elles ont aussi contribué à la mise au point de système de culture en poches ou rhizotrons permettant une visualisation ou un suivi de la nodulation ou du développement racinaire (Clemow *et al.*, 2011; Jeudy *et al.*, 2016). Enfin, les images de racines publiées dans

l'article relatif au mutant étudié chez *M. truncatula* ont servi de base au paramétrage d'un modèle de développement racinaire (Schnepf *et al.*, 2016).

Mise en évidence d'une variabilité génétique

Les trois démarches ont nécessité l'étude d'une variabilité de ressources génétiques : population de lignées recombinantes de pois, mutant induit par rayonnement γ chez *M. truncatula*, ou collection d'accessions de pois.

Chacune des descriptions de ces diversités a été innovante. En effet, aucune étude n'avait jusqu'alors exploré conjointement les variabilités de mise en place des racines et des nodosités et évalué leur impact sur l'acquisition d'N. D'autres études de populations de lignées recombinantes ont été effectuées depuis, mais elles n'ont le plus souvent examiné que les nodosités et la fixation symbiotique ; à ce jour, l'étude présentée dans le chapitre I reste la plus détaillée notamment en ce qui concerne le développement racinaire. L'étude faite sur le mutant de *M. truncatula* et son parent sauvage J5 représente, quant à elle, la première caractérisation détaillée qui ait été publiée sur l'adaptation architecturale du système racinaire d'une plante légumineuse à la disponibilité en azote externe. Enfin, aucune étude n'a encore été publiée concernant la diversité de développement racinaire et nodulaire d'une collection d'accessions de pois ou de choix entre les partenaires pois et Rlv.

Toutes les études menées ont mis en évidence une grande diversité pour les caractères mesurés, et les données acquises sont un réel enrichissement des connaissances concernant les ressources génétiques étudiées et des candidats potentiels pour d'autres recherches. TR185 est ainsi apparu un candidat intéressant pour examiner, chez *M. truncatula*, la signalétique de carence en N dans le milieu ; suspecté par M. Djordjevic (Australian National University, Canberra) comme étant muté pour un gène récepteur des peptides CEPs ; ceci pouvant expliquer la permanence de son état de carence en N. Des travaux menés depuis ont confirmés cette hypothèse : les croisements que nous avons réalisés, ainsi que les séquençages faits par F. Frugier et C. Laffont (IPS2, Gif-sur-Yvette), ont depuis montré que TR185 est allélique au mutant *cra2* ; les travaux menés en collaboration entre les collègues de l'Australian National University et ceux l'IPS2 ayant montré par ailleurs que MtCRA2 est probablement un récepteur de MtCEP1 (Mohd-Radzman *et al.*, 2016).

L'étude de la diversité des développements racinaire, nodulaire et aérien menée sur la collection de 104 accessions de pois (Annexe 5) a permis de définir des ensembles de génotypes pouvant servir à d'autres programmes de recherche ; pour exemples i) elle a contribué à choisir 3 génotypes contrastés pour leur architecture pouvant ainsi servir de

référence pour le WP3 (Exploitation de l'architecture et des interactions plantes x microorganismes du sol pour un meilleur contrôle des stress biotiques et abiotiques) du projet d'Investissement d'Avenir PeaMUST, ii) elle a permis de choisir 10 géotypes contrastés de pois qui ont été étudiés de façon plus approfondie dans différentes conditions pour, à terme, paramétrer un modèle d'architecture du système racinaire nodulé (collaboration AS Voisin, L. Pagès), iii) elle a été associée en son entier pour sa diversité d'architecture racinaire à la collection « Aphanomyces » créée à l'INRA de Rennes pour constituer la collection de 266 géotypes nécessaire à l'analyse de génétique d'association relative à l'architecture racinaire et à la tolérance à *A. euteiches*. Enfin, l'étude à partir de cette population de 104 accessions de la diversité du choix entre pois et Rlv a permis d'acquérir des résultats préliminaires nécessaires à la soumission d'un projet ANR, GRaSP (Genetics of Rhizobia Selection by Pea), visant à connaître les déterminants génétiques de ce choix ; les premières expérimentations prévues dans ce projet vont être réalisées à partir d'un sous-échantillon de géotypes de pois choisis parmi les 104 grâce à notre étude de diversité.

Mise en évidence de corrélations génétiques entre variables d'architecture racinaire nodulée et d'acquisition d'N

Dans les deux approches que nous avons menées sur pois en interaction avec des rhizobia, nous avons mis en évidence l'existence de corrélations génétiques entre les caractères d'architecture racinaire ou nodulaire et ceux d'acquisition d'N. Ses corrélations ont été examinées sur différentes ressources génétiques, sous différentes conditions de culture et à différents stade de développement des plantes.

Au cours de notre toute première expérimentation de comparaison entre 7 lignées de pois, cultivées en pots et inoculées avec la souche de Rlv 1007, nous avons mis en évidence que les corrélations étaient globalement positives mais qu'elles évoluaient au cours du cycle de la plante. Ainsi, au stade précoce de 4 feuilles visibles, la quantité d'N contenue dans les parties aériennes (elle-même très fortement corrélée à la biomasse aérienne) n'était significativement corrélée qu'à la biomasse des racines ; la corrélation entre les deux demeurant significative jusqu'à début floraison (Table I.1). La corrélation entre biomasse de nodosités (ou surface de nodosités) et quantité d'N dans les parties aériennes, quant à elle, ne s'est révélée significative qu'à partir du stade de 9 feuilles et l'est restée jusqu'à début remplissage des grains. La quantité d'N a été aussi corrélée au nombre de nodosités, mais ceci uniquement au stade de 9 feuilles.

Dans les deux expérimentations suivantes, les mesures que nous avons réalisées au stade début floraison sur la population de lignées recombinantes (inoculées par la souche P221) ou à 4 semaines, c'est-à-dire un peu avant début floraison, sur la collection de 104 accessions (inoculées par un mélange de 5 souches de Rlv) ont confirmé l'existence d'une forte corrélation à ce stade entre biomasse aérienne et biomasse racinaire ou nodulaire, la corrélation avec le nombre de nodosités étant plus faible. Cette relation entre biomasse aérienne et biomasse des organes symbiotiques est décrite dans la littérature sur différentes espèces. En effet, la croissance de la plante fait augmenter la demande en N de la plante tout en entraînant une plus forte disponibilité en ressources de C liées à la photosynthèse (Moreau *et al.*, 2008; Voisin *et al.*, 2010; Laguerre *et al.*, 2012).

Notre étude sur les 104 accessions de pois a permis de montrer que la corrélation entre biomasse aérienne et biomasse des nodosités est observée quelle que soit l'origine géographique ou la diversité d'usage des géotypes de pois. La plus faible corrélation observée entre biomasse aérienne et nombre de nodosités suggère que la formation des nodosités est moins dépendante du potentiel de croissance que l'expansion des nodosités et soumise, au moins en partie, à un contrôle génétique distinct et relatif à l'interaction entre plante et Rlv.

Cette étude a permis aussi de s'interroger également sur l'impact des souches sur l'acquisition de l'N – leur efficacité symbiotique – et sur la variabilité de leur capacité à noduler les plantes. Elle pourrait permettre aussi de déterminer si elles induisent des modifications en architecture racinaire. Une analyse en composantes principales a été réalisée avec, comme variables actives, les variables décrivant l'architecture racinaire nodulée, les biomasses et l'acquisition d'N, et comme variables supplémentaires, les proportions dans les nodosités de chacune des 5 souches (Fig. 11). Le choix préférentiel pour SF (TOM) semble associé à la présence de nodosités à forte biomasse individuelle mais peu nombreuses ; à l'inverse les accessions ayant choisi de préférence SA auraient des nodosités plus petites et plus nombreuses mais une biomasse aérienne et efficacité symbiotique plus élevées. Ceci suggère que des différences de choix de souches pourraient avoir un impact sur l'architecture racinaire nodulée ainsi qu'observé précédemment (Laguerre *et al.*, 2007). Il ne faut exclure cependant qu'inversement des différences d'architecture pourraient avoir un impact sur le choix des souches.

Néanmoins, l'étude complémentaire effectuée sur un échantillon de 18 accessions contrastées a montré que l'efficacité de la fixation de l'azote n'est pas un déterminant majeur du choix par les géotypes de pois de leur partenaire symbiotique dans un

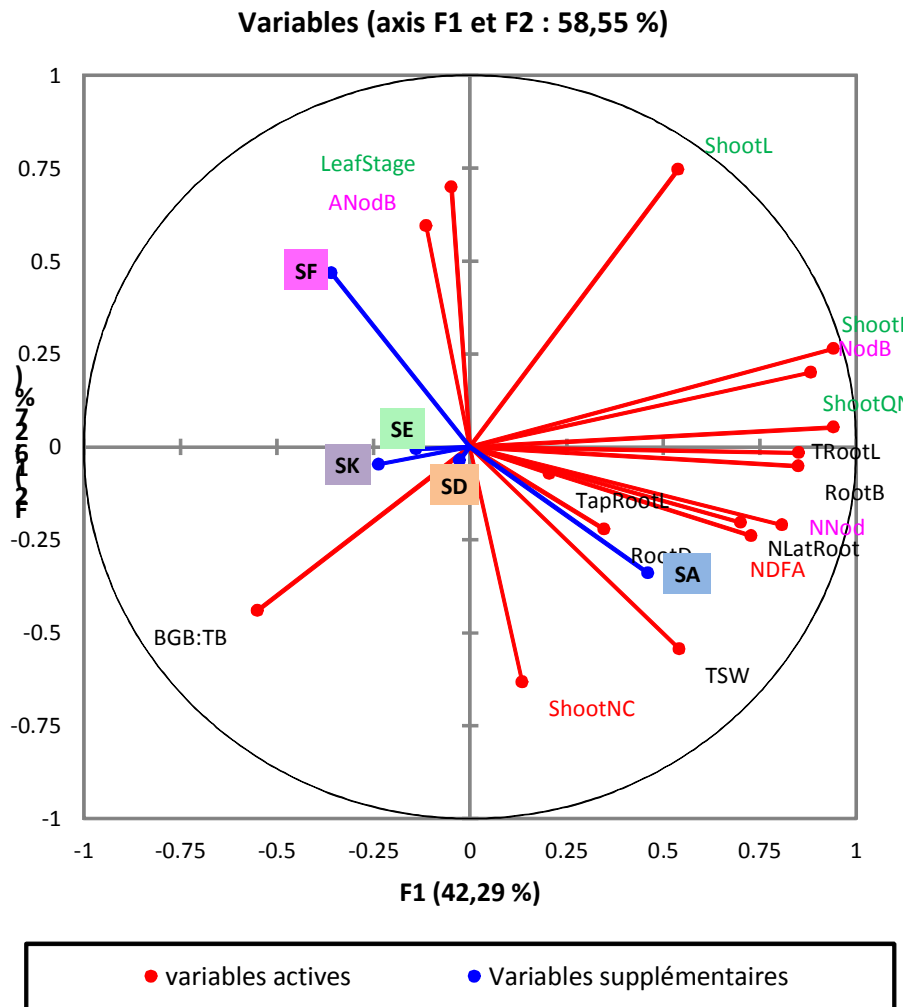


Fig. 11. Analyse en composantes principales des 104 accessions de pois avec 16 variables actives décrivant l'architecture racinaire nodulée, le développement aérien et l'acquisition d'N et 5 variables supplémentaires SA, SD, SE, SF et SK qui sont les proportions de chacune de ces souches dans les nodosités.

RootB: root biomass, RootD: mean root diameter, NLatRoot: Number of first order lateral roots, TapRootL: Tap root length, TRootL: Total root length, NodB: nodule biomass, NNod: number of nodules, ANodB average biomass per nodule, LeafStage: Number of nodes with leaves, ShootB: Shoot biomass, ShootL: Shoot length, ShootNC: Shoot nitrogen content, ShootQN: Shoot nitrogen accumulation, BGB:TB: relative part of belowground upon total biomass, NDFA: nitrogen accumulation derived from symbiotic fixation, TSW: thousand seed weight

contexte de multi-inoculation. Cette confrontation des pois à un mélange de souches de Rlv est représentatif des conditions de culture en champs. Dans les démarches de recherche et de création variétale à venir, prenant en compte les interactions avec les rhizobia, il est donc indispensable de considérer les deux composantes de la symbiose : efficacité mais aussi choix entre partenaires symbiotiques.

2. Perspectives

Chacune des trois différentes pistes abordées au cours de cette thèse pourraient être approfondies, en particulier grâce aux nouveaux outils qui existent ou vont exister très prochainement à Dijon : ressources génétiques et génomiques sur le pois et nouveaux outils de phénotypage racinaire. Deux niveaux d'approfondissement sont possibles ; le premier consiste à ré-analyser les données phénotypiques acquises en utilisant les nouvelles ressources génomiques disponibles ; le second consiste à envisager des expérimentations supplémentaires pour poursuivre certains questionnements.

Utiliser les nouveaux outils disponibles

Avec son grand génome d'environ 4,5 Gb, le pois est, comme la lentille, jusqu'à présent resté sans séquence de référence de son génome. Grâce à la diminution du coût de génotypage à haute densité et du développement des nouvelles méthodes de génotypage (Next-Generation Sequencing: NGS), dont le génotypage par séquençage (Genotyping-By-Sequencing: GBS), plusieurs programmes nationaux et internationaux ont développé ces dernières années des ressources génomiques sur les légumineuses d'intérêt agronomique ayant un plus petit génome comme par exemple le soja et le pois chiche. Plus récemment, un consortium international a été constitué pour générer la séquence complète du génome du pois (Madoui *et al.*, 2015).

Auparavant, une première étape vers la connaissance du génome de pois a été franchie, grâce à la création d'un atlas d'expression des gènes (<http://bios.dijon.inra.fr/FATAL/cgi/pscam.cgi>). Cette ressource génomique permet de rechercher, chez le pois, la séquence homologue d'un gène d'intérêt identifié chez une autre espèce et de connaître son profil d'expression dans la plante. Cet atlas a été obtenu par séquençage haut débit de banques d'ADNc produites à partir de 20 tissus provenant de différents organes (dont les racines et les nodosités) prélevés, à différents stades de développement, sur des plantes soumises à différentes conditions de nutrition azotée (Alves-Carvalho *et al.*, 2015).

La première carte génétique du pois a été, quant à elle, construite dès 1925 sur la base d'une dizaine de marqueurs morphologiques (Wellensiek, 1925). Des progrès considérables ont été fait depuis avec l'apparition des marqueurs SSR (Single Sequence Repeat) et SNP (Single Nucleotide Polymorphism), et de nombreuses cartes génétiques basées sur différentes populations biparentales existent. Récemment, une carte consensus a été construite à partir de 12 populations de RILs génotypées en utilisant 13000 SNP représentatifs d'un sous-set des transcrits mis en évidence précédemment (Tayeh *et al.*, 2015). La banque d'ADN génomique de type BAC (Bacterial Artificial Chromosome) qui a été créée permet d'accéder à l'organisation physique du génome du pois (<http://cnrgv.toulouse.inra.fr/fr>). Enfin, dans le cadre du projet d'Investissement d'Avenir PeaMUST, des données de génotypage viennent d'être acquises sur différents panels de pois, par capture d'exome et séquençage de plus de 50000 fragments. Plus d'un million de SNP avec moins de 10% de données manquantes ont ainsi été détectés pour ces panels.

Beaucoup d'efforts ont été aussi déployés ces dernières années pour faciliter et automatiser le phénotypage racinaire ; ceci conjointement à la baisse des coûts d'acquisition d'image. Les améliorations ont initialement surtout visé à élaborer des outils informatiques permettant la collecte de données de phénotypage à partir d'images en deux dimensions (2D) réalisées grâce à la culture en rhizotrons plats ; WinRHIZO[®], SmartRoot (Lobet *et al.*, 2011) et archiDART (Delory *et al.*, 2016) sont parmi les plus utilisés des logiciels dédiés. Les innovations portent aussi sur deux autres niveaux : i) la construction de plateformes de phénotypage racinaire (et aérien) à haut débit pour faciliter l'automatisation des prises d'image ; ii) l'obtention d'images en 3D des systèmes racinaires pour des cultures en pots ou en milieu naturel, utilisant des techniques de tomographie ou de résonance magnétique (Dusschoten *et al.*, 2016). La plateforme de l'INRA de Dijon, 4PMI (Jeudy *et al.*, 2016), se distingue de celle de l'Institute of Bio- and Geosciences à Juelich en Allemagne, GROWSCREEN Rhizo (Nagel *et al.*, 2012), par ses rhizotrons en forme de tubes.

Pour préciser l'architecture génétique des caractères

La mise en évidence de co-localisations entre la majeure partie des QTL relatifs à l'accumulation d'N dans les plantes ou dans les graines avec des QTL relatifs aux développements racinaire ou nodulaire réalisée dans la première approche indique qu'ils partagent des déterminismes communs. Notre détection de QTL relatifs au développement et à la croissance des racines et des parties aériennes a été validée depuis. En effet, leur projection, sur la carte génétique consensus, a mis en évidence des colocalisations entre les

QTL que nous avons détectés et ceux identifiés par analyse de génétique d'association sur une population de 266 accessions de pois ; ainsi 23 des colocalisations étaient relatives à des variables racinaires et 13 à des variables aériennes (Desgroux, 2016).

Cependant, en raison du faible nombre de marqueurs sur la carte génétique utilisée dans notre étude (152 marqueurs), la localisation génomique des QTL de développement racinaire et nodulaire et d'acquisition d'azote détectés a été très imprécise, et la détermination des gènes sous-jacents impliqués dans la variation de ces caractères n'a pas été possible. Une nouvelle recherche de QTL s'appuyant sur une carte plus dense (13000 SNP) devrait permettre une réduction significative des intervalles de confiance de ces QTL. Cette étude étant conduite sur une population de RILs, seules les variations génétiques présentes au sein des parents de cette population de RILs pourront être détectées comme impliquées dans ces caractères. Il est néanmoins possible que d'autres régions du génome soient impliquées dans la variation de ces caractères, et une approche basée sur un panel génétique plus diversifié pourrait potentiellement indiquer des zones candidates supplémentaires.

De ce fait, une étude de génétique d'association utilisant ces 13000 marqueurs SNP a été effectuée sur la collection de 104 accessions de pois inoculée par le mélange de cinq souches de Rlv. Un des SNP présentant un fort signal d'association identifié par cette approche se trouve dans le gène *LE* (impliqué dans la voie de synthèse des gibbérellines et déterminant la taille des entre-nœuds) qui était déjà connu pour être associé à la hauteur des plantes. La détection de cette association constitue une validation *post-hoc* de la méthode. De façon intéressante, les variations de ce marqueur ont également été associées à des variations de teneur en N des parties aériennes, en accord avec les colocalisations entre QTL relatifs à la hauteur des plantes et ceux relatifs à la teneur en N des graines ou des parties aériennes que nous avons trouvés dans l'étude présentée dans le chapitre I (Bourion *et al.*, 2010) et par (Burstin *et al.*, 2007). Un SNP dans un gène codant pour une LRR-RLK a aussi été identifié comme ayant un effet sur des variations du taux de fixation symbiotique. Aucun effet de ce SNP n'a été observé sur le nombre de nodosités formées, à la différence des gènes codant pour des LRR-RLKs préalablement identifiées comme impliquées dans la perception des NFs tels que *MtDMI2/PsSYM19* (Endre *et al.*, 2002) ou dans l'AON tels que *MtSUNN /PsSYM29* (Krusell *et al.*, 2002).

De façon générale, cette étude de génétique d'association n'a permis de détecter que très peu d'associations entre marqueurs SNP et caractères; ce qui peut s'expliquer par la faible taille de la collection et une densité de marqueurs qui reste faible compte-tenu de la forte décroissance du déséquilibre de liaison observée le long des groupes de liaison. L'association

entre le choix préférentiel pour la souche SF (TOM) et un SNP dans le gène *SYM2* n'a pas pu être observée, néanmoins, des variations de proportions de nodulation par SF et des variations dans des SNP situés sur d'autres groupes de liaison que LGI laissent supposer qu'il existe d'autres déterminants que le gène *SYM2* dans ce choix. Une nouvelle étude de génétique d'association impliquant une collection de pois de taille plus grande (336 accessions), inoculée par un mélange comprenant une trentaine de souches de Rlv, est prévue dans le cadre du projet ANR GRaSP qui va débuter en 2017. La collection sera génotypée avec plus d'un million de SNP à partir de captures d'exome.

Pour valider de nouveaux gènes candidats

L'expérimentation sur les 336 accessions va être réalisée sur la plateforme 4PMI de Dijon. Il sera donc possible, en plus de la détermination finale des populations de rhizobia associées à chacune des accessions, de suivre la mise en place des racines et des nodosités et la croissance aérienne de l'ensemble des plantes à partir de l'analyse des images prises de façon automatisée au cours de la culture. Des marqueurs SNP dans des gènes candidats pourront être testés pour leur association avec des variations de caractères d'architecture racinaire nodulée, d'acquisition d'N et de choix de souches Rlv. Des homologues chez le pois du gène *MtCRA2* ou de gènes impliqués dans le transport d'N dont on a vu un différentiel d'expression entre TR185 et J5 pourraient être des candidats d'architecture racinaire et nodulaire. En effet, il est possible que des différences alléliques au niveau de ces gènes induisent des différences de complémentarité entre racines et nodosités. Parmi les gènes impliqués dans la perception des NFs, ceux codant pour des LysM-RLKs, sont quant à eux des candidats de spécificité d'interaction entre pois et rhizobia retenus dans le projet GRaSP. En complément de l'analyse GWAS, il conviendra pour les gènes candidats retenus d'étudier la variabilité de leur séquence sur l'ensemble de la collection et d'étudier les associations avec des phénotypes d'architecture racinaire nodulée ou de choix de souches de Rlv. Le phénotypage de mutants TILLING (Targeting induced local lesions in genomes) pour ces gènes sera un complément nécessaire à la validation des gènes candidats. Concernant des gènes d'architecture racinaire nodulée, leur phénotypage pourrait bénéficier de l'utilisation de plateformes utilisant de la résonance magnétique ou de la tomographie qui permettent d'avoir accès à la structure du système racinaire (angles d'insertion des racines, nodosités) et à la répartition du C dans ce système (Metzner *et al.*, 2016).

Une fois l'intérêt des gènes candidats confirmé, il pourra être envisagé une amélioration de la nutrition azotée par une sélection facilitée par l'utilisation des marqueurs SNP. Une validation au champ des innovations devra aussi être réalisée.

3. Conclusion

Par ce travail de thèse, nous avons acquis une meilleure compréhension du contrôle génétique de la mise en place des racines et des nodosités et de leur impact sur la nutrition azotée. Les démarches suivies ont nécessité l'étude d'une grande variabilité de ressources génétiques et une approche pluridisciplinaire du phénotypage comportant des descriptions des structures et de leur fonctionnement prenant en compte le partenaire bactérien.

Une grande variabilité génétique pour les caractères d'architecture racinaire ou nodulaire et ceux d'acquisition d'N a ainsi été mise en évidence, ainsi que l'existence de corrélations génétiques entre eux. Une approche de génétique quantitative a permis d'identifier des zones du génome pouvant être impliquées dans les variations des caractères. Deux pistes d'amélioration de la nutrition azotée ont aussi été étudiées : l'amélioration de l'acquisition d'N par les racines à partir d'une étude détaillée d'un mutant de développement racinaire, puis l'amélioration de la symbiose avec les rhizobia en examinant la question de la capacité des géotypes de pois à favoriser les associations symbiotiques avec les souches de rhizobium les plus performantes.

Les résultats obtenus apportent des bases de réflexion concernant la conception d'un idéotype de nutrition azotée. Au-delà de la complémentarité indispensable entre les deux voies d'acquisition d'azote, il est indispensable de prendre en compte l'interaction entre les deux partenaires symbiotiques. Il reste encore un grand champ de recherche pour l'optimisation de ce mécanisme symbiotique complexe, qui met en jeu la formation des nodosités et leur fonctionnement, en lien avec une signalétique et des interactions trophiques complexes entre partenaires symbiotiques et intra-plante. Différents champs d'innovation peuvent être envisagés : combinaisons variétés x inoculum bactérien, variétés capables de sélectionner dans les sols les rhizobia les plus efficaces ou inocula efficaces et à large gamme d'hôtes. Dans tous les cas, il conviendra de vérifier au champ si cette innovation apporte un meilleur rendement et une meilleure tolérance à des stress biotiques et abiotiques.

REFERENCES

BIBLIOGRAPHIQUES

RÉFÉRENCES BIBLIOGRAPHIQUES

- Achard P, Vriezen WH, Van Der Straeten D, Harberd NP. 2003.** Ethylene regulates Arabidopsis development via the modulation of DELLA protein growth repressor function. *Plant Cell* **15**(12): 2816-2825.
- Acikgoz E. 1982.** Cold tolerance and its association with seedling morphology and chemical composition in annual forage legumes. I. Field peas (*Pisum sativum* ssp. *arvense* L. Poir.) *Zeitschrift Fur Pflanzenzuchtung-Journal of Plant Breeding* **88**(2): 118-126.
- Ali-Khan ST, Snoad B. 1977.** Root and shoot development in peas. *Annals of Applied Biology* **85**(1): 131-136.
- Alves-Carvalho S, Aubert G, Carrère S, Cruaud C, Brochot A-L, Jacquin F, Klein A, Martin C, Boucherot K, Kreplak J. 2015.** Full-length de novo assembly of RNA-seq data in pea (*Pisum sativum* L.) provides a gene expression atlas and gives insights into root nodulation in this species. *The plant journal* **84**(1): 1-19.
- Amarger N. 1981.** Competition for nodule formation between effective and ineffective strains of *Rhizobium meliloti*. *Soil Biology & Biochemistry* **13**(6): 475-480.
- Amarger N, Lobreau JP. 1982.** Quantitative study of nodulation competitiveness in *Rhizobium* strains. *Applied and Environmental Microbiology* **44**(3): 583-588.
- Amey RC, Schleicher T, Slinn J, Lewis M, Macdonald H, Neill SJ, Spencer-Phillips PTN. 2008.** Proteomic analysis of a compatible interaction between *Pisum sativum* (pea) and the downy mildew pathogen *Peronospora viciae*. *European Journal of Plant Pathology* **122**(1): 41-55.
- Andriankaja A, Boisson-Demier A, Frances L, Sauviac L, Jauneau A, Barker DG, de Carvalho-Niebel F. 2007.** AP2-ERF transcription factors mediate nod factor-dependent mt ENOD11 activation in root hairs via a novel cis-regulatory motif. *Plant Cell* **19**(9): 2866-2885.
- Ané JM, Kiss GB, Riely BK, Penmetsa RV, Oldroyd GED, Ayax C, Lévy J, Debellé F, Baek JM, Kalo P, Rosenberg C, Roe BA, Long SR, Dénarié J, Cook DR. 2004.** *Medicago truncatula* *DMII* required for bacterial and fungal symbioses in legumes. *Science* **303**(5662): 1364-1367.
- Antolín-Llovera M, Ried Martina K, Parniske M. 2014.** Cleavage of the SYMBIOSIS RECEPTOR-LIKE KINASE Ectodomain Promotes Complex Formation with Nod Factor Receptor 5. *Current Biology* **24**(4): 422-427.
- Appleby CA. 1984.** Leghemoglobin and *Rhizobium* respiration. *Annual review of plant physiology* **35**(1): 443-478.
- Aubert G, Morin J, Jacquin F, Loridon K, Quillet MC, Petit A, Rameau C, Lejeune-Hénaut I, Huguet T, Burstin J. 2006.** Functional mapping in pea, as an aid to the candidate gene selection and for investigating synteny with the model legume *Medicago truncatula*. *Theoretical and Applied Genetics* **112**(6): 1024-1041.
- Auld D, Murray G, O'Keeffe L, Campbell A, Markarian D. 1978.** Registration of Melrose Field Pea (Reg. No. 11). *Crop Science* **18**(5): 913-913.
- Auld DL, Adams KJ, Swensen JB, Murray GA. 1983.** Diallel analyses of winterhardiness in peas. *Crop Science* **23**(4): 763-766.
- Badenoch-Jones J, Parker CW, Letham DS. 1987.** Phytohormones, *Rhizobium* mutants, and nodulation in legumes. VII. Identification and quantification of cytokinins in effective and ineffective pea root nodules using radioimmunoassay. *Journal of Plant Growth Regulation* **6**(2): 97-111.
- Bagchi R, Salehin M, Adeyemo OS, Salazar C, Shulaev V, Sherrier DJ, Dickstein R. 2012.** Functional assessment of the *Medicago truncatula* NIP/LATD protein

- demonstrates that it is a high-affinity nitrate transporter. *Plant Physiology* **160**(2): 906-916.
- Bailly X, Olivieri I, Brunel B, Cleyet-Marel J-C, Béna G. 2007.** Horizontal gene transfer and homologous recombination drive the evolution of the nitrogen-fixing symbionts of *Medicago* species. *Journal of Bacteriology* **189**(14): 5223-5236.
- Baldwin I, Fred E. 1929.** Nomenclature of the root-nodule bacteria of the Leguminosae. *Journal of Bacteriology* **17**(2): 141.
- Baranger A, Aubert G, Arnau G, Laine AL, Deniot G, Potier J, Weinachter C, Lejeune-Henaut I, Lallemand J, Burstin J. 2004.** Genetic diversity within *Pisum sativum* using protein- and PCR-based markers. *Theoretical and Applied Genetics* **108**(7): 1309-1321.
- Barbulova A, Rogato A, D'Apuzzo E, Omrane S, Chiurazzi M. 2007.** Differential effects of combined N sources on early steps of the nod factor-dependent transduction pathway in *Lotus japonicus*. *Molecular Plant-Microbe Interactions* **20**(8): 994-1003.
- Barker DG, Pfaff T, Moreau D, Groves E, Ruffel S, Lepetit M, Whitehand S, Maillet F, Nair RM, Journet EP 2006.** Growing *M. truncatula*: choice of substrates and growth conditions. In Mathesius U, Journet EP, Sumner LW. *The Medicago truncatula handbook*.
- Barrett T, Troup DB, Wilhite SE, Ledoux P, Rudnev D, Evangelista C, Kim IF, Soboleva A, Tomashevsky M, Edgar R. 2007.** NCBI GEO: mining tens of millions of expression profiles--database and tools update. *Nucleic Acids Res* **35**(Database issue): D760-765.
- Beemster GTS, Baskin TI. 2000.** STUNTED PLANT 1 mediates effects of cytokinin, but not of auxin, on cell division and expansion in the root of *Arabidopsis*. *Plant Physiology* **124**(4): 1718-1727.
- Beijerinck MW. 1888.** Cultur des *Bacillus radicolica* aus den Knöllchen. *Botanische Zeitung* **46**: 46.
- Ben Ze'ev N, Zohary D. 1973.** Species relationships in the genus *Pisum* L. *Israel J. Bot* **22**(2): 73-91.
- Benkova E, Hejatko J. 2009.** Hormone interactions at the root apical meristem. *Plant Molecular Biology* **69**(4): 383-396.
- Bi YM, Wang RL, Zhu T, Rothstein SJ. 2007.** Global transcription profiling reveals differential responses to chronic nitrogen stress and putative nitrogen regulatory components in *Arabidopsis*. *Bmc Genomics* **8**.
- Black M, Moolhuijzen P, Chapman B, Barrero R, Howieson J, Hungria M, Bellgard M. 2012.** The genetics of symbiotic nitrogen fixation: comparative genomics of 14 rhizobia strains by resolution of protein clusters. *Genes* **3**(1): 138-166.
- Bloemberg GV, Kamst E, Harteveld M, Vanderdrift K, Haverkamp J, Thomasoates JE, Lugtenberg BJJ, Spaink HP. 1995.** A central domain of *Rhizobium* node protein mediates host-specificity by determining the hydrophobicity of fatty acyl moieties of nodulation factors. *Molecular Microbiology* **16**(6): 1123-1136.
- Bogdanova VS, Kosterin OE, Yadrikhinskiy AK. 2014.** Wild peas vary in their cross-compatibility with cultivated pea (*Pisum sativum* subsp. *sativum* L.) depending on alleles of a nuclear-cytoplasmic incompatibility locus. *Theoretical and Applied Genetics* **127**(5): 1163-1172.
- Boisson-Dernier A, Chabaud M, Garcia F, Becard G, Rosenberg C, Barker DG. 2001.** *Agrobacterium rhizogenes*-transformed roots of *Medicago truncatula* for the study of nitrogen-fixing and endomycorrhizal symbiotic associations. *Molecular Plant-Microbe Interactions* **14**(6): 695-700.
- Borisov AY, Danilova TN, Koroleva TA, Kuznetsova EV, Madsen L, Mofett M, Naumkina TS, Nemankin TA, Ovchinnikova ES, Pavlova ZB, Petrova NE, Pinaev AG, Radutoiu S, Rozov SM, Rychagova TS, Shtark OY, Solovov, II,**

- Stougaard J, Tikhonovich IA, Topunov AF, Tsyganov VE, Vasil'chikov AG, Voroshilova VA, Weeden NF, Zhernakov AI, Zhukov VA. 2007.** Regulatory genes of garden pea (*Pisum sativum* L.) controlling the development of nitrogen-fixing nodules and arbuscular mycorrhiza: A review of basic and applied aspects. *Applied Biochemistry and Microbiology* **43**(3): 237-243.
- Bos L, van der Want JPH. 1962.** Early browning of pea, a disease caused by a soil- and seed-borne virus. *Tijdschrift Over Plantenziekten* **68**(6): 368-390.
- Bourion V, Laguerre G, Depret G, Voisin AS, Salon C, Duc G. 2007.** Genetic variability in nodulation and root growth affects nitrogen fixation and accumulation in pea. *Annals of Botany* **100**(3): 589-598.
- Bourion V, Martin C, de Larambergue H, Jacquin F, Aubert G, Martin-Magniette ML, Balzergue S, Lescure G, Citerne S, Lepetit M, Munier-Jolain N, Salon C, Duc G. 2014.** Unexpectedly low nitrogen acquisition and absence of root architecture adaptation to nitrate supply in a *Medicago truncatula* highly branched root mutant. *Journal of Experimental Botany* **65**(9): 2365-2380.
- Bourion V, Rizvi SMH, Fournier S, de Larambergue H, Galmiche F, Marget P, Duc G, Burstin J. 2010.** Genetic dissection of nitrogen nutrition in pea through a QTL approach of root, nodule, and shoot variability. *Theoretical and Applied Genetics* **121**(1): 71-86.
- Bouwman AF, Beusen AHW, Griffioen J, Van Groenigen JW, Hefting MM, Oenema O, Van Puijenbroek P, Seitzinger S, Slomp CP, Stehfest E. 2013.** Global trends and uncertainties in terrestrial denitrification and N₂O emissions. *Philosophical Transactions of the Royal Society B-Biological Sciences* **368**(1621).
- Bremer E, Rennie RJ, Rennie DA. 1988.** Dinitrogen fixation of lentil, field pea and fababean under drylands conditions. *Canadian Journal of Soil Science* **68**(3): 553-562.
- Brewin N, Beringer J, Johnston A. 1980.** Plasmid-mediated transfer of host-range specificity between two strains of *Rhizobium leguminosarum*. *Microbiology* **120**(2): 413-420.
- Bright LJ, Liang Y, Mitchell DM, Harris JM. 2005.** The *LATD* gene of *Medicago truncatula* is required for both nodule and root development. *Molecular Plant-Microbe Interactions* **18**(6): 521-532.
- Brun F, Richard-Molard C, Pagès L, Chelle M, Ney B. 2010.** To what extent may changes in the root system architecture of *Arabidopsis thaliana* grown under contrasted homogenous nitrogen regimes be explained by changes in carbon supply? A modelling approach. *Journal of Experimental Botany* **61**(8): 2157-2169.
- Burstin J, Marget P, Huart M, Moessner A, Mangin B, Duchene C, Desprez B, Munier-Jolain N, Duc G. 2007.** Developmental genes have pleiotropic effects on plant morphology and source capacity, eventually impacting on seed protein content and productivity in pea. *Plant Physiology* **144**(2): 768-781.
- Burstin J, Salloignon P, Chabert-Martinello M, Magnin-Robert JB, Siol M, Jacquin F, Chauveau A, Pont C, Aubert G, Delaitre C, Truntzer C, Duc G. 2015.** Genetic diversity and trait genomic prediction in a pea diversity panel. *Bmc Genomics* **16**.
- Buyse L, Callebaut A, Oostveldt Pv, Parijs Rv. 1983.** The GA 3 effect on dwarf peas is clearly temperature and variety dependent. *Mededelingen van de Faculteit Landbouwwetenschappen, Rijksuniversiteit Gent* **48**(4): 1119-1127.
- Caetano-Anolles G, Gresshoff PM. 1991.** Plant Genetic-Control of Nodulation. *Annual Review of Microbiology* **45**: 345-382.
- Carroll BJ, Mathews A 1990.** Nitrate inhibition of nodulation in legumes. In: Gresshoff PM ed. *Molecular biology of symbiotic nitrogen fixation*. Boca Raton: CRC Press, 159-180.

