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Dynamique d'allocation du phosphore prélevé et de sa remobilisation pendant le remplissage du grain chez le blé dur

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Par **Mohamed EL MAZLOUZI**

**Dynamique d'allocation du phosphore prélevé et de sa
remobilisation pendant le remplissage du grain chez le
blé dur**

Sous la direction de : **Alain MOLLIER**

Soutenue le 16 décembre 2020

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École doctorale Sciences et Environnements, Université de Bordeaux

École doctorale
**Sciences et
environnements**

université
de **BORDEAUX**

Dynamique d'allocation du phosphore prélevé et de sa remobilisation pendant le remplissage du grain chez le blé dur

Résumé : Une des pistes pour gérer durablement le phosphore (P) dans les agrosystèmes consiste à diminuer les exportations répétées du P avec les récoltes. La teneur en P des produits récoltés, en particulier les grains, est alors un paramètre clé dans la gestion du cycle du P dans les agrosystèmes. Au cours de la période post-floraison, l'accumulation du P dans les grains dépend de l'absorption post-floraison et de la remobilisation du P des organes végétatifs. Cependant les processus écophysiologicals qui régissent le remplissage des grains en P et la contribution des deux sources sont encore mal connus. Les objectifs de cette thèse étaient de mieux comprendre les processus impliqués dans le remplissage des grains en P et de déterminer la contribution de la remobilisation et de l'absorption post-floraison au P des grains chez le blé dur (*Triticum turgidum*. L). Trois expérimentations ont été réalisées en conditions contrôlées avec un système de culture hydroponique permettant de contrôler l'offre en P. D'abord, les effets de la privation en P pendant la période post-floraison sur la remobilisation et les teneurs en P des grains ont été analysés chez deux cultivars de blé dur. Ensuite, l'utilisation du traçage isotopique du P exogène a permis d'étudier la dynamique fine du P durant la période post-floraison et son transport aux grains. Les résultats obtenus montrent que la croissance des grains n'est pas limitée par l'offre en P pendant la période post-floraison si les plantes sont suffisamment alimentées en P pendant la phase végétative. En effet, la privation en P appliquées à partir de la floraison n'a pas eu d'effet négatif sur le rendement et sur la qualité des grains, malgré la diminution significative dans la teneur en P des grains. Nous avons également montré que les flux de remobilisation augmentent en conditions de limitation en P. Le marquage au ^{32}P de la floraison à la maturité a permis de montrer que la remobilisation du P représente 81 % du P des grains pour les plantes cultivées avec un faible niveau en P tandis qu'elle représente 65 % pour les plantes cultivées avec un niveau élevé en P. Les deux marquages successifs au ^{32}P au cours du développement des grains révèlent que le P absorbé par les racines pendant la période post-floraison n'est pas directement alloué aux grains via le xylème. Enfin, ce travail ouvre de nouvelles perspectives pour améliorer l'efficacité d'utilisation du P en limitant les exportations répétées de P et ses conséquences environnementales et sur la qualité nutritionnelle des grains.

Mots-clés : Phosphore, blé dur, remplissage des grains, remobilisation, absorption post-floraison, agronomie

Dynamics of exogenous phosphorus partitioning and phosphorus remobilization from vegetative organs throughout grain filling in durum wheat

Abstract: Reducing the amount of phosphorus (P) exported from the field at harvest is one of the strategies to sustainably manage P inputs in agriculture. Thus, the grain P concentration is a key parameter in the management of P in agroecosystems. Grain P in wheat originates from two sources: the post-anthesis root P uptake and the remobilization of P from the vegetative parts of the plant. However, the exact contributions of both P sources to the P accumulated in the grains and the effect of P supply remains unclear. The objectives of this thesis are to provide a better understanding of the processes involved in the allocation of P to grains and to determine the contribution of post-anthesis P uptake and P remobilization to grain P in durum wheat (*Triticum turgidum*. L). The work of this thesis is based on three experiments carried out under controlled conditions in a hydroponic system that allows the controls of P supply to plants. Firstly, the effects of P deprivation during the post-anthesis period on P remobilization and grain P content were analyzed in two durum wheat cultivars. Secondly, the use of P isotope tracing was used to study the dynamics of P during the post-anthesis period. The results show that the P supply does not limit grain growth during the post-anthesis period if the plants are sufficiently supplied with P during the vegetative stage. Indeed, the P deprivation applied from anthesis onwards has no negative effect on grain yield and quality, despite the significant decrease in the P content of the grains. We have also shown that P remobilization fluxes increase under P-limiting conditions. The ^{32}P labelling from anthesis to maturity showed that the remobilization of P from vegetative organs represents 81% of grain P in low P plants while it represents 65% for high P plants. The two successive labelling experiments with ^{32}P during grain development reveal that the P absorbed by the roots during the post-anthesis period is not directly allocated to the grains via the xylem. Finally, this work opens up new perspectives for improving P use efficiency by limiting the repeated P export and hence its environmental impact and nutritional consequences.

Keywords: Phosphorus, durum wheat, grain filling, remobilization, post-anthesis uptake, agronomy

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Introduction générale

Contexte scientifique et objectifs

Le phosphore (P) est un macro-élément essentiel pour la croissance et le développement des plantes. C'est un élément indispensable dans le métabolisme, la structure cellulaire et le transfert du matériel génétique (Marschner, 2011; Raghothama, 1999). Après l'azote, il est considéré comme le deuxième élément qui limite la production végétale dans les agrosystèmes. Pour répondre aux besoins des cultures, le P est apporté sous forme d'engrais phosphatés, afin de compenser les exportations répétées de cet élément dues aux récoltes et à l'exploitation intensive du sol (Tilman et al., 2002). Actuellement, ces engrais de synthèse sont extraits à partir des roches phosphatées, qui sont une ressource non renouvelable et inégalement répartie dans le monde (Cordell and White, 2014). De plus, une augmentation de la demande mondiale en engrais phosphatés est attendue dans les prochaines décennies en raison de la croissance démographique et de la demande pour la production alimentaire (Tilman et al., 2002; Vance et al., 2003). Par conséquent, les réserves de phosphate de bonne qualité sont en cours d'épuisement (Cordell and White, 2014). Le défi de l'agriculture durable est d'augmenter la production agricole avec de bas niveaux d'intrants tout en préservant l'environnement. Cela passe à court terme par une adaptation des pratiques agronomiques et de méthodes d'utilisation raisonnée de la fertilisation phosphatée. À plus long terme, l'amélioration génétique afin d'augmenter l'efficacité d'utilisation du P par les cultures est nécessaire (Rose et al., 2013; Wang et al., 2016).

L'acquisition du phosphore est souvent limitée par la faible concentration d'ions phosphates (entre 0.1 et 10 μM) présents dans la solution du sol (Shen et al., 2011; Vance et al., 2003). Pour faire face à cette faible biodisponibilité, les plantes ont développé plusieurs stratégies qui leur permettent de se développer sur des milieux pauvres en P (White and Hammond, 2008b; Veneklaas et al., 2012). Ces stratégies peuvent être classées en deux catégories. La première correspond à la capacité de la plante à extraire le P du sol (P Acquisition Efficiency) et concerne les caractères liés au système racinaire tels que la modification de l'architecture et la morphologie racinaire, la stimulation des processus rhizosphériques ou les associations aux mycorhizes (Veneklaas et al., 2012). Cela permet une

meilleure exploration du sol et la libération de formes de P peu disponibles. La deuxième stratégie consiste à maximiser l'efficacité d'utilisation interne du P absorbé (P Utilization Efficiency), et reflète la capacité de la plante à se développer, croître et produire du rendement (biomasse ou grain) en fonction de la quantité de P absorbée (Rose and Wissuwa, 2012). Ainsi, la compréhension des mécanismes impliqués dans l'absorption, le transport et l'allocation du P chez les plantes est alors indispensable pour sélectionner des variétés plus efficaces en P (Rose and Wissuwa, 2012).

Étant donné que la faible biodisponibilité en phosphore dans les sols cultivés affecte fortement la croissance des jeunes plantes, de nombreux programmes de sélection génétique se sont focalisés sur l'amélioration de l'efficacité d'acquisition du P par les plantes pendant la phase végétative et les processus rhizosphériques sous-jacents (Hinsinger, 2001; White and Hammond, 2008b). Cela a permis une meilleure compréhension de la variabilité génotypique pour l'acquisition de P chez plusieurs espèces végétales (Horst et al., 1993; Manske et al., 2002; Osborne and Rengel, 2002b; Ozturk et al., 2005). Néanmoins on dispose de peu d'informations sur l'efficacité d'utilisation interne de P par les plantes et plus particulièrement sur les processus contrôlant l'accumulation du P dans les grains pendant la phase de remplissage (Heuer et al., 2017; Wang et al., 2016). En effet, l'exportation du P par les cultures est l'un des éléments majeurs dans le cycle du P à l'échelle des agrosystèmes (Cong et al., 2020; Wang et al., 2016). On estime que la quantité de P exportée chaque année par les cultures céréalières correspond à 85 % des engrais phosphatés appliqués aux cultures (Lott et al., 2009). À l'échelle de la plante, environ 60 à 85 % du P prélevé par la plante est alloué aux grains à maturité (Batten, 1992; Raboy, 2009; Rose et al., 2013). L'une des stratégies permettant la réduction de la dépendance envers les engrais phosphatés consiste à diminuer la quantité de P exportée avec les récoltes (Rose et al., 2013; Wang et al., 2016). En d'autres termes elle consiste à améliorer l'efficacité d'utilisation du P par la plante.

Chez les plantes à sénescence monocarpique comme le blé, le phosphore absorbé pendant la phase végétative de croissance est remobilisé, tout comme les assimilats carbonés, l'azote et les autres éléments vers les grains pendant la phase reproductrice (Maillard et al., 2015; Veneklaas et al., 2012). La dynamique temporelle de l'accumulation du carbone et du P a une grande influence sur le rendement et la qualité des grains. Les besoins en P de la plante au cours de la période post-floraison sont alors dominés par deux processus concurrents : les besoins en P des tissus végétatifs pour continuer le fonctionnement cellulaire normal (e.g. photosynthèse, métabolisme de base) et la demande en P des grains en développement. Les données de la littérature ont montré que les concentrations en P

des grains sont supérieures à celles requises pour le fonctionnement normal des cellules et qu'une diminution de celle-ci n'affecte pas la germination des semences (Nadeem et al., 2011; Pariasca-Tanaka et al., 2015; White and Veneklaas, 2012). Ces données proviennent majoritairement des études effectuées chez le riz, le maïs et l'orge. La plupart des essais visant à réduire la concentration de P dans les grains a porté sur la réduction des phytates dans les grains (Guttieri et al., 2004; Raboy, 2001). Le phytate représente généralement de 65 à 85% du P total des grains. C'est la forme de stockage majoritaire du P dans les grains. Son rôle principal est d'alimenter les plantules en P pendant les phases précoces de développement en libérant du P après son hydrolyse sous l'action des phytases (White and Veneklaas, 2012). Du point de vue de la nutrition humaine, des teneurs élevées en phytates sont considérées comme un trait indésirable en raison de la capacité des phytates à chélater les micronutriments tels que le zinc, le fer et le calcium (Gupta et al., 2015; Raboy, 2007; Sparvoli and Cominelli, 2015). Complexés sous cette forme, ces micronutriments sont indisponibles pour les humains et les animaux monogastriques, qui n'ont pas ou peu d'activité phytasique dans leurs appareils digestifs. Une fois dans l'environnement, l'excès du P dans les eaux de surface peut déclencher l'eutrophisation des écosystèmes aquatiques (Brinch-Pedersen et al., 2002; Vance et al., 2003). Par conséquent, la réduction de l'accumulation de P dans les grains réduirait non seulement la quantité de P exportée du champ, mais aussi limiterait les risques environnementaux (Rose and Wisuwa, 2012). La réduction de l'accumulation du P dans les grains requiert une meilleure compréhension des processus impliqués dans l'absorption du P et de sa remobilisation pendant la phase du développement des grains. Ce travail est nettement plus avancé chez le riz que chez les autres céréales. L'accumulation du P dans les graines dépend de deux sources : l'absorption post-floraison du P exogène par les racines et la remobilisation du P endogène à partir des tissus végétatifs. Cependant la contribution relative des deux sources de P au remplissage des grains et les effets de la disponibilité en P sont mal connus. Déterminer l'importance respective des deux sources pourrait aider à prioriser les pistes d'amélioration de l'efficacité d'utilisation du P. Ce verrou de connaissance limite les possibilités de simuler correctement sur la durée du cycle de la culture le prélèvement et la remobilisation du P et ses conséquences sur le rendement et l'exportation du P (Figure 0.1).

Dans ce cadre, l'objectif général de cette thèse est d'identifier les processus impliqués dans le transport et l'allocation du P aux grains chez le blé dur (*Triticum durum* L.). Pour répondre à cet objectif, nous avons utilisé une approche écophysiological basée sur l'étude des flux de P à l'échelle de la plante entière. Dans un premier temps, nous nous sommes intéressés à l'effet d'une privation de P en période post-floraison sur la remobilisation et

les teneurs en P des grains chez deux cultivars de blé dur contrastés dans leur capacités d'accumulation de P. Dans un second temps, la dynamique temporelle de l'accumulation du P dans les grains a été caractérisée finement grâce à l'utilisation de traçage isotopique avec du ^{32}P . Les objectifs spécifiques de ce travail de thèse ont consisté à :

- Évaluer les effets de deux niveaux de P appliqués au stade post-floraison sur la remobilisation et les teneurs en P des grains
- Déterminer la contribution de la remobilisation et de l'absorption post-floraison au P accumulé dans les grains
- Identifier et quantifier les flux de P vers les grains pendant la phase de développement des grains et analyser les effets de la disponibilité en P sur ces flux.

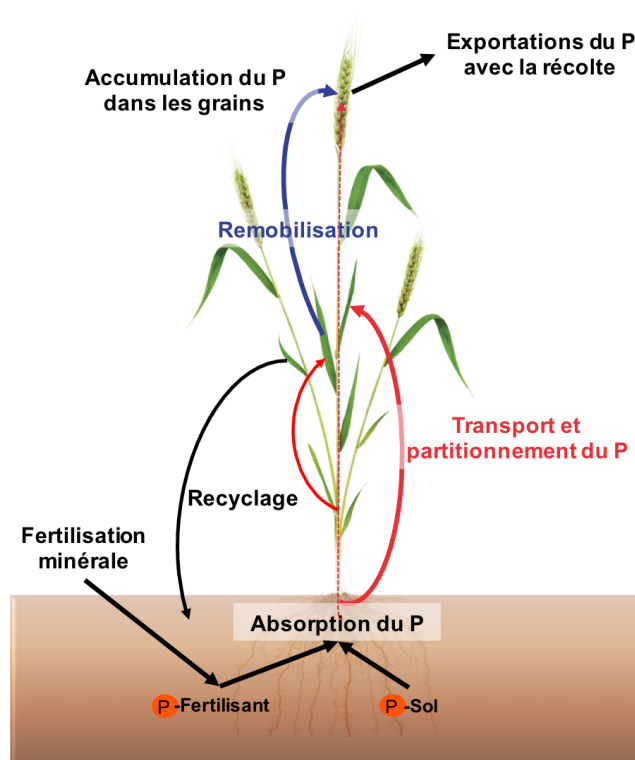


FIGURE 0.1 – Aperçu simplifiée de la dynamique de P à l'échelle de la plante entière

Organisation générale du manuscrit

Ce manuscrit est composé de six chapitres résumant les différents axes de travail menés au cours de cette thèse. Les trois chapitres résultats sont présentés sous forme d'articles scientifiques en anglais publiés ou en préparation au cours de la thèse (2 articles publiés et 1 en préparation). Ils peuvent être lus indépendamment et par conséquent quelques redites sont inévitables. Pour faciliter la lecture, un résumé en français reprenant le contexte et les résultats majeurs est ajouté au début de chaque chapitre résultats. La dernière partie

est consacrée à une discussion générale et aux conclusions. Les références bibliographiques propres aux articles sont intégrées à celles du manuscrit.

Le chapitre 1 est une synthèse bibliographique résumant l'état des connaissances actuelles sur les mécanismes impliqués dans le transport et l'allocation du phosphore dans les grains avec un focus sur les céréales. Il présente également quelques rappels concernant la biologie et le cycle de développement du blé et sur le cycle du P dans les agrosystèmes. Les effets de la disponibilité en P sur la physiologie de la plante, les composantes du rendement et les teneurs en P des grains sont également détaillés dans ce chapitre. Le chapitre 2 récapitule l'ensemble du matériel et méthode utilisés au cours des trois expérimentations pour répondre aux objectifs de recherche. Dans ce chapitre, le matériel végétal, les conditions de culture ainsi que les techniques analytiques sont décrits. Le chapitre 3 présente les résultats expérimentaux sur l'effet de la disponibilité en P pendant la période post-floraison sur l'accumulation et le partitionnement de P chez deux cultivars de blé dur ayant des ratio de biomasse aérienne/ biomasse racinaire contrastés. Dans le 4^{ème} chapitre, le traceur isotopique ^{32}P a été employé sur toute la période post-floraison pour distinguer et estimer la contribution de l'absorption post-floraison et de la remobilisation au remplissage des grains en P. Le chapitre 5 est présenté sous la forme d'un article en cours de préparation. Dans ce chapitre, la dynamique du P récemment absorbé après la floraison et son devenir dans la plante pendant la phase de développement des grains a été étudié à l'aide de deux marquages ponctuels de 24h en utilisant le ^{32}P comme traceur. Enfin, ce travail de thèse se termine sur une discussion générale des différents chapitres comprenant une synthèse des résultats et leur implication dans un contexte plus large suivie d'une mise en perspective des travaux réalisés.

Chapter 1

Review of the literature

The first part of the literature review summarizes the different agronomic aspects of durum wheat cultivation and development. Later, this review will focus on plant phosphorus (P) nutrition, particularly in cereals, including the importance of P in cropping systems, the effects P deficiency on plant growth and grain yield, the role of P remobilization and post-anthesis P uptake, and their contribution to the accumulated P in the grain. An overview of what has been done to select cereal crops with increased P use efficiency (PUE) is also presented. This review will not be limited to wheat in order to explore the diversity of mechanisms implicated in plant responses to P limitations under different experimental conditions.

1.1 Durum wheat

1.1.1 Durum wheat biology and production

Wheat is an annual plant of the *Poaceae* family and the second most widely grown cereal in the world (734 million tonnes harvested in 2018/2019) after corn (1 100 million tonnes) (Statista, 2020). The genus *Triticum* includes wheat species with varying levels of ploidy such as diploid, tetraploid and hexaploid species (Shewry, 2009). Approximately 95% of the wheat grown worldwide is hexaploid bread wheat (*Triticum aestivum*) and the remaining 5% is durum wheat (*Triticum durum*) (Shewry, 2009). Durum wheat is distinguished from common wheat by the hardness of its grains (hence the name *blé dur* in French) and its high protein content (13 to 15%, FranceAgriMer, 2020). It is a plant of major economic importance in certain regions such as southern Europe, northern Africa and northern America, where it is mainly cultivated. In those regions, durum wheat provides a good alternative to other types of wheat because of its adaptability to dry and hot climate (Grant et al., 2012b). Among the European Union (EU) countries, France is the second-largest producer behind Italy with a production of ~2 million tonnes

in 2019 (Eurostat 2019; Xynias et al., 2020). The average yield of durum wheat in France varies between 4 to 7 tonnes/ha depending on the variety and the season (FranceAgriMer, 2020). The end use of durum wheat grain is to make food products such as pasta and semolina (couscous and bulghur). Despite its lower annual production, durum wheat is considered as a bridge between bread wheat and other wild relatives of wheat (Shewry, 2009). As a result, it is one of the cereal models used for genetic improvement research, because the knowledge acquired with durum wheat can easily be applied to other cereal species (Grant et al., 2012b; Vergauwen and De Smet, 2017). Currently, breeding efforts are directed to narrow the gap between potential and current yields, increase grain quality (e.g. protein content), increase water and fertilizer use efficiency and disease resistance (Grant et al., 2012a; Xynias et al., 2020).

Most cultivated durum wheats are spring cultivars and therefore, they have limited vernalization requirements (Royo et al., 2020). They can be sown in spring as well as in autumn. Balanced nutrient management is an important factor to achieve durum wheat production with good agronomic characteristics such as grain yield and protein content (Grant et al., 2012b). Due to its high protein concentration in the grain, durum wheat requires large fertilizer input. Nitrogen application at a rate of 120 to 150 kg ha⁻¹ is a common practice for durum wheat to reach a protein concentration higher than 12% (Grant et al., 2012b). Durum wheat has an intermediate requirement for phosphorus fertilization (COMIFER, 2019). According to the recommendations proposed by COMIFER (2019), P fertilization must take into account soil P test, the requirement of the crop, fertilization history and the management of residues from the previous crop. Then, P fertilizer rate can be determined with a pre-defined scoring grid for each crop based on soil P test and P export by the crop (grain yield x P content). The objective is to maintain the soil at a P level that does not limit crop yield. For example, the amount of P removed by annual cereal crop can be up to 20–35 kg P ha⁻¹ (White and Hammond, 2008a). To balance the P offtake by the crop, a similar rate of mineral P fertilizers needs to be applied to maintain the level of available P required to optimize future crop production. A comparison of the amount of P required to produce 1 ton of yield is shown in Table 1.1. Wheat requires between 2 to 5 kg P per ton of grain yield. Recommendations based on soil P analyses are covered in sec 1.2.3.

1.1.2 Durum wheat life cycle

After germination, the durum wheat development cycle can be divided into two phases. The first one, the vegetative phase, extends from germination to the heading stage (Sreenivasulu and Schnurbusch, 2012). This phase is characterised by the production

Table 1.1 – Comparison of P uptake and P uptake per unit of yield by maize, rice, wheat and soybeans (From Rao et al., 1999)

Crop	Plant part	Yield (kg ha ⁻¹)	Total P uptake (kg P ha ⁻¹)	P uptake per unit yield (kg P t ⁻¹ yield)
Maize	Grain	9416	26	4.7
	Total	19496	44	
Rice	Grain	5380	10	3
	Total	10990	16	
Wheat	Grain	2690	12	2 - 5.6
	Total	6050	15	
Soybeans	Grain	3000	22	8.3
	Total	6700	25	

of stems (tillers), leaves and roots. The canopy of the plant grows due to the initiation of new leaves (Shewry, 2009). The carbohydrates accumulated during this period are stored in stems for later use. The second one, the reproductive phase, consists of several stages that begins with the the spike initiation (Sreenivasulu and Schnurbusch, 2012). Spike growth leads to heading followed by anthesis which is recognised by appearance of pollen from florets within the spike. Shortly after anthesis, the formed grains will gradually start to fill up with carbohydrates and nutrients (Shewry et al., 2012). The grain filling is a critical period for final grain weight which can be affected by many environmental stresses. The duration of each phase depends essentially on temperature, genotype and environmental conditions (Shewry et al., 2012). They are often standardised using the Zadoks decimal scale which is a common reference to describe the crop development and key phases (Zadoks et al., 1974). They are based on the observation of individual plants (Figure 1.1). Like other plant developmental processes, wheat and grain development can be estimated by calculating the growing degree-days (thermal time) after sowing, which is the sum of daily temperatures accumulated by the wheat plants (Bonhomme, 2000). It can be also calculated as the number of days after sowing.

In wheat, major yield components are spikes per surface area (or spike per plants in pot studies), grain number per spike and grain weight (or thousand-grain weight). Each of these components is determined successively during the plant life cycle as shown in Figure 1.1 (Sreenivasulu and Schnurbusch, 2012). The impact of P limitation on grain yield components is covered in the section 1.3.3.

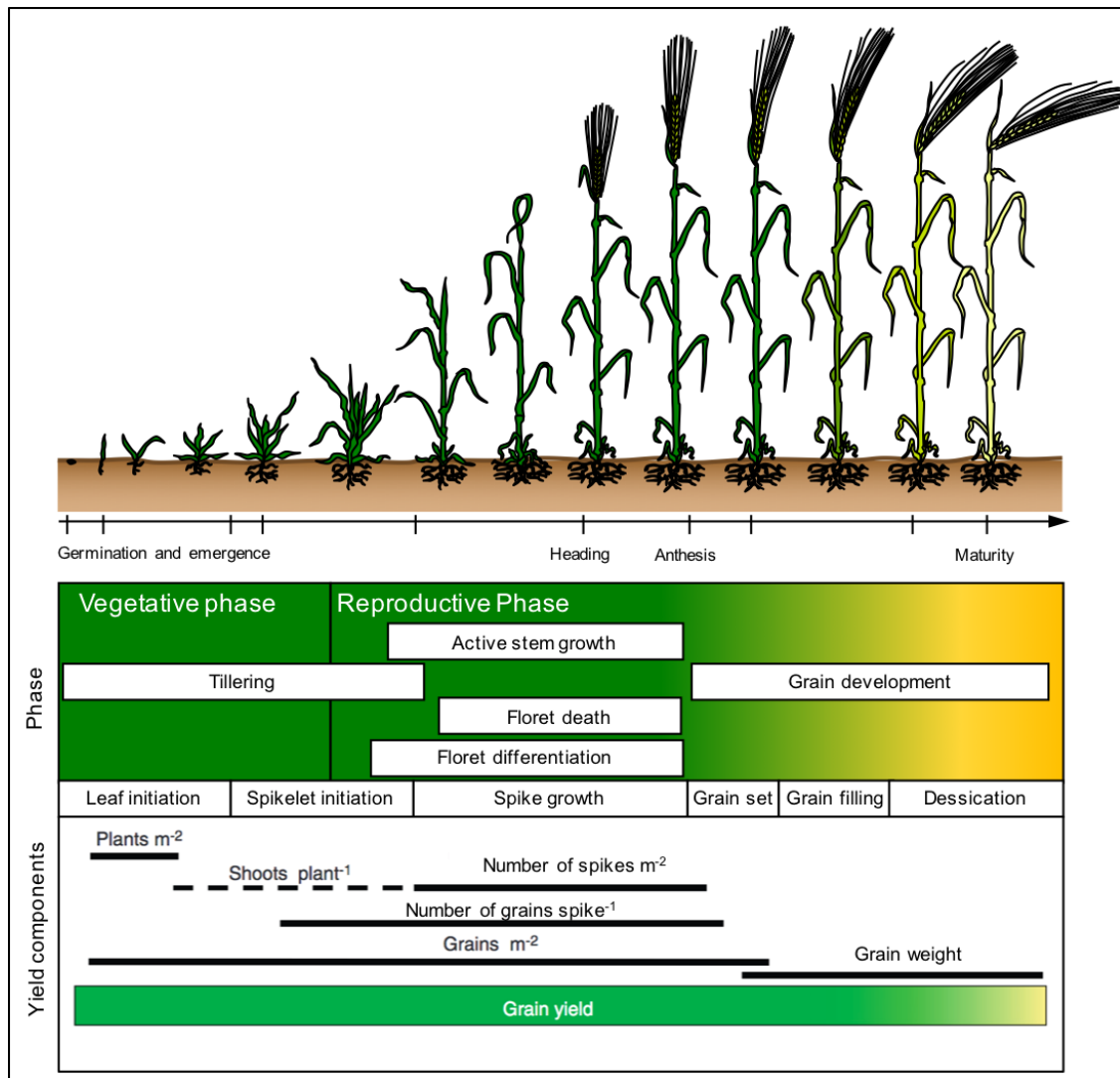


Figure 1.1 – Schematic illustration of different phases of cereal development and yield components establishment (adapted from Sreenivasulu and Schnurbusch, 2012)

1.1.3 Wheat grain development and grain characteristics

The development of the wheat grain starts after anthesis and can be divided into three main stages: cell division and expansion, grain filling, and grain maturation (Sabelli and Larkins, 2009). In the first stage, endosperm undergo a succession of cell divisions leading to grain enlargement. This stage is characterised by a rapid accumulation of water into grains and it may extend to 14 days after anthesis (Shewry et al., 2012). In the second stage, the grain growth continues through a period of almost linear increase in grain dry weight as a result of the accumulation of carbon (mainly in the form of starch), protein and nutrient in the grain (Sabelli and Larkins, 2009). This period usually lasts for 14 to 28 days, depending on the genotype and environmental conditions (e.g. temperature, water availability). The first two stages are crucial for grain yield because final grain weight will depend on the number of established endosperm cells and the rate and the duration of assimilate accumulation (Sabelli and Larkins, 2009). After grain filling is complete, which occurs at physiological maturity, maximum grain dry weight is achieved and hence final grain yield. The development of the wheat grain can be monitored by measuring grain water content and changes in fresh and dry weight (Figure 1.2). Grain water content at physiological maturity for wheat varies from 37% to 45% (Calderini et al., 2000; Ferreira et al., 2012).