- Catoira R, Galera C, de Billy F, Penmetsa RV, Journet EP, Maillet F, Rosenberg C, Cook D, Gough C, Dénarié J. 2000.** Four genes of *Medicago truncatula* controlling components of a nod factor transduction pathway. *Plant Cell* **12**(9): 1647-1665.
- Cellier P, Schneider A, Thiébeau P, Vertès F 2015.** Impacts environnementaux de l'introduction des légumineuses dans les systèmes de production. *Les légumineuses pour des systèmes agricoles et alimentaires durables* QUAE, 297-338.
- Chabaud M, Larssonaud C, Marmouget C, Huguet T. 1996.** Transformation of Barrel Medic (*Medicago truncatula* Gaertn.) by *Agrobacterium tumefaciens* and regeneration *via* somatic embryogenesis of transgenic plants with the *MtENOD12* nodulin promoter fused to the *gus* reporter gene. *Plant Cell Report* **15**: 305 - 310.
- Charcosset A, Gallais A. 1996.** Estimation of the contribution of quantitative trait loci (QTL) to the variance of a quantitative trait by means of genetic markers. *Theoretical and Applied Genetics* **93**(8): 1193-1201.
- Charpentier M, Bredemeier R, Wanner G, Takeda N, Schleiff E, Parniske M. 2008.** *Lotus japonicus* CASTOR and POLLUX Are Ion Channels Essential for Perinuclear Calcium Spiking in Legume Root Endosymbiosis. *The Plant cell* **20**(12): 3467-3479.
- Charpentier M, Sun J, Martins TV, Radhakrishnan GV, Findlay K, Soumpourou E, Thouin J, Véry A-A, Sanders D, Morris RJ, Oldroyd GED. 2016.** Nuclear-localized cyclic nucleotide-gated channels mediate symbiotic calcium oscillations. *Science* **352**(6289): 1102-1105.
- Chen X-C, Feng J, Hou B-H, Li F-Q, Li Q, Hong G-F. 2005.** Modulating DNA bending affects NodD-mediated transcriptional control in *Rhizobium leguminosarum*. *Nucleic Acids Research* **33**(8): 2540-2548.
- Chimwamurombe P, Khulbe R. 2011.** 1 History, origin and evolution. *Biology and Breeding of Food Legumes*: 1-18.
- Choi H-K, Mun J-H, Kim D-J, Zhu H, Baek J-M, Mudge J, Roe B, Ellis N, Doyle J, Kiss GB. 2004.** Estimating genome conservation between crop and model legume species. *Proceedings of the National Academy of Sciences of the United States of America* **101**(43): 15289-15294.
- Clark JSC, Spencer-Phillips PTN. 1994.** Resistance to *Peronospora viciae* expressed as differential colony growth in two cultivars of *Pisum sativum*. *Plant Pathology* **43**(1): 56-64.
- Clemow SR, Clairmont L, Madsen LH, Guinel FC. 2011.** Reproducible hairy root transformation and spot-inoculation methods to study root symbioses of pea. *Plant Methods* **7**(1): 1.
- Coque M, Gallais A. 2006.** Genomic regions involved in response to grain yield selection at high and low nitrogen fertilization in maize. *Theoretical and Applied Genetics* **112**(7): 1205-1220.
- Corre-Hellou G, Crozat Y. 2005.** N₂ fixation and N supply in organic pea (*Pisum sativum* L.) cropping systems as affected by weeds and pea weevil (*Sitona lineatus* L.). *European Journal of Agronomy* **22**(4): 449-458.
- Courty PE, Smith P, Koegel S, Redecker D, Wipf D. 2015.** Inorganic Nitrogen Uptake and Transport in Beneficial Plant Root-Microbe Interactions. *Critical Reviews in Plant Sciences* **34**(1-3): 4-16.
- Cousin R. 1976.** Amélioration génétique du pois d'hiver. *Annales De L'Amelioration Des Plantes* **26**(2): 235-263.
- Cousin R. 1997.** Peas (*Pisum sativum* L.). *Field Crops Research* **53**(1-3): 111-130.
- Crozat Y, Aveline A, Coste F, Gillet JP, Domenach AM. 1994.** Yield performance and seed production pattern of field-grown pea and soybean in relation to N nutrition. *European Journal of Agronomy* **3**(2): 135-144.
- D'Apuzzo E, Rogato A, Simon-Rosin U, El Alaoui H, Barbulova A, Betti M, Dimou M, Katinakis P, Marquez A, Marini AM, Udvardi MK, Chiurazzi M. 2004.**

- Characterization of three functional high-affinity ammonium transporters in *Lotus japonicus* with differential transcriptional regulation spatial expression. *Plant Physiology* **134**(4): 1763-1774.
- D'Haese W, Holsters M. 2002.** Nod factor structures, responses, and perception during initiation of nodule development. *Glycobiology* **12**(6): 79R-105R.
- Dangeard P-AC. 1926.** *Recherches sur les tubercules radicaux des légumineuses*: Impr. Jouve et cie.
- Davis EO, Evans IJ, Johnston AWB. 1988.** Identification of *nodX*, a gene that allows *Rhizobium leguminosarum* biovar *viciae* strain TOM to nodulate Afghanistan peas. *Molecular & General Genetics* **212**(3): 531-535.
- de Billy F, Grosjean C, May S, Bennett M, Cullimore JV. 2001.** Expression studies on *AUX1*-like genes in *Medicago truncatula* suggest that auxin is required at two steps in early nodule development. *Molecular Plant-Microbe Interactions* **14**(3): 267-277.
- De Smet I, Tetsumura T, De Rybel B, Frey NFD, Laplaze L, Casimiro I, Swarup R, Naudts M, Vanneste S, Audenaert D, Inzé D, Bennett MJ, Beeckman T. 2007.** Auxin-dependent regulation of lateral root positioning in the basal meristem of *Arabidopsis*. *Development* **134**(4): 681-690.
- Debelle F, Moulin L, Mangin B, Denarie J, Boivin C. 2001.** nod Genes and Nod signals and the evolution of the rhizobium legume symbiosis. *Acta Biochimica Polonica* **48**(2): 359-365.
- Delory BM, Baudson C, Brostaux Y, Lobet G, du Jardin P, Pagès L, Delaplace P. 2016.** archiDART: an R package for the automated computation of plant root architectural traits. *Plant and Soil* **398**(1-2): 351-365.
- Dénarié J, Cullimore J. 1993.** Lipo-Oligosaccharide Nodulation Factors - a Minireview New Class of Signaling Molecules Mediating Recognition and Morphogenesis. *Cell* **74**(6): 951-954.
- Denarie J, Debelle F, Prome JC. 1996.** Rhizobium lipo-chitooligosaccharide nodulation factors: Signaling molecules mediating recognition and morphogenesis. *Annual Review of Biochemistry* **65**: 503-535.
- Denarie J, Debelle F, Rosenberg C. 1992.** Signaling and host range variation in nodulation. *Annual Review of Microbiology* **46**: 497-531.
- Denison RF. 2000.** Legume sanctions and the evolution of symbiotic cooperation by rhizobia. *American Naturalist* **156**(6): 567-576.
- Denison RF, Hunt S, Layzell DB. 1992.** Nitrogenase activity, nodule respiration, and O₂ permeability following detopping of alfalfa and birdsfoot trefoil. *Plant Physiology* **98**(3): 894-900.
- Depret G. 2008.** *Importance de la variabilité génétique bactérienne sur le fonctionnement de la symbiose Rhizobium leguminosarum biovar viciae avec le pois (Pisum sativum L.)*. Dijon.
- Depret G, Houot S, Allard MR, Breuil MC, Nouaim R, Laguerre G. 2004.** Long-term effects of crop management on *Rhizobium leguminosarum* biovar *viciae* populations. *Fems Microbiology Ecology* **51**(1): 87-97.
- Depret G, Laguerre G. 2008.** Plant phenology and genetic variability in root and nodule development strongly influence genetic structuring of *Rhizobium leguminosarum* biovar *viciae* populations nodulating pea. *New Phytologist* **179**(1): 224-235.
- Desgroux A. 2016.** *Rôle de l'architecture racinaire dans le contrôle génétique de la diminution des symptômes de pourriture racinaire dus à Aphanomyces euteiches chez le pois (Pisum sativum)*. Rennes.
- Ding H, Hynes MF. 2009.** Plasmid transfer systems in the rhizobia. *Canadian Journal of Microbiology* **55**(8): 917-927.

- Djordjevic MA, Mohd-Radzman NA, Imin N. 2015.** Small-peptide signals that control root nodule number, development, and symbiosis. *Journal of Experimental Botany* **66**(17): 5171-5181.
- Doré C, Varoquaux F. 2006.** *Le pois. Histoire et amélioration de cinquante plantes cultivées*: Editions Quae.
- Doré T, Meynard JM, Sebillotte M. 1998.** The role of grain number, nitrogen nutrition and stem number in limiting pea crop (*Pisum sativum*) yields under agricultural conditions. *European Journal of Agronomy* **8**(1): 29-37.
- Downie JA. 2010.** The roles of extracellular proteins, polysaccharides and signals in the interactions of rhizobia with legume roots. *Fems Microbiology Reviews* **34**(2): 150-170.
- Duc G, Agrama H, Bao SY, Berger J, Bourion V, De Ron AM, Gowda CLL, Mikic A, Millot D, Singh KB, Tullu A, Vandenberg A, Patto MCV, Warkentin TD, Zong XX. 2015.** Breeding Annual Grain Legumes for Sustainable Agriculture: New Methods to Approach Complex Traits and Target New Cultivar Ideotypes. *Critical Reviews in Plant Sciences* **34**(1-3): 381-411.
- Duc G, Mariotti A, Amarger N. 1988.** Measurements of genetic variability for symbiotic dinitrogen fixation in field-grown fababean (*Vicia faba* L) using a low-level ¹⁵N-tracer technique. *Plant and Soil* **106**(2): 269-276.
- Dumont E, Fontaine V, Vuylsteker C, Sellier H, Bodèle S, Voedts N, Devaux R, Frise M, Avia K, Hilbert J-L, Bahrman N, Hanocq E, Lejeune-Hénaut I, Delbreil B. 2009.** Association of sugar content QTL and PQL with physiological traits relevant to frost damage resistance in pea under field and controlled conditions. *Theoretical and Applied Genetics* **118**(8): 1561-1571.
- Dusschoten D, Metzner R, Kochs J, Postma JA, Pflugfelder D, Buehler J, Schurr U, Jahnke S. 2016.** Quantitative 3D analysis of plant roots growing in soil using magnetic resonance imaging. *Plant Phys* **170**.
- Eckhardt MM, Baldwin I, Fred E. 1931.** Studies of the root-nodule organism of *Lupinus*. *Journal of Bacteriology* **21**(4): 273.
- Edwards A, Heckmann AB, Yousafzai F, Duc G, Downie JA. 2007.** Structural implications of mutations in the pea *SYM8* symbiosis gene, the *DMII* ortholog, encoding a predicted ion channel. *Molecular Plant-Microbe Interactions* **20**(10): 1183-1191.
- Ellis THN, Poyser SJ, Knox MR, Vershinin AV, Ambrose MJ. 1998.** Polymorphism of insertion sites of Ty1-copia class retrotransposons and its use for linkage and diversity analysis in pea. *Molecular and General Genetics* **260**(1): 9-19.
- Endre G, Kereszt A, Kevei Z, Mihacea S, Kalo P, Kiss GB. 2002.** A receptor kinase gene regulating symbiotic nodule development. *Nature* **417**(6892): 962-966.
- Esseling JJ, Lhuissier FG, Emons AMC. 2003.** Nod factor-induced root hair curling: continuous polar growth towards the point of nod factor application. *Plant Physiology* **132**(4): 1982-1988.
- Eteve G, Hiroux G, Catoir JM. 1979.** Relation entre la teneur en sucres chez différentes variétés de pois et l'adaptation à l'hiver. *Annales De L Amelioration Des Plantes* **29**(5): 557-562.
- FAOSTAT 2007.** Food and Agriculture Organization website. <http://faostat.fao.org>.
- Ferguson BJ, Indrasumunar A, Hayashi S, Lin MH, Lin YH, Reid DE, Gresshoff PM. 2010.** Molecular analysis of legume nodule development and autoregulation. *Journal of Integrative Plant Biology* **52**(1): 61-76.
- Fesenko AN, Provorov NA, Orlova IF, Orlov VP, Simarov BV. 1995.** Selection of *Rhizobium leguminosarum* bv. *viciae* strains for inoculation of *Pisum sativum* L. cultivars - Analysis of symbiotic efficiency and nodulation competitiveness. *Plant and Soil* **172**(2): 189-198.

- Firmin JL, Wilson KE, Carlson RW, Davies AE, Downie JA. 1993.** Resistance to nodulation of Cv Afghanistan peas is overcome by nodX, which mediates an O-acetylation of the *Rhizobium leguminosarum* lipo-oligosaccharide nodulation factor. *Molecular Microbiology* **10**(2): 351-360.
- Fobert P, Roy N, Nash J, Iyer V. 1991.** Procedure for obtaining efficient root nodulation of a pea cultivar by a desired *Rhizobium* strain and preempting nodulation by other strains. *Applied and Environmental Microbiology* **57**(6): 1590-1594.
- Forde BG. 2002.** Local and long-range signaling pathways regulating plant responses to nitrate. *Annual review of plant biology* **53**: 203-224.
- Fourmont R. 1956.** Les variétés de pois (*Pisum sativum* L.) cultivées en France. *INRA*: 1-253.
- Fowler D, Coyle M, Skiba U, Sutton M, Cape J, Reis S, Sheppard L, Jenkins A, Grizzetti B, Galloway J, Vitousek P, Leach A, Bouwman A, Butterbach-Bahl K, Dentener F, Stevenson D, Amann M, Voss M. 2013.** The global nitrogen cycle in the twenty-first century. *Philos Trans R Soc Lond B Biol Sci* **368**: 20130164.
- Frank B. 1889.** Ueber die Pilzsymbiose der Leguminosen. *Berichte der Deutschen Botanischen Gesellschaft* **7**(8): 332-346.
- Fraser CM, Chapple C. 2011.** The phenylpropanoid pathway in Arabidopsis. *The Arabidopsis book / American Society of Plant Biologists* **9**: e0152-e0152.
- Friesen ML, Heath KD. 2013.** One hundred years of solitude: integrating single-strain inoculations with community perspectives in the legume-rhizobium symbiosis. *New Phytologist* **198**(1): 7-9.
- Frugier F, Kosuta S, Murray JD, Crespi M, Szczyglowski K. 2008.** Cytokinin: secret agent of symbiosis. *Trends in Plant Science* **13**(3): 115-120.
- Fu XD, Harberd NP. 2003.** Auxin promotes Arabidopsis root growth by modulating gibberellin response. *Nature* **421**(6924): 740-743.
- Fukushima RS, Hatfield RD. 2001.** Extraction and isolation of lignin for utilization as a standard to determine lignin concentration using the acetyl bromide spectrophotometric method. *Journal of Agricultural and Food Chemistry* **49**(7): 3133-3139.
- Gage DJ. 2004.** Infection and invasion of roots by symbiotic, nitrogen-fixing rhizobia during nodulation of temperate legumes. *Microbiol Mol Biol Rev* **68**.
- Gagnot S, Tamby JP, Martin-Magniette ML, Bitton F, Taconnat L, Balzergue S, Aubourg S, Renou JP, Lecharny A, Brunaud V. 2008.** CATdb: a public access to Arabidopsis transcriptome data from the URGV-CATMA platform. *Nucleic Acids Research* **36**: D986-D990.
- Galloway JN, Townsend AR, Erismann JW, Bekunda M, Cai Z, Freney JR, Martinelli LA, Seitzinger SP, Sutton MA. 2008.** Transformation of the Nitrogen Cycle: Recent Trends, Questions, and Potential Solutions. *Science* **320**(5878): 889-892.
- Garcia J, Barker DG, Journet EP. 2006.** Seed storage and germination. In Mathesius U, Journet EP, Sumner LW. *The Medicago truncatula handbook*.
- Gaworzewska ET, Carlile MJ. 1982.** Positive Chemotaxis of *Rhizobium leguminosarum* and other Bacteria towards Root Exudates from Legumes and other Plants. *Microbiology* **128**(6): 1179-1188.
- Ge YC, Dudoit S, Speed TP. 2003.** Resampling-based multiple testing for microarray data analysis. *Test* **12**(1): 1-77.
- Gentleman R, Carey V. 2002.** Bioconductor. *R News* **2**(1): 11-16.
- Geurts R, Heidstra R, Hadri AE, Downie JA, Franssen H, vanKammen A, Bisseling T. 1997.** Sym2 of pea is involved in a nodulation factor-perception mechanism that controls the infection process in the epidermis. *Plant Physiology* **115**(2): 351-359.
- Geurts R, Xiao TT, Reinhold-Hurek B. 2016.** What Does It Take to Evolve A Nitrogen-Fixing Endosymbiosis? *Trends in Plant Science* **21**(3): 199-208.

- Gifford ML, Dean A, Gutierrez RA, Coruzzi GM, Birnbaum KD. 2008.** Cell-specific nitrogen responses mediate developmental plasticity. *Proceedings of the National Academy of Sciences of the United States of America* **105**(2): 803-808.
- Giraud E, Moulin L, Vallenet D, Barbe V, Cytryn E, Avarre J-C, Jaubert M, Simon D, Cartieaux F, Prin Y, Bena G, Hannibal L, Fardoux J, Kojadinovic M, Vuillet L, Lajus A, Cruveiller S, Rouy Z, Mangenot S, Segurens B, Dossat C, Franck WL, Chang W-S, Saunders E, Bruce D, Richardson P, Normand P, Dreyfus B, Pignol D, Stacey G, Emerich D, Verméglio A, Médigue C, Sadowsky M. 2007.** Legumes Symbioses: Absence of Nod Genes in Photosynthetic Bradyrhizobia. *Science* **316**(5829): 1307-1312.
- Girin T, El-Kafafi ES, Widiez T, Erban A, Hubberten HM, Kopka J, Hoefgen R, Gojon A, Lepetit M. 2010.** Identification of Arabidopsis mutants impaired in the systemic regulation of root nitrate uptake by the nitrogen status of the plant. *Plant Physiology* **153**(3): 1250-1260.
- Gojon A, Krouk G, Perrine-Walker F, Laugier E. 2011.** Nitrate transceptor(s) in plants. *Journal of Experimental Botany* **62**(7): 2299-2308.
- Gojon A, Nacry P, Davidian JC. 2009.** Root uptake regulation: a central process for NPS homeostasis in plants. *Current Opinion in Plant Biology* **12**(3): 328-338.
- Gonzalez-Rizzo S, Crespi M, Frugier F. 2006.** The *Medicago truncatula* CRE1 cytokinin receptor regulates lateral root development and early symbiotic interaction with *Sinorhizobium meliloti*. *Plant Cell* **18**(10): 2680-2693.
- Gough C, Cullimore J. 2011.** Lipo-chitooligosaccharide Signaling in Endosymbiotic Plant-Microbe Interactions. *Molecular Plant-Microbe Interactions* **24**(8): 867-878.
- Gyaneshwar P, Hirsch AM, Moulin L, Chen W-M, Elliott GN, Bontemps C, Estrada-de los Santos P, Gross E, dos Reis Jr FB, Sprent JI. 2011.** Legume-nodulating betaproteobacteria: diversity, host range, and future prospects. *Molecular Plant-Microbe Interactions* **24**(11): 1276-1288.
- Hagedorn D, Gritton E. 1973.** Inheritance of resistance to the pea seed-borne mosaic virus. *Phytopathology* **63**(9): 1130-1133.
- Hardarson G, Heichel GH, Barnes DK, Vance CP. 1982.** Rhizobial Strain Preference of Alfalfa Populations Selected for Characteristics Associated with N₂ Fixation. *Crop Science* **22**(1): 55-58.
- Heath KD, Tiffin P. 2009.** Stabilizing mechanisms in a legume–rhizobium mutualism. *Evolution* **63**(3): 652-662.
- Heidstra R, Yang WC, Yalcin Y, Peck S, Emons AM, Kammen van A, Bisseling T. 1997.** Ethylene provides positional information on cortical cell division but is not involved in Nod factor-induced root hair tip growth in *Rhizobium*-legume interaction. *Development* **124**(9): 1781-1787.
- Helz G, Baldwin I, Fred E. 1927.** Strain variations and host specificity of the root-nodule bacteria of the pea group. *J. agric. Res* **35**: 1039-1055.
- Herridge DF, Peoples MB, Boddey RM. 2008.** Global inputs of biological nitrogen fixation in agricultural systems. *Plant and Soil* **311**(1-2): 1-18.
- Hirsch AM, Fang Y, Asad S, Kapulnik Y. 1997.** The role of phytohormones in plant-microbe symbioses. *Plant and Soil* **194**(1): 171-184.
- Hogg B, Davies AE, Wilson KE, Bisseling T, Downie JA. 2002.** Competitive nodulation blocking of cv. Afghanistan pea is related to high levels of nodulation factors made by some strains of *Rhizobium leguminosarum* bv. *viciae*. *Molecular Plant-Microbe Interactions* **15**(1): 60-68.
- Holl FB. 1975.** Host plant control of the inheritance of dinitrogen fixation in the *Pisum*-*Rhizobium* symbiosis. *Euphytica* **24**(3): 767-770.

- Huault E, Laffont C, Wen J, Mysore KS, Ratet P, Duc G, Frugier F. 2014.** Local and Systemic Regulation of Plant Root System Architecture and Symbiotic Nodulation by a Receptor-Like Kinase. *PLoS genetics* **10**(12): e1004891.
- Hussey G, Gunn HV. 1984.** Plant-production in pea (*Pisum sativum* L. cvs. Puget and Upton) from long-term callus with superficial meristems. *Plant Science Letters* **37**(1-2): 143-148.
- Hwang S, Ray JD, Cregan PB, King CA, Davies MK, Purcell LC. 2014.** Genetics and mapping of quantitative traits for nodule number, weight, and size in soybean (*Glycine max* L.[Merr.]). *Euphytica* **195**(3): 419-434.
- Ikram S, Bedu M, Daniel-Vedele F, Chaillou S, Chardon F. 2012.** Natural variation of Arabidopsis response to nitrogen availability. *Journal of Experimental Botany* **63**(1): 91-105.
- Imaizumi-Anraku H, Takeda N, Charpentier M, Perry J, Miwa H, Umehara Y, Kouchi H, Murakami Y, Mulder L, Vickers K, Pike J, Allan Downie J, Wang T, Sato S, Asamizu E, Tabata S, Yoshikawa M, Murooka Y, Wu G-J, Kawaguchi M, Kawasaki S, Parniske M, Hayashi M. 2005.** Plastid proteins crucial for symbiotic fungal and bacterial entry into plant roots. *Nature* **433**(7025): 527-531.
- Imin N, Mohd-Radzman NA, Ogilvie HA, Djordjevic MA. 2013.** The peptide-encoding *CEPI* gene modulates lateral root and nodule numbers in *Medicago truncatula*. *Journal of Experimental Botany* **64**(17): 5395-5409.
- Irizarry RA, Hobbs B, Collin F, Beazer-Barclay YD, Antonellis KJ, Scherf U, Speed TP. 2003.** Exploration, normalization, and summaries of high density oligonucleotide array probe level data. *Biostatistics* **4**(2): 249-264.
- Ivanov A, Kameka A, Pajak A, Bruneau L, Beyaert R, Hernandez-Sebastia C, Marsolais F. 2012.** Arabidopsis mutants lacking asparaginases develop normally but exhibit enhanced root inhibition by exogenous asparagine. *Amino Acids* **42**(6): 2307-2318.
- Jakobsson M, Rosenberg NA. 2007.** CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics* **23**(14): 1801-1806.
- Janczarek M, Rachwał K, Marzec A, Grządziel J, Palusińska-Szyszk M. 2015.** Signal molecules and cell-surface components involved in early stages of the legume–rhizobium interactions. *Applied Soil Ecology* **85**: 94-113.
- Jarvis B, Van Berkum P, Chen W, Nour S, Fernandez M, Cleyet-Marel J, Gillis M. 1997.** Transfer of *Rhizobium loti*, *Rhizobium huakuii*, *Rhizobium ciceri*, *Rhizobium mediterraneum*, and *Rhizobium tianshanense* to *Mesorhizobium* gen. nov. *International Journal of Systematic and Evolutionary Microbiology* **47**(3): 895-898.
- Jensen ES, Hauggaard-Nielsen H. 2003.** How can increased use of biological N₂ fixation in agriculture benefit the environment? *Plant and Soil* **252**(1): 177-186.
- Jensen ES, Peoples MB, Boddey RM, Gresshoff PM, Hauggaard-Nielsen H, Alves BJR, Morrison MJ. 2012.** Legumes for mitigation of climate change and the provision of feedstock for biofuels and biorefineries. A review. *Agronomy for Sustainable Development* **32**(2): 329-364.
- Jeady C, Adrian M, Baussard C, Bernard C, Bernaud E, Bourion V, Busset H, Cabrera-Bosquet L, Cointault F, Han S, Lamboeuf M, Moreau D, Pivato B, Prudent M, Trouvelot S, Truong HN, Vernoud V, Voisin A-S, Wipf D, Salon C. 2016.** RhizoTubes as a new tool for high throughput imaging of plant root development and architecture: test, comparison with pot grown plants and validation. *Plant Methods* **12**(1): 1-18.
- Jeady C, Ruffel S, Freixes S, Tillard P, Santoni AL, Morel S, Journet EP, Duc G, Gojon A, Lepetit M, Salon C. 2010.** Adaptation of *Medicago truncatula* to nitrogen limitation is modulated via local and systemic nodule developmental responses. *New Phytologist* **185**(3): 817-828.

- Jeuffroy M-H, Warembourg FR. 1991.** Carbon transfer and partitioning between vegetative and reproductive organs in *Pisum sativum* L. *Plant Physiology* **97**(1): 440-448.
- Jin J, Watt M, Mathesius U. 2012.** The autoregulation gene *SUNN* mediates changes in root organ formation in response to nitrogen through alteration of shoot-to-root auxin transport. *Plant Physiology* **159**(1): 489-500.
- Jing R, Vershinin A, Grzebyta J, Shaw P, Smykal P, Marshall D, Ambrose MJ, Ellis THN, Flavell AJ. 2010.** The genetic diversity and evolution of field pea (*Pisum*) studied by high throughput retrotransposon based insertion polymorphism (RBIP) marker analysis. *Bmc Evolutionary Biology* **10**(1): 44.
- Jombart T, Devillard S, Balloux F. 2010.** Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. *Bmc Genetics* **11**.
- Jordan DC. 1982.** NOTES: Transfer of *Rhizobium japonicum* Buchanan 1980 to *Bradyrhizobium* gen. nov., a Genus of Slow-Growing, Root Nodule Bacteria from Leguminous Plants. *International Journal of Systematic and Evolutionary Microbiology* **32**(1): 136-139.
- Jourjon MF, Jasson S, Marcel J, Ngom B, Mangin B. 2005.** MCQTL: multi-allelic QTL mapping in multi-cross design. *Bioinformatics* **21**(1): 128-130.
- Journet E-P, Carreau V, Gouzy J, Thoquet P, Rosenberg C, Barker D, Huguet T, Denarie J, Gamas P. 2001.** The model legume *Medicago truncatula*: recent advances and perspectives in genomics. *Oléagineux Corps Gras Lipides (France)*.
- Kawaharada Y, Kelly S, Nielsen MW, Hjuler CT, Gysel K, Muszynski A, Carlson RW, Thygesen MB, Sandal N, Asmussen MH, Vinther M, Andersen SU, Krusell L, Thirup S, Jensen KJ, Ronson CW, Blaise M, Radutoiu S, Stougaard J. 2015.** Receptor-mediated exopolysaccharide perception controls bacterial infection. *Nature* **523**(7560): 308-315.
- Kiba T, Krapp A. 2016.** Plant Nitrogen Acquisition Under Low Availability: Regulation of Uptake and Root Architecture. *Plant and Cell Physiology*.
- Kiba T, Kudo T, Kojima M, Sakakibara H. 2011.** Hormonal control of nitrogen acquisition: roles of auxin, abscisic acid, and cytokinin. *Journal of Experimental Botany* **62**(4): 1399-1409.
- Kiers ET, Hutton MG, Denison RF. 2007.** Human selection and the relaxation of legume defences against ineffective rhizobia. *Proceedings of the Royal Society B-Biological Sciences* **274**(1629): 3119-3126.
- Kiers ET, Ratcliff WC, Denison RF. 2013.** Single-strain inoculation may create spurious correlations between legume fitness and rhizobial fitness. *New Phytologist* **198**(1): 4-6.
- Kiniry JR, Jones CA, Otoole JC, Blanchet R, Cabelguenne M, Spanel DA. 1989.** Radiation-Use Efficiency in Biomass Accumulation Prior to Grain-Filling for 5 Grain-Crop Species. *Field Crops Research* **20**(1): 51-64.
- Klein A, Houtin H, Rond C, Marget P, Jacquin F, Boucherot K, Huart M, Rivière N, Boutet G, Lejeune-Hénaut I. 2014.** QTL analysis of frost damage in pea suggests different mechanisms involved in frost tolerance. *Theoretical and Applied Genetics* **127**(6): 1319-1330.
- Kobayashi H, Graven YN, Broughton WJ, Perret X. 2004.** Flavonoids induce temporal shifts in gene-expression of nod-box controlled loci in *Rhizobium* sp. NGR234. *Molecular Microbiology* **51**(2): 335-347.
- Kof EM, Vinogradova IA, Oorzhak AS, Kalibernaya ZV. 2006.** The rates of shoot and root growth in intact plants of pea mutants in leaf morphology. *Russian Journal of Plant Physiology* **53**(1): 116-125.
- Kraft JM. 1992.** Registration of 90-2079, 90-2131, and 90-2322 pea germplasms. *Crop Science* **32**(4): 1076-1076.

- Kraft JM, Boge W. 2001.** Root characteristics in pea in relation to compaction and fusarium root rot. *Plant Disease* **85**(9): 936-940.
- Krouk G, Lacombe B, Bielach A, Perrine-Walker F, Malinska K, Mounier E, Hoyerova K, Tillard P, Leon S, Ljung K, Zazimalova E, Benkova E, Nacry P, Gojon A. 2010.** Nitrate-Regulated Auxin Transport by NRT1.1 Defines a Mechanism for Nutrient Sensing in Plants. *Developmental Cell* **18**(6): 927-937.
- Krouk G, Tillard P, Gojon A. 2006.** Regulation of the high-affinity NO₃⁻ uptake system by NRT1.1-mediated NO₃⁻ demand signaling in Arabidopsis. *Plant Physiology* **142**(3): 1075-1086.
- Krusell L, Madsen LH, Sato S, Aubert G, Genua A, Szczyglowski K, Duc G, Kaneko T, Tabata S, de Bruijn F, Pajuelo E, Sandal N, Stougaard J. 2002.** Shoot control of root development and nodulation is mediated by a receptor-like kinase. *Nature* **420**(6914): 422-426.
- Kuang R-B, Liao H, Yan X-L, Dong Y-S. 2005.** Phosphorus and Nitrogen Interactions in Field-Grown Soybean as Related to Genetic Attributes of Root Morphological and Nodular Traits. *Journal of Integrative Plant Biology* **47**(5): 549-559.
- Kumar N, Lad G, Giuntini E, Kaye ME, Udomwong P, Shamsani NJ, Young JPW, Bailly X. 2015.** Bacterial genospecies that are not ecologically coherent: population genomics of *Rhizobium leguminosarum*. *Open Biology* **5**(1).
- Kumar S, Stecher G, Tamura K. 2016.** MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Molecular Biology and Evolution* **33**(7): 1870-1874.
- Laffont C, Blanchet S, Lapierre C, Brocard L, Ratet P, Crespi M, Mathesius U, Frugier F. 2010.** The Compact Root Architecture1 gene regulates lignification, flavonoid production, and polar auxin transport in *Medicago truncatula*. *Plant Physiology* **153**(4): 1597-1607.
- Laffont C, Rey T, Andre O, Novero M, Kazmierczak T, Debelle F, Bonfante P, Jacquet C, Frugier F. 2015.** The CRE1 Cytokinin Pathway Is Differentially Recruited Depending on *Medicago truncatula* Root Environments and Negatively Regulates Resistance to a Pathogen. *Plos One* **10**(1).
- Laguerre G, Depret G, Bourion V, Duc G. 2007.** *Rhizobium leguminosarum* bv. *viciae* genotypes interact with pea plants in developmental responses of nodules, roots and shoots. *New Phytologist* **176**(3): 680-690.
- Laguerre G, Heulin-Gotty K, Brunel B, Klonowska A, Le Quéré A, Tillard P, Prin Y, Cleyet-Marel J-C, Lepetit M. 2012.** Local and systemic N signaling are involved in *Medicago truncatula* preference for the most efficient *Sinorhizobium* symbiotic partners. *New Phytologist* **195**(2): 437-449.
- Laguerre G, Louvrier P, Allard MR, Amarger N. 2003.** Compatibility of rhizobial genotypes within natural populations of *Rhizobium leguminosarum* biovar *viciae* for nodulation of host legumes. *Applied and Environmental Microbiology* **69**(4): 2276-2283.
- Laguerre G, Nour SM, Macheret V, Sanjuan J, Drouin P, Amarger N. 2001.** Classification of rhizobia based on *nodC* and *nifH* gene analysis reveals a close phylogenetic relationship among *Phaseolus vulgaris* symbionts. *Microbiology* **147**(4): 981-993.
- Laperche A, Devienne-Barret F, Maury O, Le Gouis J, Ney B. 2006.** A simplified conceptual model of carbon/nitrogen functioning for QTL analysis of winter wheat adaptation to nitrogen deficiency. *Theoretical and Applied Genetics* **113**(6): 1131-1146.
- Laplaze L, Benkova E, Casimiro I, Maes L, Vanneste S, Swarup R, Weijers D, Calvo V, Parizot B, Herrera-Rodriguez MB, Offringa R, Graham N, Doumas P, Friml J, Bogusz D, Beeckman T, Bennett M. 2007.** Cytokinins act directly on lateral root founder cells to inhibit root initiation. *Plant Cell* **19**(12): 3889-3900.

- Larigauderie A, Reynolds JF, Strain BR. 1994.** Root response to CO₂ enrichment and nitrogen supply in loblolly-pine. *Plant and Soil* **165**(1): 21-32.
- LaRue T, Weeden N. 1992.** The symbiosis genes of pea. *Pisum Genet* **24**: 5-12.
- LaRue TA, Temnykh S, Weeden NF. 1996.** *sym* 18. A novel gene conditioning altered strain specificity in *Pisum sativum* cv. 'Sparkle'. *Plant and Soil* **180**(2): 191-195.
- Laus MC, Logman TJ, Lamers GE, Van Brussel AA, Carlson RW, Kijne JW. 2006.** A novel polar surface polysaccharide from *Rhizobium leguminosarum* binds host plant lectin. *Molecular Microbiology* **59**(6): 1704-1713.
- Lavin M, Herendeen PS, Wojciechowski MF. 2005.** Evolutionary rates analysis of Leguminosae implicates a rapid diversification of lineages during the tertiary. *Systematic Biology* **54**(4): 575-594.
- Lejay L, Tillard P, Lepetit M, Olive FD, Filleur S, Daniel-Vedele F, Gojon A. 1999.** Molecular and functional regulation of two NO₃⁻ uptake systems by N- and C-status of Arabidopsis plants. *Plant Journal* **18**(5): 509-519.
- Lejeune-Henaut I, Bourion V, Eteve G, Cunot E, Delhay K, Desmyter C. 1999.** Floral initiation in field-grown forage peas is delayed to a greater extent by short photoperiods, than in other types of European varieties. *Euphytica* **109**(3): 201-211.
- Lejeune-Hénaut I, Hanocq E, Béthencourt L, Fontaine V, Delbreil B, Morin J, Petit A, Devaux R, Boilleau M, Stempniak J-J, Thomas M, Lainé A-L, Foucher F, Baranger A, Burstin J, Rameau C, Giauffret C. 2008.** The flowering locus *Hr* colocalizes with a major QTL affecting winter frost tolerance in *Pisum sativum* L. *Theoretical and Applied Genetics* **116**(8): 1105-1116.
- Lévy J, Bres C, Geurts R, Chalhoub B, Kulikova O, Duc G, Journet EP, Ané JM, Lauber E, Bisseling T, Dénarié J, Rosenberg C, Debelle F. 2004.** A putative Ca²⁺ and calmodulin-dependent protein kinase required for bacterial and fungal symbioses. *Science* **303**(5662): 1361-1364.
- Lewis ME, Gritton ET. 1992.** Use of one cycle of recurrent selection per year for increasing resistance to *Aphanomyces* root rot in peas. *Journal of the American Society for Horticultural Science* **117**(4): 638-642.
- Li X, Zeng R, Liao H. 2016.** Improving crop nutrient efficiency through root architecture modifications. *Journal of Integrative Plant Biology* **58**(3): 193-202.
- Liang Y, Mitchell DM, Harris JM. 2007.** Abscisic acid rescues the root meristem defects of the *Medicago truncatula latd* mutant. *Developmental Biology* **304**(1): 297-307.
- Liang Y, Toth K, Cao YR, Tanaka K, Espinoza C, Stacey G. 2014.** Lipochitooligosaccharide recognition: an ancient story. *New Phytologist* **204**(2): 289-296.
- Lie T. 1971.** Temperature-dependent root-nodule formation in pea cv. Iran. *Plant and Soil*: 751-752.
- Lie TA. 1978.** Symbiotic specialisation in pea plants: The requirement of specific *Rhizobium* strains for peas from Afghanistan. *Annals of Applied Biology* **88**(3): 462-465.
- Lie TA. 1981.** Gene centres, a source for genetic variants in symbiotic nitrogen fixation: Host-induced ineffectivity in *Pisum sativum* ecotype fulvum. *Plant and Soil* **61**(1): 125-134.
- Lie TA. 1984.** Host Genes in *Pisum sativum* L. Conferring Resistance to European *Rhizobium leguminosarum* Strains. *Plant and Soil* **82**(3): 415-425.
- Lie TA, Goktan D, Engin M, Pijnenborg J, Anlarsal E. 1987.** Coevolution of the Legume-Rhizobium Association. *Plant and Soil* **100**(1-3): 171-181.
- Lima JE, Kojima S, Takahashi H, von Wiren N. 2010.** Ammonium Triggers Lateral Root Branching in Arabidopsis in an AMMONIUM TRANSPORTER1;3-Dependent Manner. *Plant Cell* **22**(11): 3621-3633.

- Limpens E, Franken C, Smit P, Willemse J, Bisseling T, Geurts R. 2003.** LysM domain receptor kinases regulating rhizobial Nod factor-induced infection. *Science* **302**(5645): 630-633.
- Linkohr BI, Williamson LC, Fitter AH, Leyser H. 2002.** Nitrate and phosphate availability and distribution have different effects on root system architecture of Arabidopsis. *The plant journal* **29**(6): 751-760.
- Lira Junior MdA, Lima AST, Arruda JRF, Smith DL. 2005.** Effect of root temperature on nodule development of bean, lentil and pea. *Soil Biology and Biochemistry* **37**(2): 235-239.
- Lobet G, Pagès L, Draye X. 2011.** A Novel Image-Analysis Toolbox Enabling Quantitative Analysis of Root System Architecture. *Plant Physiology* **157**(1): 29-39.
- Lockwood JL, Ballard J. 1960.** Evaluation of pea introductions for resistance to Aphanomyces and Fusarium root rots. *Quarterly Bulletin. Michigan State University Agricultural Experiment Station* **42**: 704-713.
- Long S. 1996.** Rhizobium symbiosis: nod factors in perspective. *Plant Cell* **8**: 1885 - 1898.
- Loqué D, Yuan L, Kojima S, Gojon A, Wirth J, Gazzarrini S, Ishiyama K, Takahashi H, von Wiren N. 2006.** Additive contribution of AMT1;1 and AMT1;3 to high-affinity ammonium uptake across the plasma membrane of nitrogen-deficient Arabidopsis roots. *Plant Journal* **48**(4): 522-534.
- Loridon K, McPhee K, Morin J, Dubreuil P, Pilet-Nayel ML. 2005.** Microsatellite marker polymorphism and mapping in pea (*Pisum sativum* L.). *Theor Appl Genet.* **111**.
- Loudet O, Chaillou S, Krapp A, Daniel-Vedele F. 2003.** Quantitative trait loci analysis of water and anion contents in interaction with nitrogen availability in *Arabidopsis thaliana*. *Genetics* **163**(2): 711-722.
- Lu J, Knox M, Ambrose M, Brown J, Ellis T. 1996.** Comparative analysis of genetic diversity in pea assessed by RFLP-and PCR-based methods. *Theoretical and Applied Genetics* **93**(7): 1103-1111.
- MacLean AM, Finan TM, Sadowsky MJ. 2007.** Genomes of the symbiotic nitrogen-fixing bacteria of legumes. *Plant Physiology* **144**(2): 615-622.
- Madoui MA, Labadie K, Aury JM, Coyne CJ, Warkentin T, Kreplak J, Lichtenzveig J, Dolezel J, Aubert G, Berges H, Wincker P, Burstin J 2015.** The international pea genome sequencing project: sequencing and assembly progresses. *PLANT and ANIMAL Genome XXIII*. SanDiego, CA.
- Madsen EB, Madsen LH, Radutoiu S, Olbryt M, Rakwalska M, Szczyglowski K, Sato S, Kaneko T, Tabata S, Sandal N, Stougaard J. 2003.** A receptor kinase gene of the LysM type is involved in legume perception of rhizobial signals. *Nature* **425**(6958): 637-640.
- Magori S, Kawaguchi M. 2009.** Long-distance control of nodulation: Molecules and models. *Molecules and Cells* **27**(2): 129-134.
- Maj D, Wielbo J, Marek-Kozaczuk M, Skorupska A. 2010.** Response to flavonoids as a factor influencing competitiveness and symbiotic activity of *Rhizobium leguminosarum*. *Microbiological Research* **165**(1): 50-60.
- Malamy JE. 2005.** Intrinsic and environmental response pathways that regulate root system architecture. *Plant Cell and Environment* **28**(1): 67-77.
- Malamy JE, Benfey PN. 1997.** Organization and cell differentiation in lateral roots of Arabidopsis thaliana. *Development* **124**(1): 33-44.
- Malamy JE, Ryan KS. 2001.** Environmental regulation of lateral root initiation in Arabidopsis. *Plant Physiology* **127**(3): 899-909.
- Malkov N, Fliegmann J, Rosenberg C, Gascioli V, Timmers AC, Nurisso A, Cullimore J, Bono J-J. 2016.** Molecular Basis of Lipo-chitoooligosaccharide Recognition by the Lysin Motif Receptor-like Kinase LYR3 in Legumes. *Biochemical Journal*: BCJ20160073.