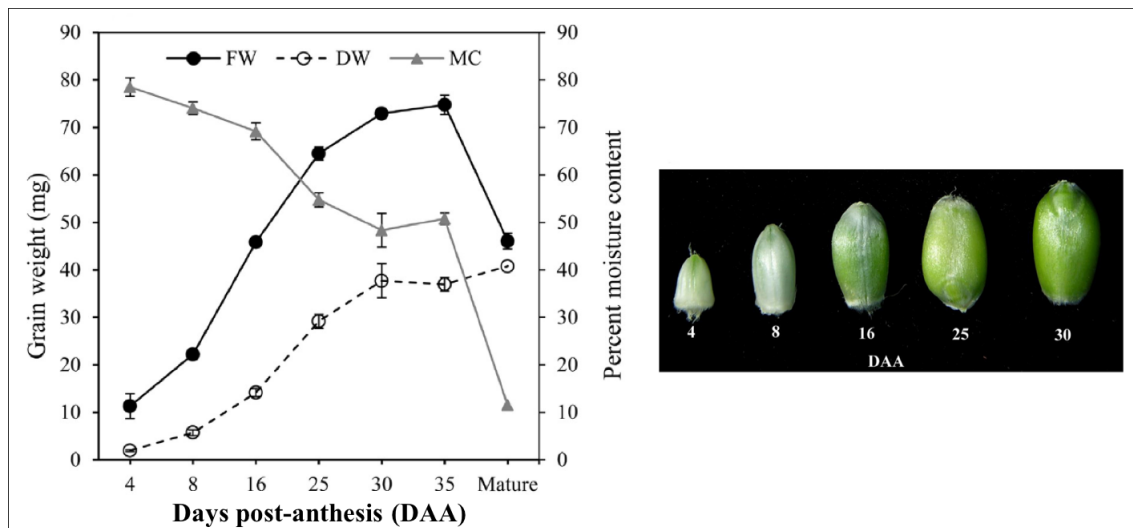


Figure 1.2 – Changes in grain fresh weight (FW), dry weight (DW) and percent water content during wheat grain development (Adpated from Mukherjee et al. 2015)

The three major tissues in mature wheat grain (also known as caryopsis) are the bran, the starchy endosperm, and the germ (Figure 1.3). These different parts have distinct roles, and therefore, their composition and structure are different. The bran is formed of many layers such as the pericarp, testa (seed coat) and hyaline layer (Brouns et al., 2012).

Those peripheric layers represent an important barrier to protect the endosperm and germ against moisture or damage during germination (Evers and Millar, 2002). The aleurone layer, the outer part of the starchy endosperm, consists of a single layer of living cells (Brouns et al., 2012). It is rich in mineral, vitamins and protein (~15% of the total wheat protein) (Brouns et al., 2012). The starchy endosperm is a storage tissue and the major part of the grain representing 80 to 85% of the wheat grain (Brouns et al., 2012; Evers and Millar, 2002). It is mainly composed of starch and protein that provide energy for the plant during germination processes and early seedling growth. The germ is composed of the plant embryo and the scutellum. It represent ~3% of the wheat grain. Accumulation of phosphorus and its localisation in the grain is covered in section 1.5.

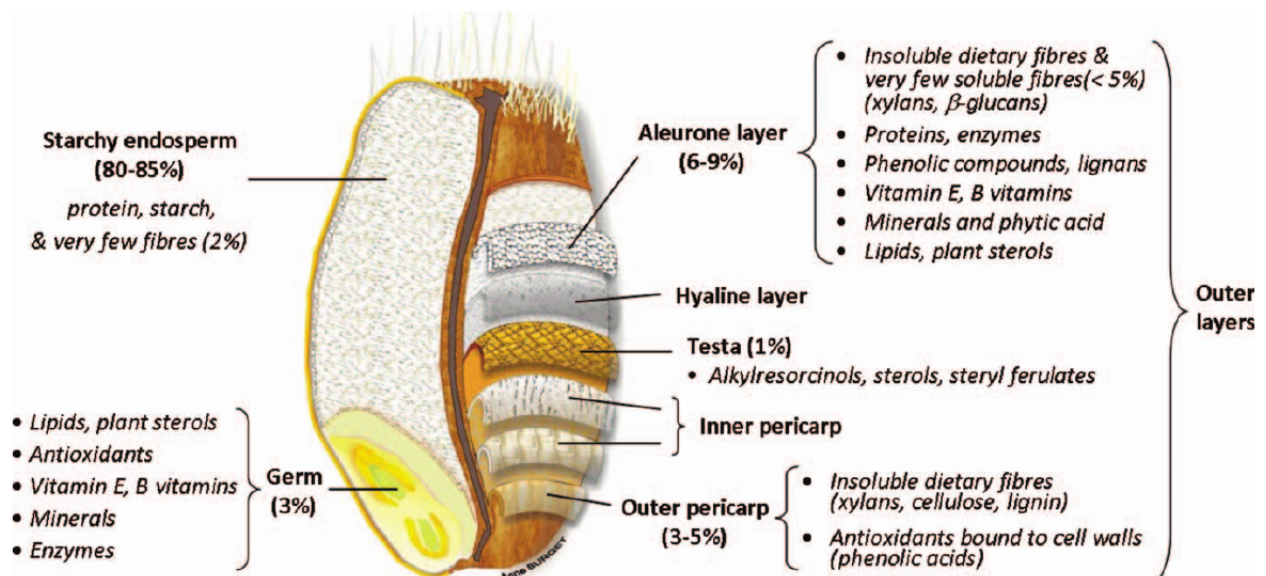


Figure 1.3 – Schematic structure of the wheat grain. The parentheses indicate the percentage of each tissue in proportion to the dry weight of the whole grain (From Brouns et al. 2012)

1.2 Phosphorus in the soil-plant continuum

1.2.1 The importance of phosphorus in the food production system

Phosphorus is at the hub of three major agronomic, economic and environmental issues in the food production systems (Vance et al., 2003). First, P is an essential element for life, including plant production. Since 1960, world cereal production has doubled while N and P fertilizer use increased by a 7 and 3.5 fold respectively (Tilman et al., 2002). Future projections expect P use to increase by 3 fold in 2050 (Tilman et al.,

2002). More than 30% of global croplands is experiencing P deficits, which means that P removed through harvest is not completely compensated with fertilizer (MacDonald et al., 2011). Second, P is a non-renewable resource on the human time scale and its global supply is likely to decrease in the future (Cordell et al., 2009). Mineral P fertilizers are extracted from a finite resource, the phosphate rock (Cordell et al., 2009). Most of the globally extracted mineral P are mined in Morocco, China and the United States (USGS, 2020). About 90% of mined P worldwide is used for food production (Cordell et al., 2009). Of this 90%, about 44% of global P fertilizer applications are for cereal production, with a large proportion being applied to wheat (14.6%), maize (13.9%) and rice (12.5%) (Heffer et al., 2017). The demand for P fertilizers is growing due to the global demand for food and animal feed (Godfray et al., 2010). Many countries are dependent on P fertilizer imports because P reserves are unequally distributed on earth (Cordell and White, 2014). This dependence raises questions about the long-term P supply for food security and it is already identified as critical raw material for the EU (100% of import reliance) (European-Commission, 2017). According to the estimation reported in Cordell and White (2014), peak P production is expected to occur between the 2030s and 2080s. This means that P fertilizer production from high quality and accessibility P reserves will reach a maximum before gradually decreasing (Cordell and White, 2014). Third, P is an element that triggers pollution problems in aquatic environment (Tiessen, 2008). The alteration of the P cycle by the over-use of P fertilizers and human activities (e.g. wastewater effluents) has led to an increase in P transfers to aquatic ecosystems (MacDonald et al., 2011; Tiessen, 2008). Excess movement of P to these ecosystems has led to the phenomenon of eutrophication defined as the excessive production of organic matter in water bodies over-enriched with nutrients. The eutrophication has negative consequences on water quality and ecosystem functioning (Tiessen, 2008).

1.2.2 Phosphorus cycle in agroecosystems

Figure 1.4 illustrates the phosphorus cycle in agroecosystems. Soil P originates from the alteration of P-rich minerals such as apatite (Shen et al., 2011). Unlike natural ecosystems, the P-cycle in agroecosystems is not closed. Although the total P content of soils may be large, only a small part of it is available for plant uptake. This causes the need to replace P exports or losses from the field by regular fertilizer applications (mineral fertilizers or manures) to maintain or increase soil P availability to fulfil plant requirements and ensure high-yield production (Schachtman et al., 1998). Plants absorb phosphorus that is dissolved in the soil solution (Pi). There are various reactions regulating the amount of Pi in soil solution. It include adsorption-desorption of phosphate on soil surfaces, diffusion, dissolution-precipitation, mineralization of organic substances and

immobilization (Hinsinger, 2001). The forms Pi in the soil is pH dependent. In cultivated soils where pH varies between 4 and 8.2, Pi forms include the negatively charged primary orthophosphate anion (H_2PO_4^-) and smaller amounts of secondary orthophosphate anion (HPO_4^{2-}). H_2PO_4^- is the predominating form in slightly acidic soils, while HPO_4^{2-} is the dominating form under alkaline pH.

Depending on the soil type and pH, more than 80% of applied P fertilizer becomes immobile and unavailable for plants (Schachtman et al., 1998). Different processes explain the low availability of P and the low efficiency of P fertilizers for plant nutrition (Schachtman et al., 1998). First, the physico-chemical component, P easily reacts with certain elements, such as iron (Fe), calcium (Ca) or aluminium (Al) to form insoluble Fe and Al-phosphates in acid soils and Ca-phosphates in alkaline soils (Shen et al., 2011). Second, rhizosphere microorganisms can modify the availability of P by varying the pH or through the mineralization of organic P in the rhizosphere (Rengel and Marschner, 2005). Organic P in the soil result from the microbial degradation of plant residues and organic amendments. It accounts for 15 to 80% of total P in the soil depending on soil age and use (Hiradate et al., 2007). Common organic P compounds in the soil are inositol phosphates (e.g. phytate), phospholipids, and nucleic acids (Hiradate et al., 2007). Another aspect to consider is the quality of the P fertilizer. Toxic element such cadmium enter the food chain through repeated application of P fertiliser (Cordell and White, 2014; Perrier et al., 2016). The regulation towards this aspect is getting harder which will cause an additional cost to get high-quality P fertilizer (European-Commission, 2017).

1.2.3 Fertilisation management and crop response to applied P

Next to nitrogen, phosphorus is the 2nd nutrient that strongly limits crop productivity in agroecosystems (Tilman et al., 2002). Optimum P nutrition is critical to reach the attainable yield potential of crops (Mueller et al., 2012; Vance et al., 2003). The recommendations for P fertilization inputs are based on a soil P test and its calibration against crop response curves (Jordan-Meille et al., 2012). The goal is to group soils into classes regarding the critical concentration of P in the soil that results in at least 90 % of the maximum achievable yield (Tang et al., 2009). Plant available P can be measured using different chemical extraction methods such as Olsen-P, P-Colwell and P-Bray methods. An example of a common response curves of wheat grain yield to Olsen-P in the soil is shown in Fig 1.5. The applicability of each method depends on the soil type. As a result, the soil-based method serves only as an approximation for the real amount of plant-available Pi. Zehetner et al. (2018) tested different soil P extraction methods in relation to plant P uptake. The authors found a weak correlation between the chemical

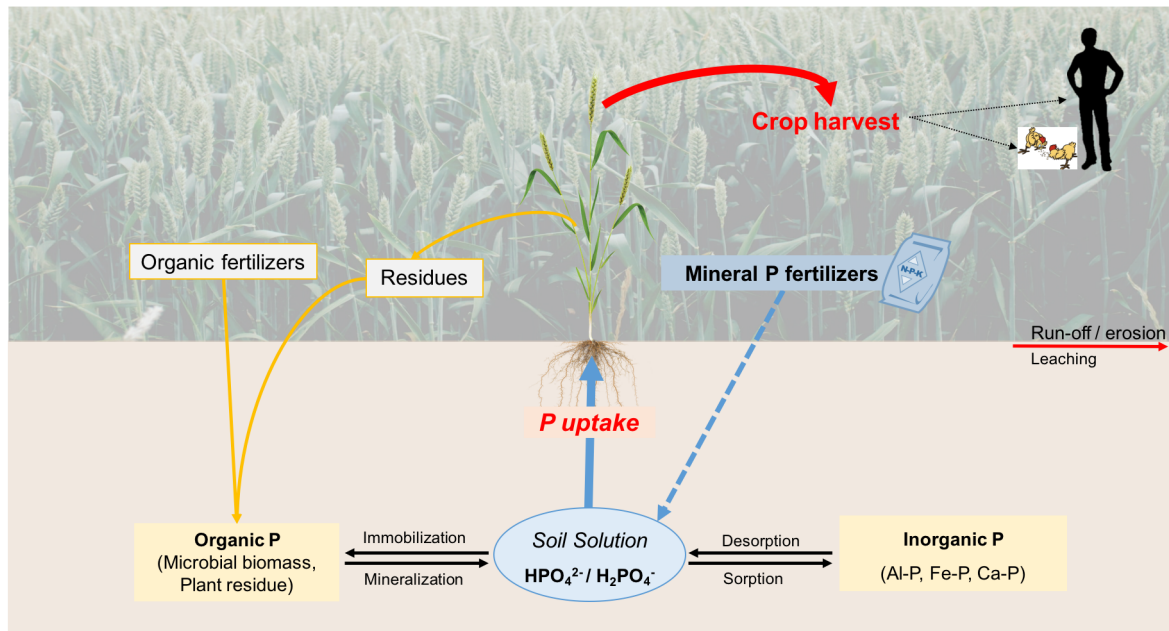


Figure 1.4 – Overview of the phosphorus cycle in cropping systems. There are three main pools of P in the soil: organic P, inorganic P and P in soil solution. Phosphate ions in the soil solution are the only source of P for plants uptake. Mineral P fertilizers are applied to compensate for the P exports in harvested products.

extractable P from soil and crop yield response. Plant-based method such P nutrition index, can give a better insight on crop P nutrition because it links effective P uptake to biomass accumulation (Cadot et al., 2018). The P nutrition index indicates whether or not the soil P supply is sufficient to cover the demand for P required for plant growth. However, this method is difficult to implement because the P nutrition index depends on the growth stage and the plant organ. It does not predict the need for P fertilisation for the next crop as it does not account for soil properties. There are other integrative approaches based on the measurement of phosphate concentration in the soil solution, the replenishment capacity by the solid phase and the P demand for growth using mechanistic modelling (Li et al., 2019; Mollier et al., 2008).

1.3 Roles of phosphorus in plant nutrition

Phosphorus is an essential plant nutrient required in diverse physiological and metabolic functions, particularly for energy transfer, nucleic acid and phospholipids membrane synthesis (Raghothama, 1999). In many of these functions, P cannot be substituted by any other elements (White and Hammond, 2008a). Its content varies between 0,2 to 1,5% of the plant dry weight (Broadley et al., 2004; Schachtman et al., 1998).

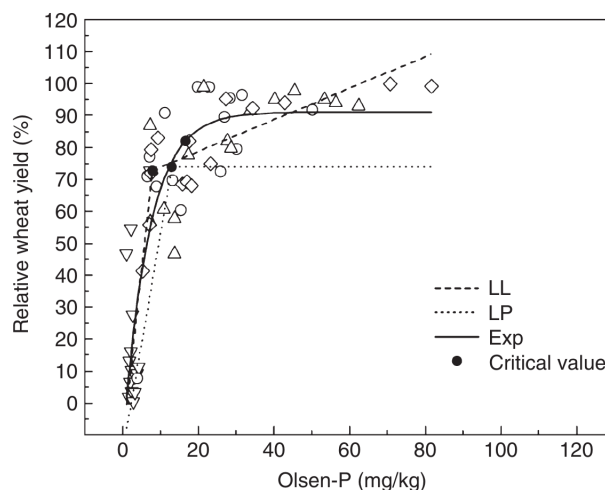


Figure 1.5 – Example of the relationship between Olsen-P and grain yield of wheat (From Jordan-Meille et al., 2012, adapted from Tang et al., 2009). In this example, threshold values corresponding to at least 90% of the relative yield are in the range 10-20 mg/kg for the Olsen-P method. LL: linear-linear model, LP: linear-plateau model, Exp: Mitscherlich model.

1.3.1 Phosphorus uptake and transport

Phosphorus is taken up by plant roots in the form of phosphate ions (referred to hereafter as Pi) (Schachtman et al., 1998). For most plant, maximal P uptake occurs between a pH range of 5 to 6, where the form H_2PO_4^- dominates. Phosphorus is considered to be the least available of all macronutrients for plant uptake and the concentration of available Pi in soil solution rarely exceeds $10\ \mu\text{M}$ (Schachtman et al., 1998). Therefore, plants need to absorb P against its electrochemical gradient, because the Pi concentration inside the roots may be 1000-fold higher (5–20 mM) than that of the soil solution (Raghothama, 1999). The uptake of P is mediated by plasma-membrane localized phosphate transporters (PHT). This energy-mediated process is necessary to overcome the electrochemical gradient between soil solution and root cells. The movement of Pi in the soil solution is prominently governed by diffusion (Hinsinger et al., 2011; Schachtman et al., 1998). Because of its low diffusion (10^{-12} to $10^{-15}\ \text{m}^2\ \text{s}^{-1}$), the replenishment rate of Pi in the soil solution is slower than root P uptake (Barber, 1995; Schachtman et al., 1998). As a result, the absorption of Pi leads to the creation of a depletion zone around the roots. The movement of Pi by mass flow (movement of water) in the soil is negligible. For example, mass flow only contributed to 5% of total Pi uptake in field-grown maize (Hinsinger, 2001).

Phosphate transporters (PHT) mediate the uptake and transport of Pi across cell membranes. To date, five families of Pi transporters have been characterized: PHT1, PHT2,

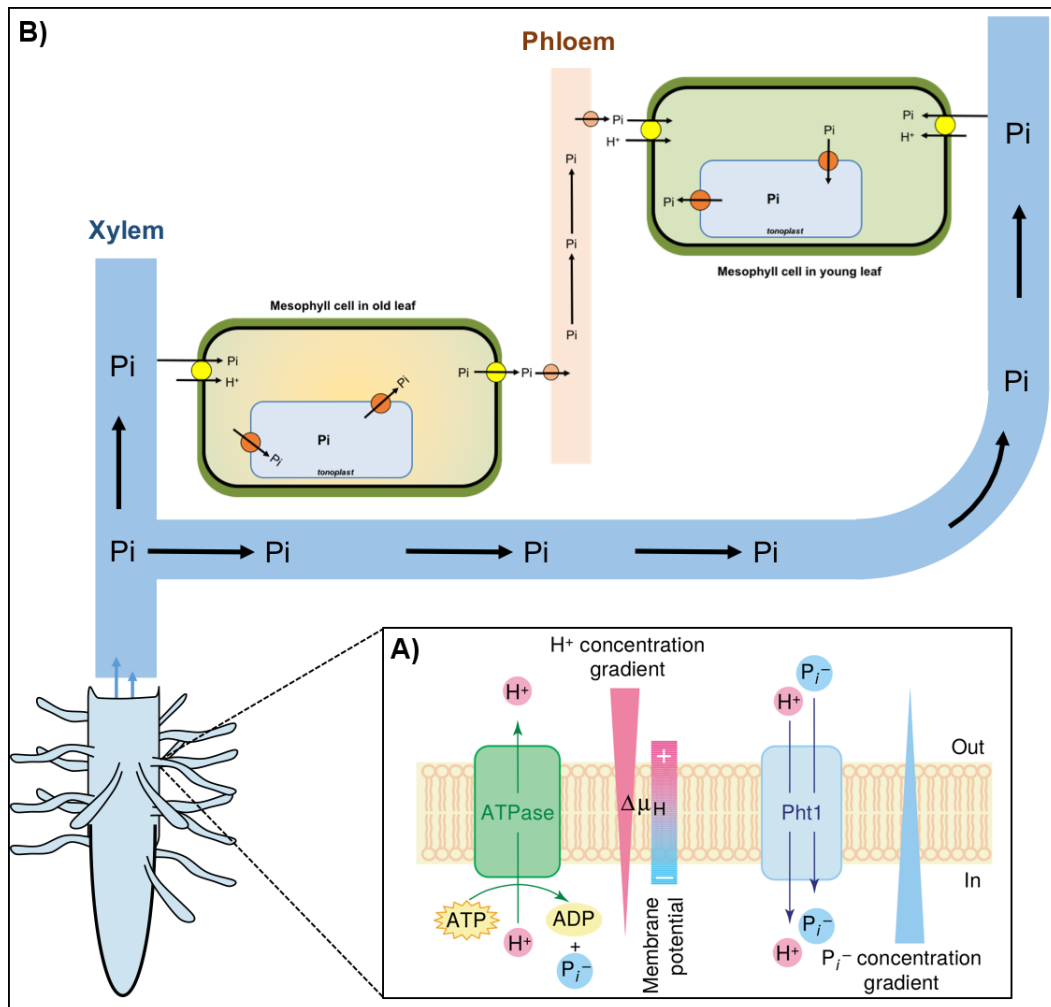


Figure 1.6 – Schematic representation of P uptake across the plant plasma membrane by the PHT1 transporter (A) and its translocation in the plant through xylem and phloem (B). The energy required for the active P uptake is obtained by an ATPase pump that forms a proton electrochemical gradient at the root-soil interface. The protons are transported in the same direction as P_i through the PHT1 transporter (Adapted from Karandashov and Bucher, 2005; Mimura, 1995)

PHT3, PHT4 and PHT5 (Kisko et al., 2018; Młodzińska and Zboińska, 2016). Most of these transporters have been identified and characterized in *Arabidopsis* and rice (*Oryza sativa* L.). They are classified into subgroups according to their sub-cellular localization and functional properties (Kisko et al., 2018). For example, the PHT1 family of transporters are expressed in the root epidermis, particularly in the root hair zone, meaning that PHT1 is associated with Pi uptake in the root–soil interface (Nussaume et al., 2011). As shown in Figure 1.6A, PHT1 operate as a proton-coupled H_2PO_4^- co-transporter. The characterization of the kinetic properties of P uptake systems revealed the presence of both high and low-affinity P transporters (Raghothama, 1999). High-affinity P transporters are operating when the external P supply is low (μM range), whereas the low-affinity transporters are sufficient when the P supply is high (Raghothama, 1999). It has been reported that high-affinity phosphate transporters such as PHT1;4 are strongly induced by low Pi availability (Liu et al., 2013; Misson et al., 2004). Moreover, several studies in *Arabidopsis* and barley have suggested the involvement of the PHT1 transporters in the translocation of P between plant organs, rather than only being involved in the uptake of P from the external soil solution (Mudge et al., 2002; Nussaume et al., 2011; Rae et al., 2003; Smith et al., 2003). In addition, it has been shown that Pht1;5 is required for the remobilisation of P from senescing leaves and its transport to reproductive tissues in mature *Arabidopsis* plants (Nagarajan et al., 2011). Under low P conditions, Pht1;5 have important roles for proper Pi loading into root xylem and its translocation from root to shoot (Nagarajan et al., 2011). Also, Rae et al. (2003) reported that PHT1;6 is involved in the remobilization of stored phosphate in old leaves and its transport in the phloem in barley.

The PHT2 family of Pi transporters are localized in chloroplasts, consistent with their role in P loading in shoot and leaf (Raghothama and Karthikeyan, 2005). The PHT3 is a small family of mitochondrial Pi transporters (Rausch and Bucher, 2002). Members of the PHT4 family transport P between cell compartments such as cytosol and chloroplast (Młodzińska and Zboińska, 2016). The vacuolar Pi transport is mediated by the PHT5 family. PHT5 transporters play a fundamental role in maintaining adequate levels of cellular Pi, indispensable for normal functioning. Using ^{31}P -magnetic resonance spectroscopy analysis, Liu et al. (2016) showed that PHT5 is localised in the tonoplast and demonstrated its role in the regulation of Pi sequestration into vacuoles (Figure 1.7).

Once imported to the root, P is subsequently loaded into the xylem for long-distance transport to different plant parts (Figure 1.6B) (Bielecki, 1973; Mimura et al., 1996). It is unloaded in priority into growing sinks such as apical meristem and leaves (Bielecki,

1973). The transport of P in the plant is facilitated by its mobility in the phloem and xylem (Bielecki, 1973; Marschner, 2011). The distribution of P between plant tissues is considered to occur predominantly in the phloem in the form of phosphate (Bielecki, 1973; Hill, 1980). Other studies have also shown the existence of a non-negligible amount of organic P such as hexose-phosphates in the phloem, but their role is not known (Rausch and Bucher, 2002). A fraction of stored P in older leaves can be remobilized and transported towards younger growing leaves (Raghothama, 1999). Young leaves are not only supplied with P from the roots, but also with P from the adult leaves that have completed their growth (Figure 1.6B).

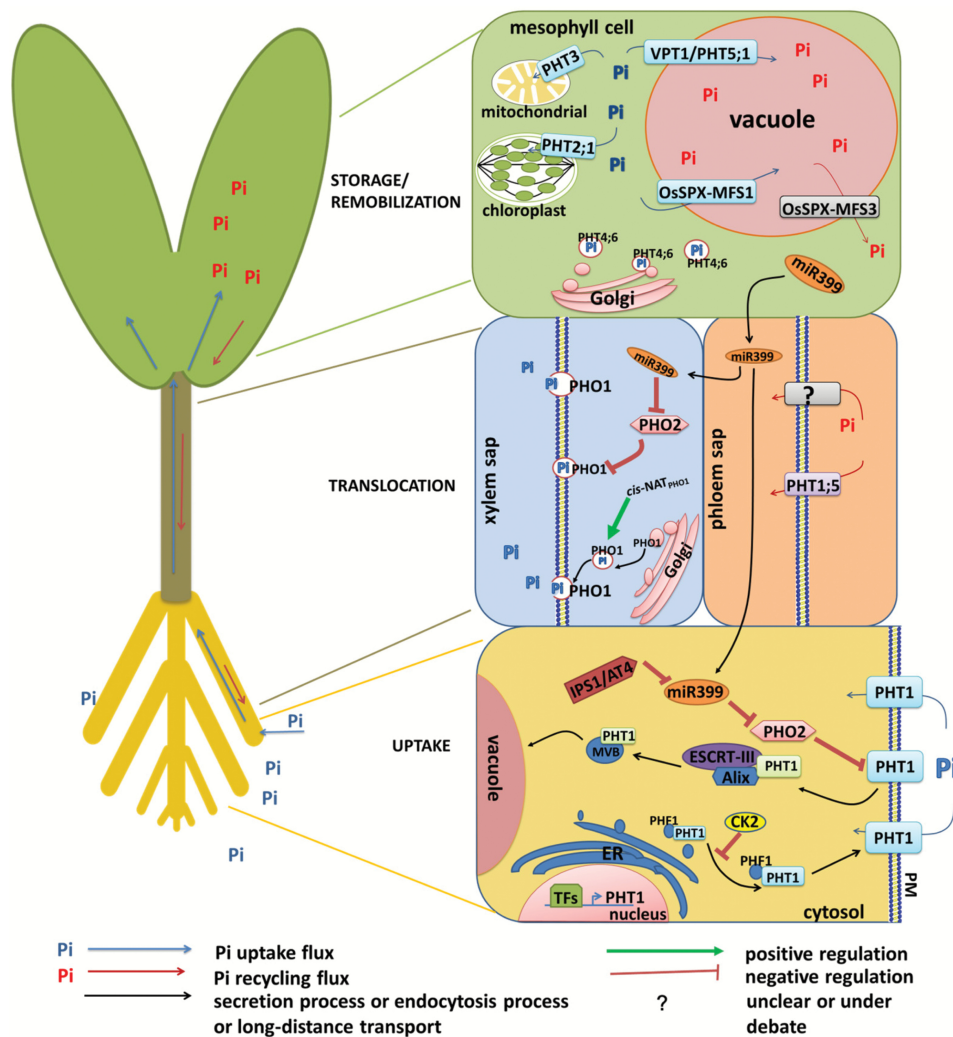


Figure 1.7 – Schematic representation of the cellular localization of main Pi transporters in plants (From Luan et al., 2016)