- Mandy G. 1974.** Fodder pea "Iregi Sarga". *Acta Agronomica Academiae Scientiarum Hungaricae* **23**(1/2): 204-205.
- Marchant A, Bhalerao R, Casimiro I, Eklof J, Casero PJ, Bennett M, Sandberg G. 2002.** AUX1 promotes lateral root formation by facilitating indole-3-acetic acid distribution between sink and source tissues in the Arabidopsis seedling. *Plant Cell* **14**(3): 589-597.
- Martin-Sanz A, Caminero C, Jing R, Flavell A, de la Vega MP. 2011.** Genetic diversity among Spanish pea (*Pisum sativum* L.) landraces, pea cultivars and the world *Pisum* sp. core collection assessed by retrotransposon-based insertion polymorphisms (RBIPs). *Spanish Journal of Agricultural Research* **9**(1): 166-178.
- Masson-Boivin C, Giraud E, Perret X, Batut J. 2009.** Establishing nitrogen-fixing symbiosis with legumes: how many rhizobium recipes? *Trends in Microbiology* **17**(10): 458-466.
- Mathesius U. 2008.** Auxin: at the root of nodule development? *Functional Plant Biology* **35**(8): 651-668.
- McKenzie RH, Middleton AB, Solberg ED, DeMulder J, Flore N, Clayton GW, Bremer E. 2001.** Response of pea to rhizobia inoculation and starter nitrogen in Alberta. *Canadian Journal of Plant Science* **81**(4): 637-643.
- McPhee K. 2005.** Variation for seedling root architecture in the core collection of pea germplasm. *Crop Science* **45**(5): 1758-1763.
- Meade J, Higgins P, Ogara F. 1985.** Studies on the inoculation and competitiveness of a *Rhizobium leguminosarum* strain in soils containing indigenous rhizobia. *Applied and Environmental Microbiology* **49**(4): 899-903.
- Melchinger A, Chandra S, Utz H, eds. 2000.** *New ways in quantitative genetics: results of QTL analyses revisited by cross validation and bootstrapping*. Les Colloques. Paris (France).
- Metzner R, Chubek A, Dautzenberg M, Breuer E, Windt C, Pflugfelder D, Jahnke S. 2016.** In vivo monitoring of the development of legume root system, nodule and pod development. *ILS2 conference. Troia, Portugal. October 12-14, 2016*: 194.
- Mikic A, Medovic A, Jovanovic Z, Stanisavljevic N. 2014a.** Integrating archaeobotany, paleogenetics and historical linguistics may cast more light onto crop domestication: the case of pea (*Pisum sativum*). *Genetic Resources and Crop Evolution* **61**(5): 887-892.
- Mikic A, Smykal P, Kenicer G, Vishnyakova M, Sarukhanyan N, Akopian JA, Vanyan A, Gabrielyan I, Smykalova I, Sherbakova E, Zoric L, Atlagic J, Zeremski-Skoric T, Cupina B, Krstic D, Jajic I, Antanasovic S, Dordevic V, Mihailovic V, Ivanov A, Ochatt S, Toker C, Zlatkovic B, Ambrose M. 2014b.** Beauty will save the world, but will the world save beauty? The case of the highly endangered *Vavilovia formosa* (Stev.) Fed. *Planta* **240**(5): 1139-1146.
- Mohd-Radzman NA, Laffont C, Ivanovici A, Patel N, Reid DE, Stougaard J, Frugier F, Imin N, Djordjevic MA. 2016.** Different pathways act downstream of the peptide receptor CRA2 to regulate lateral root and nodule development. *Plant Physiology*.
- Moreau D, Burstin J, Aubert G, Huguet T, Ben C, Prospero JM, Salon C, Munier-Jolain N. 2012.** Using a physiological framework for improving the detection of quantitative trait loci related to nitrogen nutrition in *Medicago truncatula*. *Theoretical and Applied Genetics* **124**(4): 755-768.
- Moreau D, Salon C, Munier-Jolain N. 2007.** A model-based framework for the phenotypic characterization of the flowering of *Medicago truncatula*. *Plant Cell and Environment* **30**(2): 213-224.
- Moreau D, Schneider C, Huguet T, Salon C, Munier-Jolain N. 2009.** Can differences of nitrogen nutrition level among *Medicago truncatula* genotypes be assessed non-

- destructively? Probing with a recombinant inbred lines population: Probing with a recombinant inbred lines population. *Plant Signaling & Behavior* **4**(1): 30-32.
- Moreau D, Voisin AS, Salon C, Munier-Jolain N. 2008.** The model symbiotic association between *Medicago truncatula* cv. Jemalong and *Rhizobium meliloti* strain 2011 leads to N-stressed plants when symbiotic N₂ fixation is the main N source for plant growth. *Journal of Experimental Botany* **59**(13): 3509-3522.
- Morère-Le Paven M-C, Viau L, Hamon A, Vandecasteele C, Pellizzaro A, Bourdin C, Laffont C, Lapied B, Lepetit M, Frugier F. 2011.** Characterization of a dual-affinity nitrate transporter MtNRT1.3 in the model legume *Medicago truncatula*. *Journal of Experimental Botany* **62**(15): 5595-5605.
- Mortier V, Den Herder G, Whitford R, Van de Velde W, Rombauts S, D'haeseleer K, Holsters M, Goormachtig S. 2010.** CLE Peptides Control *Medicago truncatula* Nodulation Locally and Systemically. *Plant Physiology* **153**(1): 222-237.
- Moulin L, Munive A, Dreyfus B, Boivin-Masson C. 2001.** Nodulation of legumes by members of the [beta]-subclass of Proteobacteria. *Nature* **411**(6840): 948-950.
- Muday GK, DeLong A. 2001.** Polar auxin transport: controlling where and how much. *Trends in Plant Science* **6**(11): 535-542.
- Mulligan JT, Long SR. 1985.** Induction of *Rhizobium meliloti nodC* expression by plant exudate requires *nodD*. *Proceedings of the National Academy of Sciences* **82**(19): 6609-6613.
- Munier-Jolain N, Carrouee B. 2003.** Considering pea in sustainable agriculture: agricultural and environmental arguments. *Cahiers Agricultures* **12**(2): 111-120.
- Murray GA, Swensen JB. 1991.** Winterhardiness of pea. *Pisum Genetics* **23**: 4-13.
- Mutch LA, Young JPW. 2004.** Diversity and specificity of *Rhizobium leguminosarum* biovar *viciae* on wild and cultivated legumes. *Molecular Ecology* **13**(8): 2435-2444.
- Nagel KA, Putz A, Gilmer F. 2012.** GROWSCREEN-Rhizo is a novel phenotyping robot enabling simultaneous measurements of root and shoot growth for plants grown in soil-filled rhizotrons. *Funct Plant Biol* **39**.
- Naseer S, Lee Y, Lapierre C, Franke R, Nawrath C, Geldner N. 2012.** Casparian strip diffusion barrier in Arabidopsis is made of a lignin polymer without suberin. *Proceedings of the National Academy of Sciences of the United States of America* **109**(25): 10101-10106.
- Näsholm T, Kielland K, Ganeteg U. 2009.** Uptake of organic nitrogen by plants. *New Phytologist* **182**(1): 31-48.
- Ney B, Turc O. 1993.** Heat-unit-based description of the reproductive development of pea. *Crop Science* **33**(3): 510-514.
- Ng JLP, Hassan S, Truong TT, Hocart CH, Laffont C, Frugier F, Mathesius U. 2015.** Flavonoids and Auxin Transport Inhibitors Rescue Symbiotic Nodulation in the *Medicago truncatula* Cytokinin Perception Mutant cre1. *Plant Cell* **27**(8): 2210-2226.
- Nicolas MF, Hungria M, Arias CAA. 2006.** Identification of quantitative trait loci controlling nodulation and shoot mass in progenies from two Brazilian soybean cultivars. *Field Crops Research* **95**(2-3): 355-366.
- Nishimura R, Hayashi M, Wu G-J, Kouchi H, Imaizumi-Anraku H, Murakami Y, Kawasaki S, Akao S, Ohmori M, Nagasawa M, Harada K, Kawaguchi M. 2002.** HAR1 mediates systemic regulation of symbiotic organ development. *Nature* **420**(6914): 426-429.
- Nodari RO, Tsai SM, Guzmán P, Gilbertson RL, Gepts P. 1993.** Toward an integrated linkage map of common bean. III. Mapping genetic factors controlling host-bacteria interactions. *Genetics* **134**(1): 341-350.
- Novak K, Biedermannova E, Vondryš J. 2009.** Symbiotic and growth performance of supernodulating forage pea lines. *Crop Science* **49**(4): 1227-1234.

- Novak K, Chovanec P, Skrdleta V, Kropacova M, Lisa L, Nemcova M. 2002.** Effect of exogenous flavonoids on nodulation of pea (*Pisum sativum* L.). *Journal of Experimental Botany* **53**(375): 1735-1745.
- Oka-Kira E, Kawaguchi M. 2006.** Long-distance signaling to control root nodule number. *Current Opinion in Plant Biology* **9**(5): 496-502.
- Okamoto S, Kawaguchi M. 2015.** Shoot HAR1 mediates nitrate inhibition of nodulation in *Lotus japonicus*. *Plant Signaling & Behavior* **10**(5).
- Okamoto S, Ohnishi E, Sato S, Takahashi H, Nakazono M, Tabata S, Kawaguchi M. 2009.** Nod Factor/Nitrate-Induced *CLE* Genes that Drive HAR1-Mediated Systemic Regulation of Nodulation. *Plant and Cell Physiology* **50**(1): 67-77.
- Okamoto S, Shinohara H, Mori T, Matsubayashi Y, Kawaguchi M. 2013.** Root-derived *CLE* glycopeptides control nodulation by direct binding to HAR1 receptor kinase. *Nat Commun* **4**.
- Okamoto S, Tabata R, Matsubayashi Y. 2016.** Long-distance peptide signaling essential for nutrient homeostasis in plants. *Current Opinion in Plant Biology* **34**: 35-40.
- Oldroyd GED. 2013.** Speak, friend, and enter: signalling systems that promote beneficial symbiotic associations in plants. *Nature Reviews Microbiology* **11**(4): 252-263.
- Omrane S, Ferrarini A, D'Apuzzo E, Rogato A, Delledonne M, Chiurazzi M. 2009.** Symbiotic competence in *Lotus japonicus* is affected by plant nitrogen status: transcriptomic identification of genes affected by a new signalling pathway. *New Phytologist* **183**(2): 380-394.
- Oono R, Anderson CG, Denison RF. 2011.** Failure to fix nitrogen by non-reproductive symbiotic rhizobia triggers host sanctions that reduce fitness of their reproductive clonemates. *Proceedings of the Royal Society B-Biological Sciences* **278**(1718): 2698-2703.
- Ott T, van Dongen JT, Gu C, Krusell L, Desbrosses G, Vigeolas H, Bock V, Czechowski T, Geigenberger P, Udvardi MK. 2005.** Symbiotic leghemoglobins are crucial for nitrogen fixation in legume root nodules but not for general plant growth and development. *Current Biology* **15**(6): 531-535.
- Ovtsyna AO, Rademaker GJ, Esser E, Weinman J, Rolfe BG, Tikhonovich IA, Lugtenberg BJJ, Thomas-Oates JE, Spaink HP. 1999.** Comparison of characteristics of the *nodX* genes from various *Rhizobium leguminosarum* strains. *Molecular Plant-Microbe Interactions* **12**(3): 252-258.
- Pate JS, Herridge DF. 1978.** Partitioning and Utilization of Net Photosynthate in a Nodulated Annual Legume. *Journal of Experimental Botany* **29**(2): 401-412.
- Peer WA, Bandyopadhyay A, Blakeslee JJ, Makam SI, Chen RJ, Masson PH, Murphy AS. 2004.** Variation in expression and protein localization of the PIN family of auxin efflux facilitator proteins in flavonoid mutants with altered auxin transport in *Arabidopsis thaliana*. *Plant Cell* **16**(7): 1898-1911.
- Peer WA, Murphy AS. 2007.** Flavonoids and auxin transport: modulators or regulators? *Trends in Plant Science* **12**(12): 556-563.
- Peix A, Ramirez-Bahena MH, Velazquez E, Bedmar EJ. 2015.** Bacterial Associations with Legumes. *Critical Reviews in Plant Sciences* **34**(1-3): 17-42.
- Penmetsa R, Cook D. 1997.** A legume ethylene-insensitive mutant hyperinfected by its rhizobial symbiont. *Science* **275**: 527 - 530.
- Peret B, De Rybel B, Casimiro I, Benkova E, Swarup R, Laplaze L, Beeckman T, Bennett MJ. 2009.** Arabidopsis lateral root development: an emerging story. *Trends in Plant Science* **14**(7): 399-408.
- Perret X, Staehelin C, Broughton WJ. 2000.** Molecular basis of symbiotic promiscuity. *Microbiology and Molecular Biology Reviews* **64**(1): 180-+.
- Philippot L, Raaijmakers JM, Lemanceau P, van der Putten WH. 2013.** Going back to the roots: the microbial ecology of the rhizosphere. *Nat Rev Micro* **11**(11): 789-799.

- Phillips DA. 1980.** Efficiency of symbiotic nitrogen fixation in legumes. *Annual review of plant physiology* **31**(1): 29-49.
- Pilet-Nayel ML, Muehlbauer FJ, McGee RJ, Kraft JM, Baranger A, Coyne CJ. 2002.** Quantitative trait loci for partial resistance to *Aphanomyces* root rot in pea. *Theoretical and Applied Genetics* **106**(1): 28-39.
- Plet J, Wasson A, Ariel F, Le Signor C, Baker D, Mathesius U, Crespi M, Frugier F. 2011.** MtCRE1-dependent cytokinin signaling integrates bacterial and plant cues to coordinate symbiotic nodule organogenesis in *Medicago truncatula*. *Plant Journal* **65**(4): 622-633.
- Postma JG, Jacobsen E, Feenstra WJ. 1988.** Three pea mutants with an altered nodulation studied by genetic analysis and grafting. *Journal of Plant Physiology* **132**(4): 424-430.
- Prell J, Poole P. 2006.** Metabolic changes of rhizobia in legume nodules. *Trends in Microbiology* **14**(4): 161-168.
- Prudent M, Vernoud V, Girodet S, Salon C. 2016.** How nitrogen fixation is modulated in response to different water availability levels and during recovery: A structural and functional study at the whole plant level. *Plant and Soil* **399**(1-2): 1-12.
- Radutoiu S, Madsen LH, Madsen EB, Felle HH, Umehara Y, Gronlund M, Sato S, Nakamura Y, Tabata S, Sandal N, Stougaard J. 2003.** Plant recognition of symbiotic bacteria requires two LysM receptor-like kinases. *Nature* **425**(6958): 585-592.
- Rahi P, Kapoor R, Young JPW, Gulati A. 2012.** A genetic discontinuity in root-nodulating bacteria of cultivated pea in the Indian trans-Himalayas. *Molecular Ecology* **21**(1): 145-159.
- Raj A, Stephens M, Pritchard JK. 2014.** fastSTRUCTURE: Variational Inference of Population Structure in Large SNP Data Sets. *Genetics* **197**(2): 573-U207.
- Ramaekers L, Galeano CH, Garzo'n N, Vanderleyden J, Blair MW. 2013.** Identifying quantitative trait loci for symbiotic nitrogen fixation capacity and related traits in common bean. *Mol Breeding* **31**: 163-180.
- Ramírez-Bahena MH, García-Fraile P, Peix A, Valverde A, Rivas R, Igual JM, Mateos PF, Martínez-Molina E, Velázquez E. 2008.** Revision of the taxonomic status of the species *Rhizobium leguminosarum* (Frank 1879) Frank 1889^{AL}, *Rhizobium phaseoli* Dangeard 1926^{AL} and *Rhizobium trifolii* Dangeard 1926^{AL}. *R. trifolii* is a later synonym of *R. leguminosarum*. Reclassification of the strain *R. leguminosarum* DSM 30132 (=NCIMB 11478) as *Rhizobium pisi* sp. nov. *International Journal of Systematic and Evolutionary Microbiology* **58**(11): 2484-2490.
- Redmond JW, Batley M, Innes RW, Kuempel PL, Djordjevic MA, Rolfe BG 1986.** Flavones Induce Expression of the Nodulation Genes in *Rhizobium*. In: Lugtenberg B ed. *Recognition in Microbe-Plant Symbiotic and Pathogenic Interactions*. Berlin, Heidelberg: Springer Berlin Heidelberg, 115-121.
- Reid DE, Ferguson BJ, Hayashi S, Lin Y-H, Gresshoff PM. 2011.** Molecular mechanisms controlling legume autoregulation of nodulation. *Annals of Botany* **108**(5): 789-795.
- Remans T, Nacry P, Pervent M, Filleur S, Diatloff E, Mounier E, Tillard P, Forde BG, Gojon A. 2006a.** The Arabidopsis NRT1.1 transporter participates in the signaling pathway triggering root colonization of nitrate-rich patches. *Proceedings of the National Academy of Sciences of the United States of America* **103**(50): 19206-19211.
- Remans T, Nacry P, Pervent M, Girin T, Tillard P, Lepetit M, Gojon A. 2006b.** A central role for the nitrate transporter NRT2.1 in the integrated morphological and physiological responses of the root system to nitrogen limitation in Arabidopsis. *Plant Physiology* **140**(3): 909-921.
- Remmler L, Clairmont L, Rolland-Lagan A-G, Guinel FC. 2014.** Standardized mapping of nodulation patterns in legume roots. *New Phytologist*: n/a-n/a.

- Rey T, Nars A, Bonhomme M, Bottin A, Huguet S, Balzergue S, Jardinaud MF, Bono JJ, Cullimore J, Dumas B, Gough C, Jacquet C. 2013.** NFP, a LysM protein controlling Nod factor perception, also intervenes in *Medicago truncatula* resistance to pathogens. *New Phytologist* **198**(3): 875-886.
- Riely BK, Lougnon G, Ané JM, Cook DR. 2007.** The symbiotic ion channel homolog DMI1 is localized in the nuclear membrane of *Medicago truncatula* roots. *The plant journal* **49**(2): 208-216.
- Rivas R, Willems A, Palomo JL, García-Benavides P, Mateos PF, Martínez-Molina E, Gillis M, Velázquez E. 2004.** *Bradyrhizobium betae* sp. nov., isolated from roots of *Beta vulgaris* affected by tumour-like deformations. *International Journal of Systematic and Evolutionary Microbiology* **54**(4): 1271-1275.
- Robertson BK, Åman P, Darvill AG, McNeil M, Albersheim P. 1981.** Host-symbiont interactions V. The structure of acidic extracellular polysaccharides secreted by *Rhizobium leguminosarum* and *Rhizobium trifolii*. *Plant Physiology* **67**(3): 389-400.
- Rogato A, D'Apuzzo E, Barbulova A, Omrane S, Parlati A, Carfagna S, Costa A, Lo Schiavo F, Esposito S, Chiurazzi M. 2010.** Characterization of a Developmental Root Response Caused by External Ammonium Supply in *Lotus japonicus*. *Plant Physiology* **154**(2): 784-795.
- Rogel MA, Ormeno-Orrillo E, Romero EM. 2011.** Symbiovars in rhizobia reflect bacterial adaptation to legumes. *Systematic and Applied Microbiology* **34**(2): 96-104.
- Ruffel S, Freixes S, Balzergue S, Tillard P, Jeudy C, Martin-Magniette ML, van der Merwe MJ, Kakar K, Gouzy J, Fernie AR, Udvardi M, Salon C, Gojon A, Lepetit M. 2008.** Systemic signaling of the plant nitrogen status triggers specific transcriptome responses depending on the nitrogen source in *Medicago truncatula*. *Plant Physiology* **146**(4): 2020-2035.
- Ruffel S, Krouk G, Ristova D, Shasha D, Birnbaum KD, Coruzzi GM. 2011.** Nitrogen economics of root foraging: Transitive closure of the nitrate-cytokinin relay and distinct systemic signaling for N supply vs. demand. *Proceedings of the National Academy of Sciences of the United States of America* **108**(45): 18524-18529.
- Ruzicka K, Simaskova M, Duclercq J, Petrsek J, Zazimalova E, Simon S, Friml J, Van Montagu MCE, Benkova E. 2009.** Cytokinin regulates root meristem activity via modulation of the polar auxin transport. *Proceedings of the National Academy of Sciences of the United States of America* **106**(11): 4284-4289.
- Sachs JL, Ehinger MO, Simms EL. 2010.** Origins of cheating and loss of symbiosis in wild *Bradyrhizobium*. *Journal of Evolutionary Biology* **23**(5): 1075-1089.
- Sachs JL, Kembel SW, Lau AH, Simms EL. 2009.** In Situ Phylogenetic Structure and Diversity of Wild *Bradyrhizobium* Communities. *Applied and Environmental Microbiology* **75**(14): 4727-4735.
- Sagan M, Duc G. 1996.** *Sym28* and *Sym29*, two new genes involved in regulation of nodulation in pea (*Pisum sativum* L.). *Symbiosis* **20**(3): 229-245.
- Sagan M, Messenger A, Duc G. 1993a.** Specificity of the *Rhizobium*-legume symbiosis obtained after mutagenesis in pea (*Pisum sativum* L.). *New Phytologist* **125**(4): 757-761.
- Sagan M, Morandi D, Tarenghi E, Duc G. 1995.** Selection of nodulation and mycorrhizal mutants in the model plant *Medicago truncatula* (Gaertn.) after gamma-ray mutagenesis. *Plant Science* **111**(1): 63-71.
- Sagan M, Ney B, Duc G. 1993b.** Plant symbiotic mutants as a tool to analyse nitrogen nutrition and yield relationship in field-growth peas (*Pisum sativum* L.). *Plant and Soil* **153**(1): 33-45.
- Saito K, Yoshikawa M, Yano K, Miwa H, Uchida H, Asamizu E, Sato S, Tabata S, Imaizumi-Anraku H, Umehara Y, Kouchi H, Murooka Y, Szczyglowski K, Downie JA, Parniske M, Hayashi M, Kawaguchi M. 2007.** NUCLEOPORIN85 Is

- Required for Calcium Spiking, Fungal and Bacterial Symbioses, and Seed Production in *Lotus japonicus*. *The Plant cell* **19**(2): 610-624.
- Salon C, Lepetit M, Gamas P, Jeudy C, Moreau S, Moreau D, Voisin AS, Duc G, Bourion V, Munier-Jolain N. 2009.** Analysis and modeling of the integrative response of *Medicago truncatula* to nitrogen constraints. *Comptes Rendus Biologies* **332**(11): 1022-1033.
- Salon C, Munier-Jolain NG, Duc G, Voisin AS, Grandgirard D, Larmure A, Emery RJN, Ney B. 2001.** Grain legume seed filling in relation to nitrogen acquisition: A review and prospects with particular reference to pea. *Agronomie* **21**(6-7): 539-552.
- Santalla M, Amurrio JM, De Ron AM. 2001.** Symbiotic Interactions between *Rhizobium leguminosarum* Strains and Elite Cultivars of *Pisum sativum* L. *Journal of Agronomy and Crop Science* **187**(1): 59-68.
- Sato S, Nakamura Y, Kaneko T, Asamizu E, Kato T, Nakao M, Sasamoto S, Watanabe A, Ono A, Kawashima K. 2008.** Genome structure of the legume, *Lotus japonicus*. *DNA research* **15**(4): 227-239.
- Scheible WR, Morcuende R, Czechowski T, Fritz C, Osuna D, Palacios-Rojas N, Schindelasch D, Thimm O, Udvardi MK, Stitt M. 2004.** Genome-wide reprogramming of primary and secondary metabolism, protein synthesis, cellular growth processes, and the regulatory infrastructure of Arabidopsis in response to nitrogen. *Plant Physiology* **136**(1): 2483-2499.
- Schmutz J, Cannon SB, Schlueter J, Ma J, Mitros T, Nelson W, Hyten DL, Song Q, Thelen JJ, Cheng J, Xu D, Hellsten U, May GD, Yu Y, Sakurai T, Umezawa T, Bhattacharyya MK, Sandhu D, Valliyodan B, Lindquist E, Peto M, Grant D, Shu S, Goodstein D, Barry K, Futrell-Griggs M, Abernathy B, Du J, Tian Z, Zhu L, Gill N, Joshi T, Libault M, Sethuraman A, Zhang X-C, Shinozaki K, Nguyen HT, Wing RA, Cregan P, Specht J, Grimwood J, Rokhsar D, Stacey G, Shoemaker RC, Jackson SA. 2010.** Genome sequence of the palaeopolyploid soybean. *Nature* **463**(7278): 178-183.
- Schmutz J, McClean PE, Mamidi S, Wu GA, Cannon SB, Grimwood J, Jenkins J, Shu S, Song Q, Chavarro C, Torres-Torres M, Geffroy V, Moghaddam SM, Gao D, Abernathy B, Barry K, Blair M, Brick MA, Chovatia M, Gepts P, Goodstein DM, Gonzales M, Hellsten U, Hyten DL, Jia G, Kelly JD, Kudrna D, Lee R, Richard MMS, Miklas PN, Osorno JM, Rodrigues J, Thareau V, Urrea CA, Wang M, Yu Y, Zhang M, Wing RA, Cregan PB, Rokhsar DS, Jackson SA. 2014.** A reference genome for common bean and genome-wide analysis of dual domestications. *Nat Genet* **46**(7): 707-713.
- Schnabel E, Journet EP, de Carvalho-Niebel F, Duc G, Frugoli J. 2005.** The *Medicago truncatula* *SUNN* gene encodes a *CLVI*-like leucine-rich repeat receptor kinase that regulates nodule number and root length. *Plant Molecular Biology* **58**(6): 809-822.
- Schnabel EL, Kassaw TK, Smith LS, Marsh JF, Oldroyd GE, Long SR, Frugoli JA. 2011.** The *ROOT DETERMINED NODULATION1* gene regulates nodule number in roots of *Medicago truncatula* and defines a highly conserved, uncharacterized plant gene family. *Plant Physiology* **157**(1): 328-340.
- Schneider A, Huygue C, Maleplate T, Labalette F, Peyronnet C, Carrouée B 2015.** Rôles des légumineuses dans l'agriculture française. *Les légumineuses pour des systèmes agricoles et alimentaires durables* QUAE, 11-78.
- Schnepf A, Leitner D, Schweiger PF, Scholl P, Jansa J. 2016.** L-System model for the growth of arbuscular mycorrhizal fungi, both within and outside of their host roots. *Journal of the Royal Society Interface* **13**(117).
- Schultze M, Kondorosi A. 1998.** Regulation of symbiotic root nodule development. *Annual review of genetics* **32**(1): 33-57.

- Schulze J, Adgo E, Merbach W. 1999.** Carbon costs associated with N₂ fixation in *Vicia faba* L and *Pisum sativum* L. over a 14-day period. *Plant Biology* **1**(6): 625-631.
- Searle IR, Men AE, Laniya TS, Buzas DM, Iturbe-Ormaetxe I, Carroll BJ, Gresshoff PM. 2003.** Long-Distance Signaling in Nodulation Directed by a CLAVATA1-Like Receptor Kinase. *Science* **299**(5603): 109-112.
- Segovia L, Pinero D, Palacios R, Martinez-Romero E. 1991.** Genetic structure of a soil population of nonsymbiotic *Rhizobium leguminosarum*. *Applied and Environmental Microbiology* **57**(2): 426-433.
- Seifert GJ, Roberts K 2007.** The biology of arabinogalactan proteins. *Annual review of plant biology*. Palo Alto: Annual Reviews, 137-161.
- Shi HZ, Kim Y, Guo Y, Stevenson B, Zhu JK. 2003.** The Arabidopsis SOS5 locus encodes a putative cell surface adhesion protein and is required for normal cell expansion. *Plant Cell* **15**(1): 19-32.
- Simms EL, Taylor DL. 2002.** Partner choice in nitrogen-fixation mutualisms of legumes and rhizobia. *Integrative and Comparative Biology* **42**(2): 369-380.
- Sinclair TR, Horie T. 1989.** Leaf Nitrogen, Photosynthesis, and Crop Radiation Use Efficiency - a Review. *Crop Science* **29**(1): 90-98.
- Singh S, Katzer K, Lambert J, Cerri M, Parniske M. 2014.** CYCLOPS, A DNA-Binding Transcriptional Activator, Orchestrates Symbiotic Root Nodule Development. *Cell Host & Microbe* **15**(2): 139-152.
- Smit G, Kijne J, Lugtenberg B. 1987.** Involvement of both cellulose fibrils and a Ca²⁺-dependent adhesin in the attachment of *Rhizobium leguminosarum* to pea root hair tips. *Journal of Bacteriology* **169**(9): 4294-4301.
- Smith GB, Wollum AG. 1989.** Nodulation of *Glycine max* by six *Bradyrhizobium japonicum* strains with different competitive abilities. *Applied and Environmental Microbiology* **55**(8): 1957-1962.
- Smykal P, Coyne CJ, Ambrose MJ, Maxted N, Schaefer H, Blair MW, Berger J, Greene SL, Nelson MN, Besharat N, Vymyslicky T, Toker C, Saxena RK, Roorkiwal M, Pandey MK, Hu JG, Li YH, Wang LX, Guo Y, Qiu LJ, Redden RJ, Varshney RK. 2015.** Legume Crops Phylogeny and Genetic Diversity for Science and Breeding. *Critical Reviews in Plant Sciences* **34**(1-3): 43-104.
- Smykal P, Kenicer G, Flavell AJ, Corander J, Kosterin O, Redden RJ, Ford R, Coyne CJ, Maxted N, Ambrose MJ, Ellis NTH. 2011.** Phylogeny, phylogeography and genetic diversity of the *Pisum* genus. *Plant Genetic Resources-Characterization and Utilization* **9**(1): 4-18.
- Sohlenkamp C, Wood CC, Roeb GW, Udvardi MK. 2002.** Characterization of Arabidopsis AtAMT2, a high-affinity ammonium transporter of the plasma membrane. *Plant Physiology* **130**(4): 1788-1796.
- Sorin C, Bussell JD, Camus I, Ljung K, Kowalczyk M, Geiss G, McKhann H, Garcion C, Vaucheret H, Sandberg G, Bellini C. 2005.** Auxin and light control of adventitious rooting in Arabidopsis require ARGONAUTE1. *Plant Cell* **17**(5): 1343-1359.
- Souza AA, Boscariol RL, Moon DH, Camargo LE, Tsai SM. 2000.** Effects of *Phaseolus vulgaris* QTL in controlling host-bacteria interactions under two levels of nitrogen fertilization. *Genetics and Molecular Biology* **23**(1): 155-161.
- Spaink HP, Sheeley DM, Vanbrussel AAN, Glushka J, York WS, Tak T, Geiger O, Kennedy EP, Reinhold VN, Lugtenberg BJJ. 1991.** A novel highly unsaturated fatty-acid moiety of lipo-oligosaccharide signals determines host specificity of *Rhizobium*. *Nature* **354**(6349): 125-130.
- Spaink HP, Wijffelman CA, Pees E, Okker RJ, Lugtenberg B. 1987.** *Rhizobium* nodulation gene *nodD* as a determinant of host specificity.

- Sprent JI. 2007.** Evolving ideas of legume evolution and diversity: a taxonomic perspective on the occurrence of nodulation. *New Phytologist* **174**(1): 11-25.
- Sprent JI, Sutherland J, De Faria S, Dilworth M, Corby H, Becking J, Materon L, Drozd J. 1987.** Some aspects of the biology of nitrogen-fixing organisms [and discussion]. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*: 111-129.
- Stedle E, Peterson CA. 1998.** How does water get through roots? *Journal of Experimental Botany* **49**(322): 775-788.
- Stougaard J. 2001.** Genetics and genomics of root symbiosis. *Current Opinion in Plant Biology* **4**(4): 328-335.
- Stracke S, Kistner C, Yoshida S, Mulder L, Sato S, Kaneko T, Tabata S, Sandal N, Stougaard J, Szczyglowski K, Parniske M. 2002.** A plant receptor-like kinase required for both bacterial and fungal symbiosis. *Nature* **417**: 959 - 962.
- Sullivan JT, Patrick HN, Lowther WL, Scott DB, Ronson CW. 1995.** Nodulating strains of *Rhizobium loti* arise through chromosomal symbiotic gene transfer in the environment. *Proceedings of the National Academy of Sciences of the United States of America* **92**(19): 8985-8989.
- Syñoño K, Torrey JG. 1976.** Identification of Cytokinins of Root Nodules of the Garden Pea, *Pisum sativum* L. *Plant Physiology* **57**(4): 602-606.
- Tabata R, Sumida K, Yoshii T, Ohyama K, Shinohara H, Matsubayashi Y. 2014.** Perception of root-derived peptides by shoot LRR-RKs mediates systemic N-demand signaling. *Science* **346**(6207): 343-346.
- Tar'an B, Warkentin T, Somers DJ, Miranda D, Vandenberg A, Blade S, Bing D. 2004.** Identification of quantitative trait loci for grain yield, seed protein concentration and maturity in field pea (*Pisum sativum* L.). *Euphytica* **136**(3): 297-306.
- Tayeh N, Aluome C, Falque M, Jacquin F, Klein A, Chauveau A, Berard A, Houtin H, Rond C, Kreplak J, Boucherot K, Martin C, Baranger A, Pilet-Nayel M-L, Warkentin TD, Brunel D, Marget P, Le Paslier M-C, Aubert G, Burstin J. 2015.** Development of two major resources for pea genomics: the GenoPea 13.2K SNP Array and a high-density, high-resolution consensus genetic map. *The plant journal* **84**(6): 1257-1273.
- Tellstrom V, Usadel B, Thimm O, Stitt M, Kuster H, Niehaus K. 2007.** The lipopolysaccharide of *Sinorhizobium meliloti* suppresses defense-associated gene expression in cell cultures of the host plant *Medicago truncatula*. *Plant Physiology* **143**(2): 825-837.
- Thimm O, Blasing O, Gibon Y, Nagel A, Meyer S, Kruger P, Selbig J, Muller LA, Rhee SY, Stitt M. 2004.** MAPMAN: a user-driven tool to display genomics data sets onto diagrams of metabolic pathways and other biological processes. *Plant Journal* **37**(6): 914-939.
- Thorup-Kristensen K. 1998.** Root growth of green pea (*Pisum sativum* L.) genotypes. *Crop Sci* **38**.
- Tian QY, Sun P, Zhang WH. 2009.** Ethylene is involved in nitrate-dependent root growth and branching in *Arabidopsis thaliana*. *New Phytologist* **184**(4): 918-931.
- Timmerman-Vaughan GM, McCallum JA, Frew TJ, Weeden NF, Russell AC. 1996.** Linkage mapping of quantitative trait loci controlling seed weight in pea (*Pisum sativum* L.). *Theoretical and Applied Genetics* **93**(3): 431-439.
- Timmerman-Vaughan GM, Mills A, Whitfield C, Frew T, Butler R, Murray S, Lakeman M, McCallum J, Russell A, Wilson D. 2005.** Linkage mapping of QTL for seed yield, yield components, and developmental traits in pea. *Crop Science* **45**(4): 1336-1344.
- Tirichine L, Imaizumi-Anraku H, Yoshida S, Murakami Y, Madsen LH, Miwa H, Nakagawa T, Sandal N, Albrektsen AS, Kawaguchi M, Downie A, Sato S, Tabata**

- S, Kouchi H, Parniske M, Kawasaki S, Stougaard J. 2006.** Deregulation of a Ca²⁺/calmodulin-dependent kinase leads to spontaneous nodule development. *Nature* **441**(7097): 1153-1156.
- Tirichine L, Sandal N, Madsen LH, Radutoiu S, Albrektsen AS, Sato S, Asamizu E, Tabata S, Stougaard J. 2007.** A Gain-of-Function Mutation in a Cytokinin Receptor Triggers Spontaneous Root Nodule Organogenesis. *Science* **315**(5808): 104-107.
- Tominaga A, Gondo T, Akashi R, Zheng S-h, Arima S, Suzuki A. 2012.** Quantitative trait locus analysis of symbiotic nitrogen fixation activity in the model legume *Lotus japonicus*. *Journal of Plant Research* **125**(3): 395-406.
- Trainer MA, Charles TC. 2006.** The role of PHB metabolism in the symbiosis of rhizobia with legumes. *Applied Microbiology and Biotechnology* **71**(4): 377-386.
- Tricot F, Crozat Y, Pellerin S. 1997.** Root system growth and nodule establishment on pea (*Pisum sativum* L.). *Journal of Experimental Botany* **48**(11): 1935-1941.
- Triplett EW, Sadowsky MJ. 1992.** Genetics of competition for nodulation of legumes. *Annual Review of Microbiology* **46**: 399-428.
- Tuberosa R, Sanguinetti MC, Landi P, Giuliani MM, Salvi S, Conti S. 2002.** Identification of QTLs for root characteristics in maize grown in hydroponics and analysis of their overlap with QTLs for grain yield in the field at two water regimes. *Plant Mol Biol* **48**.
- Ubeda-Tomas S, Swarup R, Coates J, Swarup K, Laplaze L, Beemster GTS, Hedden P, Bhalerao R, Bennett MJ. 2008.** Root growth in Arabidopsis requires gibberellin/DELLA signalling in the endodermis. *Nature Cell Biology* **10**(5): 625-628.
- Udvardi MK, Day DA. 1997.** Metabolite transport across symbiotic membranes of legume nodules. *Annual review of plant biology* **48**(1): 493-523.
- van Hengel AJ, Roberts K. 2002.** Fucosylated arabinogalactan-proteins are required for full root cell elongation in arabidopsis. *Plant Journal* **32**(1): 105-113.
- van Zeijl A, Op den Camp Rik HM, Deinum Eva E, Charnikhova T, Franssen H, Op den Camp Huub JM, Bouwmeester H, Kohlen W, Bisseling T, Geurts R. 2015.** Rhizobium Lipo-chitoooligosaccharide Signaling Triggers Accumulation of Cytokinins in Medicago truncatula Roots. *Molecular Plant* **8**(8): 1213-1226.
- Vandamme P, Pot B, Gillis M, De Vos P, Kersters K, Swings J. 1996.** Polyphasic taxonomy, a consensus approach to bacterial systematics. *Microbiological reviews* **60**(2): 407-438.
- Varshney RK, Chen W, Li Y, Bharti AK, Saxena RK, Schlueter JA, Donoghue MTA, Azam S, Fan G, Whaley AM, Farmer AD, Sheridan J, Iwata A, Tuteja R, Penmetsa RV, Wu W, Upadhyaya HD, Yang S-P, Shah T, Saxena KB, Michael T, McCombie WR, Yang B, Zhang G, Yang H, Wang J, Spillane C, Cook DR, May GD, Xu X, Jackson SA. 2012.** Draft genome sequence of pigeonpea (*Cajanus cajan*), an orphan legume crop of resource-poor farmers. *Nat Biotech* **30**(1): 83-89.
- Varshney RK, Song C, Saxena RK, Azam S, Yu S, Sharpe AG, Cannon S, Baek J, Rosen BD, Tar'an B, Millan T, Zhang X, Ramsay LD, Iwata A, Wang Y, Nelson W, Farmer AD, Gaur PM, Soderlund C, Penmetsa RV, Xu C, Bharti AK, He W, Winter P, Zhao S, Hane JK, Carrasquilla-Garcia N, Condie JA, Upadhyaya HD, Luo M-C, Thudi M, Gowda CLL, Singh NP, Lichtenzweig J, Gali KK, Rubio J, Nadarajan N, Dolezel J, Bansal KC, Xu X, Edwards D, Zhang G, Kahl G, Gil J, Singh KB, Datta SK, Jackson SA, Wang J, Cook DR. 2013.** Draft genome sequence of chickpea (*Cicer arietinum*) provides a resource for trait improvement. *Nat Biotech* **31**(3): 240-246.
- Vasse J, De Billy F, Camut S, Truchet G. 1990.** Correlation between ultrastructural differentiation of bacteroids and nitrogen fixation in alfalfa nodules. *Journal of Bacteriology* **172**(8): 4295-4306.