1.3.2 Phosphorus pools

Unlike N and other nutrients, the absorbed P is not reduced in plants but utilized or incorporated into metabolic processes in its oxidized form (Marschner, 2011). Orthophosphate and pyrophosphate are the principal forms of inorganic P in plant cells (Maathuis, 2009). The assimilation of P in plants can take several forms depending on the nature of the liaisons created. It can be bound to hydroxyl groups of sugars and alcohols by esterification (Maathuis, 2009). It is involved in the structure of nucleic acids, ATP and ADP, phospholipids, and many proteins and intermediate molecules. The main P-containing molecules found in plant are (Figure 1.8):

- **Nucleic acid:** P is a major structural element for nucleic acids (DNA & RNA) that carry the genetic information and its translation into proteins (Marschner, 2011). Nucleic acid-P is the largest organic P pool in the plant, representing up to 40–60% of total organic P with RNA (mostly ribosomal RNA, rRNA) containing 85% of this P (Veneklaas et al., 2012). The requirement for DNA and RNA differs among tissues, it is high in tissues with rapid cell division and/or cell expansion and low in mature and senescing tissues (Marschner, 2011). The reduction of rRNA contents due to P deficiency negatively impacts plant growth because rRNA content is positively correlated with protein synthesis capacity and growth rate (Raven, 2008; Veneklaas et al., 2012).
- **Phospholipids:** P is involved in the composition of phospholipids, which are essential constituents of cell membranes (Marschner, 2011). Phospholipids are required in abundance by growing tissues to meet the cell division and membranes synthesis demands (White and Hammond, 2008b). Phospholipids contain a significant part of cellular P and as such constitute a P reserve, which can be used by the plant when P supply is low (Veneklaas et al., 2012). The replacement of phospholipids by galactolipids and/or sulfolipids is a common response to P deficiency (Andersson et al., 2003; Smith et al., 2015). Although this modification may affect cell membrane characteristics (permeability), it allows the plant to switch to other metabolic pathways that consume less P to maintain photosynthesis and other vital plant functions (Hammond et al., 2004; Lambers et al., 2011; Veneklaas et al., 2012).
- **ATP & ADP:** P plays a vital role in energy transfer through its involvement in the structure of ATP, ADP and other intermediate molecules (Marschner, 2011). The released energy via the hydrolysis of ATP is essential for photosynthesis, respiration and all active metabolic processes (Marschner, 2011). Plants under P deficiency can use alternative pathways to reduce reliance on ATP/ADP by directing metabolic processes toward using pyrophosphate as an energy substrate (Hammond et al., 2004; White and Hammond, 2008b). These pathways are however less efficient in

terms of energy supply and lead to altered P metabolism and plant growth (Hammond et al., 2004). ATP and ADP are also required to modulate protein activity through reversible protein phosphorylation which is considered as a key player in signal transduction (Marschner, 2011).

- **Phytate:** Phytic acid (PA, *myo*-inositol hexaphosphate) is the major P storage compound in many plants. About 75-85% of total P in mature grains or seeds may be stored in the form of phytic acid or its salt form, phytate (Lott et al., 2000; Raboy, 1997). It is mostly located in the aleurone layer for wheat and barley or in the embryo for maize (O'Dell et al., 1972). With six negatively charged phosphate groups, phytate has the capacity to bind numerous cations such as iron (Fe), zinc (Zn), calcium (Ca) and magnesium (Mg) (Lott et al., 2000). The salt formed, cations-phytate, is insoluble and the complexed minerals are poorly bioavailable for humans and monogastric animals (Raboy, 2001). Phytate is considered a major factor that control the homeostasis of inorganic P during grain development (Iwai et al., 2012). It has also a vital role in supplying plant seedling with P and mineral nutrients during germination and early growth (Azeke et al., 2011; Nadeem et al., 2011; White and Veneklaas, 2012).
- **Inorganic P (Pi):** The vacuole is the main storage compartment for P in vegetative tissues (Bielecki, 1973). Generally, the Pi concentration in plant tissues reflects their level of P nutrition (White and Hammond, 2008b). Most P in plant tissues is in the form of Pi (Figure 1.8). In plant cells, Pi can be either metabolically active or stored to maintain the cellular Pi homeostasis (Veneklaas et al., 2012). Under adequate P supply, over 85% of the cellular Pi is stored in the vacuole. Variations in vacuolar Pi generally reflect variations in external P supply. When P supply is low, the imports of Pi from the vacuole to the cytoplasm is observed to keep cytoplasmic Pi concentration in the range 5–10 mM (Marschner, 2011; Schachtman et al., 1998), hence the low variation of P concentration in this compartment. In barley grown in hydroponic under high P concentration, the predominant form of P is found to be the inorganic P (79%) followed by nucleic acid P (13%) and lipid-P (4%) (Chapin and Bielecki, 1982). However, when grown under low P supply, nucleic acids comprised 42% of the total P and the remaining P was equally distributed among P fraction with inorganic P (21%), ester-P (23%), and lipid-P (15%) (Chapin and Bielecki, 1982).

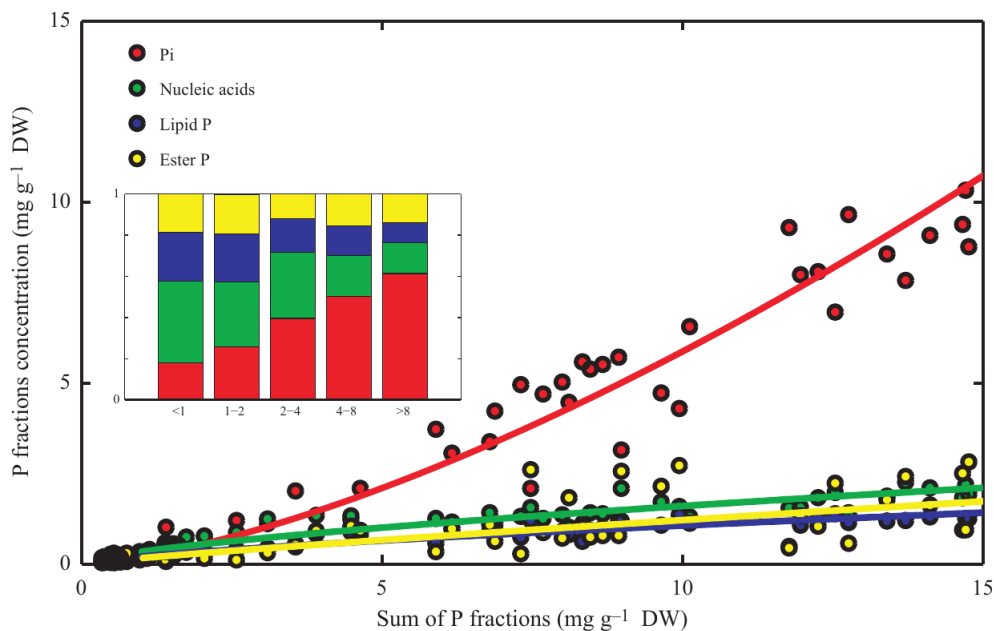


Figure 1.8 – Phosphorus pools found in plants grown at different P supply. The inset shows the relative proportions of different P pools with the increase of total P concentration (Reproduced from Veneklaas et al., 2012)

1.3.3 Effects of phosphorus deficiency on plant growth and grain yield

Phosphorus deficiency has wide ranging consequences on plant growth and development due to its irreplaceable role in plant structure and metabolism (Bielecki, 1973; Hammond et al., 2004). The deficiency occurs when the supply of Pi does not satisfy the plant's demand for normal functioning. The impact of P deficiency on the plant's growth and physiology depends on the level of P deficiency and the stage of development (Römer and Schilling, 1986). It also varies markedly between species and among genotypes (Heuer et al., 2017; White and Hammond, 2008b). Visually, it manifests by purple or brown coloration in older leaves which is the consequence of anthocyanin production (White and Hammond, 2008b). Phosphorus deficiency during the early growth stage generally has a drastic negative impact on crop productivity than P limitation during later stages (Grant et al., 2001). Rodríguez et al. (1998a) showed that P deficiency slows leaf expansion and elongation and therefore reduces the total dry weight of young wheat plants (Rodríguez et al., 1998a). Comparable results were obtained in maize (*Zea mays* L.) where the decrease in the growth of P deficient plants was related to a lower cell production (Assuero et al., 2004). In wheat, the time needed to reach anthesis is delayed by P deficiency (Rodríguez and Goudriaan, 1995; Rodríguez et al., 1998b). Many authors reported that P deficiency limits the yield of wheat by reducing the number of tillers as a result of

the low rate of tiller emergence (Elliott et al., 1997; Rodríguez et al., 1999; Su et al., 2009; Wang et al., 2010a). Lázaro et al. (2010) investigated the relationship between yield component and spike weight in wheat under phosphorus deficiency and found that changes in spike dry weight were mainly due to differences in spike growth rate and grain number. Similarly, the reduction in grain yield in barley (*Hordeum vulgare* L.) grown under severe P conditions was mainly due to a decrease in the number of tillers and the number of grains per spike (Grant et al., 2001; Hoppo et al., 1999). In soybeans (*Glycine max*), seed yield was decreased through a reduction in the number of seeds and pods per plant, but seed size remained stable (Crafts-Brandner, 1992b). This indicates that P deficiency reduced seed set, but did not negatively affect the growth of seeds that were set.

In many crop species, the allocation of more biomass to the root system at the expense of the shoot is an immediate response to P deficiency (Cakmak et al., 1994; Marschner, 2011). This preferential allocation of biomass towards the roots increases the root:shoot ratio in P deficient plants which is generally associated with higher capacity for P uptake (Cakmak et al., 1994; Shen et al., 2018). Higher root:shoot ratio can be the result of greater export of assimilates to the roots and efficient utilization of carbohydrates in the roots to increase P uptake (Cakmak et al., 1994). In addition, the short term effect of P deficiency can be manifested by a temporary increase in root growth (Mollier and Pellerin, 1999). The P status of the plant is an important factor to stimulate root growth. The comparison of two rice genotypes with different tolerance to P deficiency revealed that the more tolerant genotype preferentially distributed P to roots (Wissuwa et al., 2005).

The probability of P toxicity increases when tissue P concentrations exceed 10 mg g^{-1} . However, P toxicity in crops is rare because P homeostasis is tightly regulated by many processes such as the down-regulation of P transporters, the conversion of P_i into organic storage form (e.g. phytate) and P_i loss by efflux (Schachtman et al., 1998). Depending on the levels of P supply, P efflux can be between 8 and 70% of the influx (Cogliatti and María, 1990; Schachtman et al., 1998).

1.3.4 Plant responses to phosphorus limitations

Plants have developed several mechanisms to cope with the low availability of phosphorus in the soil and to optimize its internal use. Roots are the main entry point for P in the plant and as a result plants have developed several root traits to maximize its acquisition (Figure 1.9). Root architecture adaptation to P deficiency is shown to be decisive for P uptake under low P conditions (Lynch and Brown, 2001). These adaptations refer to the root spatial distribution and root disposition in P deficient plants (Lynch,

2011). Yuan et al. (2016) showed that wheat plants produce longer and denser root hairs under low P conditions. Egle et al. (1999) compared modern wheat genotypes with older genotypes and reported that improved P uptake was found in new wheat genotypes with higher root length density (root length per cm^3 of soil volume, cm cm^{-3}) and uptake rate per unit root. More fines roots, shallower basal roots, more dispersed lateral roots and greater root biomass with denser root hairs are among the most desirable trait for increasing P acquisition in crops (Gahoonia et al., 1997; Lynch, 2011; Richardson et al., 2011). Cereals often have a more pronounced root morphological responses to P deficiency than legumes, which tend to alter the root physiological processes to mobilize more P from the soil (Lyu et al., 2016).

Numerous root-induced chemical and biological changes in the rhizosphere contribute to increase the availability of P in the soil (Hinsinger, 2001; Shen et al., 2011). The exudation of organic acids, mainly malate and citrate (Pearse et al., 2006), as well as the acidification of the rhizosphere promote the dissolution and mobilization of P in the soil (Hinsinger, 2001). Exudation of high rates of organic acids in the rhizosphere is associated with a large capacity for P-mobilization in many plant species including barley, rice and rapeseed (Gahoonia et al., 2000; Kirk et al., 1999; Zhang et al., 1997). Shen et al. (2018) demonstrated that P-mobilizing exudation in wheat showed different behaviours with increasing P deficiency stress. In particular, the author pointed out that citrate but not malate exudation was enhanced by P deficiency (Shen et al., 2018).

The association with arbuscular mycorrhizal fungi allows the plant to explore greater soil volume, increase the root surface area and access P beyond the root depletion zone (Karandashov and Bucher, 2005). Schachtman et al. (1998) reported that roots colonized by mycorrhizal fungi can have a P influx 3 to 5 times greater than non-mycorrhizal roots. Inoculation of durum wheat with mycorrhizal fungus increased grain yield up to 5.4 times and shoot P to 4 times as compared to non-inoculated plants (Karagiannidis and Hadjisavva-Zinoviadi, 1998). In their study, Pandey et al. (2005) tested wheat, rye and triticale (a hybrid of wheat and rye) for growth and P uptake characteristics on low P soil. They found that rye had greater root colonization by mycorrhiza which resulted in higher root and shoot biomass and total P uptake, but had a lower PUE per unit of P absorbed. This is probably due to higher carbon costs associated with maintaining mycorrhiza symbiosis. The vast majority of studies shows that the association with mycorrhiza greatly improve plant P uptake and growth for many species including wheat (Manske et al., 2000), barley (Criado et al., 2015), rice and maize (Battini et al., 2017) under low P availability.