- Veitenheimer EE, Gritton ET. 1984.** Preliminary root studies of *Pisum sativum*. *Pisum Newsletter* **16**: 73-74.
- Velázquez E, Peix A, Zurdo-Piñero JL, Palomo JL, Mateos PF, Rivas R, Muñoz-Adelantado E, Toro N, García-Benavides P, Martínez-Molina E. 2005.** The Coexistence of Symbiosis and Pathogenicity-Determining Genes in *Rhizobium rhizogenes* Strains Enables Them to Induce Nodules and Tumors or Hairy Roots in Plants. *Molecular Plant-Microbe Interactions* **18**(12): 1325-1332.
- Venkateshwaran M, Jayaraman D, Chabaud M, Genre A, Balloon AJ, Maeda J, Forshey K, den Os D, Kwiecien NW, Coon JJ. 2015.** A role for the mevalonate pathway in early plant symbiotic signaling. *Proceedings of the National Academy of Sciences* **112**(31): 9781-9786.
- Vernié T, Kim J, Frances L, Ding Y, Sun J, Guan D, Niebel A, Gifford ML, de Carvalho-Niebel F, Oldroyd GED. 2015.** The NIN Transcription Factor Coordinates Diverse Nodulation Programs in Different Tissues of the *Medicago truncatula* Root. *The Plant cell* **27**(12): 3410-3424.
- Vershinin AV, Allnut TR, Knox MR, Ambrose MJ, Ellis THN. 2003.** Transposable elements reveal the impact of introgression, rather than transposition, in *Pisum* diversity, evolution, and domestication. *Molecular Biology and Evolution* **20**(12): 2067-2075.
- Vilmorin-Andrieux. 1883.** Les Plantes potagères. Chapitre : Pois. *Vilmorin-Andrieux & Cie*: 423-474.
- Virtanen AI, von Hausen S, Laine T. 1937.** Investigations on the root nodule bacteria of leguminous plants: XX. Excretion of nitrogen in associated cultures of legumes and non-legumes. *The Journal of Agricultural Science* **27**(04): 584-610.
- Vocanson A, Jeuffroy M-H, Roger-Estrade J. 2006.** Effect of Sowing Date and Cultivar on Root System Development in Pea (*Pisum sativum* L.). *Plant and Soil* **283**(1): 339-352.
- Voisin AS, Bourion V, Duc G, Salon C. 2007.** Using an ecophysiological analysis to dissect genetic variability and to propose an ideotype for nitrogen nutrition in pea. *Annals of Botany* **100**(7): 1525-1536.
- Voisin AS, Munier-Jolain NG, Salon C. 2010.** The nodulation process is tightly adjusted to plant growth. An analysis using environmentally and genetically induced variation of nodule number and biomass in pea. *Plant and Soil* **337**(1-2): 399-412.
- Voisin AS, Salon C, Jeudy C, Warembourg FR. 2003a.** Symbiotic N₂ fixation activity in relation to C economy of *Pisum sativum* L. as a function of plant phenology. *Journal of Experimental Botany* **54**(393): 2733-2744.
- Voisin AS, Salon C, Jeudy C, Warembourg FR. 2003b.** Root and nodule growth in *Pisum sativum* L. in relation to photosynthesis: Analysis using ¹³C-labelling. *Annals of Botany* **92**(4): 557-563.
- Voisin AS, Salon C, Munier-Jolain NG, Ney B. 2002a.** Effect of mineral nitrogen on nitrogen nutrition and biomass partitioning between the shoot and roots of pea (*Pisum sativum* L.). *Plant and Soil* **242**(2): 251-262.
- Voisin AS, Salon C, Munier-Jolain NG, Ney B. 2002b.** Quantitative effects of soil nitrate, growth potential and phenology on symbiotic nitrogen fixation of pea (*Pisum sativum* L.). *Plant and Soil* **243**(1): 31-42.
- Wachsman G, Sparks EE, Benfey PN. 2015.** Genes and networks regulating root anatomy and architecture. *New Phytologist* **208**(1): 26-38.
- Wais RJ, Keating DH, Long SR. 2002.** Structure-function analysis of nod factor-induced root hair calcium spiking in *Rhizobium-legume* symbiosis. *Plant Physiology* **129**(1): 211-224.
- Walch-Liu P, Forde BG. 2008.** Nitrate signalling mediated by the NRT1.1 nitrate transporter antagonises L-glutamate-induced changes in root architecture. *Plant Journal* **54**(5): 820-828.

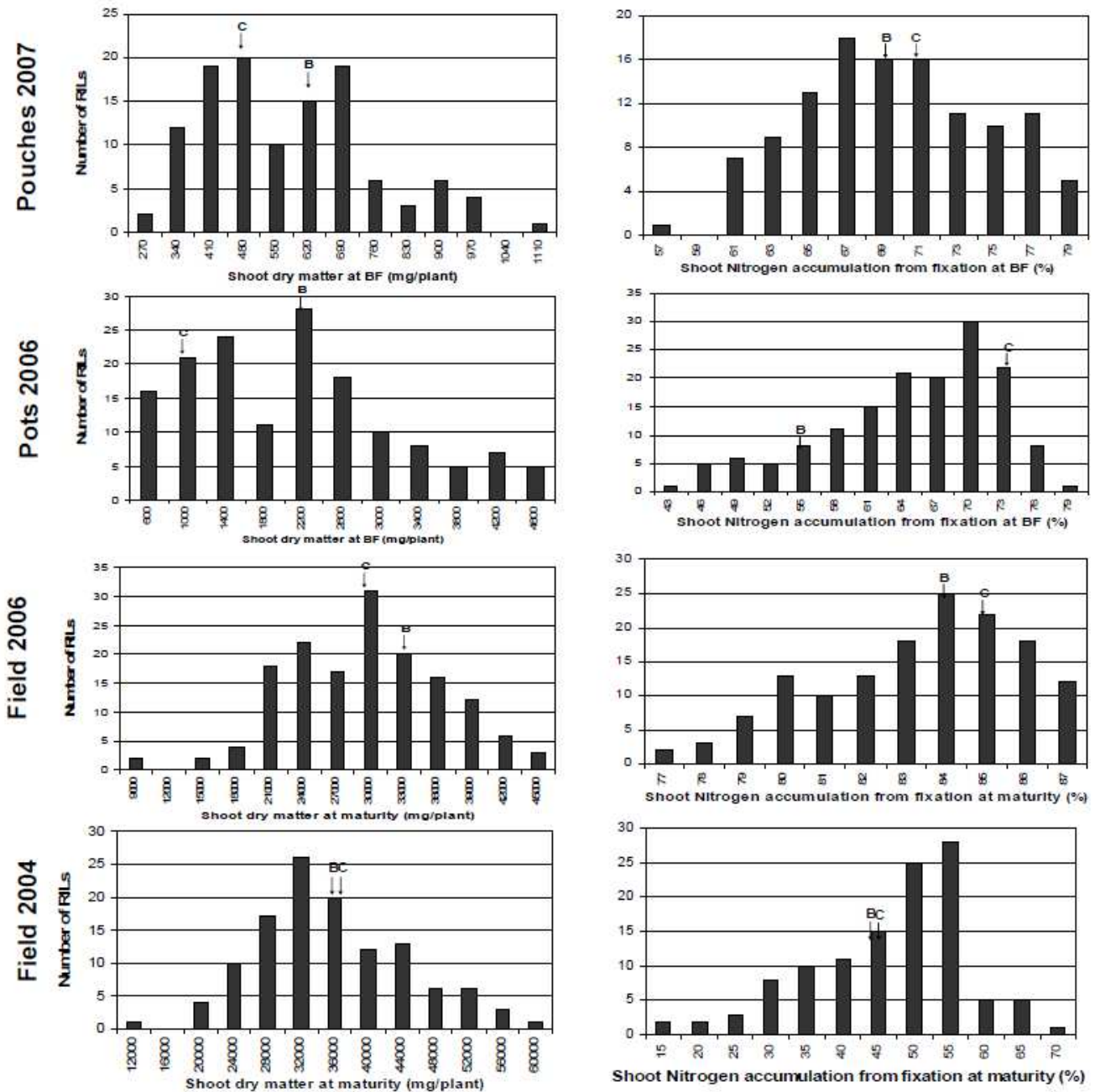
- Walch-Liu P, Ivanov, II, Filleur S, Gan YB, Remans T, Forde BG. 2006a.** Nitrogen regulation of root branching. *Annals of Botany* **97**(5): 875-881.
- Walch-Liu P, Liu LH, Remans T, Tester M, Forde BG. 2006b.** Evidence that L-glutamate can act as an exogenous signal to modulate root growth and branching in *Arabidopsis thaliana*. *Plant and Cell Physiology* **47**(8): 1045-1057.
- Walker SA, Viprey V, Downie JA. 2000.** Dissection of nodulation signaling using pea mutants defective for calcium spiking induced by Nod factors and chitin oligomers. *Proceedings of the National Academy of Sciences of the United States of America* **97**(24): 13413-13418.
- Wang RC, Okamoto M, Xing XJ, Crawford NM. 2003.** Microarray analysis of the nitrate response in *Arabidopsis* roots and shoots reveals over 1,000 rapidly responding genes and new linkages to glucose, trehalose-6-phosphate, iron, and sulfate metabolism. *Plant Physiology* **132**(2): 556-567.
- Weeden N, Brauner S, Przyborowski J. 2002.** Genetic analysis of pod dehiscence in pea (*Pisum sativum* L.). *Cellular and Molecular Biology Letters* **7**(2B): 657-664.
- Weeden NF, Moffet M. 2002.** Identification of genes affecting root mass and root/shoot ratio in a JI 1794 x 'Slow' RIL population. *Pisum Genetics* **34**: 28-31.
- Weeden NF, Tonguc M, Boone WE. 1999.** Mapping coding sequences in pea by PCR. *Pisum Genet* **31**: 30-32.
- Wellensiek S. 1925.** Genetic monograph on *Pisum*. *Bibl. Genetica* **2**: 343-476.
- Wellensiek S. 1951.** *Pisum*-crosses IX: The new flower colour "cerise". *Genetica* **25**(1): 525-529.
- Wicker E, Moussart A, Duparque M, Rouxel F. 2003.** Further Contributions to the Development of a Differential Set of Pea Cultivars (*Pisum sativum*) to Investigate the Virulence of Isolates of *Aphanomyces euteiches*. *European Journal of Plant Pathology* **109**(1): 47-60.
- Wilson PW, Burton JC. 1938.** Excretion of nitrogen by leguminous plants. *The Journal of Agricultural Science* **28**(02): 307-323.
- Winarno R, Lie TA. 1979.** Competition between *Rhizobium* strains in nodule formation: Interaction between nodulating and non-nodulating strains. *Plant and Soil* **51**(1): 135-142.
- Woese CR. 1987.** Bacterial evolution. *Microbiological reviews* **51**(2): 221.
- Wopereis J, Pajuelo E, Dazzo FB, Jiang QY, Gresshoff PM, de Bruijn FJ, Stougaard J, Szczyglowski K. 2000.** Short root mutant of *Lotus japonicus* with a dramatically altered symbiotic phenotype. *Plant Journal* **23**(1): 97-114.
- Wu G, Lewis DR, Spalding EP. 2007.** Mutations in *Arabidopsis* multidrug resistance-like ABC transporters separate the roles of acropetal and basipetal auxin transport in lateral root development. *Plant Cell* **19**(6): 1826-1837.
- Yendrek CR, Lee YC, Morris V, Liang Y, Pislariu CI, Burkart G, Meckfessel MH, Salehin M, Kessler H, Wessler H, Lloyd M, Lutton H, Teillet A, Sherrier DJ, Journet EP, Harris JM, Dickstein R. 2010.** A putative transporter is essential for integrating nutrient and hormone signaling with lateral root growth and nodule development in *Medicago truncatula*. *Plant Journal* **62**(1): 100-112.
- Young J. 2003.** The genus name *Ensifer* Casida 1982 takes priority over *Sinorhizobium* Chen *et al.* 1988, and *Sinorhizobium morelense* Wang *et al.* 2002 is a later synonym of *Ensifer adhaerens* Casida 1982. Is the combination '*Sinorhizobium adhaerens*' (Casida 1982) Willems *et al.* 2003 legitimate? Request for an opinion. *International Journal of Systematic and Evolutionary Microbiology* **53**(6): 2107-2110.
- Young JPW, Johnston AWB, Brewin NJ. 1982.** A search for peas (*Pisum sativum* L.) showing strain specificity for symbiotic *Rhizobium leguminosarum*. *Heredity* **48**(APR): 197-201.

- Young JPW, Matthews P. 1982.** A distinct class of peas (*Pisum sativum* L.) from Afghanistan that show strain specificity for symbiotic Rhizobium. *Heredity* **48**(APR): 203-210.
- Young ND, Debelle F, Oldroyd GED, Geurts R, Cannon SB, Udvardi MK, Benedito VA, Mayer KFX, Gouzy J, Schoof H, Van de Peer Y, Proost S, Cook DR, Meyers BC, Spannagl M, Cheung F, De Mita S, Krishnakumar V, Gundlach H, Zhou SG, Mudge J, Bharti AK, Murray JD, Naoumkina MA, Rosen B, Silverstein KAT, Tang HB, Rombauts S, Zhao PX, Zhou P, Barbe V, Bardou P, Bechner M, Bellec A, Berger A, Berges H, Bidwell S, Bisseling T, Choisine N, Couloux A, Denny R, Deshpande S, Dai XB, Doyle JJ, Dudez AM, Farmer AD, Fouteau S, Franken C, Gibelin C, Gish J, Goldstein S, Gonzalez AJ, Green PJ, Hallab A, Hartog M, Hua A, Humphray SJ, Jeong DH, Jing Y, Jocker A, Kenton SM, Kim DJ, Klee K, Lai HS, Lang CT, Lin SP, Macmil SL, Magdelenat G, Matthews L, McCorrison J, Monaghan EL, Mun JH, Najjar FZ, Nicholson C, Noirot C, O'Bleness M, Paule CR, Poulain J, Prion F, Qin BF, Qu CM, Retzel EF, Riddle C, Sallet E, Samain S, Samson N, Sanders I, Saurat O, Scarpelli C, Schiex T, Segurens B, Severin AJ, Sherrier DJ, Shi RH, Sims S, Singer SR, Sinharoy S, Sterck L, Viollet A, Wang BB, Wang KQ, Wang MY, Wang XH, Warfsmann J, Weissenbach J, White DD, White JD, Wiley GB, Wincker P, Xing YB, Yang LM, Yao ZY, Ying F, Zhai JX, Zhou LP, Zuber A, Denarie J, Dixon RA, May GD, Schwartz DC, Rogers J, Quetier F, Town CD, Roe BA. 2011.** The Medicago genome provides insight into the evolution of rhizobial symbioses. *Nature* **480**(7378): 520-524.
- Yuan LX, Loque D, Ye FH, Frommer WB, von Wiren N. 2007.** Nitrogen-dependent posttranscriptional regulation of the ammonium transporter AtAMT1;1. *Plant Physiology* **143**(2): 732-744.
- Zehner S, Schober G, Wenzel M, Lang K, Göttfert M. 2008.** Expression of the *Bradyrhizobium japonicum* type III secretion system in legume nodules and analysis of the associated tts box promoter. *Molecular Plant-Microbe Interactions* **21**(8): 1087-1093.
- Zhang HM, Forde BG. 1998.** An Arabidopsis MADS box gene that controls nutrient-induced changes in root architecture. *Science* **279**(5349): 407-409.
- Zhang HM, Forde BG. 2000.** Regulation of Arabidopsis root development by nitrate availability. *Journal of Experimental Botany* **51**(342): 51-59.
- Zhang HM, Jennings A, Barlow PW, Forde BG. 1999.** Dual pathways for regulation of root branching by nitrate. *Proceedings of the National Academy of Sciences of the United States of America* **96**(11): 6529-6534.
- Zhang HM, Rong HL, Pilbeam D. 2007.** Signalling mechanisms underlying the morphological responses of the root system to nitrogen in *Arabidopsis thaliana*. *Journal of Experimental Botany* **58**(9): 2329-2338.
- Zhu H, Choi H-K, Cook DR, Shoemaker RC. 2005.** Bridging Model and Crop Legumes through Comparative Genomics. *Plant Physiology* **137**(4): 1189-1196.
- Zhukov V, Radutoiu S, Madsen LH, Rychagova T, Ovchinnikova E, Borisov A, Tikhonovich I, Stougaard J. 2008.** The Pea *Sym37* Receptor Kinase Gene Controls Infection-Thread Initiation and Nodule Development. *Molecular Plant-Microbe Interactions* **21**(12): 1600-1608.
- Zohary D, Hopf M. 1973.** Domestication of pulses in the Old World. *Science* **182**(4115): 887-894.
- Zohary D, Hopf M. 1973.** Domestication of Pulses in the Old World Legumes were companions of wheat and barley when agriculture began in the Near East. *Science* **182**(4115): 887-894.

ANNEXES

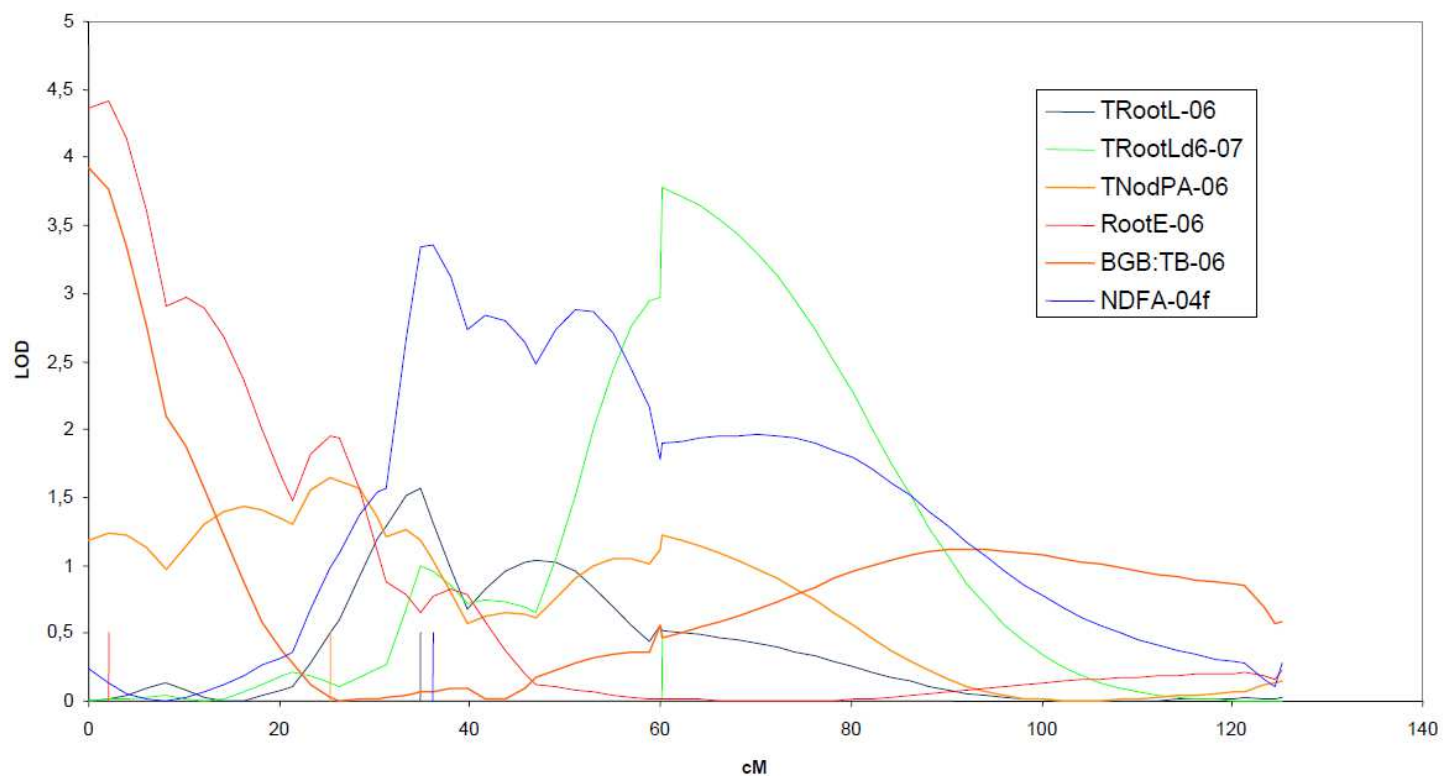
ANNEXES

Annexe 1 : Fichiers additionnels du chapitre I



Supplemental Fig. I.1. Frequency distribution of RIL4 population for shoot dry matter per plant and percentage of nitrogen derived from fixation, at BF in two glasshouse experiments; Pouches in 2007 and Pots in 2006, and at maturity in two field experiments; 2006 and 2004. Arrows indicate the mean value of the parental lines: B, 'Ballet'; C, 'Cameor'

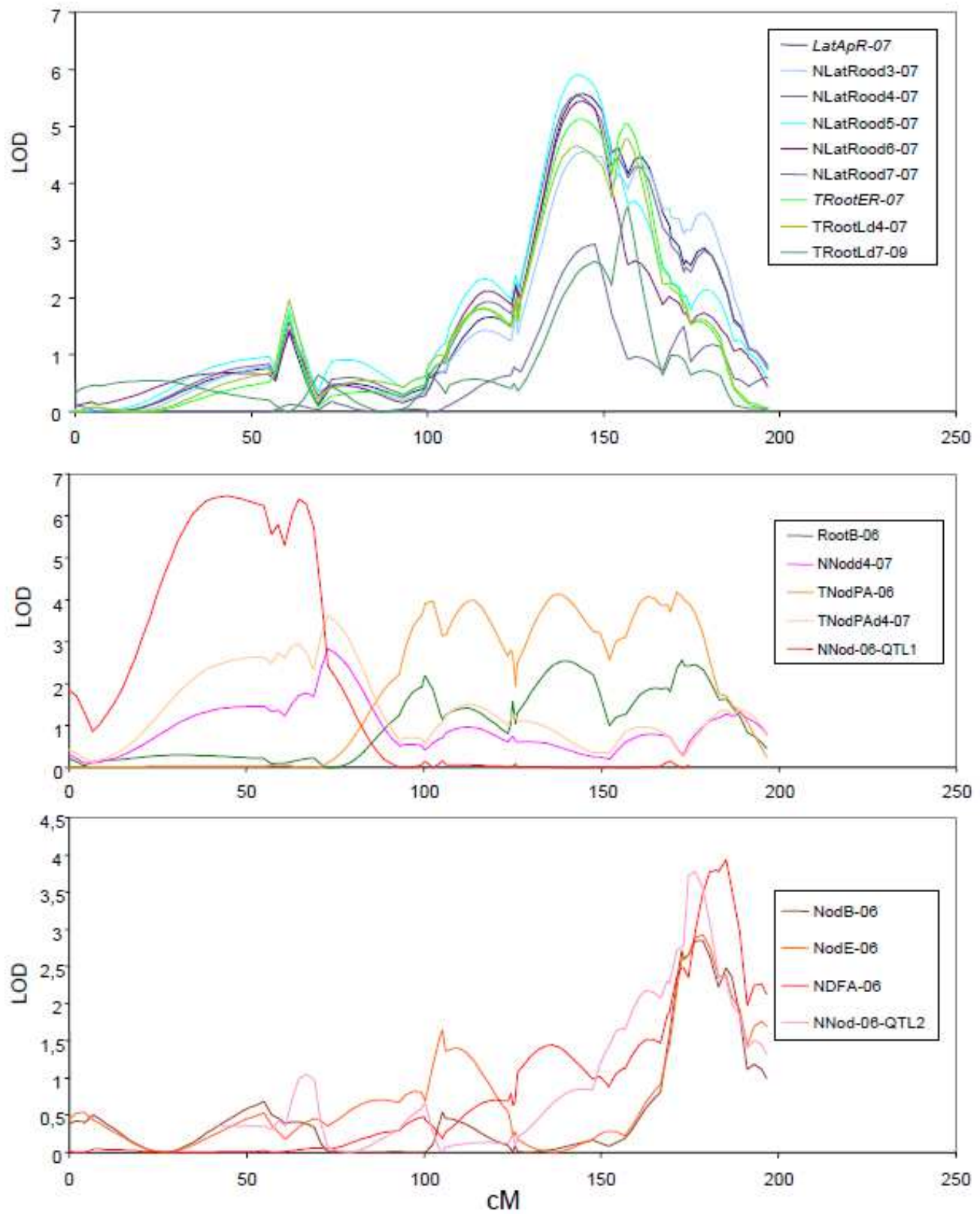
LGII



Supplemental Fig. I.2. LOD score curve on LGII, LGIII and LGVII for QTL associated with nitrogen acquisition structure or functioning, in two glasshouse experiments; Pouches in 2007 and Pots in 2006, and in a field experiment in 2004

Continued

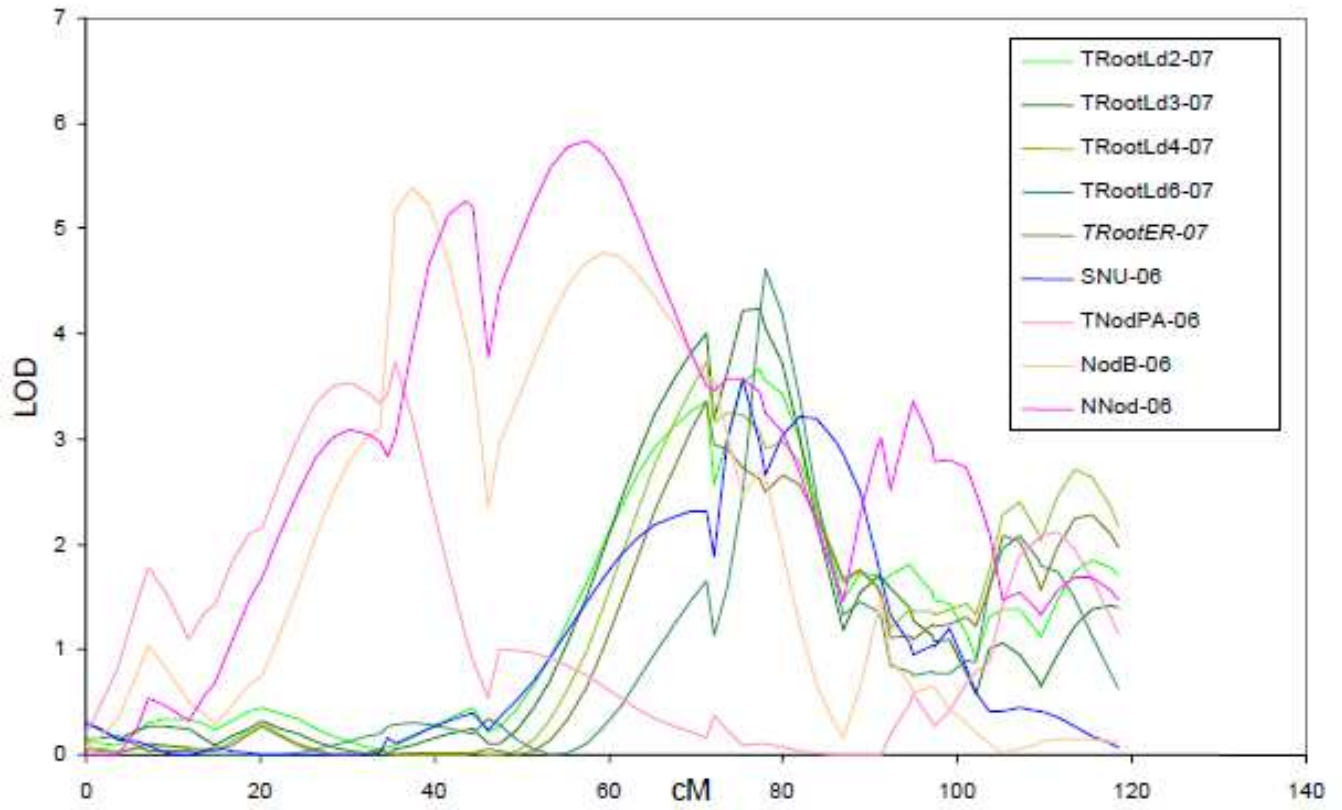
LGIII



Supplemental Fig. I.2 suite

Continued

LGVII



Supplemental Fig. I.2 suite

Supplemental Table I.1. Effects of the different alleles of Le and Af on root and nodule traits evaluated at four developmental stages on seven pea genotypes

| Stage | Trait | gene Le | | gene Af | |
|---------------------------|----------|---------------------|---------------------|---------------------|---------------------|
| | | Le | le | Af | af |
| 4-leaf | NLatRoot | 85.8 ^a | 81.3 ^a | 85.9 ^a | 76.5 ^b |
| | TRootL | 744.4 ^a | 743.7 ^a | 786.5 ^a | 638.1 ^b |
| | RootB | 122.7 ^a | 121.1 ^a | 129.1 ^a | 103.5 ^b |
| | NNod | 33.9 ^a | 24.4 ^b | 32.5 ^a | 18.4 ^b |
| | TNodPA | 1.84 ^a | 1.46 ^b | 1.76 ^a | 1.29 ^b |
| | NodB | 5.68 ^a | 3.89 ^b | 5.77 ^a | 1.87 ^b |
| 9-leaf | NLatRoot | 117.3 ^a | 104.2 ^a | 113.0 ^a | 101.8 ^b |
| | TRootL | 2122.1 ^a | 2089.6 ^a | 2173.4 ^a | 1228.9 ^b |
| | RootB | 381.3 ^a | 351.3 ^a | 402.7 ^a | 267.9 ^b |
| | NNod | 253.4 ^a | 199.5 ^b | 265.9 ^a | 114.2 ^b |
| | TNodPA | 7.23 ^a | 4.61 ^b | 6.51 ^a | 3.8 ^b |
| | NodB | 12.29 ^a | 6.73 ^a | 13.34 ^a | 1.32 ^b |
| Beginning of flowering | NLatRoot | 132.2 ^a | 108.2 ^b | 124.4 ^a | 103.9 ^a |
| | TRootL | 2863.0 ^a | 2690.5 ^a | 2896.0 ^a | 2711.8 ^a |
| | RootB | 792.1 ^a | 603.5 ^b | 691.3 ^a | 666.9 ^a |
| | NNod | 582.5 ^a | 380.2 ^b | 511.2 ^a | 356.2 ^b |
| | TNodPA | 18.28 ^a | 13.65 ^b | 20.48 ^a | 13.70 ^b |
| | NodB | 68.97 ^a | 51.04 ^b | 72.57 ^a | 53.18 ^b |
| Beginning of seed filling | NLatRoot | 149.2 ^a | 116.3 ^b | 135.9 ^a | 116.7 ^b |
| | TRootL | 3267.9 ^a | 2852.5 ^b | 3126.6 ^a | 2790.3 ^a |
| | RootB | 947.2 ^a | 717.9 ^b | 819.3 ^a | 808.5 ^a |
| | NNod | 776.6 ^a | 506.0 ^b | 647.8 ^a | 557.3 ^b |
| | TNodPA | 28.18 ^a | 19.04 ^b | 25.94 ^a | 21.76 ^a |
| | NodB | 126.03 ^a | 71.71 ^b | 102.1 ^a | 92.15 ^a |

NLatRoot, number of first order lateral roots; RootB, root dry matter; TRootL, total root length; NNod, number of nodules; NodB, nodule dry matter; TNodPA, total nodule projected area; ShootQN, shoot nitrogen accumulation. Indicated for each gene, the mean value of the genotypes carrying one of the two different alleles. Means followed by different letters are significantly different based on multiple comparisons (LSD test) at the 0.05 probability level.

Supplemental Table I.2. Mean parental values, broad sense heritability (h^2), significance of genotype effect, Mean, Min and Max values for nitrogen acquisition structure traits recorded in RIL4 population in two glasshouse experiments (Pouches 2007, Pots 2006)

| Trait | | Pouches 2007 | | | | | | Pots 2006 | | | | | | | |
|---|------------|--------------|--------|-------|---------|-------|-------|-----------|--------|--------|-------|---------|--------|--------|--------|
| | | Ballet | Cameor | h^2 | Pr > F | Mean | Min | Max | Ballet | Cameor | h^2 | Pr > F | Mean | Min | Max |
| Number of first order Lateral Roots at d1 | NLatRootd1 | 7.3 | 0.0 | 0.35 | 0.003 | 1.3 | 0.0 | 5.7 | - | - | - | - | - | - | - |
| Number of first order Lateral Roots at d2 | NLatRootd2 | 35.0 | 9.3 | 0.69 | <0.0001 | 24.3 | 3.0 | 45.0 | - | - | - | - | - | - | - |
| Number of first order Lateral Roots at d3 | NLatRootd3 | 63.3 | 54.7 | 0.67 | <0.0001 | 44.2 | 9.7 | 87.0 | - | - | - | - | - | - | - |
| Number of first order Lateral Roots at d4 | NLatRootd4 | 97.0 | 76.3 | 0.68 | <0.0001 | 69.1 | 20.3 | 118.7 | - | - | - | - | - | - | - |
| Number of first order Lateral Roots at d5 | NLatRootd5 | 99.3 | 88.3 | 0.59 | <0.0001 | 79.1 | 27.7 | 126.7 | - | - | - | - | - | - | - |
| Number of first order Lateral Roots at d6 | NLatRootd6 | 98.3 | 95.0 | 0.50 | <0.0001 | 86.9 | 30.3 | 131.3 | - | - | - | - | - | - | - |
| Number of first order Lateral Roots at BF | NLatRoot | 112.0 | 101.7 | 0.12 | ns | 104.8 | 39.3 | 150.7 | 92.8 | 86.3 | 0.15 | ns | 91.9 | 56.3 | 141.0 |
| Tap Root Length at d0 (cm) | TRootLd0 | 3.2 | 2.6 | 0.88 | <0.0001 | 3.2 | 1.6 | 5.8 | - | - | - | - | - | - | - |
| Total Root Length at d1 (cm) | TRootLd1 | 12.1 | 7.0 | 0.67 | <0.0001 | 8.7 | 2.6 | 13.1 | - | - | - | - | - | - | - |
| Total Root Length at d2 (cm) | TRootLd2 | 74.8 | 31.3 | 0.56 | <0.0001 | 45.2 | 9.4 | 80.3 | - | - | - | - | - | - | - |
| Total Root Length at d3 (cm) | TRootLd3 | 194.6 | 101.6 | 0.61 | <0.0001 | 127.9 | 24.1 | 201.9 | - | - | - | - | - | - | - |
| Total Root Length at d4 (cm) | TRootLd4 | 305.3 | 244.9 | 0.76 | <0.0001 | 253.2 | 68.2 | 407.1 | - | - | - | - | - | - | - |
| Total Root Length at d6 (cm) | TRootLd6 | 437.4 | 396.4 | 0.76 | <0.0001 | 425.2 | 190.7 | 577.5 | - | - | - | - | - | - | - |
| Total Root Length at BF (cm) | TRootL | 826.8 | 728.4 | 0.84 | <0.0001 | 812.2 | 485.3 | 1272.3 | 1818.9 | 1906.7 | 0.78 | <0.0001 | 1955.1 | 1215.9 | 3226.6 |
| First order Lateral Root Appearance Rate | LatApR | 0.454 | 0.415 | 0.67 | <0.0001 | 0.341 | 0.102 | 0.584 | - | - | - | - | - | - | - |
| Total Root Elongation Rate (cm/°C.day) | TRootER | 1.766 | 1.662 | 0.76 | <0.0001 | 1.608 | 0.461 | 2.622 | - | - | - | - | - | - | - |
| Root Biomass at BF (mg) | RootB | 151.9 | 121.5 | 0.85 | <0.0001 | 157.2 | 89.4 | 271.1 | 321.6 | 259.5 | 0.9 | <0.0001 | 340.6 | 135.3 | 749.5 |
| Number of Nodules at d4 | NNodd4 | 0.7 | 5.7 | 0.50 | <0.0001 | 2.1 | 0.0 | 11.3 | - | - | - | - | - | - | - |
| Number of Nodules at d5 | NNodd5 | 6.0 | 12.3 | 0.46 | <0.0001 | 10.1 | 0.0 | 39.0 | - | - | - | - | - | - | - |
| Number of Nodules at d6 | NNodd6 | 24.0 | 35.3 | 0.51 | <0.0001 | 31.0 | 0.3 | 80.0 | - | - | - | - | - | - | - |
| Number of Nodules at BF | NNod | 56.3 | 68.3 | 0.40 | 0.0005 | 56.3 | 9.7 | 117.7 | 122.2 | 209.3 | 0.74 | <0.0001 | 177.9 | 45.8 | 458.0 |
| Total Nodule Projected Area at d4 (cm ²) | TNodPA d4 | 0.007 | 0.068 | 0.44 | <0.0001 | 0.022 | 0.000 | 0.112 | - | - | - | - | - | - | - |
| Total Nodule Projected Area at d6 (cm ²) | TNodPA d6 | 0.239 | 0.402 | 0.55 | <0.0001 | 0.294 | 0.003 | 0.682 | - | - | - | - | - | - | - |
| Total Nodule Projected Area at BF (cm ²) | TNodPA | 1.745 | 0.994 | 0.77 | <0.0001 | 1.631 | 0.532 | 3.510 | 12.26 | 3.82 | 0.91 | <0.0001 | 8.97 | 1.02 | 24.36 |
| Nodule Appearance Rate (nb/°C.day) | NodApR | 0.161 | 0.205 | 0.43 | 0.0002 | 0.200 | 0.010 | 0.506 | - | - | - | - | - | - | - |
| Nodule Projected Area Increase Rate (mm ² /°C.day) | NodPAR | 0.156 | 0.225 | 0.49 | <0.0001 | 0.184 | 0.004 | 0.451 | - | - | - | - | - | - | - |
| Nodule Biomass at BF (mg) | NodB | 13.87 | 16.38 | 0.70 | <0.0001 | 14.61 | 3.95 | 34.76 | 19.07 | 30.74 | 0.8 | <0.0001 | 34.50 | 5.41 | 108.92 |
| Nodule Biomass:Belowground Biomass at BF | NB:BGB | 0.081 | 0.118 | 0.62 | <0.0001 | 0.085 | 0.026 | 0.169 | 0.056 | 0.107 | 0.49 | <0.0001 | 0.088 | 0.031 | 0.219 |
| Belowground Biomass:Total Biomass at BF | BGB:TB | 0.208 | 0.218 | 0.69 | <0.0001 | 0.224 | 0.159 | 0.309 | 0.126 | 0.194 | 0.83 | <0.0001 | 0.146 | 0.194 | 0.83 |

Supplemental Table I.3. Pearson correlation coefficients between nitrogen acquisition structure traits recorded in RIL4 population in two glasshouse experiments

| | NLatRoot-06 | TRootL-06 | RootB-06 | NNod-06 | TNodpA-06 | NodB-06 | NLatRoot-07 | TRootL-07 | RootB-07 | NNod-07 | TNodpA-07 | NodB-07 | LatApR-07 | TRootER-07 | NodApR-07 | TNodPAR-07 |
|-------------|-------------|-----------|----------|---------|-----------|---------|-------------|-----------|----------|---------|-----------|---------|-----------|------------|-----------|------------|
| NLatRoot-06 | | 0.36*** | ns | 0.23* | ns | ns | 0.20* | 0.22* | ns | ns | 0.24* | 0.24* | 0.20* | ns | ns | ns |
| TRootL-06 | | | 0.78*** | 0.75*** | 0.70*** | 0.63*** | 0.28** | 0.67*** | 0.61*** | 0.30** | 0.51*** | 0.50*** | 0.40*** | 0.44*** | 0.41*** | 0.36*** |
| RootB-06 | | | | 0.76*** | 0.94*** | 0.76*** | 0.21* | 0.75*** | 0.76*** | 0.33*** | 0.68*** | 0.41*** | 0.34*** | 0.40*** | 0.47*** | 0.42*** |
| NNod-06 | | | | | 0.71*** | 0.75*** | ns | 0.57*** | 0.53*** | 0.30** | 0.45*** | 0.41*** | 0.26** | 0.31** | 0.40*** | 0.32** |
| TNodpA-06 | | | | | | 0.77*** | ns | 0.74*** | 0.77*** | 0.35*** | 0.68*** | 0.39*** | 0.34*** | 0.40*** | 0.48*** | 0.44*** |
| NodB-06 | | | | | | | ns | 0.55*** | 0.58*** | 0.28** | 0.50*** | 0.42*** | 0.27** | 0.29** | 0.42*** | 0.36*** |
| NLatRoot-07 | | | | | | | | 0.33*** | ns | ns | 0.23* | 0.24* | 0.67*** | 0.50*** | ns | ns |
| TRootL-07 | | | | | | | | | 0.89*** | 0.44*** | 0.74*** | 0.50*** | 0.45*** | 0.57*** | 0.38*** | 0.27** |
| RootB-07 | | | | | | | | | | 0.35*** | 0.84*** | 0.44*** | 0.31** | 0.41*** | 0.36*** | 0.29** |
| NNod-07 | | | | | | | | | | | 0.25* | 0.37*** | 0.20* | 0.35*** | 0.78*** | 0.60*** |
| TNodpA-07 | | | | | | | | | | | | 0.42*** | 0.26** | 0.28** | 0.28** | 0.24* |
| NodB-07 | | | | | | | | | | | | | 0.22* | 0.31** | 0.30** | 0.26** |
| LatApR-07 | | | | | | | | | | | | | | 0.85*** | 0.24* | 0.33*** |
| TRootER-07 | | | | | | | | | | | | | | | 0.38*** | 0.42*** |
| NodApR-07 | | | | | | | | | | | | | | | | 0.88*** |
| TNodPAR-07 | | | | | | | | | | | | | | | | |

*, **, ***: significant correlation at the 0.05, 0.01, 0.001 probability level, respectively.

Supplemental Table I.4. Mean parental values, broad sense heritability (h^2) and significance of genotype effect for carbon accumulation, nitrogen acquisition functioning traits and developmental variables recorded in RIL4 population in two glasshouse and two field experiments

| Trait | | Pouches 2007 | | | | Pots 2006 | | | | Field 2006 | | | | Field 2004 | |
|---|---------|--------------|--------|-------|---------|-----------|--------|-------|---------|------------|---------|-------|---------|------------|---------|
| | | Ballet | Cameor | h^2 | Pr > F | Ballet | Cameor | h^2 | Pr > F | Ballet | Cameor | h^2 | Pr > F | Ballet | Cameor |
| Shoot Biomass or maturity (mg) | ShootB | 635 | 498.4 | 0.90 | <0.0001 | 2366.9 | 1203.5 | 0.96 | <0.0001 | 33979.3 | 30515.6 | 0.58 | <0.0001 | 37264.4 | 38628.9 |
| Straw Biomass (mg) | StrawB | - | - | - | - | - | - | - | - | 12431.3 | 10080.0 | 0.61 | <0.0001 | 14466.3 | 13313.8 |
| Seed Biomass (mg) | SeedB | - | - | - | - | - | - | - | - | 21548.1 | 20435.6 | 0.58 | <0.0001 | 22798.1 | 25315.1 |
| Seed Number | SeedNb | - | - | - | - | - | - | - | - | 83.1 | 95.9 | 0.64 | <0.0001 | 90.3 | 123.0 |
| Thousand Seed Weight (mg) | TSW | - | - | - | - | - | - | - | - | 258.8 | 213.5 | 0.83 | <0.0001 | 252.5 | 205.8 |
| Percentage of N derived from fixation | NDFA | 69.9 | 71.1 | 0.53 | <0.0001 | 55.3 | 74.6 | 0.9 | <0.0001 | 84.0 | 84.9 | 0.49 | <0.0001 | 46.8 | 49.1 |
| Shoot N content (%) | ShootNC | 3.17 | 4.14 | 0.84 | <0.0001 | 3.28 | 4.66 | 0.9 | <0.0001 | 2.77 | 3.32 | 0.6 | <0.0001 | 2.44 | 3.01 |
| Straw N content (%) | StrawNC | - | - | - | - | - | - | - | - | 1.04 | 1.01 | 0.7 | <0.0001 | 1.02 | 1.02 |
| Seed N content (%) | SeedNC | - | - | - | - | - | - | - | - | 3.76 | 4.46 | 0.67 | <0.0001 | 3.34 | 4.05 |
| Shoot N accumulation (mg) | ShootQN | 20.1 | 20.6 | 0.77 | <0.0001 | 77.5 | 56.2 | 0.95 | <0.0001 | 943.6 | 1016.6 | 0.52 | <0.0001 | 910.2 | 1160.7 |
| Straw N accumulation (mg) | StrawQN | - | - | - | - | - | - | - | - | 129.1 | 103.0 | 0.71 | <0.0001 | 147.0 | 135.5 |
| Seed N accumulation (mg) | SeedQN | - | - | - | - | - | - | - | - | 814.5 | 913.6 | 0.53 | <0.0001 | 763.2 | 1025.2 |
| Specific Nitrogen Uptake (g ShootQN/g belowground) | SNU | 0.121 | 0.150 | 0.38 | 0.0013 | 0.245 | 0.194 | 0.68 | <0.0001 | - | - | - | - | - | - |
| Root Efficiency (g ShootQNabs/g root) | RootE | 0.039 | 0.050 | 0.48 | <0.0001 | 0.113 | 0.055 | 0.81 | <0.0001 | - | - | - | - | - | - |
| Nodule Efficiency (g ShootQNfix/g nodule) | NodE | 1.088 | 0.907 | 0.39 | 0.0004 | 3.034 | 1.392 | 0.57 | <0.0001 | - | - | - | - | - | - |
| Leaf Area at d3 (cm ²) | LeafAd3 | 4.5 | 4.4 | 0.60 | <0.0001 | - | - | - | - | - | - | - | - | - | - |
| Leaf Area at d4 (cm ²) | LeafAd4 | 10.0 | 14.2 | 0.56 | <0.0001 | - | - | - | - | - | - | - | - | - | - |
| Leaf Area at d5 (cm ²) | LeafAd5 | 17.2 | 24.4 | 0.66 | <0.0001 | - | - | - | - | - | - | - | - | - | - |
| Leaf Area at d6 (cm ²) | LeafAd6 | 33.4 | 40.2 | 0.75 | <0.0001 | - | - | - | - | - | - | - | - | - | - |
| Leaf Area (cm ²) | LeafA | 87.4 | 104.4 | 0.92 | <0.0001 | - | - | - | - | - | - | - | - | - | - |
| Leaf Area Increase Rate before BF (cm ² /°C.day) | LeafAR | 0.16 | 0.175 | 0.73 | <0.0001 | - | - | - | - | - | - | - | - | - | - |
| Radiation Use Efficiency (g TotalB/MJ) | RUE | 2.191 | 1.801 | 0.91 | <0.0001 | - | - | - | - | - | - | - | - | - | - |
| Leaf Chlorophyll content (SPAD) | SPAD | 33.18 | 38.86 | 0.73 | <0.0001 | - | - | - | - | - | - | - | - | - | - |
| Specific Leaf Nitrogen (g ShootQN/m ² leaf) | SLN | 2.29 | 1.97 | 0.90 | <0.0001 | - | - | - | - | - | - | - | - | - | - |
| Shoot Length or maturity (cm) | ShootL | 22.3 | 20.8 | 0.89 | <0.0001 | 74.9 | 47.5 | 0.97 | <0.0001 | 78.8 | 66.7 | 0.87 | <0.0001 | 84.4 | 78.8 |
| Number of basal branches | NBranch | 0.00 | 0.00 | 0.00 | ns | 0.000 | 0.056 | 0.64 | <0.0001 | 0.475 | 0.850 | 0.61 | <0.0001 | 0.775 | 0.963 |
| Leaf Appearance Rate before BF (nb/°C.day) | LeafApR | 0.02 | 0.016 | 0.43 | 0.0002 | 0.0167 | 0.0148 | 0.45 | <0.0001 | - | - | - | - | - | - |
| Beginning of flowering date (°C.day) | BegFlo | 883.0 | 788.4 | 0.99 | <0.0001 | 1032 | 799.8 | 0.99 | <0.0001 | 840.3 | 746.4 | 0.94 | <0.0001 | 849.9 | 736.7 |

Supplemental Table I.5. Mean, Minimum and Maximum values of carbon accumulation, nitrogen acquisition functioning traits and developmental variables recorded in RIL4 population in two glasshouse and two field experiments

| Trait | | Pouches 2007 | | | Pots 2006 | | | Field 2006 | | | Field 2004 | | |
|---|---------|--------------|-------|--------|-----------|-------|--------|------------|--------|---------|------------|---------|---------|
| | | Mean | Min | Max | Mean | Min | Max | Mean | Min | Max | Mean | Min | Max |
| Shoot Biomass or maturity (mg) | ShootB | 613.0 | 279.5 | 1137.4 | 2298.4 | 682.8 | 4857.4 | 30936.5 | 9399.0 | 46157.2 | 37182.7 | 12357.0 | 62767.0 |
| Straw Biomass (mg) | StrawB | - | - | - | - | - | - | 11329.4 | 2875.0 | 22965.0 | 14745.8 | 4010.0 | 25400.0 |
| Seed Biomass (mg) | SeedB | - | - | - | - | - | - | 19607.1 | 6524.0 | 29229.2 | 22429.1 | 8347.0 | 38507.0 |
| Seed Number | SeedN | - | - | - | - | - | - | 80.6 | 22.4 | 132.5 | 99.2 | 42.0 | 161.9 |
| Thousand Seed Weight (mg) | TSW | - | - | - | - | - | - | 244.8 | 185.2 | 305.8 | 226.7 | 128.5 | 305.9 |
| Percentage of N derived from fixation | NDFA | 70.6 | 57.0 | 80.4 | 66 | 43.4 | 79.3 | 84.1 | 77.1 | 89.6 | 49.0 | 17.8 | 71.9 |
| Shoot N content (%) | ShootNC | 3.19 | 1.97 | 4.43 | 3.89 | 3.12 | 5.14 | 2.99 | 2.56 | 3.58 | 2.69 | 2.01 | 3.29 |
| Straw N content (%) | StrawNC | - | - | - | - | - | - | 1.10 | 0.78 | 1.77 | 1.15 | 0.77 | 1.71 |
| Seed N content (%) | SeedNC | - | - | - | - | - | - | 4.07 | 3.59 | 4.72 | 3.69 | 3.07 | 4.41 |
| Shoot N accumulation (mg) | ShootQN | 18.9 | 10.1 | 31.1 | 87.8 | 25.1 | 182.4 | 924.0 | 339.0 | 1399.0 | 999.7 | 407.2 | 1691.5 |
| Straw N accumulation (mg) | StrawQN | - | - | - | - | - | - | 123.5 | 43.6 | 364.5 | 169.5 | 57.7 | 410.8 |
| Seed N accumulation (mg) | SeedQN | - | - | - | - | - | - | 800.6 | 287.7 | 1243.4 | 829.4 | 349.5 | 1483.1 |
| Specific Nitrogen Uptake (g ShootQN/g belowground) | SNU | 0.113 | 0.072 | 0.200 | 0.238 | 0.113 | 0.398 | - | - | - | - | - | - |
| Root Efficiency (g ShootQNabs/g root) | RootE | 0.036 | 0.021 | 0.059 | 0.089 | 0.030 | 0.207 | - | - | - | - | - | - |
| Nodule Efficiency (g ShootQNfix/g nodule) | NodE | 1.334 | 0.413 | 8.137 | 2.080 | 0.669 | 4.930 | - | - | - | - | - | - |
| Leaf Area at d3 (cm ²) | LeafAd3 | 4.4 | 0.8 | 11.4 | - | - | - | - | - | - | - | - | - |
| Leaf Area at d4 (cm ²) | LeafAd4 | 13.1 | 4.3 | 27.6 | - | - | - | - | - | - | - | - | - |
| Leaf Area at d5 (cm ²) | LeafAd5 | 23.8 | 10.3 | 42.3 | - | - | - | - | - | - | - | - | - |
| Leaf Area at d6 (cm ²) | LeafAd6 | 40.7 | 20.1 | 67.9 | - | - | - | - | - | - | - | - | - |
| Leaf Area (cm ²) | LeafA | 100.9 | 47.5 | 183.8 | - | - | - | - | - | - | - | - | - |
| Leaf Area Increase Rate before BF (cm ² /°C.day) | LeafAR | 0.185 | 0.096 | 0.325 | - | - | - | - | - | - | - | - | - |
| Radiation Use Efficiency (g TotalB/MJ) | RUE | 2.03 | 1.31 | 3.65 | - | - | - | - | - | - | - | - | - |
| Leaf Chlorophyll content (SPAD) | SPAD | 32.60 | 23.31 | 45.15 | - | - | - | - | - | - | - | - | - |
| Specific Leaf Nitrogen (g ShootQN/m ² leaf) | SLN | 1.98 | 1.30 | 2.87 | - | - | - | - | - | - | - | - | - |
| Shoot Length or maturity (cm) | ShootL | 21.8 | 12.7 | 30.5 | 66.7 | 36.3 | 105.5 | 69.7 | 30 | 100 | 84.3 | 45.0 | 115.0 |
| Number of basal branches | NBranch | 0.01 | 0.00 | 1.00 | 0.11 | 0.00 | 1.00 | 0.79 | 0.05 | 2.20 | 0.91 | 0.10 | 2.20 |
| Leaf Appearance Rate before BF (nb/°C.day) | LeafApR | 0.015 | 0.009 | 0.018 | 0.016 | 0.012 | 0.017 | - | - | - | - | - | - |
| Beginning of flowering date (°C.day) | BegFlo | 866.8 | 694.0 | 1038.6 | 952.2 | 699.9 | 1090.7 | 830.3 | 720.1 | 1007.3 | 808.3 | 717.4 | 872.5 |

Supplemental Table I.6. Characteristics of the QTL for nitrogen acquisition structure and functioning traits detected in RIL4 population from both glasshouse and field experiments

| | Trait | Linkage group | Position (cM) | Marker flanking the QTL; marker at the | LOD ^a | R ² (%) ^b | Additive effect ^c |
|-------------|---------------|---------------|---------------|--|------------------|---------------------------------|------------------------------|
| Root traits | NLatRoot-06 | LGI | 134.5 | Phos4kin | 3.4 | 9.5 | 2.71 |
| | LatApR-07 | LGIII | 144.4 | CdK3, AA374 | 5.5 | 18.3 | -0.02 |
| | NLatRootd3-07 | LGIII | 144.4 | CdK3, AA374 | 4.5 | 15.6 | -2.78 |
| | NLatRootd4-07 | LGIII | 142.4 | CdK3, AA374 | 5.4 | 18.2 | -4.83 |
| | NLatRootd5-07 | LGIII | 142.4 | CdK3, AA374 | 5.8 | 19.1 | -4.96 |
| | NLatRootd6-07 | LGIII | 144.4 | CdK3, AA374 | 5.3 | 17.9 | -4.51 |
| | NLatRootd7-07 | LGIII | 147.6 | AA374 | 2.9 | 10.5 | -3.04 |
| | NLatRootd3-07 | LGIV | 43 | AA386, AD186_2 | 2.8 | 10.3 | -2.28 |
| | TRootLd6-07 | LGI | 136.5 | Phos4kin, <i>Af</i> | 7.3 | 23.4 | 18.22 |
| | TRootLd7-07 | LGI | 138.2 | Af | 2.9 | 10.6 | 12.69 |
| | TRootL-06 | LGI | 138.2 | Af | 21 | 39.7 | 106.21 |
| | TRootL-07 | LGI | 138.2 | Af | 10.6 | 30.2 | 35.79 |
| | TRootL-06 | LGII | 34.8 | Dioxase, AB72 | 1.5 | 4.6 | -28.45 |
| | TRootLd6-07 | LGII | 60.2 | AB33 | 3.7 | 13.4 | -12.42 |
| | TRootER-07 | LGIII | 142.4 | CdK3, AA374 | 5 | 17.4 | -0.09 |
| | TRootLd4-07 | LGIII | 156.8 | AB68 | 4.7 | 16.5 | -11.97 |
| | TRootLd7-07 | LGIII | 156.8 | AB68 | 3.5 | 12.8 | -14.12 |
| | TRootER-07 | LGIV | 39 | AA386 | 5.9 | 19.9 | -0.1 |
| | TRootLd3-07 | LGIV | 45 | AA386, AD186_2 | 3 | 11 | -6.25 |
| | TRootLd4-07 | LGIV | 41 | AA386, AD186_2 | 5.2 | 17.9 | -14.14 |
| | TRootER-07 | LGV | 39 | AA255_3, AD158 | 5.4 | 18.6 | 0.09 |
| | TRootLd4-07 | LGV | 39 | AA255_3, AD158 | 5.3 | 18.1 | 14.09 |
| | TRootLd7-07 | LGV | 41 | AA255_3, AD158 | 3.2 | 11.7 | 14.44 |
| | TRootLd0-07 | LGV | 114.5 | AB146, AD155 | 4.1 | 14.3 | 0.19 |
| | TRootER-07 | LGVII | 71.1 | Acetisom | 3.3 | 12.2 | -0.07 |
| | TRootLd2-07 | LGVII | 77.3 | SuTMem, AD159_4 | 3.6 | 12.8 | -2.71 |
| | TRootLd3-07 | LGVII | 77.3 | SuTMem, AD159_4 | 4.1 | 14.6 | -6.86 |
| | TRootLd4-07 | LGVII | 71.1 | Acetisom | 3.7 | 13.3 | -10.75 |
| | TRootLd6-07 | LGVII | 77.9 | AD159_4 | 4.5 | 15.8 | -13.84 |
| | RootB-06 | LGI | 138.2 | Af | 30.7 | 49 | 38.09 |
| | RootB-07 | LGI | 138.2 | Af | 17 | 41.1 | 9.19 |
| | RootB-06 | LGIII | 172.8 | ERDP | 2.4 | 7 | -11.37 |
| | Nodule traits | NodApR-07 | LGI | 138.2 | Af | 2.9 | 10.5 |
| NNodd5-07 | | LGI | 138.2 | Af | 3.3 | 11.7 | 1.45 |
| NNodd6-07 | | LGI | 138.2 | Af | 3.8 | 13.3 | 2.99 |
| NNod-06 | | LGI | 136.5 | Phos4kin- <i>Af</i> | 17.1 | 35.2 | 21.42 |
| NNod-06 | | LGIII | 44.7 | AB104_2, Uni | 6.2 | 16.5 | 17.88 |
| NNodd4-07 | | LGIII | 72.9 | AAP1 | 2.8 | 10.1 | -0.4 |
| NNod-06 | | LGIII | 176.6 | AB53, bfruct | 3.7 | 10.5 | -10.25 |
| NNod-07 | | LGV | 37 | AA255_3 | 3.6 | 12.9 | 4.11 |
| NNod-06 | | LGVII | 57.4 | Htrans, Acetisom | 5.7 | 15.4 | 15.03 |
| NodPAR-07 | | LGI | 138.2 | Af | 4.6 | 15.7 | 0.019 |
| NodPAd6-07 | | LGI | 138.2 | Af | 5.4 | 18.1 | 0.032 |
| NodPA-06 | | LGI | 138.2 | Af | 25.9 | 45.1 | 1.332 |
| NodPA-07 | | LGI | 138.2 | Af | 9 | 26.9 | 0.099 |
| NodPA-06 | | LGII | 25.3 | AA18 | 1.5 | 4.6 | -0.334 |
| NodPAd4-07 | | LGIII | 72.9 | AAP1 | 3.5 | 12.7 | -0.005 |

Annexes du Chapitre I

| | | | | | | | |
|------------------------------------|-----------|-------|-------|---------------------|------|------|---------|
| | NodPA-06 | LGIII | 171.3 | AD270, ERDP | 4 | 11.2 | -0.557 |
| | NodPA-06 | LGVII | 35.5 | AD146 | 3.7 | 10.4 | 0.49 |
| | NodB-06 | LGI | 136.5 | <i>Af</i> | 20.9 | 39.7 | 5.88 |
| | NodB-07 | LGI | 138.2 | Af | 6.8 | 21.7 | 1.4 |
| | NodB-06 | LGIII | 178.6 | AB53, bfruct | 2.8 | 8.1 | -2.24 |
| | NodB-06 | LGVII | 37.5 | AD146, B14 | 5.1 | 13.9 | 3.02 |
| | NB:BGB-06 | LGI | 133.2 | D21, Phos4kin | 3.8 | 10.5 | 0.53 |
| | NB:BGB-06 | LGVII | 65.4 | Htrans, Acetisom | 3 | 8.6 | 0.55 |
| | NB:BGB-07 | LGVII | 35.5 | AD146 | 3 | 11 | -0.5 |
| Belowground dry matter | BGB:TB-06 | LGI | 38.7 | ACCox, PSU81288 | 4.6 | 12.8 | -0.0036 |
| | BGB:TB-06 | LGI | 138.2 | Af | 3.3 | 9.5 | 0.0023 |
| | BGB:TB-07 | LGI | 138.2 | Af | 12.2 | 33.2 | 0.0076 |
| | BGB:TB-06 | LGII | 0 | ThiolP | 3.8 | 10.9 | -0.0023 |
| | BGB:TB-06 | LGVII | 83.9 | AD159_4, Sym29 | 4.9 | 13.5 | -0.0028 |
| Nitrogen accumulation efficiency | SNU-07 | LGI | 138.2 | Af | 2.4 | 8.8 | -0.003 |
| | SNU-06 | LGVII | 75.3 | SuTMem | 3.5 | 9.8 | 0.0072 |
| | RootE-06 | LGI | 136.5 | Phos4kin, <i>Af</i> | 8.5 | 21 | -0.006 |
| | RootE-07 | LGI | 138.2 | Af | 4.5 | 15.5 | -0.0015 |
| | RootE-06 | LGII | 2 | ThiolP | 4.3 | 11.9 | 0.0044 |
| | NodE-06 | LGI | 136.5 | <i>Af</i> | 3.8 | 10.7 | -0.119 |
| | NodE-06 | LGIII | 178.6 | AB53, bfruct | 2.9 | 8.2 | 0.1075 |
| Relative part of nitrogen fixation | NDFA-06 | LGI | 136.5 | Phos4kin, <i>Af</i> | 7.8 | 19.5 | 1.83 |
| | NDFA-04f | LGII | 36.1 | AB109 | 3.3 | 12 | -1.9 |
| | NDFA-06f | LGIII | 185.2 | bfruct, AB141 | 3.9 | 10.7 | -0.55 |

^a LOD is the Log_likelihood at the position

^b R² is the percentage of the phenotypic variation explained by the QTL

^c Additive allelic value of 'Cameor'

Supplemental Table I.7. Characteristics of the QTL for carbon accumulation and seed traits

| | Trait | Linkage group | Position (cM) | Marker flanking the QTL; | | | Additive effect ^c |
|----------------------------|------------|---------------|---------------|--------------------------|------------------|---------------------------------|------------------------------|
| | | | | marker at the QTL peak | LOD ^a | R ² (%) ^b | |
| Carbon accumulation traits | LeafA-07 | LGI | 138.2 | Af | 94.1 | 79.4 | 13.19 |
| | LeafAd3-07 | LGI | 136.5 | Phos4kin, <i>Af</i> | 4.8 | 16.5 | 0.46 |
| | LeafAd4-07 | LGI | 138.2 | Af | 12 | 32.8 | 1.47 |
| | LeafAd5-07 | LGI | 138.2 | Af | 18.5 | 43 | 2.49 |
| | LeafAd6-07 | LGI | 138.2 | Af | 41.4 | 62.8 | 4.88 |
| | LeafAd7-07 | LGI | 138.2 | Af | 48.8 | 66.6 | 7.81 |
| | LeafAR-07 | LGI | 138.2 | Af | 49.6 | 67 | 0.02 |
| | LeafAd3-07 | LGVI | 193.5 | AC76b, Dmi | 2.6 | 9.6 | 0.35 |
| | RUE-07 | LGI | 138.2 | Af | 75.9 | 75.9 | -0.04 |
| | RUE-07 | LGVI | 191.5 | AC76b, Dmi | 1.3 | 5.2 | -0.01 |
| | RUE-07 | LGVII | 30.3 | AB133, AD56 | 4.6 | 16.1 | 0.01 |
| | ShootB-06 | LGI | 138.2 | Af | 23 | 42.3 | 186.14 |
| | ShootB-06 | LGII | 57 | AA372_1, AA1 | 2.7 | 8.1 | -70.6 |
| | ShootB-06 | LGIII | 160.8 | AB68, AA355 | 2.9 | 8.5 | -71.28 |
| | ShootB-06 | LGV | 65.4 | AA163_2 | 3.7 | 10.5 | 74.4 |
| | ShootB-06 | LGVII | 39.5 | AD146, B14 | 7.7 | 19.7 | 116.72 |
| | ShootB-07 | LGVII | 39.5 | AD146, B14 | 2.8 | 10.4 | 15.36 |
| | StrawB-06f | LGI | 77.9 | AA155 | 3.6 | 10.2 | -392.88 |
| | StrawB-04f | LGII | 58.9 | AA1 | 6 | 13.9 | -635.4 |
| | StrawB-06f | LGII | 68.2 | AB33, AB149 | 8.6 | 21.4 | -815.59 |
| | StrawB-04f | LGIII | 95 | AAP1, AB44_2 | 3.5 | 8.6 | -521.67 |
| | StrawB-06f | LGIV | 219.9 | OEE3 | 3.5 | 9.9 | -387.54 |
| | StrawB-04f | LGV | 46.3 | AD158, AB83 | 3.1 | 7.8 | 472.3 |
| | SeedB-06f | LGI | 136.5 | Phos4kin, <i>Af</i> | 6.8 | 17.6 | 873.69 |
| | SeedB-04f | LGI | 138.2 | Af | 7.6 | 17 | 978.33 |
| | SeedB-06f | LGII | 72.2 | AB33, AB149 | 8 | 20 | -1357.43 |
| | SeedB-04f | LGIII | 99 | AA278 | 7.3 | 16.4 | -989.63 |
| | SeedB-04f | LGIV | 37 | AA386 | 3.7 | 9.1 | -701.33 |
| | SeedB-04f | LGVII | 37.5 | AD146, B14 | 5.8 | 13.6 | 898.9 |
| | TSW-06f | LGI | 21.4 | Agps2, ACCox | 7 | 18.3 | -5.59 |
| | TSW-04f | LGIII | 156.8 | AB68 | 4.6 | 11 | -4 |
| | TSW-06f | LGIII | 158.8 | AB68, AA355 | 4.1 | 11.7 | -3.58 |
| | TSW-04f | LGIV | 32.6 | AA219, AA386 | 5.6 | 13.1 | -5.04 |
| | TSW-06f | LGIV | 39 | AA386, AD186_2 | 3.9 | 11 | -3.61 |
| | TSW-06f | LGV | 0 | OPT | 3.5 | 9.9 | -3.12 |
| | TSW-04f | LGV | 124.6 | AC58 | 3.6 | 8.8 | -3.58 |
| | TSW-06f | LGV | 137.2 | COLa1, Rbcs4 | 4.6 | 12.8 | -3.95 |
| | TSW-04f | LGVII | 7.1 | AA416 | 3.8 | 9.3 | 3.73 |
| | SeedN-04f | LGI | 129.2 | D21, Phos4kin | 7.8 | 17.3 | 4.71 |
| | SeedN-06f | LGI | 134.5 | Phos4kin | 4.7 | 12.9 | 2.91 |
| | SeedN-06f | LGII | 66.2 | AB33, AB149 | 5.6 | 15 | -4.07 |

Annexes du Chapitre I

| | | | | | | | |
|--|-------------|-------|-----------------|------------------|------|-------|--------|
| | SeedN-04f | LGIII | 95 | AB44_2, AA278 | 6.8 | 15.3 | -4.59 |
| | SeedN-06f | LGV | 133.2 | COLa1, Rbcs4 | 3.5 | 10 | 3 |
| | SeedN-04f | LGVII | 35.5 | AD146 | 4.9 | 11.5 | 3.45 |
| Developmental and morphological traits | BegFlo-06f | LGII | 4 | ThiolP, AA473 | 3 | 8.6 | 6.33 |
| | BegFlo-07 | LGII | 57 | AA372_1, AA1 | 61.7 | 72.1 | -41.16 |
| | BegFlo-04f | LGII | 59.9 | AD83 | 54.2 | 59 | -15.29 |
| | BegFlo-06f | LGII | 59.9 | AD83 | 36.7 | 53.6 | -20.88 |
| | BegFlo-07 | LGIII | 95 | AB44_2, AA278 | 4.8 | 16.8 | 11.52 |
| | BegFlo-07 | LGIII | 169.3 | AD270 | 12.3 | 34.1 | -16.71 |
| | BegFlo-04f | LGV | 41 | AA255_3, AD158 | 3.5 | 8.6 | 4.17 |
| | BegFlo-06f | LGVI | 132.3 | FVE, Akin2 | 3.7 | 10.4 | -7.51 |
| | BegFlo-07 | LGVII | 59.4 | Htrans, Acetisom | 5.5 | 18.8 | -14.12 |
| | NBranch-06 | LGI | 138.2 | Af | 4.7 | 12.9 | 0.03 |
| | NBranch-06f | LGII | 4 | ThiolP, AA473 | 6.1 | 16.2 | -0.07 |
| | NBranch-06f | LGIII | 82.9 | AAP1, AB44_2 | 3.1 | 9.1 | 0.06 |
| | NBranch-04f | LGIII | 84.9 | AAP1, AB44_2 | 6.4 | 14.5 | 0.09 |
| | NBranch-04f | LGIII | 120.2 | AB44_3, AB111 | 5.4 | 12.7 | -0.07 |
| | NBranch-06 | LGIV | 0 | L1L | 4.2 | 11.5 | -0.03 |
| | NBranch-06f | LGV | 129.2 | COLa1, Rbcs4 | 5.4 | 14.6 | 0.07 |
| | NBranch-04f | LGVI | 120.8 | PsAGO, FVE | 5.9 | 13.5 | -0.07 |
| | NBranch-06f | LGVI | 126.8 | PsAGO, FVE | 13.6 | 30.1 | -0.1 |
| | ShootL-06 | LGI | 46.7 | ACCox, PSU81288 | 3.8 | 10.8 | 1.89 |
| | ShootL-06 | LGII | 59.9 | AD83 | 3.9 | 11.2 | -1.23 |
| | ShootL-06f | LGII | 59.9 | AD83 | 6.5 | 17.1 | -1.82 |
| | ShootL-04f | LGII | 59.9 | AD83 | 3.4 | 8.5 | -1.72 |
| ShootL-04f | LGIII | 84.9 | AAP1, AB44_2 | 10.5 | 21.9 | -3.65 | |
| ShootL-07 | LGIII | 93 | AB44_2 | 19.3 | 44.4 | -1.01 | |
| ShootL-06 | LGIII | 95 | AB44_2, AA278 | 29.6 | 48.8 | -3.76 | |
| ShootL-06f | LGIII | 95 | AB44_2, AA278 | 19.5 | 38.2 | -3.44 | |
| ShootL-06 | LGV | 60.8 | AB83, AA163_2 | 5.9 | 16.1 | 1.59 | |
| ShootL-06f | LGV | 69.4 | AA163_2, AC10_2 | 5.7 | 15.3 | 1.72 | |
| ShootL-07 | LGVII | 35.5 | AD146 | 48 | 66.4 | 1.43 | |
| ShootL-06 | LGVII | 35.5 | AD146 | 17.1 | 35.6 | 2.65 | |
| ShootL-04f | LGVII | 35.5 | AD146 | 3.1 | 7.7 | 1.58 | |
| ShootL-06f | LGVII | 35.5 | AD146 | 11 | 25.9 | 2.31 | |
| ShootL-06 | LGVII | 72 | AA317 | 5.7 | 15.6 | 1.51 | |

^a LOD is the Log_likelihood at the position

^b R² is the percentage of the phenotypic variation explained by the QTL

^c Additive allelic value of 'Cameor'

Supplemental Table I.8. Characteristics of the QTL for nitrogen accumulation and content detected in RIL4 population from both glasshouse and field experiments

| | Trait | Linkage group | Position (cM) | Marker flanking the QTL; marker at the QTL peak | LOD ^a | R ² (%) ^b | Additive effect ^c |
|------------------------------|-------------|---------------|---------------|--|------------------|---------------------------------|------------------------------|
| Nitrogen accumulation traits | ShootQN-07 | LGI | 136.5 | Phos4kin, <i>Af</i> | 6 | 19.8 | 0.7 |
| | ShootQN-06 | LGI | 138.2 | Af | 29.9 | 48.6 | 8.55 |
| | ShootQN-06 | LGI | 55 | AA372_1, AA1 | 3 | 8.6 | -3.1 |
| | ShootQN-07 | LGI | 84.9 | AAP1, AB44_2 | 2.6 | 9.6 | 0.56 |
| | ShootQN-06 | LGI | 57.4 | Htrans, Acetisom | 3.6 | 10.2 | 3.79 |
| | StrawQN-06f | LGI | 77.9 | AA155 | 3.8 | 10.8 | -5.53 |
| | StrawQN-04f | LGI | 58.9 | AA1 | 5.1 | 12 | -9.37 |
| | StrawQN-06f | LGI | 74.2 | AB33, AB149 | 4.4 | 12.2 | -9.31 |
| | StrawQN-06f | LGI | 219.9 | OEE3 | 3.4 | 9.8 | -5.24 |
| | SeedQN-06f | LGI | 136.5 | Phos4kin, <i>Af</i> | 6.8 | 17.6 | 38.01 |
| | SeedQN-04f | LGI | 138.2 | Af | 8.6 | 18.7 | 40.75 |
| | SeedQN-06f | LGI | 68.2 | AB33 | 5.5 | 14.6 | -43.7 |
| | SeedQN-04f | LGI | 99 | AB44_2, AA278 | 4.4 | 10.5 | -30.06 |
| | SeedQN-04f | LGI | 37 | AA386 | 3.7 | 9.1 | -27.54 |
| | SeedQN-04f | LGI | 37.5 | AD146, B14 | 7.9 | 17.5 | 41 |
| | SLN-07 | LGI | 138.2 | Af | 127.4 | 83.9 | -0.02 |
| Nitrogen content traits | ShootNC-06 | LGI | 124.6 | AB56_1, D21 | 4 | 11.1 | 0.07 |
| | ShootNC-07 | LGI | 136.5 | Phos4kin, <i>Af</i> | 3.1 | 11.6 | 0.06 |
| | ShootNC-06 | LGI | 82.9 | AAP1, AB44_2 | 3.7 | 10.3 | 0.08 |
| | ShootNC-07 | LGI | 95 | AB44_2, AA278 | 6.9 | 22.2 | 0.09 |
| | ShootNC-07 | LGI | 34.6 | Gs3b | 12.4 | 33.9 | -0.11 |
| | StrawNC-06f | LGI | 99.3 | AA278 | 5.9 | 15.5 | 0.04 |
| | StrawNC-04f | LGI | 99.3 | AA278 | 10.4 | 21.8 | 0.05 |
| | SeedNC-06f | LGI | 97 | AB44_2, AA278 | 5.6 | 15 | 0.04 |
| | SeedNC-04f | LGI | 99.3 | AA278 | 3.8 | 9.2 | 0.03 |
| | SeedNC-06f | LGI | 50.3 | AD158, AB83 | 4.8 | 13.1 | 0.04 |
| | SeedNC-06f | LGI | 141.2 | Rbcs4 | 5.2 | 14.3 | -0.03 |
| | SeedNC-06f | LGI | 57.4 | Htrans, Acetisom | 3.1 | 8.9 | 0.03 |
| | SeedNC-04f | LGI | 57.4 | Htrans, Acetisom | 6.8 | 15.3 | 0.05 |
| | SPAD-07 | LGI | 99 | AB44_2, AA278 | 12 | 33.2 | 0.93 |
| | SPAD-07 | LGI | 121.1 | AD55 | 3.2 | 11.8 | -0.47 |

^a LOD is the Log_e likelihood at the position

^b R² is the percentage of the phenotypic variation explained by the QTL

^c Additive allelic value of 'Cameor'

Supplemental Table I.9. Broad sense heritability (h^2), global R^2 , part of genetic variance explained by QTL (p) for nitrogen acquisition structure traits recorded in RIL4 population in two glasshouse experiments (Pouches 2007, Pots 2006)

| Trait | Pouches 2007 | | | Pots 2006 | | |
|------------|--------------|---------------------------|----------------------|-----------|--------------|---------|
| | h^2 | Global R^2 ^a | p (%) ^b | h^2 | Global R^2 | p (%) |
| NLatRootd1 | 0.35 | | | - | - | - |
| NLatRootd2 | 0.69 | | | - | - | - |
| NLatRootd3 | 0.67 | 0.24 | 35.7 | - | - | - |
| NLatRootd4 | 0.68 | 0.18 | 26.7 | - | - | - |
| NLatRootd5 | 0.59 | 0.19 | 32.4 | - | - | - |
| NLatRootd6 | 0.50 | 0.18 | 35.8 | - | - | - |
| NLatRoot | 0.12 | | | 0.15 | 0.09 | 63.3 |
| TRootLd0 | 0.88 | | | - | - | - |
| TRootLd1 | 0.67 | | | - | - | - |
| TRootLd2 | 0.56 | 0.13 | 22.9 | - | - | - |
| TRootLd3 | 0.61 | 0.26 | 42.8 | - | - | - |
| TRootLd4 | 0.76 | 0.44 | 57.7 | - | - | - |
| TRootLd6 | 0.76 | 0.41 | 54.3 | - | - | - |
| TRootL | 0.84 | | | 0.78 | 0.45 | 57.2 |
| LatApR | 0.67 | 0.18 | 27.3 | - | - | - |
| TRootER | 0.76 | 0.45 | 58.7 | - | - | - |
| RootB | 0.85 | 0.41 | 48.3 | 0.90 | 0.53 | 58.8 |
| | | | | | | |
| NNodd4 | 0.50 | 0.10 | 20.3 | - | - | - |
| NNodd5 | 0.46 | 0.12 | 25.5 | - | - | - |
| NNodd6 | 0.51 | 0.13 | 26.1 | - | - | - |
| NNod | 0.40 | 0.13 | 32.2 | 0.74 | 0.50 | 68.1 |
| TNodPAd4 | 0.44 | 0.13 | 28.8 | - | - | - |
| TNodPAd6 | 0.55 | 0.18 | 32.8 | - | - | - |
| TNodPA | 0.77 | 0.27 | 34.9 | 0.91 | 0.55 | 60.8 |
| NodApR | 0.43 | 0.10 | 24.4 | - | - | - |
| TNodPAR | 0.49 | 0.16 | 32.1 | - | - | - |
| NodB | 0.70 | 0.22 | 31.1 | 0.80 | 0.48 | 60.4 |
| NB:BGB | 0.62 | 0.11 | 17.7 | 0.49 | 0.16 | 32.1 |
| BGB:TB | 0.69 | 0.33 | 48.1 | 0.83 | 0.39 | 47.0 |

^a Global R^2 is the percentage of phenotypic variation explained by the all the QTL detected for each trait

^b p is the part of genetic variance explained by all the detected QTL for the trait; $p = \text{global } R^2 / h^2$

Supplemental Table I.10. Broad sense heritability (h^2), global R^2 , part of genetic variance explained by QTL (p) for carbon accumulation, nitrogen acquisition functioning traits and developmental variables recorded in RIL4 population in two glasshouse and two field experiments