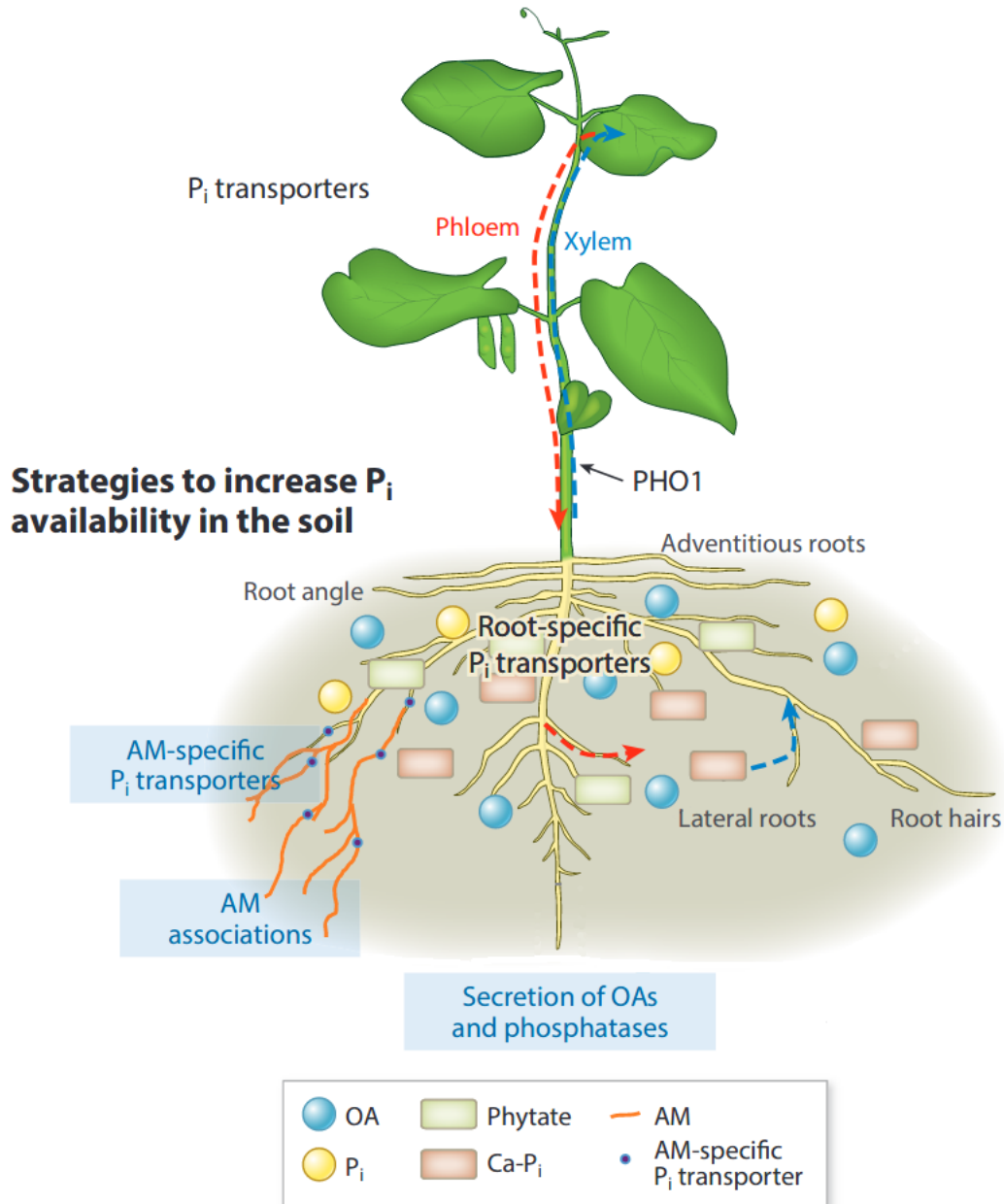


Figure 1.9 – Plant strategies to increase the availability of phosphate (P_i) in the soil (Modified from López-Arredondo et al., 2014). OA:organic acids, AM: arbuscular mycorrhizal.

The secretion of enzymes such as phosphatases and nucleases accelerate the release of P from organic sources (López-Arredondo et al., 2014). In their screening of spring semi-dwarf wheat from CIMMYT, Manske et al. (2000) showed that acid phosphatase released under P deficiency was positively correlated with P uptake at anthesis although this trait was not significantly correlated to grain yield at maturity.

In plant species such as *Fabaceae* and *Proteaceae*, the formation of specialized roots called "cluster roots" are commonly observed in severely P impoverished soils (Shane and Lambers, 2005). Cluster roots are composed of roots with dense lateral branch roots covered by large numbers of root hairs (Shane and Lambers, 2005). The ability of these specialized roots to exude large amounts of carboxylates and phosphatases, which solubilise P that is tightly bound to soil particles, allows these plants to grow in environments where other plant species cannot (Lambers et al., 2011; Shane and Lambers, 2005).

1.3.5 Interaction of P nutrition with other elements

Balanced nutrient supply is important to obtain a high yield with high quality parameters. The availability of P in the soil is affected by pH and by the presence of other minerals such as Fe, Al and Ca. As pointed in Section 1.2.2, P interacts with these elements to form insoluble compounds which decrease the availability of Pi to plants. The interaction of P with other nutrients *in planta* has been reported in a wide variety of crops because P plays a key role in many metabolic pathways in plants (Briat et al., 2015; Marschner, 2011). It was shown that high rate of P fertilizer reduces grain protein content in winter wheat, partially due to the dilution effect (Zhang et al., 2017). Several studies have reported a negative relationship between P and Zn accumulation, in which the deficiency of one results in over-accumulation of the other (Cakmak and Marschner, 1986). A close relationship between P and sulphur (S) exist in when plants are subjected to nutritional stresses (Briat et al., 2015). The metabolism of P may be less affected during P deficiency because phospholipids could be replaced by sulfolipids and vice versa (Briat et al., 2015; Siebers et al., 2015). Several relationships between P and micro-nutrients exists in grains (White and Veneklaas, 2012). Histological and imaging techniques such as X-Ray fluorescence imaging and laser ablation inductively coupled mass spectrometry have demonstrated the co-location Fe and Zn of in grain of wheat, rice and barley grain, suggesting that the storage form of P is a very efficient Fe and Zn chelator (Brier et al., 2016; Iwai et al., 2012; Wu et al., 2013).

1.4 Phosphorus use efficiency in annual crops

1.4.1 The concept of phosphorus use efficiency

Improving phosphorus use efficiency (PUE) is a major issue in agronomy and crop nutrition. This term can be defined in several ways depending on the objective and the context under consideration. PUE can be expressed in terms of economic yield or biomass produced per unit of P applied or taken up by the plant (Rose and Wissuwa, 2012). It might include the mechanisms involved in the uptake, transport, storage and utilisation of P for plant growth (Batten, 1992; Richardson et al., 2011). The most frequent definitions of PUE in the literature are given in Table 1.2. The comparison of the definition used to assess PUE as reported in Table 1.2 shows the extent to which indications of PUE can be misleading because they are not consistent across the literature. This multitude of terms fragmented our understanding of PUE and slowed down the breeding of crops with enhanced PUE (Bovill et al., 2013; Rose and Wissuwa, 2012). PUE is generally separated into two components:

1. **Phosphorus acquisition efficiency (PAE):** PAE is associated with the plant capacity to acquire P from the soil. It includes all the root architectural and physiological adaptations that support a better exploration of the soil or the release of soil-bound P due to its low mobility in the rhizosphere (Richardson et al., 2011; Wang et al., 2010b). Accordingly, root length density, root exudation of organic acid or phosphatase, colonization by mycorrhizal fungi are important determinants to enhance PAE in crop plants (c.f. section 1.3.4). In a study conducted on 42 wheat genotypes to investigate genotypic variation in P use efficiency, Manske et al. (2000) found that PAE provided more gain in term of grain yield than the efficiency of P utilization. The most valuable trait for improved P acquisition was the root density in the first 20 cm of soil. However, this conclusion is context dependent because another study by the same authors reported that P utilization was positively correlated with grain yield especially under adequate P supply (Manske et al., 2001).
2. **Phosphorus utilization efficiency (PUtiE):** This component is associated with the capacity of the plant to produce maximum biomass or grain yield using the acquired P (Batten, 1992; Veneklaas et al., 2012). Several studies reported that high remobilization of P from senescing organs, remodelling membrane composition and low grain P are strongly linked with improved PUtiE (Aziz et al., 2014; Crafts-Brandner, 1992a; Lambers et al., 2015; Wang et al., 2016). For example, it has been shown that the activities of various enzymes such as acid phosphatase and RNase are increased during senescence and under P deficient conditions. These en-

zymes are responsible for P hydrolysis from nucleic acid and organic P-compounds to provide a greater supply of P to the new growing sinks (Jeong et al., 2017b; Veneklaas et al., 2012). Hence under P deficiency, plants are able to improve PUE by adjusting internal Pi recycling, limiting P consumption, and reallocating P from old tissues to young or actively growing tissues.

Table 1.2 – Some common terms used to assess phosphorus use efficiency (PUE) in the literature (Adapted from Bovill et al., 2013; Rose and Wissuwa, 2012)

Term	Definition	References
Agronomic PUE	Yield increase per unit P applied	Hammond et al. (2009)
P use efficiency (I)	Grain yield per nutrient supplied	Manske et al. (2001)
P use efficiency (II)	Shoot biomass per unit P uptake	Wissuwa et al. (1998)
P uptake efficiency (I)	Total above ground nutrient per unit P applied	Osborne and Rengel (2002a)
P uptake efficiency (II)	Total P accumulated per unit root weight or length	Liao et al. (2008)
P acquisition efficiency	Total P in the plant per unit P applied	Osborne and Rengel (2002a)
P utilisation efficiency	Grain yield per unit P uptake	Manske et al. (2002)
Biomass utilisation efficiency	Biomass yield per unit P uptake	Su et al. (2009)
P harvest index	Grain P concentration per total P uptake	Batten (1992)
P efficiency ratio	Grain yield per unit P uptake	Jones et al. (1989)
Photosynthetic P use efficiency	Leaf photosynthesis expressed per unit leaf P	Veneklaas et al. (2012)
Physiological P use efficiency	Tissue P concentration required for 90% maximum yield	White and Hammond (2008a)

PUE is a complex trait due to genotypic variation and the effects of P supply on crop physiological and morphological characteristics (Heuer et al., 2017; McDonald et al., 2015; Rose and Wissuwa, 2012). It can be increased via greater PAE, greater PUE or both. However, the two components do not have the same importance for enhancing crop P use efficiency (Lyu et al., 2016; Wang et al., 2010b). Phosphorus utilization seems to be more critical for enhancing P efficiency at adequate P supply while P acquisition is more critical when P supply is limited (Wang et al., 2010a). Until now, most breeding efforts have only focused on identifying genotypes with higher PAE to overcome yield loss in low-phosphorus soils encountered in many regions of the world where access to fertilizer is not always possible (Lynch, 2011; Rose and Wissuwa, 2012; Wang et al., 2016).

1.4.2 Strategies to improve phosphorus use efficiency

Phosphorus fertilizers are regularly applied to ensure high yields and to replace the P that has been removed with the harvested products. However, the fertilizer efficiency

use is generally low, because only 20-30% of annually applied fertilizer is used by crops (Cordell and White, 2014; Schachtman et al., 1998). The remainder of the applied P interacts with soil component and becomes unavailable for plant uptake (Shen et al., 2011). Agronomic practices, when possible, can contribute to raising the amount of available fertiliser. These practices include the use of more effective fertilizer strategies that are more site-specific adapted: appropriate source, rate, timing and placement of P fertilizers (Richardson et al., 2011). The use of slow-release fertilisers, localising and coating seed with P fertilizer are also useful strategies that increase P use efficiency by reducing P losses to the environment (Richardson et al., 2011; Syers et al., 2008). In the long-term perspective, recycling and recovering P from wastes can help reduce requirements for mined P (Cordell and White, 2014).

The selection of crops with enhanced P acquisition or P utilisation capacities seem to be a promising strategy for the long-term when P fertilizer will be scarce. In their recent review, Cong et al. (2020) proposed four strategies to exploit genetic variation in cereals to enhance the PUE (Figure 1.10). Crops genotypes with high capacity to use the P already present in the soil is a highly desirable trait because of the large quantities of P in the soil (strategy 1, Figure 1.10). Minimising crop P requirements for growth and yield production can bring efficiency gains in the utilization of P. Either by producing similar yields by acquiring less P from the soil or by exporting less P for a given level of grain yield (Veneklaas et al., 2012). This is possible through higher photosynthetic P use efficiency (PPUE), greater efficiency of P remobilization and low grain P (strategy 2,3 and 4, Figure 1.10).

Photosynthetic P use efficiency (PPUE) refers to the rate of photosynthesis per unit P present in leaves (Hidaka and Kitayama, 2013). The P concentration in well fertilized plants are generally high and fully satisfy photosynthetic P requirements (Veneklaas et al., 2012). Some plants are more efficient in using the acquired P than other. For example, plants from the *Proteaceae* family function at very low P concentrations through low investment of P in rRNA and the replacement of most of their phospholipids by galactolipids and sulfolipids (Lambers et al., 2015).

Remobilization of internal P is a important processes in enhancing P utilization. The remobilization of P from old organs provides a non-negligible source of P to growing sinks during P deficiency (Maillard et al., 2015). In P-deficient wheat, between 50 to 90% of grain P can be attributed to P remobilization (Batten et al., 1986; Maillard et al., 2015). This proportion diminished considerably to up 20% under sufficient P supply (Batten

et al., 1986).