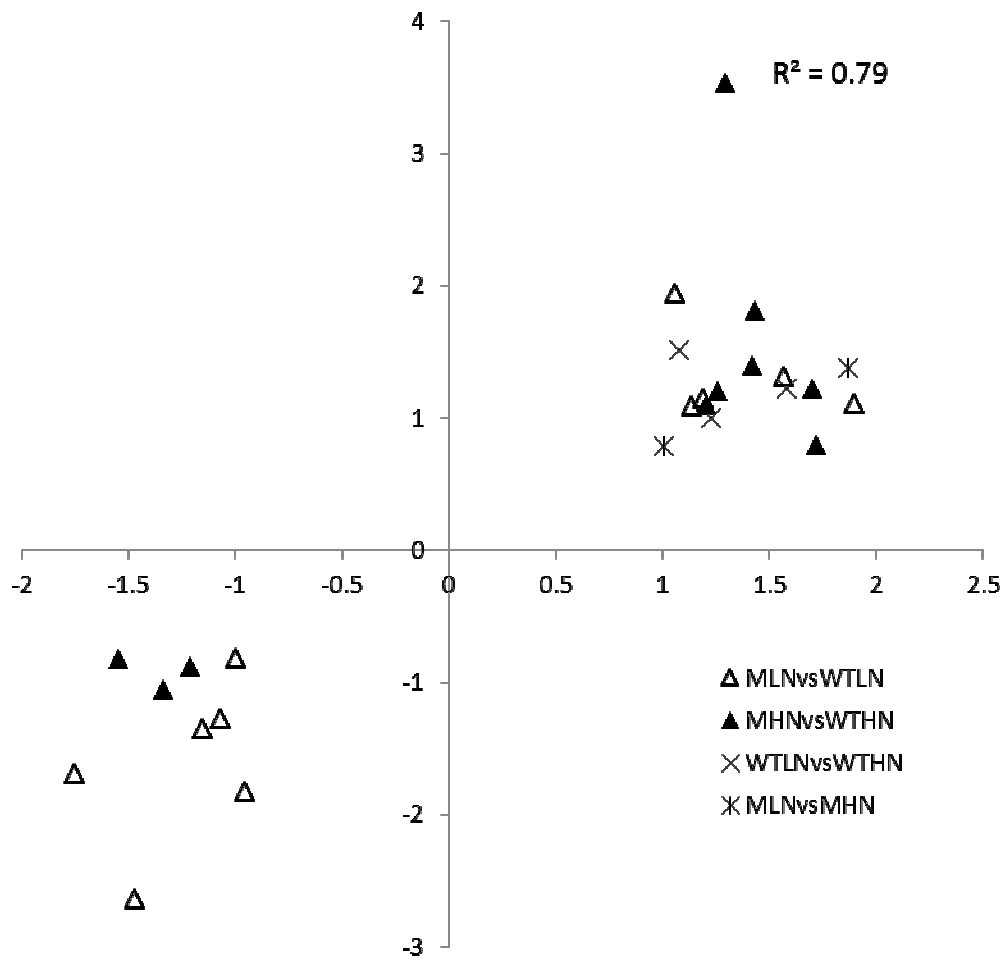
| Trait | Pouches 2007 | | | Pots 2006 | | | Field 2006 | | | Field 2004 | | |
|---------|--------------|---------------------------|----------------------|-----------|--------------|---------|------------|--------------|---------|--------------------|--------------|---------|
| | h^2 | Global R^2 ^a | p (%) ^b | h^2 | Global R^2 | p (%) | h^2 | Global R^2 | p (%) | h^2 ^c | Global R^2 | p (%) |
| ShootB | 0.90 | 0.10 | 11.6 | 0.96 | 0.60 | 62.3 | 0.58 | 0.31 | 54.3 | 0.46 | 0.41 | 88.9 |
| StrawB | - | - | - | - | - | - | 0.61 | 0.35 | 57.5 | 0.74 | 0.26 | 35.5 |
| SeedB | - | - | - | - | - | - | 0.58 | 0.31 | 53.8 | 0.44 | 0.42 | 95.3 |
| SeedN | - | - | - | - | - | - | 0.64 | 0.34 | 52.6 | 0.95 | 0.32 | 33.7 |
| TSW | - | - | - | - | - | - | 0.83 | 0.41 | 49.0 | 0.99 | 0.33 | 33.6 |
| NDFA | 0.53 | | | 0.90 | 0.20 | 21.7 | 0.49 | 0.11 | 21.9 | 0.95 | 0.12 | 12.7 |
| ShootNC | 0.84 | 0.50 | 59.5 | 0.90 | 0.20 | 22.0 | 0.60 | 0.31 | 51.8 | 0.96 | 0.33 | 34.1 |
| StrawNC | - | - | - | - | - | - | 0.70 | 0.16 | 22.2 | 0.76 | 0.22 | 28.6 |
| SeedNC | - | - | - | - | - | - | 0.67 | 0.39 | 58.5 | 0.98 | 0.21 | 21.6 |
| ShootQN | 0.77 | 0.27 | 35.5 | 0.95 | 0.54 | 56.5 | 0.52 | 0.34 | 66.1 | 0.95 | 0.34 | 35.8 |
| StrawQN | - | - | - | - | - | - | 0.71 | 0.28 | 39.9 | 0.89 | 0.12 | 13.5 |
| SeedQN | - | - | - | - | - | - | 0.53 | 0.27 | 51.7 | 0.95 | 0.42 | 44.2 |
| SNU | 0.38 | 0.09 | 23.2 | 0.68 | 0.10 | 14.4 | - | - | - | - | - | - |
| RootE | 0.48 | 0.16 | 32.4 | 0.81 | 0.31 | 38.7 | - | - | - | - | - | - |
| NodE | 0.39 | | | 0.57 | 0.19 | 33.7 | - | - | - | - | - | - |
| LeafAd3 | 0.6 | 0.26 | 42.9 | - | - | - | - | - | - | - | - | - |
| LeafAd4 | 0.56 | 0.33 | 58.7 | - | - | - | - | - | - | - | - | - |
| LeafAd5 | 0.66 | 0.43 | 65.1 | - | - | - | - | - | - | - | - | - |
| LeafAd6 | 0.75 | 0.63 | 83.8 | - | - | - | - | - | - | - | - | - |
| LeafA | 0.92 | 0.79 | 86.3 | - | - | - | - | - | - | - | - | - |
| LeafAR | 0.73 | 0.67 | 91.8 | - | - | - | - | - | - | - | - | - |
| RUE | 0.91 | 0.79 | 87.1 | - | - | - | - | - | - | - | - | - |
| SPAD | 0.73 | 0.39 | 53.9 | - | - | - | - | - | - | - | - | - |
| SLN | 0.90 | 0.84 | 93.2 | - | - | - | - | - | - | - | - | - |
| ShootL | 0.89 | 0.74 | 82.7 | 0.97 | 0.73 | 75.4 | 0.87 | 0.62 | 70.9 | 0.98 | 0.34 | 34.7 |
| NBranch | 0.00 | | | 0.64 | 0.23 | 35.4 | 0.61 | 0.44 | 72.8 | 0.67 | 0.28 | 41.2 |
| LeafApR | 0.43 | 0.18 | 42.5 | 0.45 | 0.25 | 56.4 | - | - | - | - | - | - |
| BegFlo | 0.99 | 0.78 | 78.8 | 0.99 | 0.73 | 73.4 | 0.94 | 0.57 | 60.6 | 0.99 | 0.62 | 62.4 |

^a Global R^2 is the percentage of phenotypic variation explained by the all the QTL detected for each trait

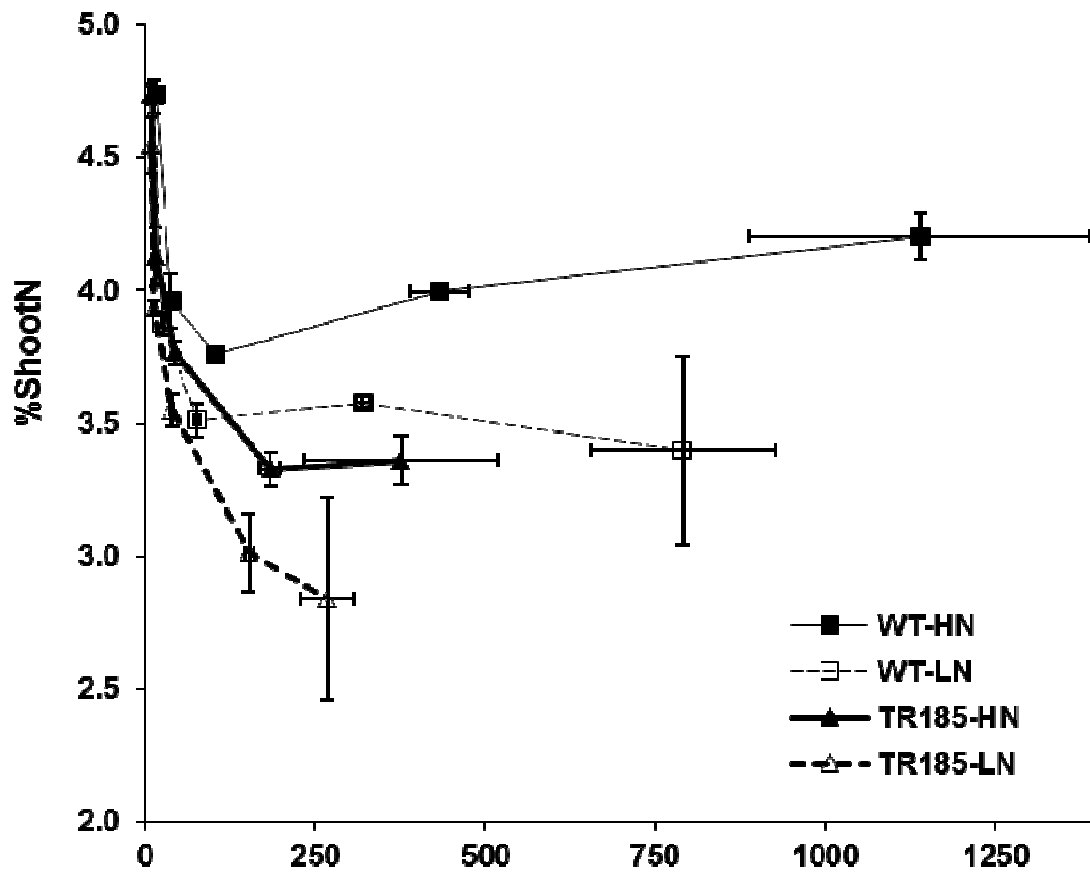
^b p is the part of genetic variance explained by all the detected QTL for the trait; $p = \text{global } R^2 / h^2$

^c h^2 for the field experiment in 2004 has been calculated from the ANOVA performed on three genotypes experimented in eight replicates

Annexe 2 : Fichiers additionnels du chapitre II

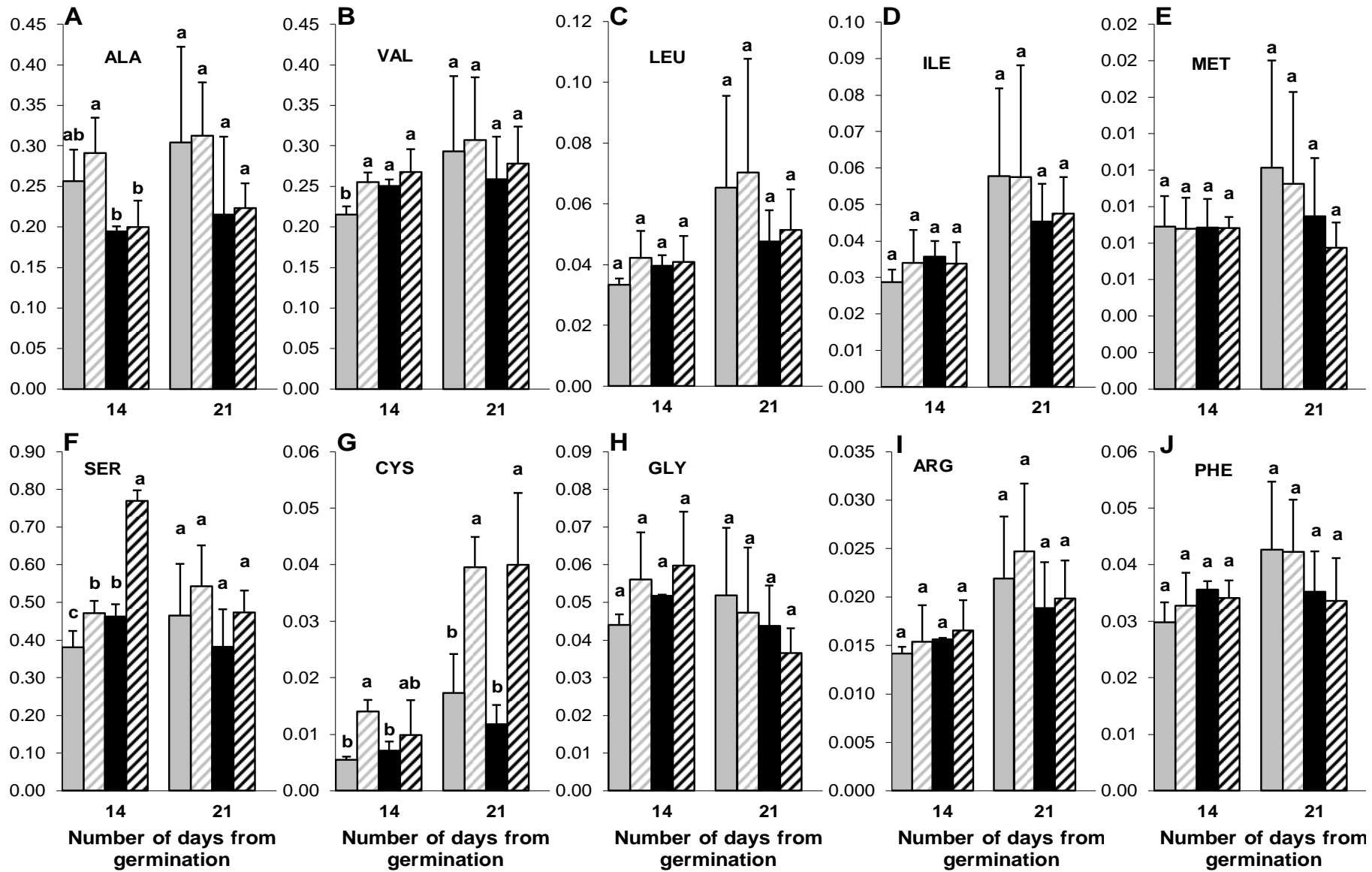


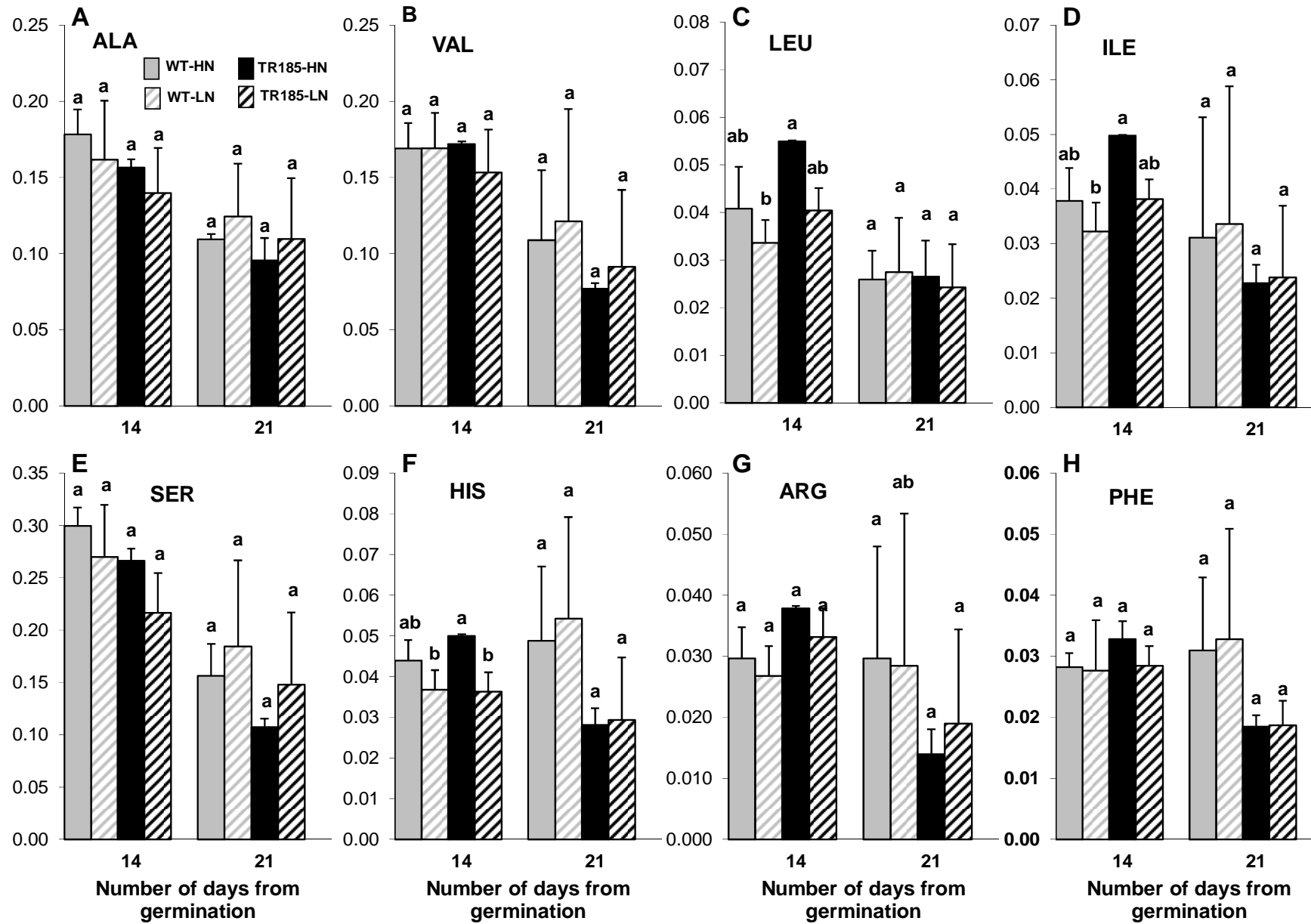
Supplemental Fig. II.1. Q-RT-PCR validation of differentially accumulated transcripts initially identified by Affymetrix GeneChip analysis



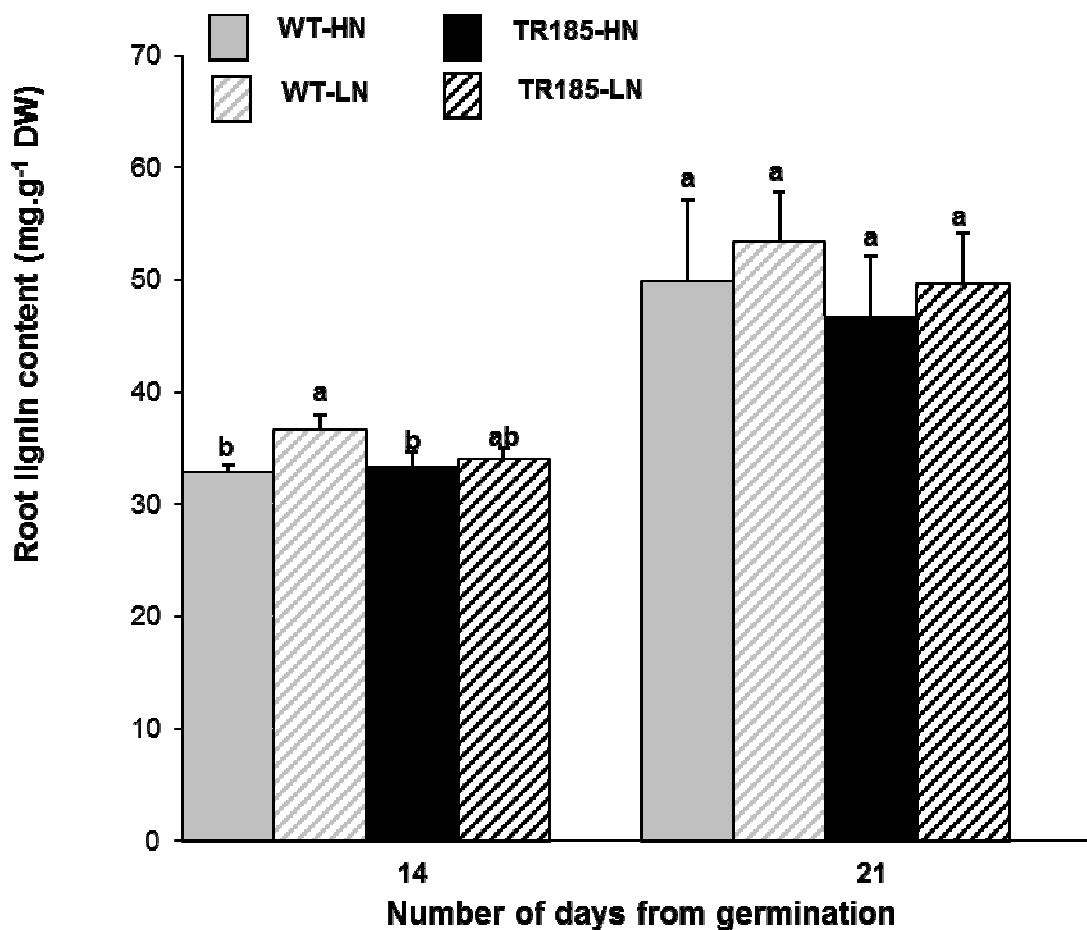
Supplemental Fig. II.2. Relationship between shoot N concentration (%ShootN) and shoot dry weight (SDW). At each date, data are means from three biological replicates of six plants each \pm SE

Supplemental Fig. II.3.
Shoot content of ten amino acids





Supplemental Fig. II.4.
 Root content of eight amino acids



Supplemental Fig. II.5. Root lignin content of the wild-type (WT) and the mutant (TR185) under high (10 mM, [HN]) or low (1 mM, [LN]) nitrate supply at 14 and 21 days after germination. At each date, data are means from three biological replicates of six plants each \pm SE and means followed by different letters are significantly different based on multiple comparisons (LSD test) at $p < 0.05$

Supplemental Table II.1. Genetic analyses of the high-branched mutant TR185

| Generation of ♀ x ♂ cross | High-branched root phenotype^a | Wild-type root phenotype^a | χ² value for 1:3 ratio |
|----------------------------------|---|---|--|
| BC1 (TR185 x J5) | 0 | 7 | |
| BC1 (J5 x TR185) | 0 | 5 | |
| BC1S1 (TR185 x J5) | 6 | 28 | 0.98 |
| BC1S1 (J5 x TR185) | 28 | 75 | 0.26 |

^a Number of plants

Supplemental Table II.2. Primers used for Real Time RT-Q-PCR assays

| Target Id ^a | primer sequence (5' -> 3') |
|------------------------|---|
| Mtr.10626.1.S1_at | TGCGACGAAGATAAAGGAATGTGG GAACTGGGGGATTAACACAAAAGG |
| Mtr.14314.1.S1_at | GGGCAAAAAGCAAAGAAGGAATCAC TACATATATTTTGGCTCCAGAGGAG |
| Mtr.16885.1.S1_at | GATCATGAAGCATCTTTCCTGAGAG GGCAGATTACAAAGCTCAAAATTCG |
| Mtr.18380.1.S1_at | TATGGAAAGTGGATGTCAGTTGGAC GTAAGAAGAACGTGCGTGTCTGAAG |
| Mtr.20107.1.S1_at | AAGCTGGGTCTGTTTATGACTCAC AGCACTAGAGATAACATTCTTGGAC |
| Mtr.20354.1.S1_at | CTTGATTCAGTTGATTGATGAGCTG GGTGTTCATCATGCTACACCTCC |
| Mtr.22383.1.S1_x_at | GAAGAGTCTCCAAGTGGATGTG ATGAATCAGTACCATTCCAAATCCC |
| Mtr.23663.1.S1_at | ACACCACCGTGCTCTGTTTCCTC TTGGATAGTGAGTGGAGGTGAAGC |
| Mtr.25576.1.S1_at | TACATAACCCTCTCGATCGATTTC TGATGGAGAGAACTTAGTCTCTAC |
| Mtr.26011.1.S1_at | GAGGAAGGTTTGAAAAAGTTGGTTTC TGAACAGATTTTCACTTACGCAACC |
| Mtr.37034.1.S1_at | CTCTCTACTTGATCAATCGTCTTGG CTAAGTTATGTCAACATAGTCAACG |
| Mtr.4076.1.S1_at | ATGGAAGCACCTCTTCGTTGAAGC ACTACCAAATCCATACGACCGTATC |
| Mtr.40997.1.S1_s_at | GCGGCTTTCTTGATTGCCTCTAC CTGGATGTAGAAATAGAAGAAGGTG |
| Mtr.41610.1.S1_at | GCACTCACACTCCTCTCATTCC TAGGGTTTGGACAGTCAGAACTC |
| Mtr.43680.1.S1_at | CCGAACACATTGCCTTCAAGTGC GGGGTAAAAGGATTGGTGCCAG |
| Mtr.43830.1.S1_at | CAGTTTTCACTTCATTTGCTTTGCC CCCAATGAATTAGACCAACTAGAG |
| Mtr.51607.1.S1_at | GGAAAAGAATGGAAGCACCTCTTCG ACTACCAAATCCATACGACCGTATC |
| Mtr.8452.1.S1_at | CTGTGAAGATGCCATATACACTGC CAACAACACAACAACGACGATGAC |
| EF1B | GGTTGAGGATCGTCTTACTGCTG AATGTCTGCCACTACCATGATC |
| Ubiquitin | CCAGAAGGAATCCACTCTTCA CTTCCCACAATAATGACGATC |

^a Target identifier (Affymetrix) or reference genes for Q-RT-PCR

Supplemental Table II.3. The 75 differentially accumulated transcripts responsive to N effect and common to those identified by Ruffel *et al.* (2008) as responsive to either local nitrate starvation (LNO3vs-NO3) or systemic signals (LNO3vsSNO3)

| Affymetrix probe | WTLNvsWTHN ^a | MLNvsMHN ^a | LNvsHN ^a | MXLN ^a | LNO3vs-NO3 ^b | LNO3vsSNO3 ^b | Annotation Ruffel |
|---------------------|-------------------------|-----------------------|---------------------|-------------------|-------------------------|-------------------------|--|
| Mtr.24492.1.S1_s_at | 1.57 | - | 1.25 | - | -37.27 | - | NAD(P)H-quinone oxidoreductase chain H, chloroplast |
| Mtr.40997.1.S1_s_at | - | 1.87 | 1.55 | - | -29.04 | - | Photosystem I P700 chlorophyll A apoprotein A1 |
| Mtr.14625.1.S1_at | 1.44 | - | 1.14 | - | -18.77 | - | Photosystem I assembly Ycf4 |
| Mtr.14636.1.S1_s_at | - | - | 1.11 | - | -18.77 | - | RNA polymerase beta subunit Rpb2-like |
| Mtr.45608.1.S1_s_at | 1.74 | - | 0.99 | - | -18.00 | - | Unknown |
| Mtr.45626.1.S1_s_at | 1.60 | - | 1.21 | - | -15.67 | - | GAP atpH |
| Mtr.49039.1.S1_s_at | - | 1.60 | 0.99 | - | -15.35 | - | H ⁺ -transporting two-sector ATPase, alpha/beta subunit |
| Mtr.14042.1.S1_s_at | - | 1.46 | - | - | -12.47 | - | GAP petD |
| Msa.1055.1.S1_at | 1.69 | - | 1.08 | - | -12.13 | -1.07 | NADH-ubiquinone oxidoreductase chain |
| Mtr.45639.1.S1_s_at | 1.40 | - | 1.09 | - | -12.13 | - | GAP atpB |
| Mtr.14644.1.S1_s_at | - | - | 0.96 | - | -11.24 | - | Photosystem I psaA and psaB |
| Msa.3141.1.S1_at | - | 1.94 | 1.21 | - | -11.08 | - | ATP synthase subunit alpha |
| Mtr.14061.1.S1_s_at | - | - | 0.95 | - | -9.85 | - | GAP psaA |
| Mtr.45615.1.S1_s_at | - | - | 1.03 | - | -9.19 | - | from 54343 to 54705 |
| Mtr.31123.1.S1_at | 1.38 | - | 0.98 | - | -8.69 | - | similar to UP Q9SYP1 (Q9SYP1) F9H16.5 protein |
| Mtr.18232.1.S1_s_at | 1.51 | - | 1.16 | - | -8.34 | - | AAA ATPase; H ⁺ -transporting two-sector ATPase |
| Msa.2071.1.S1_s_at | - | 1.80 | 1.18 | - | -8.17 | -1.09 | Unknown |
| Mtr.45637.1.S1_s_at | - | - | 1.23 | - | -7.46 | - | GAP ndhK |
| Mtr.36986.1.S1_s_at | - | - | 0.97 | - | -6.92 | - | NADH dehydrogenase 19kDa subunit |
| Mtr.29056.1.S1_at | - | - | 0.95 | - | -6.28 | - | Unknown |
| Mtr.37266.1.S1_s_at | - | 2.86 | 1.85 | -2.02 | -5.21 | - | Unknown |
| Mtr.10097.1.S1_at | - | - | 0.98 | - | -5.17 | - | Unknown |
| Mtr.44795.1.S1_at | - | - | 1.10 | - | -5.06 | - | similar to UP Q76JT3 (Q76JT3) RelA-SpoT like protein PsRSH1 |
| Mtr.43508.1.S1_at | 1.86 | - | 0.98 | - | -4.72 | 1.82 | Auxin-induced protein |
| Mtr.31694.1.S1_at | 1.44 | - | - | - | -4.72 | - | similar to UP Q84KJ4 (Q84KJ4) Myosin XI (Fragment), partial (12%) |
| Mtr.29652.1.S1_at | 1.96 | - | 1.19 | - | -4.66 | - | Unknown |
| Mtr.14053.1.S1_s_at | - | 1.51 | - | - | -4.26 | - | GAP atpF |
| Mtr.33189.1.S1_s_at | - | - | 1.13 | - | -3.94 | - | AML1 |
| Mtr.34841.1.S1_s_at | - | 1.57 | 1.14 | - | -3.76 | - | CP43 chlorophyll apoprotein of photosystem II |
| Mtr.33045.1.S1_at | - | - | 0.96 | - | -3.41 | - | similar to UP Q94KD1 (Q94KD1) At1g05960 T21E18_20 |
| Msa.1917.1.S1_at | - | 1.66 | 0.99 | - | -3.01 | - | Unknown |
| Mtr.44175.1.S1_at | - | - | 0.95 | - | -3.01 | - | similar to UP Q8RY22 (Q8RY22) AT3g03380/T21P5_20 |
| Mtr.38167.1.S1_at | 1.41 | - | - | - | -2.85 | - | Cationic peroxidase 1 precursor |
| Mtr.39306.1.S1_at | 1.40 | - | 1.26 | - | -2.79 | - | Sob protein |
| Mtr.33172.1.S1_at | - | - | 1.04 | - | -2.62 | - | weakly similar to UP Q5ZCB9 (Q5ZCB9) Ubiquitin-conjugating enzyme |
| Mtr.44875.1.S1_at | 1.37 | - | - | - | -2.45 | - | weakly similar to UP Q6K6B1 (Q6K6B1) CLIP-associating protein |
| Mtr.44882.1.S1_at | - | 1.47 | 1.08 | - | -2.36 | - | Unknown |
| Mtr.29714.1.S1_at | - | 1.34 | - | - | -2.31 | - | Unknown |
| Mtr.14599.1.S1_x_at | 1.37 | - | - | - | -2.16 | - | LQGC hypothetical protein |

Annexes du Chapitre II

| | | | | | | | |
|---------------------|-------|-------|-------|------|--------|-------|---|
| Mtr.28638.1.S1_at | - | - | 0.97 | - | -2.04 | - | Unknown |
| Mtr.13860.1.S1_at | - | - | 0.97 | - | -2.04 | - | weakly similar to UP WIS1_SCHPO (P33886) Protein kinase wis1 (sty2) |
| Mtr.33744.1.S1_s_at | 1.34 | - | - | - | -1.92 | - | similar to UP Q6AVJ1 (Q6AVJ1) Expressed protein |
| Mtr.13713.1.S1_at | - | - | 1.02 | - | -1.89 | - | Unknown |
| Mtr.4087.1.S1_at | 1.54 | - | 0.96 | - | -1.83 | - | OMNI NTL01LJ12 ABC transporter ATPase component |
| Mtr.35635.1.S1_at | 1.49 | - | - | 1.92 | -1.79 | - | Unknown |
| Mtr.38820.1.S1_at | - | - | 0.97 | - | -1.68 | - | cytochrome P450 |
| Mtr.1527.1.S1_at | 1.37 | - | - | - | -1.65 | - | Unknown |
| Mtr.5936.1.S1_at | - | - | 0.95 | - | -1.58 | - | Unknown |
| Mtr.10025.1.S1_at | 1.71 | - | 0.98 | - | -1.53 | - | Phosphoribosylanthranilate transferase-like protein |
| Mtr.5823.1.S1_a_at | - | - | 0.99 | - | -1.49 | - | similar to UP Q7Y0H3 (Q7Y0H3) CLV1-like receptor kinase |
| Mtr.6021.1.S1_at | 1.37 | - | - | - | -1.48 | - | Unknown |
| Mtr.32291.1.S1_at | 1.72 | - | 1.00 | - | -1.47 | - | similar to PIR JC4209 JC4209 GTP cyclohydrolase II |
| Mtr.6373.1.S1_at | - | - | 0.99 | - | -1.37 | - | weakly similar to UP Q40326 (Q40326) Acetyl-CoA carboxylase |
| Mtr.39559.1.S1_at | 1.64 | - | - | 1.96 | -1.33 | - | Unknown |
| Mtr.8799.1.S1_at | 1.56 | - | - | - | -1.32 | -1.41 | Raffinose synthase |
| Mtr.32561.1.S1_at | - | - | 1.06 | - | -1.32 | - | weakly similar to UP Q9FHH2 (Q9FHH2) 101 kDa heat shock protein |
| Mtr.9569.1.S1_at | - | 1.40 | - | - | 3.05 | 1.71 | Thaumatococcus-like protein 1 |
| Mtr.39456.1.S1_at | 1.63 | 1.58 | 1.61 | - | 3.10 | - | weakly similar to Q9LW87 Coatomer protein complex |
| Mtr.8550.1.S1_s_at | - | 1.71 | 1.22 | - | 127.12 | 9.06 | Leghemoglobin |
| Mtr.8284.1.S1_s_at | - | 1.82 | 1.32 | - | 196.72 | - | Leghemoglobin |
| Mtr.10382.1.S1_at | 1.91 | - | 0.98 | - | - | 32.56 | B12D-like protein |
| Mtr.4531.1.S1_s_at | 1.83 | - | 1.03 | - | - | 30.48 | B12D-like protein |
| Mtr.8645.1.S1_at | 1.57 | - | 1.20 | - | - | -1.06 | Riboflavin biosynthesis protein ribA, chloroplast precursor |
| Mtr.48109.1.S1_at | 1.36 | - | - | - | - | -1.25 | Putative WRKY4 transcription factor |
| Mtr.25557.1.S1_at | - | 1.39 | 1.13 | - | - | -1.34 | 1-aminocyclopropane-1-carboxylate oxidase |
| Mtr.31237.1.S1_at | - | - | 0.98 | - | - | 1.09 | Hydrolase-like protein |
| Mtr.48070.1.S1_at | - | - | 0.95 | - | - | 1.24 | Unknown |
| Mtr.42876.1.S1_at | - | 1.38 | - | - | - | -1.15 | Pathogenesis-related protein 4A |
| Mtr.23567.1.S1_x_at | - | 1.36 | - | - | - | -1.26 | Unknown |
| Mtr.7380.1.S1_at | -1.42 | - | - | - | - | 1.69 | Unknown |
| Msa.905.1.S1_at | -1.67 | - | - | - | 9.51 | 3.73 | Leghemoglobin |
| Mtr.29429.1.S1_at | - | -1.41 | - | 2.12 | - | -1.12 | Unknown |
| Mtr.49372.1.S1_at | - | -2.30 | - | 3.19 | -1.48 | - | Unknown |
| Mtr.12474.1.S1_at | - | - | -1.02 | - | - | -1.13 | Isoflavonoid glucosyltransferase |
| Mtr.43282.1.S1_at | -1.37 | - | -1.15 | - | 2.60 | -5.48 | S-adenosyl-L-methionine: 2,7,4'-trihydroxyisoflavanone 4'-O-methyltransferase |

^a Only significant differential expressions are shown. in a log₂ scale. ^b Fold-change of transcript accumulation, as indicated by Ruffel *et al.* (2008)

Annexe 3: Fichiers additionnels du chapitre III

Supplemental Table III.1. Pea collection. Passport and structure data.

| AccNb | Accession Name / Donor Code | Species/sspecies (a) | Cultivation status (b) | End use (c) | Sowing type (d) | Country of origin | Registration or creation year | Breeding compaigny or donor | Foliage type (e) | Flower colour (f) | Seed Appearance (g) | Testa Ornementation (h) | DAPC | Struct | Reference |
|-------|--------------------------------|-------------------------|---------------------------|-------------|-----------------|-------------------|-------------------------------|-----------------------------|------------------|-------------------|---------------------|-------------------------|------|--------|--|
| R001 | KOROZA | Ps | Cv | Gd | Sp | Netherlands | 1955 | Cebecco | NI | Pu | Wr | Ma | D2 | K07 | Bos & van der Want, 1962 |
| R002 | PISUM SATIVUM-AFGHANISTAN JI86 | Ps | Lr | | | Afghanistan | | JIC | NI | Pu | Sm | Ma | D1 | K02 | Young <i>et al.</i> , 1982; Jing <i>et al.</i> , 2007; Martin-Sanz <i>et al.</i> , 2011 |
| R003 | PISUM SATIVUM-BOLIVIA JI228 | Ps | SWd/Wd | | | Bolivia | | JIC | NI | Pu | Sm | Sp | D2 | K04 | Jing <i>et al.</i> , 2007 |
| R004 | ALASKA | Ps | Cv | Gd | Sp | United States | 1884 | Sharpe | NI | Wh | Sm | Ab | D2 | NA | Wilson & Burton, 1938; Murray & Swensen, 1991; Jing <i>et al.</i> , 2007; Martin-Sanz <i>et al.</i> , 2011 |
| R005 | PISUM SATIVUM-INDIA JI1267 | Ps | Lr | Gd | | India | | JIC | NI | Pu | Sm | Ma | D1 | K02 | Jing <i>et al.</i> , 2007; Martin-Sanz <i>et al.</i> , 2011 |
| R006 | PESOL | Ps | Lr | | | Spain | | JIC | NI | Wh | Wr | Ab | D2 | K06 | |
| R007 | PISUM SATIVUM-MEXICO JI1844 | Ps | Lr | | Win | Mexico | | JIC | NI | Pu | Sm | Sp | D3 | K09 | Jing <i>et al.</i> , 2007; Martin-Sanz <i>et al.</i> , 2011 |
| R008 | PISUM SATIVUM-ZAIRE JI2376 | Ps | SWd/Wd | | | DR Congo | | JIC | NI | Wh | Sm | Ab | D2 | K04 | Jing <i>et al.</i> , 2007; Martin-Sanz <i>et al.</i> , 2011 |
| R009 | PISUM SATIVUM-ZAMBIA JI2383 | Ps | Lr | | | Zambia | | JIC | NI | Wh | Sm | Ab | D2 | K04 | Martin-Sanz <i>et al.</i> , 2011 |
| R010 | PISUM ELATIUS JI1075 | Pse | Wd | | | Turkey | | JIC | NI | Pu | Sm | Sp | D1 | K01 | Young <i>et al.</i> , 1982; Martin-Sanz <i>et al.</i> , 2011 |
| R011 | PISUM ELATIUS JI1703 | Pse | Wd | | | | | JIC | NI | Pi | Wr | Ma | D2 | K07 | Jing <i>et al.</i> , 2007; Martin-Sanz <i>et al.</i> , 2011 |
| R012 | PISUM HUMILE JI1794 | Psh | Wd | | | Israel | | JIC | NI | Pu | Sm | Ma | D1 | K01 | LaRue & Weeden, 1992; Ellis <i>et al.</i> , 1998; Weeden <i>et al.</i> , 2002; Jing <i>et al.</i> , 2007; Martin-Sanz <i>et al.</i> , 2011; Borisov <i>et al.</i> , 2007 |
| R013 | PISUM ABYSSINICUM JI2202 | Psa | Lr | | | Yemen | | JIC | NI | Pi | Sm | Ab | D1 | K01 | Martin-Sanz <i>et al.</i> , 2011 |
| R014 | PISUM FULVUM JI2473 | Pf | Wd | | | Israel | | JIC | NI | Or | Sm | Ab | D1 | K01 | Martin-Sanz <i>et al.</i> , 2011 |
| R015 | PISUM FULVUM JI2523 | Pf | Wd | | | Syria | | JIC | NI | Or | Sm | Ab | D1 | K01 | Jing <i>et al.</i> , 2007; Martin-Sanz <i>et al.</i> , 2011 |
| R016 | PISUM TRANSCAUCASICUM JI2546 | Ps | Wd | | | Georgia | | JIC | NI | Pu | Sm | Ma | D1 | NA | Ellis <i>et al.</i> , 1998; Jing <i>et al.</i> , 2007 |
| R017 | PISUM SPECIOSUM-LIBYA JI2605 | Ps | SWd/Wd | | | Libya | | JIC | NI | Pi | Sm | Ab | D1 | K03 | Jing <i>et al.</i> , 2007; Martin-Sanz <i>et al.</i> , 2011 |
| R018 | AURALIA | Ps | Cv | Gd | Sp | Germany | 1974 | SAALESAATE N | NI | Wh | Sm | Ab | D2 | NA | |
| R019 | COTE D'OR | Ps | Lr | Fd | Win | France | | INRA, Versailles | NI | Pu | Sm | Sp | D3 | K09 | Cousin, 1976 |
| R020 | MISTRAL | Ps | Cv | Dry | Win | France | 1991 | Serasem | NI | Wh | Sm | Ab | D3 | K10 | Cousin, 1997; Wicker <i>et al.</i> , 2003 |
| R021 | WNC 23Z SPP ARVENSE 1809 | Ps | Lr | Fd | Win | | | INRA, Mons | NI | Wh | Sm | Ab | D3 | NA | |

Annexes du Chapitre III

| | | | | | | | | | | | | | | |
|------|---------------------------|-----|----|-----|-----|---------------|--------------------------|----|----|----|-------|----|-----|---|
| R022 | WNC Z61Z SPP ARVENSE Z009 | Ps | Lr | Fd | Win | | INRA, Mons | NI | Wh | Sm | Ab | D3 | NA | |
| R023 | PI273279 | Ps | Lr | Fd | | Peru | USDA, WA | NI | Pu | Sm | Sp | D2 | K05 | |
| R024 | PI180693 | Ps | Cv | Gd | | Germany | <1949 USDA, WA | NI | Pi | Sm | Ab | D2 | K05 | Lockwood & Ballard, 1960; Kraft & Bodge, 2001; Wicker et al, 2003 |
| R025 | 552 | Ps | Bl | Gd | Sp | United States | <1992 Gritton, Univ. Wis | NI | Wh | Wr | Ab | D2 | K06 | Wicker et al., 2003 |
| R026 | DARK SKIN PERFECTION | Ps | Cv | Gd | Sp | Great Britain | 1960 Unilever Ld | NI | Wh | Wr | Ab | D2 | K06 | Hagedorn & Gritton, 1973; Cousin, 1997; Kraft & Bodge, 2001 |
| R027 | 90-2131 | Ps | Bl | Gd | Sp | United States | 1990 Kraft, USDA | NI | Wh | Sm | Ab | D2 | NA | Kraft, 1992; Wicker et al, 2003 |
| R028 | SHRAT | Ps | Lr | Gd | | India | USDA, WA | NI | Pu | Sm | Ma-Sp | D1 | K02 | Lockwood & Ballard, 1960; |
| R029 | PI212112 | Ps | Lr | Gd | | Afghanistan | USDA, WA | NI | Pu | Sm | Ma | D1 | K02 | Lockwood & Ballard, 1960; Young et al., 1982; |
| R030 | ENGLISH | Ps | Bl | Fd | | Great Britain | <2001 INRA, Versailles | NI | Pu | Sm | Ma | D2 | K05 | |
| R031 | IREGI SARGA | Ps | Cv | Fd | Sp | Hungary | 1963 VIR | NI | Wh | Sm | Ab | D2 | NA | Mandy, 1974 |
| R032 | KIRIN 40 | Ps | Bl | Gd | Sp | China | <2001 INRA, Versailles | NI | Wh | Wr | Ab | D2 | K06 | |
| R033 | CUZCO 1 | Ps | Lr | Gd | | Peru | Lenoble | NI | Wh | Sm | Ab | D2 | K04 | |
| R034 | HAITI COLORE | Ps | Lr | Fd | | Haiti | INRA, Versailles | NI | Pu | Sm | Sp | D2 | NA | |
| R035 | AFGHANISTAN ASIATICUM | Ps | Lr | Fd | | Afghanistan | INRA | NI | Pu | Sm | Sp | D1 | NA | |
| R036 | NEPAL A | Ps | Lr | Fd | | Nepal | INRA | NI | Pu | Sm | Ma | D1 | K02 | |
| R037 | ABYSSINICUM VAVILOVANUM | Psa | Lr | Fd | | Ethiopia | IPK | NI | Pu | Sm | Ab | D1 | K01 | |
| R038 | CAPSICUM | Pse | Lr | Fd | | Azerbaijan | VIR | NI | Pu | Sm | Ma | D2 | NA | |
| R039 | HATIVER | Ps | Cv | Gd | Win | Netherlands | 1975 Sluis & Groot | NI | Wh | Sm | Ab | D2 | NA | Eteve et al., 1979; Buysse et al., 1983; Cousin, 1997 |
| R040 | TELEPHONE A RAMES | Ps | Cv | Gd | Sp | Great Britain | 1878 Carter | NI | Wh | Wr | Ab | D2 | K06 | Fourmont, 1956 |
| R041 | FIN DE LA BIEVRE | Ps | Cv | Gd | Sp | France | <1952 Vilmorin | NI | Wh | Sm | Ab | D2 | NA | Fourmont, 1956 |
| R042 | MERVELLE D'ETAMPES | Ps | Cv | Gd | Sp | France | 1880 Bonnemain | NI | Wh | Sm | Ab | D2 | K04 | Vilmorin-Andrieux & Cie, 1883 |
| R043 | NFG KRUPP PELUSCHKE | Ps | Cv | Fd | Sp | Germany | 1968 DSV | NI | Pu | Sm | Sp | D2 | K05 | Clark & Spencer-Phillips, 1994 |
| R044 | PETIT PROVENCAL | Ps | Cv | Gd | Sp | Great Britain | 1918 Sharpe | NI | Wh | Sm | Ab | D2 | K06 | Fourmont, 1956 |
| R045 | DESIREE | Ps | Cv | Fd | Sp | Netherlands | <1990 Naktuinbouw | NI | Pu | Sm | Ab | D2 | NA | |
| R046 | LIVIOLETTA | Ps | Cv | Fd | Sp | Germany | 1994 DSV | NI | Pu | Sm | Sp | D2 | K05 | Amey et al., 2008 |
| R047 | BINGEFORS | Ps | Bl | Fd | | Sweden | <2001 INRA, Versailles | NI | Pu | Sm | Ab | D2 | K04 | |
| R048 | MULTIRESISTANT | Ps | Bl | Gd | Sp | UnitedStates | <2001 INRA, Versailles | NI | Wh | Wr | Ab | D2 | K06 | |
| R049 | AMINO | Ps | Cv | Dry | Sp | France | 1977 Blondeau | NI | Wh | Sm | Ab | D2 | NA | Cousin, 1997; Baranger et al., 2004 |
| R050 | K4626 | Ps | Lr | Fd | Sp | Lithuania | VIR | NI | Pu | Sm | Sp | D2 | K05 | Baranger et al., 2004 |

Annexes du Chapitre III

| | | | | | | | | | | | | | | | |
|------|---------------------------------|-----|--------|-----|-----|---------------|-------|-------------------|----|----|----|----|----|-----|---|
| R051 | CAMEOR | Ps | Cv | Gd | Sp | France | 1973 | Seminor | NI | Wh | Sm | Ab | D2 | NA | Baranger <i>et al.</i> , 2004 |
| R052 | BALLET | Ps | Cv | Dry | Sp | Great Britain | 1988 | Nickerson | af | Wh | Sm | Ab | D2 | NA | Baranger <i>et al.</i> , 2004; Martin-Sanz <i>et al.</i> , 2011 |
| R053 | CERISE- <i>ce</i> ,CR | Ps | Gm | Gd | | Netherlands | 1951 | Wellensiek | NI | Pi | Sm | Ma | D2 | K07 | Wellensiek, 1951; Baranger <i>et al.</i> , 2004 |
| R054 | CHINA | Ps | Lr | Fd | Win | China | | JIC | NI | Wh | Sm | Ab | D1 | NA | Baranger <i>et al.</i> , 2004 |
| R055 | SOMMETTE | Ps | Cv | Gd | Sp | Netherlands | 1972 | Sluis & Groot | NI | Wh | Sm | Ab | D2 | NA | Baranger <i>et al.</i> , 2004 |
| R056 | MESSIRE | Ps | Cv | Dry | Sp | France | 1989 | Serasem | NI | Wh | Sm | Ab | D2 | NA | Baranger <i>et al.</i> , 2004; Martin-Sanz <i>et al.</i> , 2011 |
| R057 | ZP141 | Ps | Lr | Gd | Sp | Spain | | Univ. Valladolid | NI | Wh | Sm | Ab | D2 | NA | Baranger <i>et al.</i> , 2004 |
| R058 | ZP126 | Ps | Lr | Gd | Sp | Spain | | Univ. Valladolid | NI | Wh | Sm | Ab | D2 | NA | Baranger <i>et al.</i> , 2004 |
| R059 | PISUM SATIVUM-ETHIOPIA JI1594 | Ps | Lr | Gd | | Ethiopia | | JIC | NI | Pu | Sm | Sp | D2 | NA | Baranger <i>et al.</i> , 2004 |
| R060 | MONGOLIA JI1345 | Ps | Lr | | | Mongolia | | JIC | NI | Wh | Sm | Ab | D2 | K05 | Baranger <i>et al.</i> , 2004; Jing <i>et al.</i> , 2012 |
| R061 | PISUM SATIVUM-ETHIOPIA JI1431 | Ps | Lr | | Win | Ethiopia | | JIC | NI | Pu | Sm | Ma | D3 | NA | Baranger <i>et al.</i> , 2004 |
| R062 | COSTA RICA | Ps | Lr | Gd | Sp | Costa Rica | | JIC | NI | Wh | Sm | Ab | D2 | K04 | Baranger <i>et al.</i> , 2004; Jing <i>et al.</i> , 2007; Martin-Sanz <i>et al.</i> , 2011 |
| R063 | K4269 | Ps | Lr | Dry | Sp | Lithuania | | VIR | NI | Wh | Sm | Ab | D2 | K05 | Baranger <i>et al.</i> , 2004 |
| R064 | K1666 | Ps | Lr | | Sp | Russia | | VIR | NI | Wh | Sm | Ab | D2 | K05 | Baranger <i>et al.</i> , 2004 |
| R065 | K8290/NORD | Ps | Cv | Dry | Sp | Russia | 1998 | VIR | af | Wh | Sm | Ab | D2 | K08 | Baranger <i>et al.</i> , 2004 |
| R066 | K4088 | Ps | Lr | | Sp | Ukraine | | VIR | NI | Wh | Sm | Ab | D2 | K05 | Baranger <i>et al.</i> , 2004 |
| R067 | PISUM SATIVUM-HIBERNICUM JI1846 | Ps | Cv | Gd | Win | Egypt | <1961 | JIC | NI | Pu | Sm | Sp | D3 | NA | Ellis <i>et al.</i> , 1998; Baranger <i>et al.</i> , 2004 |
| R068 | YANGWAN | Ps | Lr | Gd | Sp | China | | CAAS | NI | Wh | Sm | Ab | D2 | NA | Baranger <i>et al.</i> , 2004 |
| R069 | PISUM HUMILE JI241 | Psh | SWd/Wd | | | Israel | | JIC | NI | Pu | Sm | Ma | D1 | K02 | Brewin <i>et al.</i> , 1980; Young <i>et al.</i> , 1982; Ellis <i>et al.</i> , 1998; Baranger <i>et al.</i> , 2004; Jing <i>et al.</i> , 2007; Martin-Sanz <i>et al.</i> , 2011 |
| R070 | PISUM ELATIUS JI1089 | Pse | SWd/Wd | | | Turkey | | JIC | NI | Pu | Sm | Sp | D1 | NA | Ellis <i>et al.</i> , 1998; Baranger <i>et al.</i> , 2004 |
| R071 | CE101=FP | Ps | Bl | Dry | Win | France | <2001 | INRA, Versailles | NI | Wh | Sm | Ab | D3 | K10 | Baranger <i>et al.</i> , 2004 |
| R072 | CHAMPAGNE | Ps | Lr | Fd | Win | France | | INRA, Versailles | NI | Pu | Sm | Sp | D3 | K09 | Cousin, 1976; Lejeune-Henaut <i>et al.</i> , 1999; Baranger <i>et al.</i> , 2004 |
| R073 | DP | Ps | Gm | Fd | Win | France | <2001 | INRA, Versailles | NI | Pu | Sm | Sp | D3 | K09 | Baranger <i>et al.</i> , 2004 |
| R074 | WIRAIG JI190 | Ps | Lr | | | Sudan | | JIC | NI | Pu | Sm | Ab | D1 | K03 | Baranger <i>et al.</i> , 2004 |
| R075 | PISUM ELATIUS JI261 | Pse | SWd/Wd | | | Turkey | | JIC | NI | Pu | Sm | Ma | D1 | K01 | Lie, 1984; Ellis <i>et al.</i> , 1998; Baranger <i>et al.</i> , 2004; Jing <i>et al.</i> , 2007; Martin-Sanz <i>et al.</i> , 2011; Borisov <i>et al.</i> , 2007 |
| R076 | MELROSE | Ps | Cv | Fd | Win | United States | 1977 | Univ. Idaho | NI | Pu | Sm | Sp | D3 | K09 | Auld <i>et al.</i> , 1978; Murray & Swensen, 1991; Baranger <i>et al.</i> , 2004 |
| R077 | TERESE | Ps | Cv | Dry | Sp | Denmark | 1988 | Pajgergfonden | af | Wh | Sm | Ab | D2 | K08 | Baranger <i>et al.</i> , 2004 |
| R078 | BACCARA | Ps | Cv | Dry | Sp | France | 1991 | Florimond-Desprez | af | Wh | Sm | Ab | D2 | K08 | Cousin, 1997; Baranger <i>et al.</i> , 2004; Wicker <i>et al.</i> , 2003; Martin-Sanz <i>et al.</i> , 2011 |
| R079 | CHEYENNE | Ps | Cv | Dry | Win | France | 1998 | GAE | af | Wh | Sm | Ab | D2 | NA | Baranger <i>et al.</i> , 2004; Martin-Sanz <i>et al.</i> , 2011 |

Annexes du Chapitre III

| | | | | | | | | | | | | | | | |
|------|------------------------------|----|----|-----|-----|---------------|-------|---------------------|----|----|----|----|----|-----|--|
| R080 | GLACIER | Ps | Cv | Dry | Win | United States | 1983 | Univ. Idaho | NI | Pu | Wr | Sp | D3 | K09 | Auld <i>et al.</i> , 1983; Baranger <i>et al.</i> , 2004 |
| R081 | HOLLY11 | Ps | Lr | Fd | Win | Hungary | | INRA, Versailles | NI | Pu | Sm | Sp | D3 | K09 | Baranger <i>et al.</i> , 2004 |
| R082 | KARNOBAT | Ps | Cv | Dry | Win | Bulgaria | <2001 | INRA, Mons | NI | Pu | Sm | Sp | D3 | NA | Baranger <i>et al.</i> , 2004 |
| R083 | KAZAR | Ps | Cv | Dry | Win | France | 1988 | Clause | NI | Wh | Sm | Ab | D3 | K10 | Baranger <i>et al.</i> , 2004 |
| R084 | TORS DAG | Ps | Cv | Gd | Sp | Sweden | 1925 | VIR | NI | Wh | Sm | Ab | D2 | K05 | Virtanen <i>et al.</i> , 1937; Wilson & Burton, 1938; Baranger <i>et al.</i> , 2004 |
| R085 | WINTERBERGER | Ps | Lr | Fd | Win | Germany | | INRA, Versailles | NI | Pu | Sm | Sp | D3 | K09 | Lejeune-Henaut <i>et al.</i> , 1999; Baranger <i>et al.</i> , 2004 |
| R086 | DU CHEMIN LONG | Ps | Cv | Gd | Sp | France | 1905 | Tézier | NI | Wh | Sm | Ab | D2 | NA | Fourmont, 1956; Baranger <i>et al.</i> , 2004 |
| R087 | CLAMART HATIF | Ps | Cv | Gd | Sp | France | <1883 | INRA, Versailles | NI | Wh | Sm | Ab | D2 | K04 | Vilmorin-Andrieux & Cie, 1883 |
| R088 | MICHAUX DE PARIS | Ps | Cv | Gd | Sp | Netherlands | 1660 | Michaux | NI | Wh | Sm | Ab | D2 | NA | Vilmorin-Andrieux & Cie, 1883; Fourmont, 1956 |
| R089 | CHAMPION D'ANGLETERRE | Ps | Cv | Gd | Sp | Great Britain | 1853 | Fairbeard | NI | Wh | Wr | Ab | D2 | NA | Vilmorin-Andrieux & Cie, 1883; Fourmont, 1956 |
| R090 | PLEIN LE PANIER | Ps | Cv | Gd | Sp | Great Britain | 1870 | Laxton | NI | Wh | Sm | Ab | D2 | NA | Fourmont, 1956 |
| R091 | SERPETTE D'AUVERGNE | Ps | Cv | Gd | Sp | France | 1829 | INRA, Versailles | NI | Wh | Sm | Ab | D2 | K04 | Vilmorin-Andrieux & Cie, 1883; Fourmont, 1956 |
| R092 | CAROUBY DE MAUSSANE | Ps | Cv | Gd | Sp | France | 1931 | Clause | NI | Pu | Sm | Sp | D2 | K04 | Fourmont, 1956 |
| R093 | CORNE DE BELIER | Ps | Cv | Gd | Sp | France | 1818 | GEVES, France | NI | Wh | Sm | Ab | D2 | K04 | Vilmorin-Andrieux & Cie, 1883; Fourmont, 1956 |
| R094 | 90-2079 | Ps | Bl | Gd | Sp | United States | 1990 | Kraft, USDA | af | Wh | Sm | Ab | D2 | NA | Kraft, 1992; Pilet-Nayel <i>et al.</i> , 2002; Wicker <i>et al.</i> , 2003 |
| R095 | FRISSON | Ps | Cv | Dry | Win | France | 1979 | INRA | NI | Wh | Sm | Ab | D3 | K10 | Eteve <i>et al.</i> , 1979; Cousin, 1997; Lejeune-Henaut <i>et al.</i> , 1999; Baranger <i>et al.</i> , 2004 |
| R096 | L1073 | Ps | Bl | Gd | | Sweden | <1960 | Lamprecht | NI | Pu | Sm | Ma | D2 | NA | Veitenheimer & Gritton, 1984; Lewis & Gritton, 1992 |
| R097 | AUSTIN | Ps | Cv | Dry | Sp | France | 1998 | Nickerson | af | Wh | Sm | Ab | D2 | K08 | Bourion <i>et al.</i> , 2007 |
| R098 | 271-134 | Ps | Lr | | | Afghanistan | | INRA, Versailles | NI | Pu | Sm | Ma | D1 | K02 | |
| R099 | PISUM SATIVUM-ETHIOPIA JI281 | Ps | Lr | | | Ethiopia | | JIC | NI | Pu | Sm | Ab | D1 | K03 | Ellis <i>et al.</i> , 1998; Lu <i>et al.</i> , 1996; Baranger <i>et al.</i> , 2004; Martin-Sanz <i>et al.</i> , 2011 |
| R100 | PUGET | Ps | Cv | Gd | Sp | Great Britain | 1967 | Brotherton | NI | Wh | Wr | Ab | D2 | K06 | Hussey & Gunn, 1984; Pilet-Nayel <i>et al.</i> , 2002 |
| R101 | ISARD | Ps | Cv | Dry | Win | France | 2005 | Agriobtenton | af | Wh | Sm | Ab | D3 | K10 | Martin-Sanz <i>et al.</i> , 2011 |
| R102 | KAYANNE | Ps | Cv | Dry | Sp | France | 2008 | Momont | af | Wh | Sm | Ab | D2 | K08 | |
| R103 | ASTRONAUTE | Ps | Cv | Dry | Sp | France | 2012 | Serasem | af | Wh | Sm | Ab | D2 | K08 | |
| R104 | TIL336/11 | Ps | Bl | Dry | Win | France | <2012 | INRA, Mons | af | Wh | Sm | Ab | D3 | K10 | |

(a) Pf: *Pisum fulvum*, Ps: *Pisum sativum*, Psa: *Pisum sativum* subsp. *abyssinicum*, Pse: *Pisum sativum* subsp. *elatius*, Psh: *Pisum sativum* subsp. *humile*; (b) Bl: Breeding line, Cv: Cultivar, Gm: Germplasm, Lr: Landrace, SWd: Semi-Wild, Wd: Wild; (c) Fd: Fodder, Gd: Garden, Dry: Dry pea; (d) Sp: Spring, Win: Winter; (e) af: afila, NI: Normal leaf; (f) Or: Orange, Pi: Pink, Pu: Purple, Wh: White; (g) Sm: Smooth, Wr: Wrinkled; (h) Ab: Absent, Ma: Marbling, Sp: Spots

(Amey *et al.*, 2008)
(Auld *et al.*, 1978)
(Auld *et al.*, 1983)
(Bos & van der Want, 1962)
(Buysse *et al.*, 1983)
(Clark & Spencer-Phillips, 1994)
(Eteve *et al.*, 1979)
(Hagedorn & Gritton, 1973)
(Hussey & Gunn, 1984)
(Kraft, 1992)
(Kraft & Boge, 2001)
(Lejeune-Henaut *et al.*, 1999)
(Lewis & Gritton, 1992)
(Lie, 1984)
(Lockwood & Ballard, 1960)
(Lu *et al.*, 1996)
(Mandy, 1974)
(Martin-Sanz *et al.*, 2011)
(Murray & Swensen, 1991)
(Virtanen *et al.*, 1937)
(Veitenheimer & Gritton, 1984)
(Wellensiek, 1951)
(Wicker *et al.*, 2003)
(Wilson & Burton, 1938)

Supplemental Table III.2. Description of the *Rhizobium leguminosarum* symbiovar *viciae* strains

| Strain name | Strain code | Culture conditions | Species | Origin | Reference |
|-------------|-------------|-----------------------|---|--|-------------------------------|
| TOM | | YM, 28°C | <i>Rhizobium leguminosarum</i> sbv. <i>viciae</i> | Turkey | Winarno R and Lie T.A., 1979 |
| 3841 | | YM St 500, 28°C | <i>Rhizobium leguminosarum</i> sbv. <i>viciae</i> | United Kingdom | |
| P1NP2H | SA | YM, 28°C | <i>Rhizobium leguminosarum</i> sbv. <i>viciae</i> | France | Laguerre <i>et al.</i> , 2003 |
| P1NP2K | SD | YM, 28°C | <i>Rhizobium leguminosarum</i> sbv. <i>viciae</i> | France | Laguerre <i>et al.</i> , 2003 |
| SI16 | SE | YM, 28°C | <i>Rhizobium leguminosarum</i> sbv. <i>viciae</i> | Algeria | Riah <i>et al.</i> , 2014 |
| SF | SF | Rif 300, 28°C | <i>Rhizobium leguminosarum</i> sbv. <i>viciae</i> | Spontaneous Rif ^r mutant derived from TOM | Laguerre, unpublished |
| 3841-sp2 | SK | YM St 300 Sp300, 28°C | <i>Rhizobium leguminosarum</i> sbv. <i>viciae</i> | Sp ^r mutant derived from 3841 | Gift of Philip Poole, JIC, UK |

Supplemental Table III.3. Oligonucleotide primers used to identify specific strains

| Name | | Sequence | Targeted sequence | Targeted strain |
|---------|-------------------|-------------------------|-------------------|-----------------|
| nodjfw | forward | TTGGAACGTATGCATTCGGTCC | IGS nodD-nodF | SA |
| noddfw | forward | ATTGGAAACTACGCATTCGCTGT | IGS nodD-nodF | SD |
| nodugfw | forward | ACAGCCCCAGTAATTAGATCCAT | IGS nodD-nodF | SE |
| NBF12 | reverse universal | GGATCRAAAGCATCCRCATATGG | | |

Primers concentration was 400nM. Each cycle consisted of 30 sec denaturation at 95°C , 30 sec annealing at 59°C and 30 sec extension at 72°C

Supplemental Table III.4. Summary statistics of the ANOVA analyses performed on the phenotypic data collected in the E1 experiment

| Phenotypic variable | Nb of accessions | Nb of observations | Mean | Min | Max | Adjusted R ² | Genotype effect | | Block effect | |
|----------------------------------|------------------|--------------------|-------|-------|--------|-------------------------|-----------------|-----|--------------|-----|
| | | | | | | | Pr(>F) | | Pr(>F) | |
| Shoot dry weight (mg per plant) | 104 | 624 | 958.0 | 148.4 | 2226.3 | 0.84 | < 2.2e-16 | *** | < 2.2e-16 | *** |
| Nodule dry weight (mg per plant) | 104 | 312 | 57.2 | 1.0 | 146.1 | 0.77 | < 2.2e-16 | *** | 0.00171 | ** |
| Nodule number per plant | 104 | 312 | 155.2 | 3.0 | 520.0 | 0.66 | < 2.2e-16 | *** | < 2.2e-16 | *** |

Significance p-value codes: 0 < '***' < 0.001 ≤ '**' < 0.01 ≤ '*' < 0.05 ≤ 'ns' < 1

Supplemental Table III.5. Summary statistics of the ANCOVA analyses of the relationship between shoot and nodule dry weight and between shoot and nodule number in E1

| Categorical variable | Nb of obs | Nodule dry weight effect | | | Categorical variable effect | | | Interaction effect | | | Nodule number effect | | | Categorical variable effect | | | Interaction effect | | |
|-----------------------|-----------|--------------------------|--------|-----|-----------------------------|-----|---------|--------------------|--------|----------|----------------------|--------------------|--------|-----------------------------|--------|--------------------|--------------------|--|--|
| | | Adj R ² | Pr(>F) | | Pr(>F) | | Pr(>F) | Adj R ² | Pr(>F) | | Pr(>F) | Adj R ² | Pr(>F) | | Pr(>F) | Adj R ² | Pr(>F) | | |
| Status | 104 | 0.75 | <2E-16 | *** | 0.52350 | ns | 0.71260 | ns | 0.48 | 1.20E-15 | *** | 0.11620 | ns | 0.68460 | ns | | | | |
| Registration | 49 | 0.84 | <2E-16 | *** | 0.01359 | * | 0.88843 | ns | 0.61 | 5.32E-10 | *** | 0.00633 | ** | 0.43845 | ns | | | | |
| Sowing type | 66 | 0.82 | <2E-16 | *** | 0.00036 | *** | 0.29087 | ns | 0.51 | 2.13E-11 | *** | 1.62E-01 | ns | 0.20040 | ns | | | | |
| DAPC group | 104 | 0.80 | <2E-16 | *** | 0.00020 | *** | 0.02114 | * | 0.47 | 9.21E-16 | *** | 0.16110 | ns | 0.43070 | ns | | | | |
| fastSTRUCTURE cluster | 54 | 0.87 | <2E-16 | *** | 0.00020 | *** | 0.82838 | ns | 0.80 | <2E-16 | *** | 5.55E-08 | *** | 0.07213 | . | | | | |
| Origin | 104 | 0.81 | <2E-16 | *** | 0.24930 | ns | 0.00150 | ** | 0.60 | <2E-16 | *** | 0.00131 | ** | 0.07820 | . | | | | |
| Use | 72 | 0.75 | <2E-16 | *** | 0.01884 | * | 0.14405 | ns | 0.48 | 5.74E-12 | *** | 0.01256 | * | 0.19967 | ns | | | | |

Significance p-value codes: 0 < '***' < 0.001 ≤ '**' < 0.01 ≤ '*' < 0.05 ≤ 'ns' < 1

Supplemental Table III.6. Median, Mean, Min and Max values of relative frequencies of the five Rlv strains within the DAPC or cluster groups

| | Nb of accessions | SA | | | | SD | | | | SE | | | | SF | | | | SK | | | | |
|-----------------------|------------------|--------|-------|-------|-------|--------|-------|-------|-------|--------|--------|-------|-------|--------|-------|-------|-------|--------|-------|-------|-------|-------|
| | | Median | Mean | Min | Max | Median | Mean | Min | Max | Median | Mean | Min | Max | Median | Mean | Min | Max | Median | Mean | Min | Max | |
| Pea collection | 104 | 0.730 | 0.675 | 0.015 | 0.953 | 0.121 | 0.138 | 0.000 | 0.551 | 0.000 | 0.011 | 0.000 | 0.156 | 0.000 | 0.049 | 0.000 | 0.971 | 0.094 | 0.128 | 0.000 | 0.621 | |
| DAPC group | D1 | 21 | 0.377 | 0.481 | 0.015 | 0.950 | 0.077 | 0.118 | 0.000 | 0.551 | 0.000 | 0.018 | 0.000 | 0.156 | 0.015 | 0.220 | 0.000 | 0.971 | 0.079 | 0.163 | 0.000 | 0.621 |
| | D2 | 63 | 0.741 | 0.724 | 0.348 | 0.953 | 0.121 | 0.143 | 0.000 | 0.400 | 0.000 | 0.008 | 0.000 | 0.089 | 0.00 | 0.006 | 0.000 | 0.406 | 0.106 | 0.119 | 0.000 | 0.400 |
| | D3 | 19 | 0.741 | 0.724 | 0.433 | 0.897 | 0.143 | 0.143 | 0.000 | 0.304 | 0.000 | 0.013 | 0.000 | 0.079 | 0.00 | 0.000 | 0.000 | 0.000 | 0.094 | 0.119 | 0.016 | 0.330 |
| fastSTRUCTURE cluster | K01 | 7 | 0.306 | 0.283 | 0.022 | 0.492 | 0.053 | 0.141 | 0.015 | 0.551 | 0.000 | 0.014 | 0.000 | 0.095 | 0.102 | 0.225 | 0.000 | 0.761 | 0.327 | 0.337 | 0.041 | 0.621 |
| | K02 | 7 | 0.344 | 0.393 | 0.015 | 0.825 | 0.156 | 0.134 | 0.000 | 0.310 | 0.000 | 0.025 | 0.000 | 0.156 | 0.017 | 0.347 | 0.000 | 0.971 | 0.079 | 0.102 | 0.015 | 0.344 |
| | K03 | 3 | 0.923 | 0.929 | 0.914 | 0.950 | 0.000 | 0.026 | 0.000 | 0.077 | 0.000 | 0.006 | 0.000 | 0.017 | 0.000 | 0.000 | 0.000 | 0.000 | 0.050 | 0.040 | 0.000 | 0.069 |
| | K04 | 11 | 0.778 | 0.758 | 0.559 | 0.877 | 0.136 | 0.137 | 0.015 | 0.273 | 0.000 | 0.003 | 0.000 | 0.018 | 0.000 | 0.000 | 0.000 | 0.000 | 0.091 | 0.103 | 0.018 | 0.225 |
| | K05 | 11 | 0.730 | 0.709 | 0.400 | 0.929 | 0.147 | 0.165 | 0.000 | 0.320 | 0.000 | 0.013 | 0.000 | 0.048 | 0.000 | 0.000 | 0.000 | 0.000 | 0.082 | 0.114 | 0.017 | 0.250 |
| | K06 | 8 | 0.741 | 0.746 | 0.580 | 0.887 | 0.161 | 0.173 | 0.037 | 0.380 | 0.0147 | 0.013 | 0.000 | 0.043 | 0.000 | 0.000 | 0.000 | 0.000 | 0.064 | 0.068 | 0.016 | 0.118 |
| | K07 | 3 | 0.622 | 0.624 | 0.488 | 0.762 | 0.206 | 0.234 | 0.178 | 0.317 | 0.000 | 0.030 | 0.000 | 0.089 | 0.000 | 0.000 | 0.000 | 0.000 | 0.111 | 0.113 | 0.032 | 0.195 |
| | K08 | 6 | 0.728 | 0.721 | 0.600 | 0.862 | 0.100 | 0.105 | 0.000 | 0.200 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.167 | 0.174 | 0.017 | 0.400 |
| | K09 | 8 | 0.787 | 0.763 | 0.594 | 0.897 | 0.132 | 0.142 | 0.000 | 0.304 | 0.000 | 0.018 | 0.000 | 0.067 | 0.000 | 0.000 | 0.000 | 0.000 | 0.066 | 0.078 | 0.031 | 0.172 |
| | K10 | 6 | 0.725 | 0.702 | 0.433 | 0.895 | 0.113 | 0.124 | 0.018 | 0.227 | 0.000 | 0.005 | 0.000 | 0.017 | 0.000 | 0.000 | 0.000 | 0.000 | 0.154 | 0.169 | 0.088 | 0.330 |

Supplemental Table III.7. Summary statistics of the ANOVA analyses performed on the phenotypic data collected in the E2 experiment

| Phenotypic variable | Nb of accessions | Nb of observations | | | | Adjusted R ² | Pea effect | | | Rlv effect | | | Pea x Rlv effect | | |
|---------------------------------|------------------|--------------------|--------|------|--------|-------------------------|------------|-----|-----------|------------|-----------|-----|------------------|-----|--|
| | | | Mean | Min | Max | | Pr(>F) | | | Pr(>F) | | | Pr(>F) | | |
| Shoot dry weight (mg per plant) | 18 | 689 | 1588.7 | 19.8 | 6302.0 | 0.89 | < 2.2e-16 | *** | < 2.2e-16 | *** | < 2.2e-16 | *** | < 2.2e-16 | *** | |
| Nodule number per plant | 18 | 179 | 172.0 | 0.0 | 589.0 | 0.93 | < 2.2e-16 | *** | < 2.2e-16 | *** | < 2.2e-16 | *** | < 2.2e-16 | *** | |

Significance p-value codes: 0 < '***' < 0.001 ≤ '***' < 0.01 ≤ '*' < 0.05 ≤ 'ns' < 1

Supplemental Table III.8. Summary statistics of the linear regression analyses performed for the five strains between nodulation index evaluated in E2 and competitiveness evaluated in E1

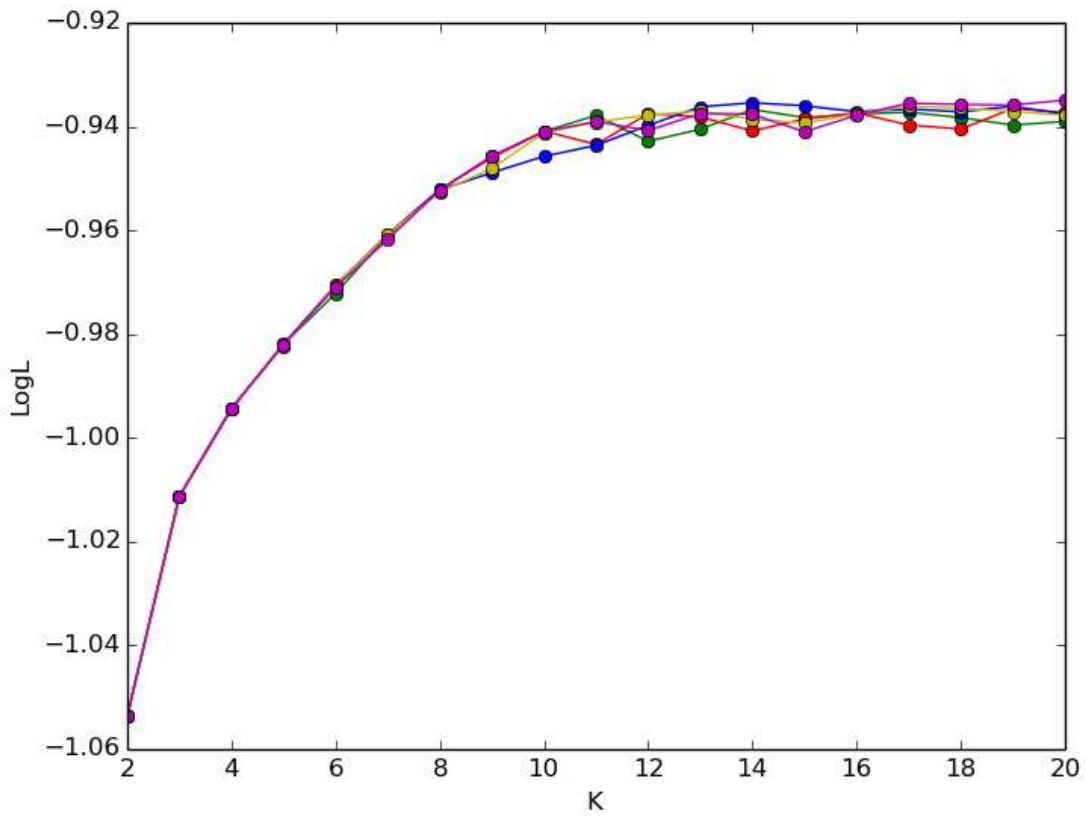
| Rlv strain | Nb of accessions | Adj R ² | Pr(>F) |
|------------|------------------|--------------------|---------------|
| SA | 18 | 0 | 0.449 ns |
| SD | 18 | 0 | 0.923 ns |
| SE | 18 | 0 | 0.271 ns |
| SF | 18 | 0.79 | 5.512E-07 *** |
| SK | 18 | 0 | 0.381 ns |

Significance p-value codes: 0 < '****' < 0.001 ≤ '**' < 0.01 ≤ '*' < 0.05 ≤ 'ns' < 1

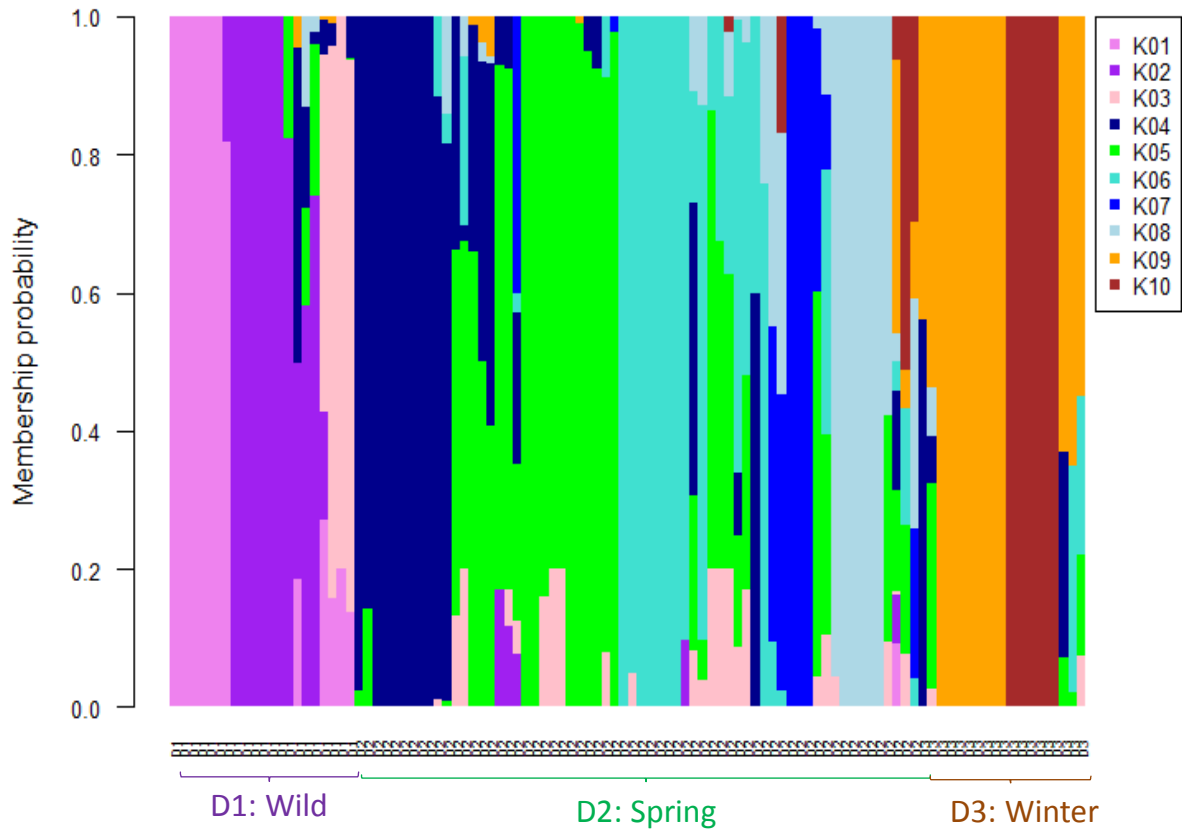
Supplemental Table III.9. Summary statistics of the linear regression analyses performed for the five strains between shoot dry matter index evaluated in E2 and competitiveness evaluated in E1

| Rlv strain | Nb of accessions | Adj R ² | Pr(>F) |
|------------|------------------|--------------------|----------|
| SA | 18 | 0.41 | 0.002 ** |
| SD | 18 | 0 | 0.343 ns |
| SE | 18 | 0 | 0.963 ns |
| SF | 18 | 0.47 | 0.001 ** |
| SK | 18 | 0 | 0.980 ns |