Tightening P cycle through four strategies of P-efficient crop genotypes

- 4 Seed P (phytate) concentration**
 - Low phytate P concentration
 - Low P translocation to grain
- 3 P-remobilisation efficiency**
 - High transfer to active organs
 - Cell P pools (lower P-containing lipid)
- 2 Photosynthetic P-use efficiency**
 - Leaf cell types (lower epidermal P)
 - Cell P pools (higher metabolic P/lipid P)
- 1 P-acquisition efficiency**
 - Architecture and morphology (e.g., root hair)
 - Physiology (e.g., carboxylates)
 - Symbioses (e.g., AM fungi)
 - Plant- and microbe-derived enzymes

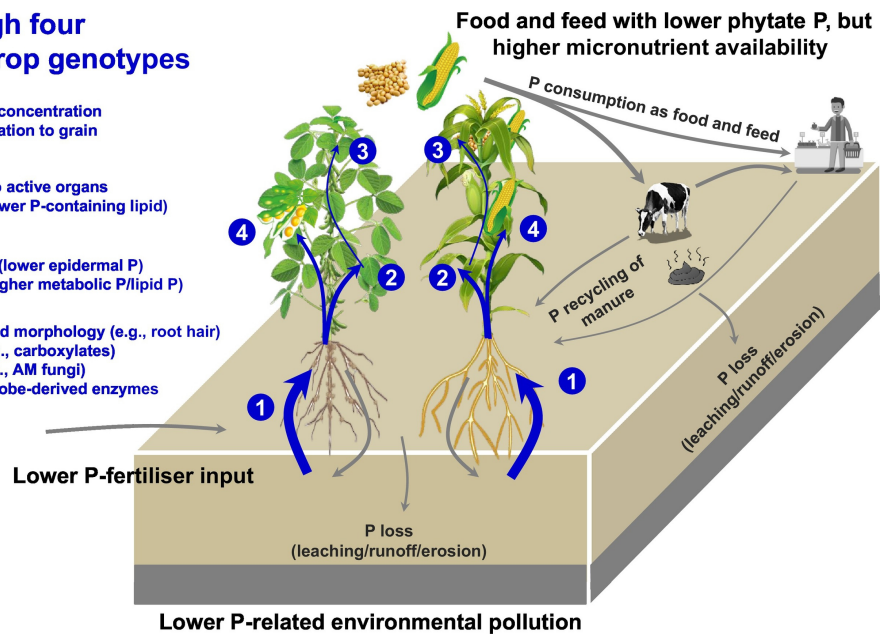


Figure 1.10 – Strategies to improve PUE in cropping systems (From Cong et al., 2020). The four proposed strategies contribute to obtain crops with enhanced P use through optimizing P acquisition, utilization and remobilization, and by reducing total P and phytate P concentrations in cereal grains.

Lowering grain P is an interesting trait since the P cycle in agroecosystems is driven by the amount of P exported at harvest (Rose et al., 2013; Wang et al., 2016). At maturity, 60-85% of aboveground P is found in grains in cereals crops (Batten, 1992; Wang et al., 2016). The selection of genotypes with a low concentration of P without yield penalties would remove less P from the soil and reduce the cost associated with P fertilizer inputs. It has been shown that breeding efforts that aimed at increasing wheat grain yield resulted in decreasing P concentration (dilution effect) (Calderini et al., 1995). This decrease in P concentration was also observed during the domestication of wheat, from wild relative to durum and bread wheat (Batten, 1986a; Veneklaas et al., 2012). Modern wheat cultivars generally have higher P harvest index which mean that more P is partitioned to the grain at maturity.

1.4.3 Low grain P and low-phytate crops

1.4.3.1 Grain P and its implication to improve PUE

Grain P concentrations in major cereals crop including wheat and rice can range from 3 to 5 mg g⁻¹ (White and Veneklaas, 2012). Lowering grain P concentrations has been

proposed as a potential mean to minimize P export in harvested grains and improve P efficiency in cereal crops (Rose et al., 2013). This will also reduce the concentration of the anti-nutritional factor phytic acid. Low grain P was initially considered undesirable because low grain P reserves may impact plant emergence and vigour after germination (White and Veneklaas, 2012). Indeed, very low grain P concentration may influence plant emergence especially in highly P-deficient soils and in the absence of P fertilization (e.g. natural ecosystems) (Pariasca-Tanaka et al., 2015). Early observations have also shown that high concentrations of P in grain enhances early seedling development and P uptake (Batten, 1992; White and Veneklaas, 2012; Zhu and Smith, 2001). However, those early observations are not fully conclusive because many low grain P concentrations were found in stressed plants and therefore the observed effect is likely to be due to other factor and not only to low P content (Bregitzer and Raboy, 2006; Julia et al., 2018; Rose et al., 2012; White and Veneklaas, 2012). Also, various comparisons between low and high P grain were mainly performed at the seedling stage without consideration of P fertilization strategies and genotypic variation (Rose et al., 2012; Vandamme et al., 2016a). Recent evidence in rice and maize have demonstrated that grain of cereal crops contain much more P than what they require for normal functioning (Nadeem et al., 2011; Vandamme et al., 2016b; Pariasca-Tanaka et al., 2015). Pariasca-Tanaka et al. (2015) and Vandamme et al. (2016b) have demonstrated that it is possible to obtain high rice yields with lower grain P concentrations in the range of 1–1.7 mg g⁻¹. Other strategies such as P fertiliser placement and seed P coatings can also be implemented to ensure the vigour of low grain P. The awareness of the finite nature of the P resource has given significant importance to reconsider developing crops with reduced grain P (Brinch-Pedersen et al., 2002; Rose et al., 2013). Therefore, a better understanding of the mechanism involved in the allocation of P to cereals is needed to achieve this goal and to improve P use efficiency in crops.

Recent research suggest that lowering rice grain P concentration by 20% is possible by exploiting the exiting genotypic variation within rice cultivars (Rose et al., 2010; Vandamme et al., 2016a,b). This may also be possible in wheat because considerable genotypic variation in grain P concentration and PUE was found amongst wheat genotypes (McDonald et al., 2015; Osborne and Rengel, 2002b; Ozturk et al., 2005). Furthermore, Yamaji et al. (2017) identified a new phosphorus transporter that controls the allocation of P to the grain in rice. This transporter, called sulphate transporter (SULTR)-like phosphorus distribution transporter (SPDT), is shown to be expressed at the rice nodes (Figure 1.11). Knock-out of this gene reduced the distribution of P to the grain without affecting parameters related to growth and grain yield (Yamaji et al., 2017). It does not affect the translocation of P from old leaves to new leaves but it does modify the parti-

tioning of taken P. The *spdt* mutants had a 20% reduction in grains P with increased P concentration in the straw (Yamaji et al., 2017). A reduction in phytic acid matched by an increase in the concentration of Fe and Zn was also reported (Yamaji et al., 2017). This example shows that the manipulation of P transporters can be a very effective strategy to lower P allocation to grains without negatively impacting agronomic performance.

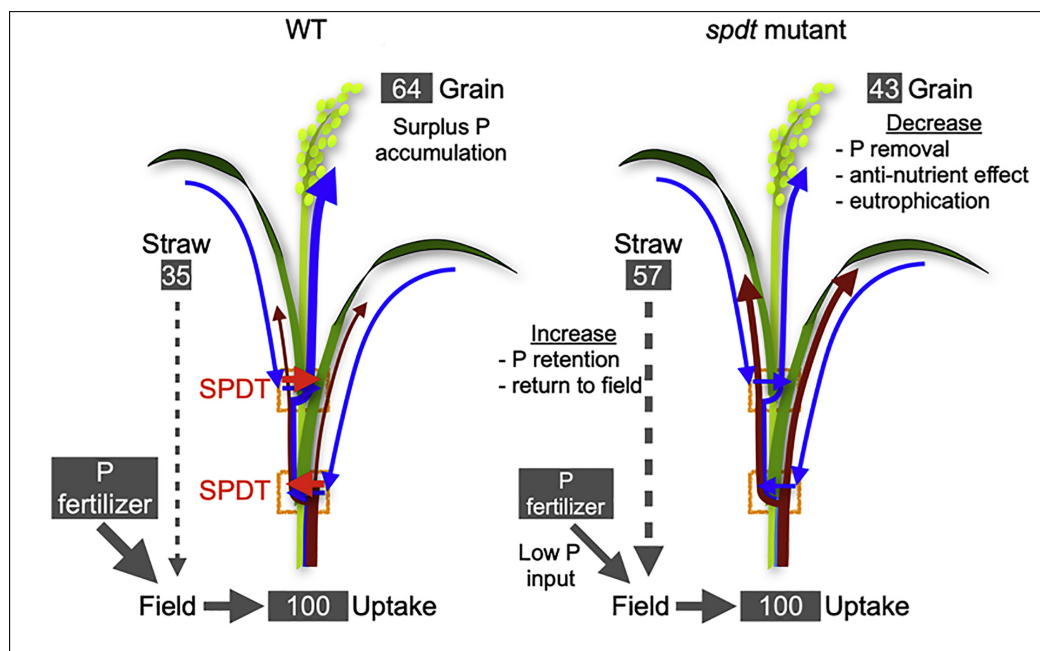


Figure 1.11 – Schematic diagram showing the role of node-localized SPDT in P distribution within the rice plant and the effect of SPDT knock-out on P allocation to grain (Reproduced from Yamaji et al., 2017)

1.4.3.2 Low-phytate crops

Phytic acid, the hexaphosphate ester of *myo*-inositol, is the primary storage form of P in cereal grains, accounting for 75 to 85% of total P (Raboy, 1997, 2009). It is mainly found as phytate salts complexing with divalent cations such as Zn^{2+} , Fe^{2+} , Mg^{2+} and Ca^{2+} (Brinch-Pedersen et al., 2002; Raboy, 2009). The quantity and localization of phytates in grains varies greatly depending on species. In wheat, barley and rice, phytates are essentially (up to 80%) present in the aleurone layer and bran while a small amount accumulates in the embryo and endosperm (O'Dell et al., 1972; Sparvoli and Cominelli, 2015). On the contrary, phytates are mainly present in the embryo and scutellum in maize seeds (Brinch-Pedersen et al., 2002; Sparvoli and Cominelli, 2015). Inside the grain, phytate is deposited in protein bodies named globoids (Raboy, 2009). It has two major roles in grains: the first is to provide minerals and energy for the seedling during germination before root emergence (Azeke et al., 2011; Julia et al., 2018; Nadeem et al., 2011). The

second is the control of P homeostasis during grain development by acting as a reservoir of Pi (Iwai et al., 2012). High Pi concentration in grains can inhibit starch synthesis by affecting the activity of ADP-glucose pyrophosphorylase, which is the key enzyme of starch synthesis (Iwai et al., 2012; Marschner, 2011). Some studies suggest a potential health-beneficial role for phytate as an antioxidant agent (Raboy, 2009).

Humans and non-ruminants are unable to efficiently digest phytates because they lack the phytases which are responsible for the release of Pi and the complexed micro-nutrients from phytate, reasons for which it is regarded as anti-nutritional (Gupta et al., 2015; Vashishth et al., 2017). This potentially contributed to nutritional deficiency (Zn and Fe) observed in human populations in some regions (Gupta et al., 2015; Vashishth et al., 2017). In addition to the inefficient absorption of P contained in phytates, high-P wastes are widely excreted leading to an increase in P levels in water bodies, which raises concerns about water quality and resource management (Brinch-Pedersen et al., 2002; Rose et al., 2013). Lott et al. (2000) reported that the amount of P exported as phytate can account for up to about 65% of the annual P fertilizer application worldwide.

To overcome nutritional deficiencies associated with phytate and its environmental implications, grain with low phytate content is a highly desirable trait (Figure 1.12) (Rose et al., 2013; Sparvoli and Cominelli, 2015; Raboy, 2001). Phytate is formed during grain development (Raboy, 1997). Although pathways leading to phytic acid biosynthesis in grain are complex and non-linear, two main pathways can be distinguished: a lipid-dependent pathway operating in all plant tissues, and a lipid-independent pathway operating principally in seeds (Sparvoli and Cominelli, 2015). The synthesis of phytic acid begins with the conversion of D-glucose-6-phosphate to *myo*-inositol-3-phosphate. This reaction is catalysed by a D-*myo*-inositol-3-phosphate synthase (MIPS) (Raboy, 2007). The two pathways of phytic synthesis differ in their early steps leading to the synthesis of *myo*-inositol trisphosphates (Raboy, 2009). Then, routes to phytic acid synthesis progress through sequential phosphorylation of *myo*-inositol (Figure 1.12). Grains with low phytate content can be obtained through varietal screening for grain phytate or by targeting genes encoding enzymes involved in the synthesis or transport and storage of phytates (Guttieri et al., 2004; Raboy, 2009).

Development of crop genotypes with low phytate content in grain is considered to be a very promising approach to obtain grains with higher nutritional quality (Brinch-Pedersen et al., 2002; Raboy, 2001, 2020). Maize *low phytic acid (lpa)* was among the first low phytate cereals to be identified by Raboy et al. (2000). It was obtained through

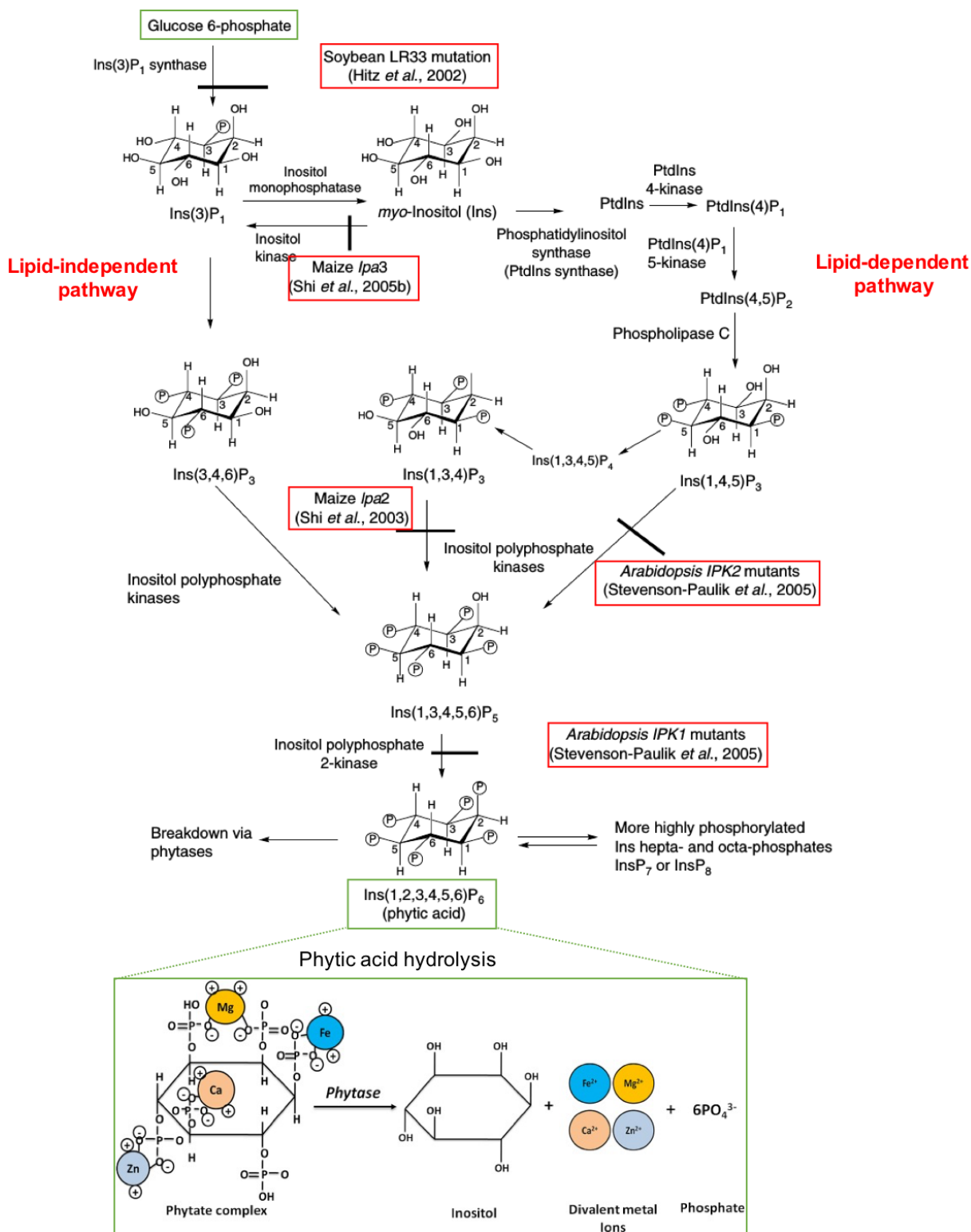


Figure 1.12 – Summary of the proposed phytic acid biosynthesis pathways in developing seeds and targets for engineering the low-phytate crops. The red box indicates known mutations with the published reference. The green box shows phytate hydrolysis during germination (Modified from Raboy, 2007; Vashishth *et al.*, 2017)