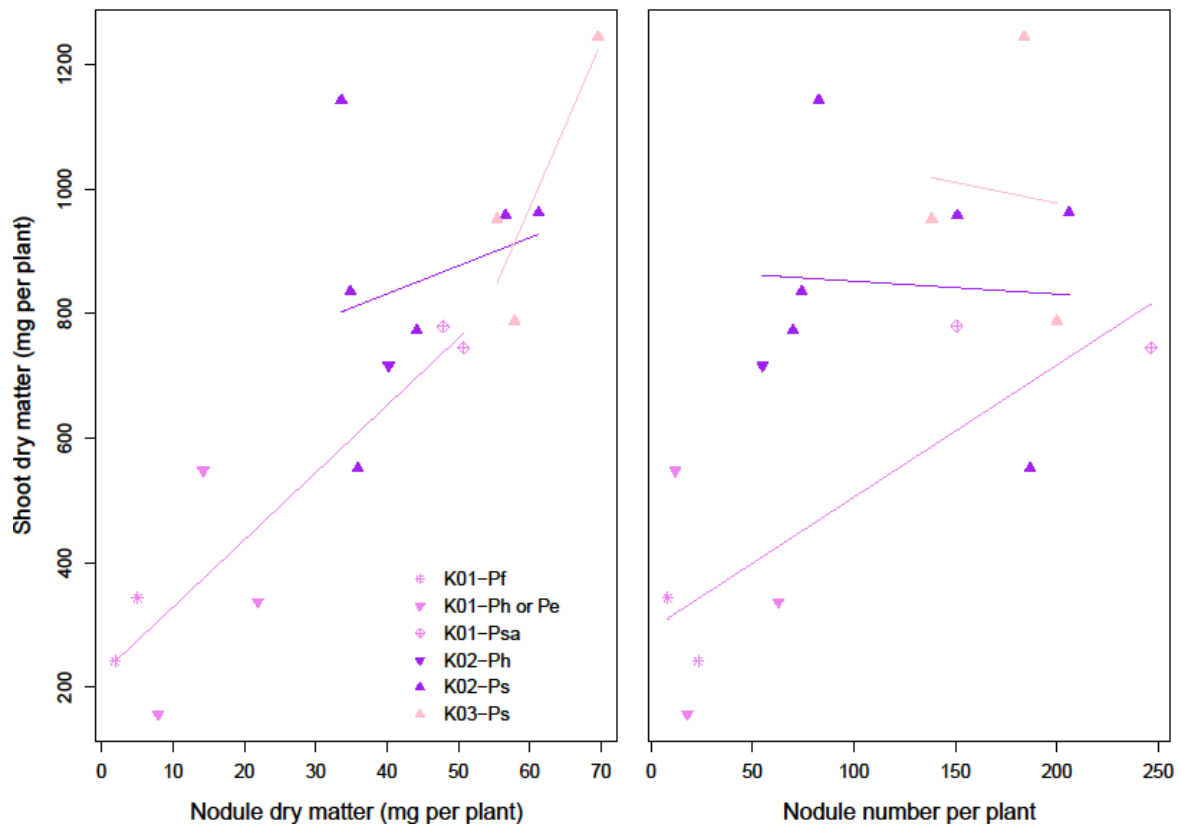
Significance p-value codes: 0 < '****' < 0.001 ≤ '**' < 0.01 ≤ '*' < 0.05 ≤ 'ns' < 1



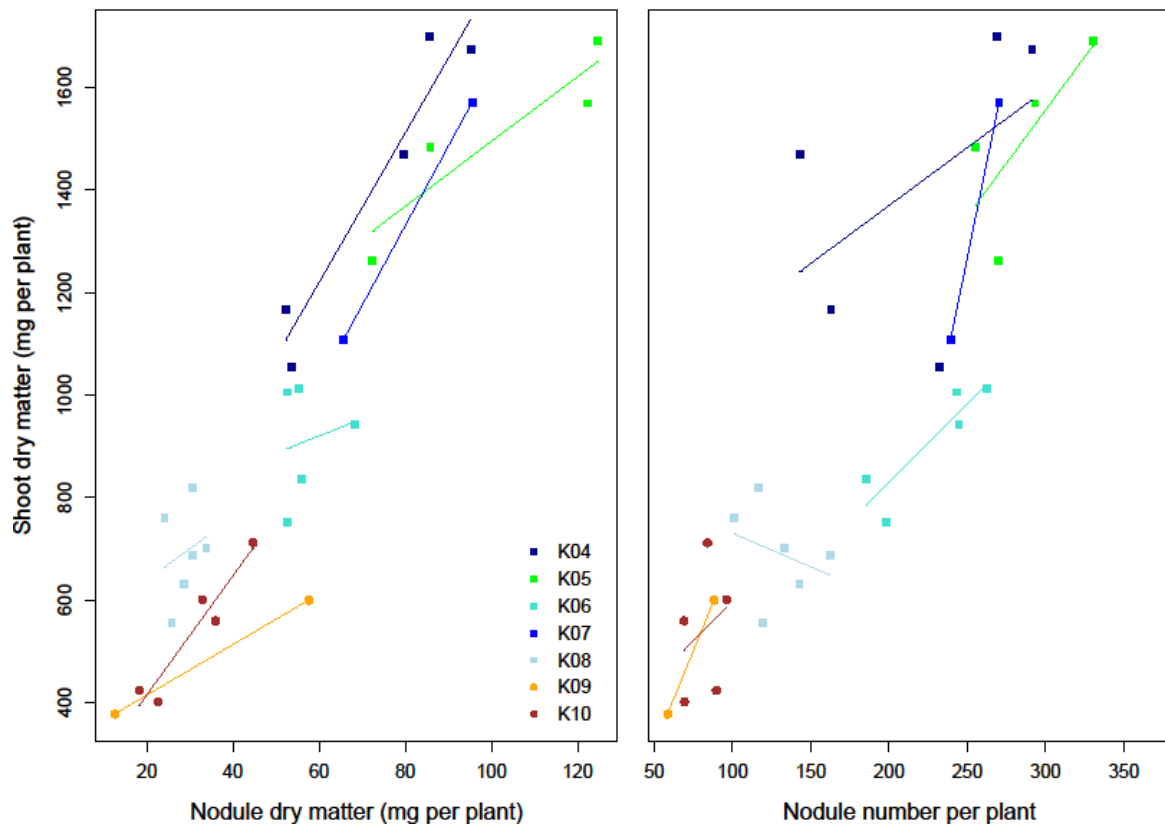
Supplemental Fig. III.1. FastSTRUCTURE analysis. Log Likelihood of the model as a function of the number of clusters for each of the five runs



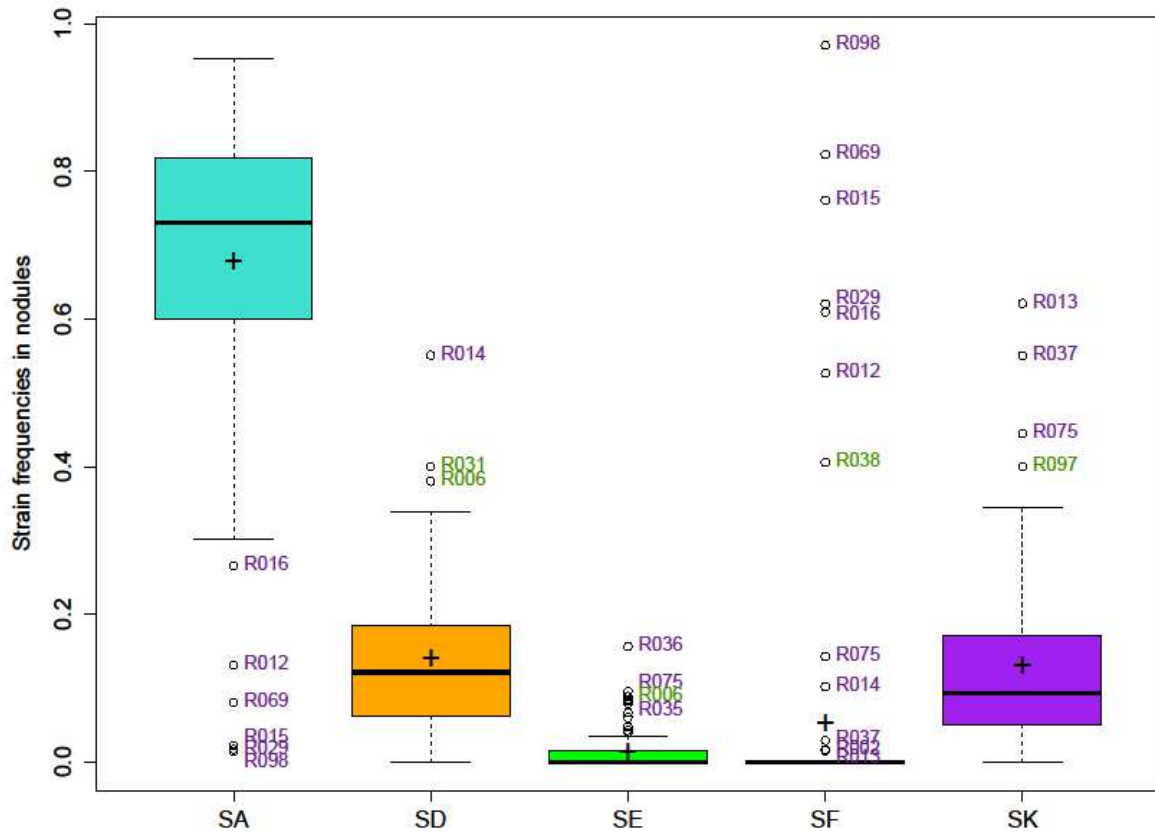
Supplemental Fig. III.2. Genetic structure of the pea collection using the fastSTRUCTURE analysis



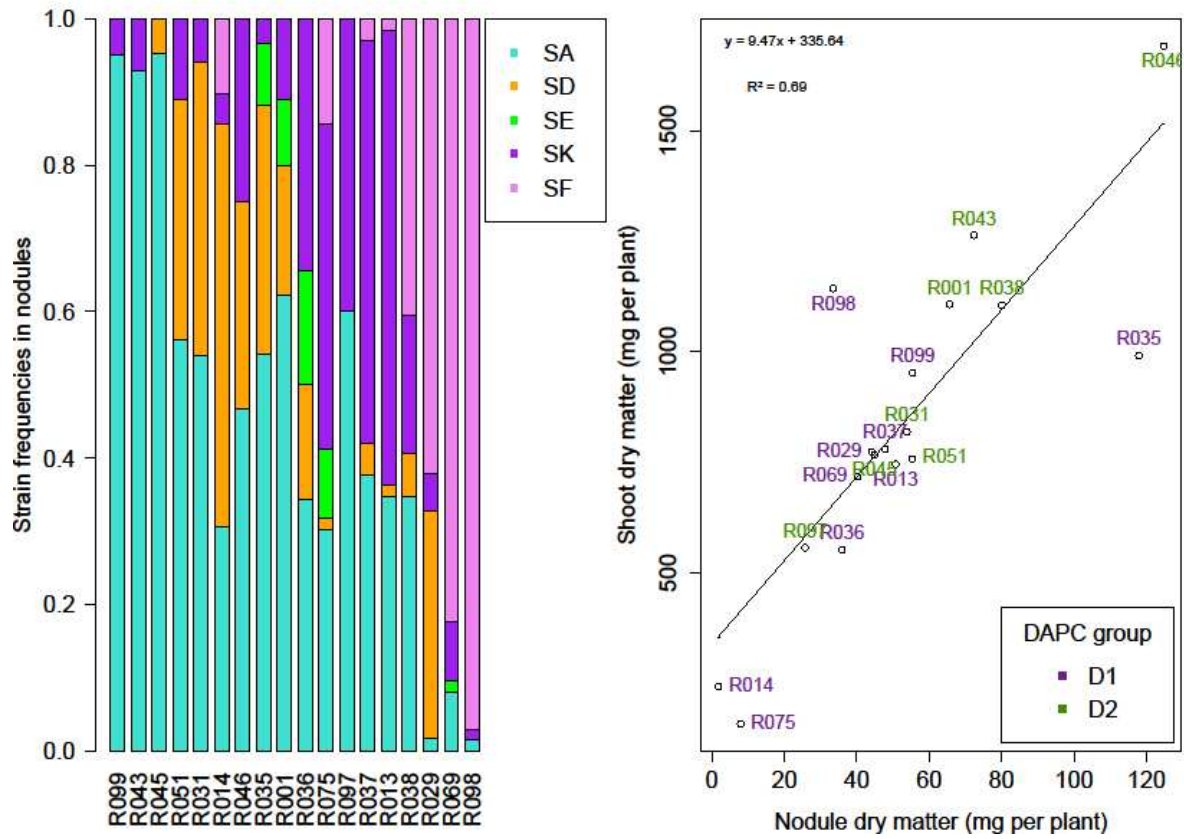
Supplemental Fig. III.3. Relationship between shoot dry matter and nodule dry matter (a) or nodule number per plant (b), for the 17 pea accessions belonging to D1 according to their membership to kluster groups in the multi-inoculation experiment (E1)



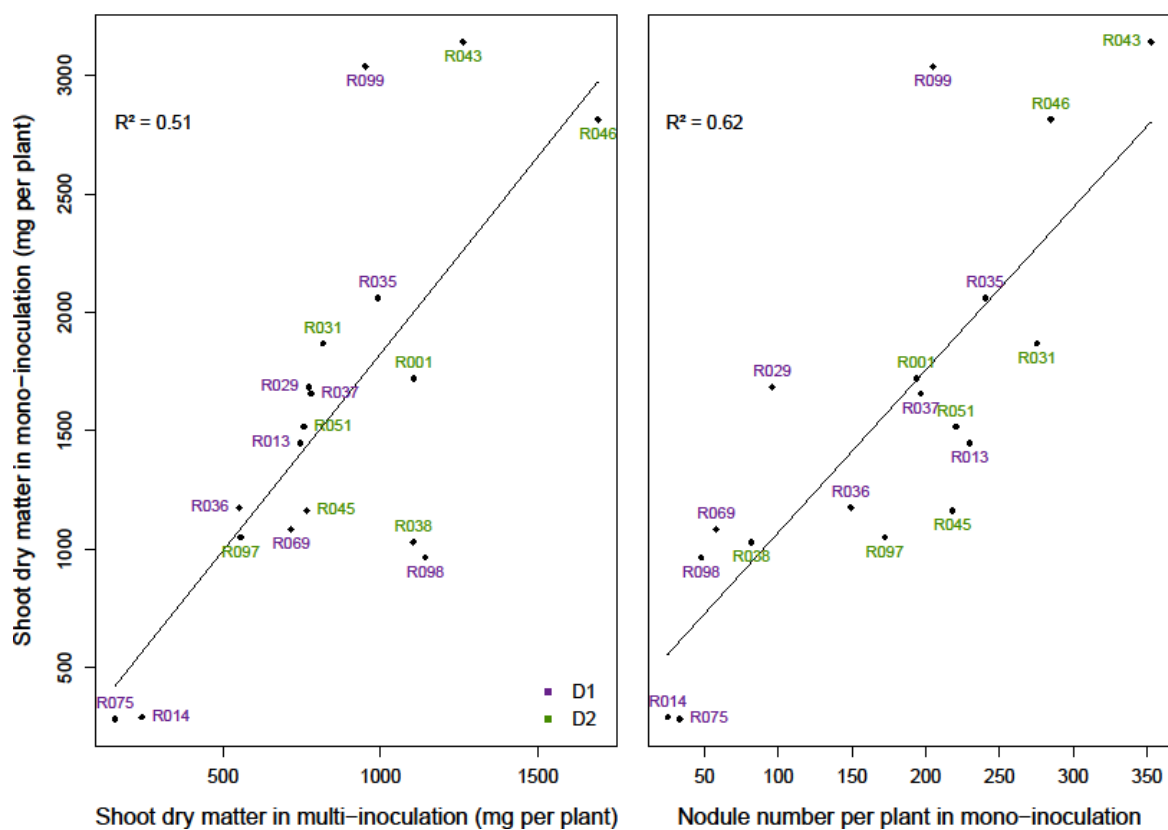
Supplemental Fig. III.4. Relationship between shoot dry matter and nodule dry matter (a) or nodule number per plant (b), for the 29 pea cultivars belonging to D2 or D3 according to their membership to kluster groups in the multi-inoculation experiment (E1)



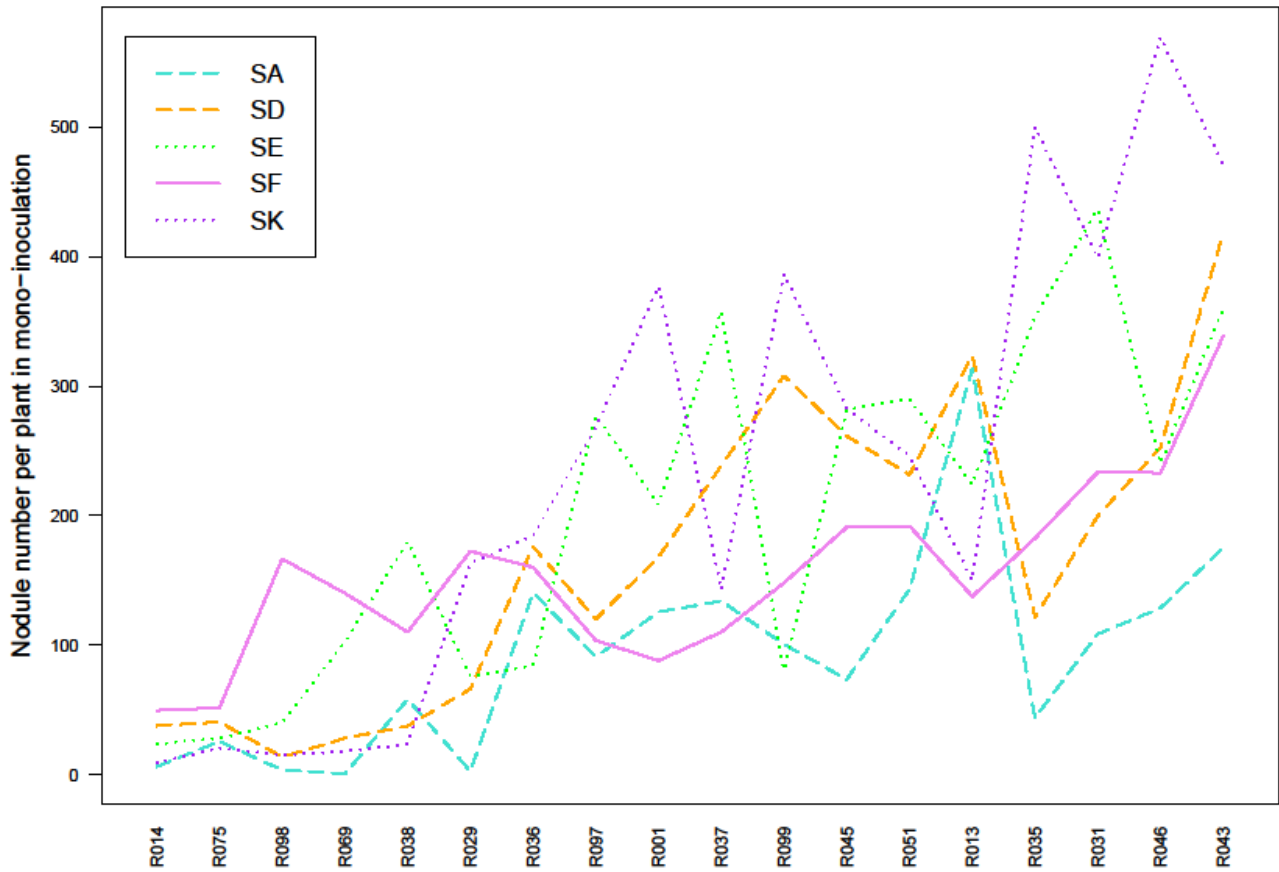
Supplemental Fig. III.5. Strain frequencies in the nodules of 104 pea accessions (E1 experiment). + indicates the mean frequency for each of the five strain. All outliers are identified by accession numbers. Colour indicates DAPC group affiliation: violet for D, green for D2 and brown for D3



Supplemental Fig. III.6. For 18 pea accessions multi-inoculated with a mixture of five Rlv strains (E1 experiment): (a) Strain frequencies in the nodules (b) Relationship between shoot dry matter and nodule dry matter



Supplemental Fig. III.7. Relationship between shoot dry matter in the mono-inoculation experiment (E2) and (a) shoot dry matter in multi-inoculation (E1 experiment) or (b) nodule number per plant in mono-inoculation (E2 experiment), for 18 pea accessions



Supplemental Fig. III.8. Nodule number per plant of 18 pea accessions with each of the five Rlv strains in mono-inoculation (E2 experiment)

Annexe 4 : Liste des jeux de données utilisés au cours de la thèse

Concernant la première approche :

- des données de phénotypage racinaire, nodulaire et aérien, acquises à 4 stades successifs de développement, sur 10 accessions de pois, dont 7 lignées parentes de populations de lignées recombinantes, cultivées en pots, soumises à faible dose de nitrate (2,5 mM) et inoculées avec une souche de Rlv (1007)
- des données de phénotypage racinaire, nodulaire et aérien, acquises sur 3 accessions de pois parmi les 7 lignées parentes, cultivées en pots et en poches, sans apport de nitrate ou avec faible apport (2,5 mM) et mono-inoculées avec une des 2 souches de Rlv (1007, P221) pour comparaison
- des données phénotypiques acquises au champ (Epoisses) pendant deux années sur la population de 153 lignées recombinantes RIL4 avec 1 kg N/ha d'azote marqué (15N): biomasse des pailles et des graines, poids de mille grains, teneur en N des pailles et des graines, mesures de $\delta^{15}\text{N}$ pour chaque lignée et sur de l'orge (= plante de référence non fixatrice)
- des données de phénotypage racinaire, nodulaire et aérien, acquises à début floraison sur cette population, cultivée en serre et en pots, soumise à faible dose de nitrate (2,5 mM, 1% 15N) et inoculée avec une souche de Rlv (P221)
- des données de phénotypage racinaire, nodulaire et aérien, acquises en cinétique jusqu'à début floraison sur cette population, cultivée en serre et en poches, soumise à faible dose de nitrate (2,5 mM, 1% 15N) et inoculée avec une souche de Rlv (P221)
- des mesures de $\delta^{15}\text{N}$ pour chaque lignée cultivée en serre, en pots ou en poches
- des images scannées de systèmes racinaires nodulés au stade début floraison des plantes cultivées en pots ou en poches et de dessins des systèmes racinaires nodulés faits en cinétique à partir des poches, pour l'ensemble des lignées de la population
- des images scannées des différents étages de feuilles des plantes cultivées en poches, pour l'ensemble des lignées de la population
- des données de cartographie génétique obtenues sur cette population, comprenant 152 marqueurs essentiellement microsattellites et couvrant 1140 cM (Loridon *et al.*, 2005; Aubert *et al.*, 2006)

Concernant la deuxième approche :

- des données de phénotypage racinaire, nodulaire et aérien, acquises à 5 dates successives sur deux géotypes de *M. truncatula*, le mutant TR185 et le sauvage J5, cultivés en hydroponie et en absence d'inoculation par du *Rhizobium*, soumis à deux doses de nitrate (1 mM et 10mM)
- des images scannées de systèmes racinaires acquises aux 5 dates de prélèvement pour les 2 géotypes de *M. truncatula* et les 2 doses de nitrate
- des photos vues de dessus des parties aériennes acquises aux 5 dates de prélèvement pour les 2 géotypes de *M. truncatula* et les 2 doses de nitrate
- des données de teneur en acides aminés des parties aériennes et des racines, acquises à 2 des 5 dates de prélèvement pour les 2 géotypes de *M. truncatula* et les 2 doses de nitrate
- des données de teneur en lignine des racines, acquises à 2 des 5 dates de prélèvement pour les 2 géotypes de *M. truncatula* et les 2 doses de nitrate
- des données normalisées d'expression de gènes obtenues sur puces Affymetrix à une des 5 dates de prélèvement pour les 2 géotypes de *M. truncatula* et les 2 doses de nitrate

Concernant la troisième approche :

- des données passeport mises à jour sur une collection de 104 accessions de pois
- des données de phénotypage racinaire, nodulaire et aérien, acquises à deux dates (8 jours et 4 semaines), sur cette collection de 104 accessions de pois, cultivée en serre et en pots, soumise à faible dose de nitrate (0.625 mM) et inoculée avec un mélange de 5 souches de Rlv
- des images scannées de systèmes racinaires nodulés et des nodules isolés pour l'ensemble de cette collection de 104 accessions de pois
- des images scannées des différents étages de feuilles des plantes pour l'ensemble de cette collection de 104 accessions de pois
- des données de composition de la population de souches associée, à 4 semaines, sur cette collection de 104 accessions de pois, cultivée en serre et en pots, soumise à faible dose de nitrate et inoculée avec un mélange de 5 souches de Rlv
- des données de phénotypage nodulaire et aérien, sur 18 accessions de pois constituant un sous-échantillon de la collection des 104, cultivées en serre et en pots, sans apport de nitrate, mono-inoculées par chacune des 5 souches ou non-inoculées (contrôle)
- des données de structuration génétique de la collection des 104 accessions de pois, utilisant les données génotypiques obtenues à partir de la puce GenoPea Illumina Infinium développée récemment (Tayeh *et al.*, 2015), incluant 13.2 K marqueurs SNP couvrant l'ensemble du génome.

Annexe 5 : Poster décrivant la diversité de la collection de pois

Genetic diversity of nodulated root structure and nitrogen nutrition in a core collection of pea

V. Bourion, H. de Larambergue, V. Aubert, C. Martin, M. Martinello, C. Delaitre, G. Aubert, M. Siol, G. Duc, J. Burstin

INRA, UMR1347 Agroécologie, GEAPSI, BP 86510, 21000 Dijon, France

Corresponding author: virginie.bourion@dijon.inra.fr

Poster. 7th EPSO Conference Porto Heli , 01-04 September 2013.

Introduction: Pea (*Pisum sativum*) is the third most important grain legume worldwide and the increasing demand for protein-rich raw material for animal feed or human nutrition has led to a greater interest in this crop as a protein source.

Legumes have the natural ability to use, as main nitrogen (N) resource, the atmospheric N₂ from symbiosis in nodules with Rhizobiaceae spp. Legumes thus do not need N fertilizers. However, N nutrition can still be a limiting factor of yield and seed quality in legumes. Nodules are very sensitive to their local environment, in particular to nitrate, and root systems of N₂ fixing legumes are poorly developed, which makes them unable to explore a large soil volume and sensitive to unfavourable conditions.

This study assessed the potential of naturally occurring genetic variability of nodulated root structure and functioning traits to improve yield pea performance.



Figure 1: Experimental setup in the glasshouse

Material and Methods: One hundred and four pea accessions were selected from the French pea collection based on their genetic diversity and agronomic traits. This core-collection includes 44 cultivars, 10 inbred lines, 28 landraces, and 22 wild genotypes. Two among the accessions are *Pisum fulvum* and all others are *Pisum sativum*. Most of the *P. sativum* belong to subsp *sativum* but 2 belong to subsp *abyssinicum* and 4 to subsp *elatius*.

A glasshouse experiment was performed in a three-block randomised design, with one pot per genotype and four plants per pot in each block (Fig.1). The 2-L pots were filled with a 1:1 (v/v) mixture of sterilized atapulgite and clay balls, with a cell suspension of a *Rhizobium leguminosarum* bv. *viciae* (10⁸ per seed) and supplied with a 0,625 mM ¹⁵N labelled nitrate (1% ¹⁵N).

The thousand seed weight (TSW) of each accession was determined before sowing. Two plants per pot were harvested for measurements at two different dates: 8-day and 4-week after sowing. The two 8-day old plants were spread onto a transparent sheet and scanned as digital images with an A3 colour scanner (Fig.2A).

The root system of each 4-week old plant was carefully spread onto a transparent sheet to minimise root overlapping and scanned (Fig.2B). All nodules were then peeled and scanned (Fig.2C). Total root length

(TRootL), mean root diameter (RootD) and nodule projected area (TNodPA) were further determined by image analysis using WinRhizo® Software. All first order lateral roots and nodules were counted (NLatRoot, NNod). Roots and nodules were oven-dried separately at 80°C for 48 and weighed (RootB, NodB) and the average nodule biomass calculated (ANodB).

In addition, shoot length (ShootL) was measured in all experiments and leaves of 4-week old plants were scanned (Fig.2D) and further analysed to determine total leaf area (LeafA). Then shoots were oven-dried for dry matter measurement (ShootB). Shoot nitrogen content (ShootNC) was estimated by mass spectrometry and the part of nitrogen accumulation derived from symbiotic fixation (NDFA) was calculated using the isotope dilution technique (Duc et al. 1988).

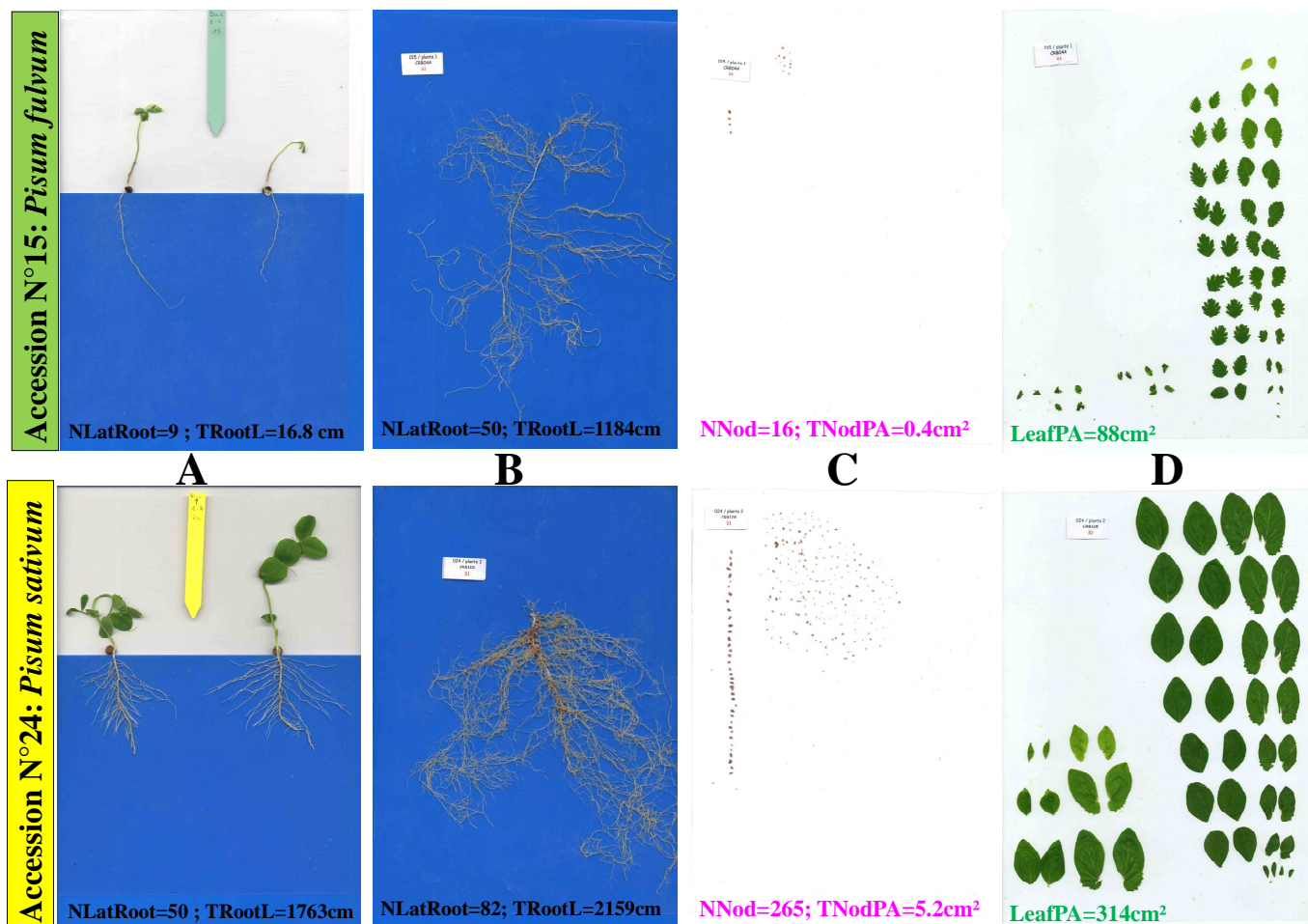


Figure 2: Successive scanned images for two different accessions.

A Whole 8-day old plants. B Whole 4-week root system. C All nodules of 4-week day plant.

Results: Significant variations between accessions were observed for most traits describing the nodulated root structure and which were obtained from measurements and root image analysis (NLatRoot, TapRootL, RootD, RootB, TRootL, NNod, NodB, ANodB, TNodPA) (Fig.3).

Significant variations between accessions were also observed for carbon and N acquisition traits, estimated respectively from leaf surface area, shoot biomass (LeafA, ShootB) and N content and nitrogen fixation measurement (ShootNC, NDFA) (Fig.3).

A significant positive relationship was found between NNod, NodB, NLatRoot, RootB and NDFA, with significant correlations ranging from 0.45 to 0.66 (Fig.4).

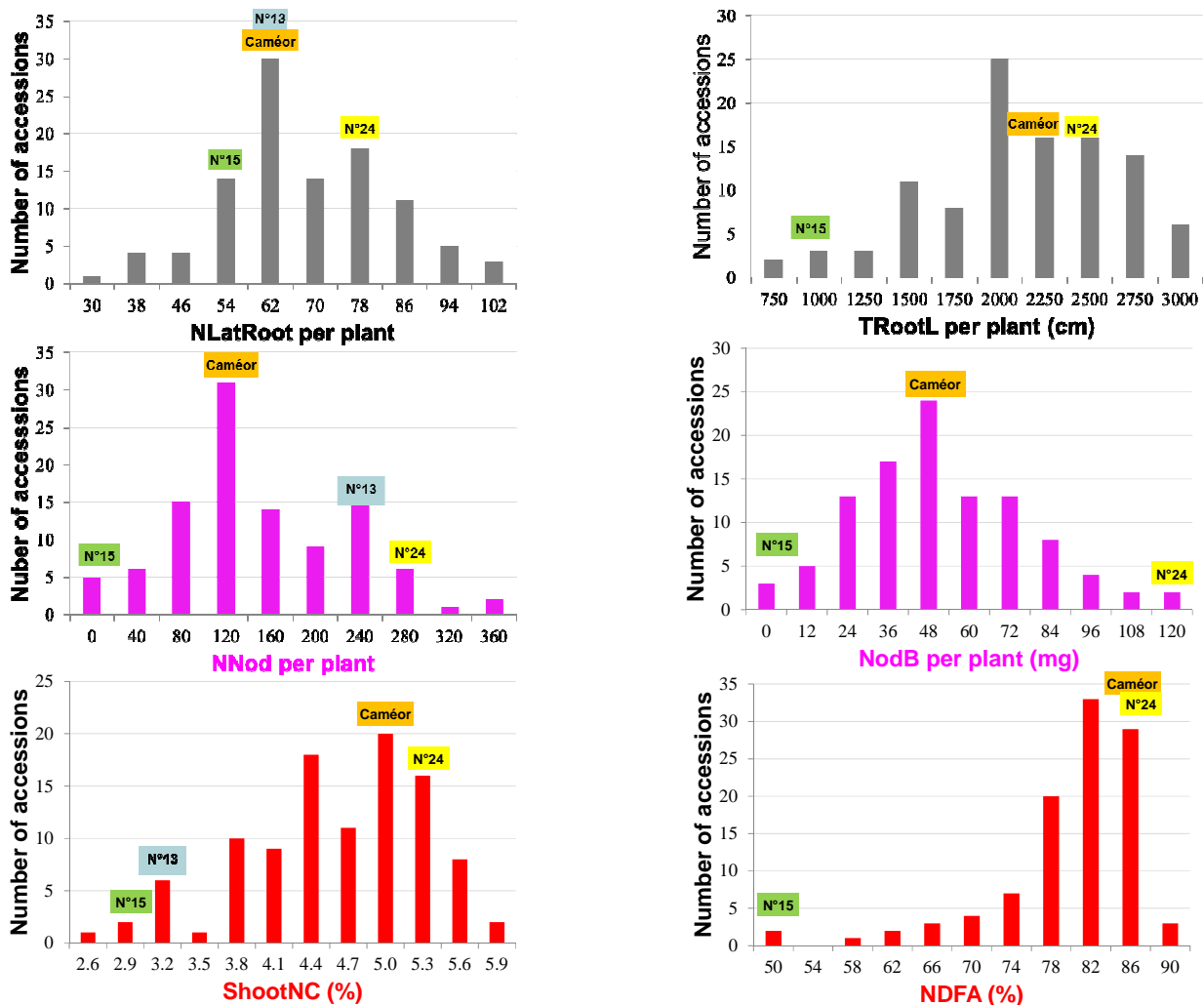


Figure 3: Frequency distribution of the 104 pea accessions for traits describing the nodulated root structure and the nitrogen nutrition at the 4-week stage

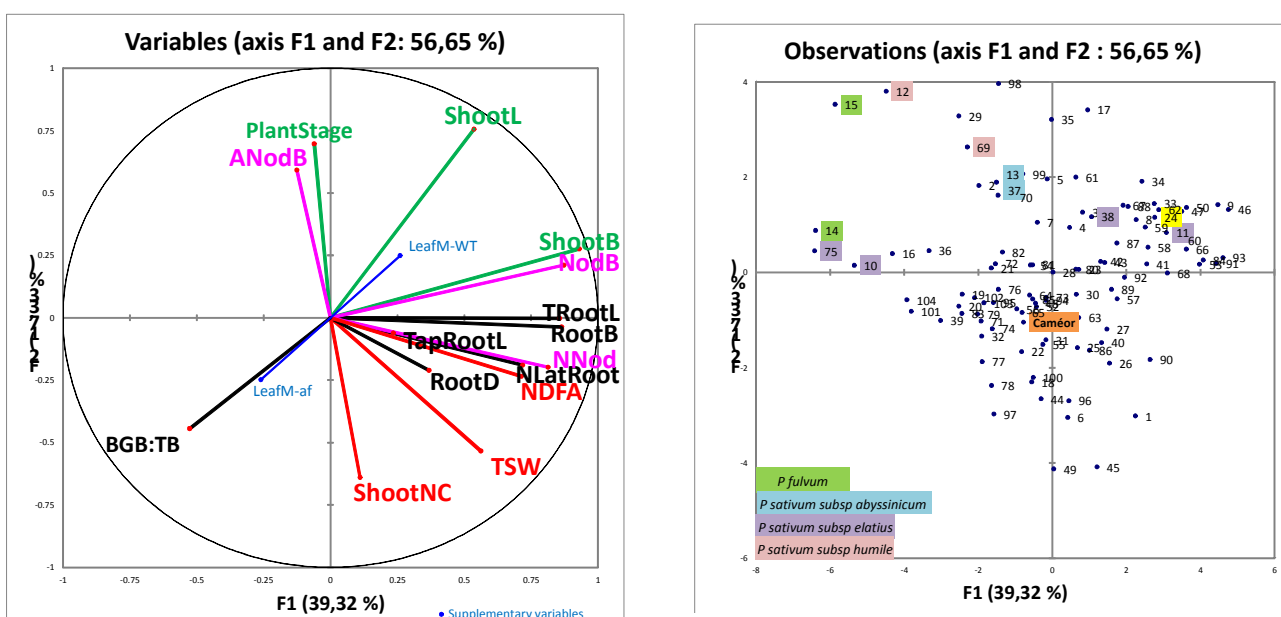


Figure 4: PCA analysis of the 104 accessions using 14 active & 1 supplementary variables

Conclusion-Perspectives: This analysis highlighted the high variability for the nodulated root structure and the nitrogen acquisition among the 104 pea accessions. A highly significant correlation was observed between lateral root number, nodule number and nitrogen acquisition traits. Further analyses will be performed to identify associations between molecular and nodulated root structure or functioning diversity. An analysis of the structure of the French pea collection has been performed with the software DaPC (Jombart et al., 2010) based on 353 SNPs and 32 SSR markers, showing that the material in the core collection could be assigned to 6 distinct genetic groups.

References:

- Bourion V, Rizvi SMH, Fournier S, de Larambergue H, Galmiche F, Marget P, Duc G, Burstin J. 2010.** Genetic dissection of nitrogen nutrition in pea through a QTL approach of root, nodule, and shoot variability. *Theoretical and Applied Genetics* **121**, 71-86.
- Duc G, Mariotti A, Amarger N. 1988.** Measurements of genetic variability for symbiotic dinitrogen fixation in field-grown faba bean (*Vicia faba* L.) using a low level ¹⁵N-tracer technique. *Plant and Soil* **106**, 269-276.
- Jombart T, Devillard S, Balloux F. 2010.** Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. *BMC Genetics* 11:94
- WinRHIZO Pro 2013c** Regent Instruments Inc, Quebec, Canada