the screening for variation in phytic acid content in a large collection of maize mutants (Raboy et al., 2000). As shown in figure 1.13, *low phytic acid (lpa)* mutants typically have a reduced phytate concentration matched by an increase in inorganic-P (Pi) with limited change in grain total P (Raboy, 2007). For example, grains of *lpa* wheat displayed a 20% decrease in phytic acid concentrations with concurrent increases in Pi but without any significant change in total P (Figure 1.13). Wheat low phytic acid mutants were identified since 2004 (Guttieri et al., 2004). Hence, research efforts since the identification of the first stable low phytic acid mutants have resulted in the release of the first stable low phytic acid mutants in the USA in 2018 (Guttieri et al., 2004; Venegas et al., 2018). With the exception of these outcomes, there are only few research studies that addressed the importance of grain P and its related traits in wheat.

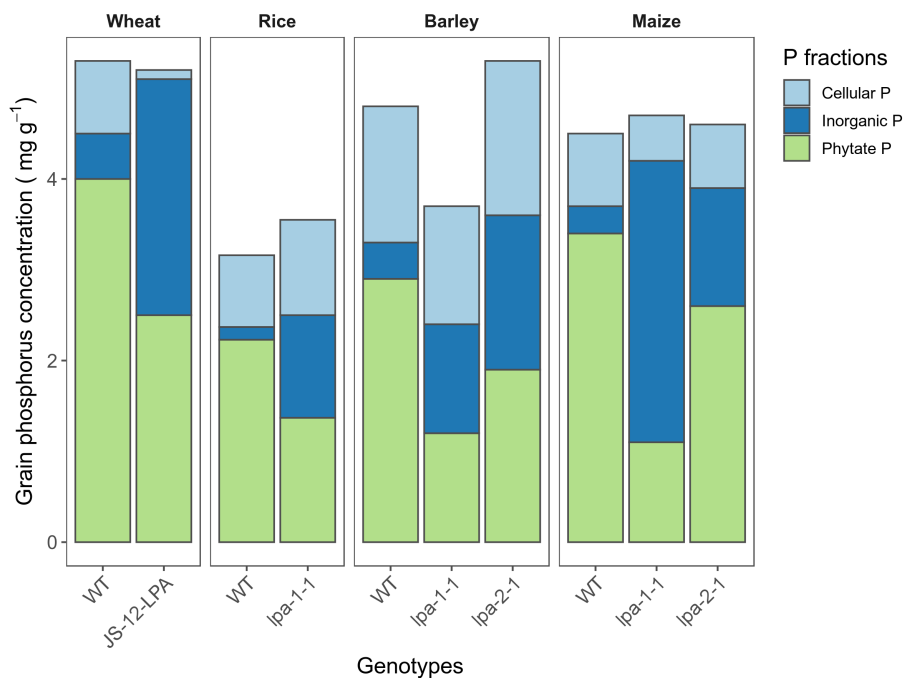


Figure 1.13 – Grain P fractions in wild-type (WT) and *low phytic-acid (lpa)* lines of wheat, rice, barley and maize (Data source: Raboy, 2007)

1.5 Accumulation of P in the grains

After the onset of anthesis, the developing grains progressively become the main sink for P. The P transferred to the grain is supplied from two distinct sources: post-anthesis uptake from external P and internal remobilization of P accumulated by leaves and other organs of the plant before anthesis (Figure 1.14).

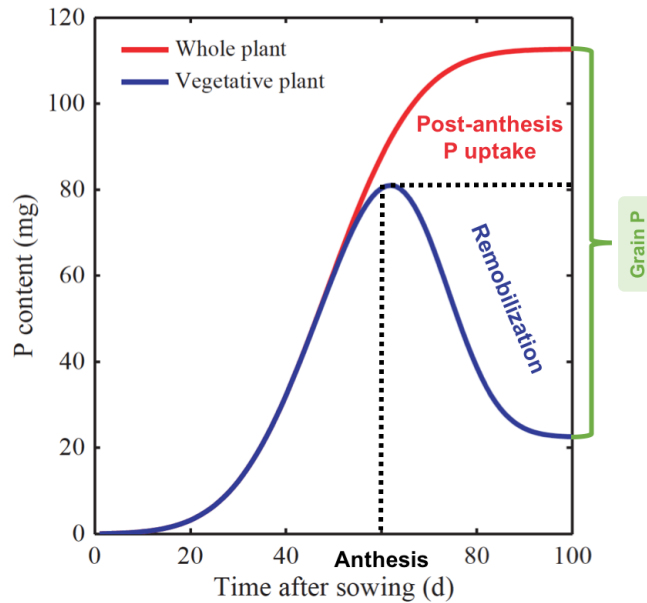


Figure 1.14 – Schematic representation of P accumulation in a cereal plant during its life cycle (Modified from Veneklaas et al., 2012). Phosphorus is supplied to grains through post-anthesis P uptake (red line) and the the remobilization of endogenous P sources from vegetative tissues (blue line)

1.5.1 Remobilization of P from vegetative organs

As described in the section 1.3.1, P remobilization is a common response that allows the plant to recycle and supply growing tissues with P when root P uptake is limited or during senescence. The onset of anthesis is the main trigger of leaf senescence in annual crops, also known as monocarpic senescence (Distelfeld et al., 2014; Gregersen et al., 2008). Other stresses such as drought, heat and nutrient deficiency can also be responsible for inducing leaf senescence (Etienne et al., 2018; Havé et al., 2017; Maillard et al., 2015). Throughout this process, nutrients accumulated in the vegetative tissues are remobilized and transported to growing tissues or to developing grains (Gregersen et al., 2008; Marschner, 2011). In wheat, senescence is controlled at the level of the individual leaf and proceeds from the oldest to the youngest leaf (Gregersen et al., 2008). Senescence in plants is generally accompanied by a strong enzymatic activity which is responsible for the degradation and the recycling of nutrients contained in macromolecules including proteins, lipids, and nucleic acid (Gregersen et al., 2008; Maillard et al., 2015). The cellular process involved in nutrient recycling is called autophagy (Masclaux-Daubresse et al., 2017). It refers to the degradation of cytoplasmic components that makes the nutrient available for the remobilization during senescence (Havé et al., 2017; Masclaux-Daubresse et al., 2017). The remobilization of P represents an important source of P to sustain new sink (e.g. grain growth) during leaf senescence and under limited external P supply

(Batten and Wardlaw, 1987; Veneklaas et al., 2012). In a hydroponic experiment, Jeong et al. (2017b) analysed the remobilisation of P fractions in rice flag leaves during grain filling and after removing P from the nutrient solution. They showed that rice plants first remobilized less essential P pools contained in vacuole and phospholipid before remobilizing nucleic acid-P and cytosolic-P to minimise the impact on photosynthesis (Jeong et al., 2017b). Indeed, the vacuole is the major storage compartment of Pi in vegetative plant cells (Raghothama, 1999; Yang et al., 2017). Vacuolar-P is mainly involved in maintaining P homeostasis inside plant cells but it is not critical for cellular functioning (Marschner, 2011; Yang et al., 2017). The activity of acid phosphatases and ribonucleases (RNases) has been shown to increase during senescence and under P deficient conditions (Smith et al., 2015; Dissanayaka et al., 2018). These enzymes are responsible for the breakdown of P from RNA (mainly rRNA) and replacing phospholipids in membranes with the non-phosphorus lipids such galactolipid, sulfolipids and digalactosyldiacylglycerol (DGDG) to provide a greater supply of P to the new growing sinks (Figure 1.15) (Siebers et al., 2015; Stigter and Plaxton, 2015). Specifically, purple acid phosphatases have been reported to play role in liberating P from phospholipids for export to developing grains in rice (Jeong et al., 2017a; Smith et al., 2015).

The extent of P remobilization rates reported in the literature is highly variable depending on genotypes and growth conditions. In wheat, P remobilization from senescent leaves can be up to 90% (Etienne et al., 2018; Maillard et al., 2015). Reducing the source of endogenous P by defoliation resulted in a reduction in grain P content with little impact on grain yield (Mohamed and Marshall, 1979). In a pot experiment, Rose et al. (2007) reported that P remobilization from leaves and stems were the main contributor to the accumulated P in the grains. They also showed that the roots did not act as a source of P for the grain since it retains 22% of the plant's P at maturity (Rose et al., 2007). However, information on root P remobilization are scarce. In contrast, P remobilization is shown to account for 56%–63% of grain P in durum wheat (Masoni et al., 2007). A better understanding of the physiological bases of P remobilization from plant tissues and its loading into grains could provide useful insight to plant breeding programs aiming at increasing phosphorus use efficiency in crops.

1.5.2 Post-anthesis P uptake and long-distance P transport

The requirement for P vary remarkably from one species to another and within cultivars of the same specie. Optimal metabolic P requirement for most crops is about 3-4 mg g⁻¹ (Veneklaas et al., 2012). Spring wheat and winter wheat differ in their critical growth stages for P uptake for maximum biomass production (Sutton et al., 1983). Phosphorus

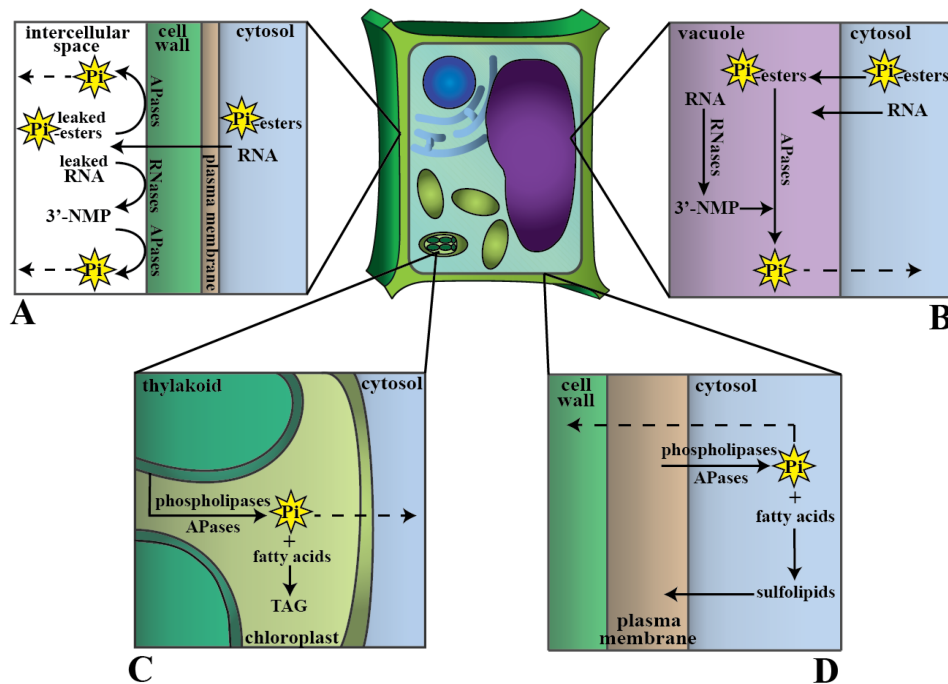


Figure 1.15 – Primary sources of phosphorus remobilization in senescent plant cells. Degradation of the plasma membrane by cell wall-localized RNases and acid phosphatases (APases) (A). RNA and P_i -esters are hydrolyzed in the vacuole by intracellular RNases and APases (B). The action of phospholipases and APases degrades phospholipids in the chloroplasts (C) and liberate P_i and fatty acids (D). A fraction of the liberated fatty acids from phospholipids is incorporated into the plasma membrane in the form of sulfolipids (From Stigter and Plaxton, 2015).

supply until the first node stage is critical for winter wheat to produce maximum grain yields, whereas adequate P supply until heading is required for spring wheat (Sutton et al., 1983). Römer and Schilling (1986) found that maximum daily P uptake rate occurred at the end of tillering. Although plant demand for P is high during the vegetative stages of wheat growth, P uptake continues during grain filling (Grant et al., 2001; Römer and Schilling, 1986). Wheat plants are particularly efficient at remobilizing P from senescent organs and redistributing it to the grain after anthesis (Abbas et al., 2018; Maillard et al., 2015). As a result, P harvest index are generally greater than carbon harvest index in many crop species including wheat (Veneklaas et al., 2012). It has been estimated that 50-90% of the P present in the mature grain comes from the remobilization of P present in other organs of the plant (Batten et al., 1986). However, the contribution of pre-stored P to grain P is decreased to 21% under high P supply (Batten et al., 1986). Although this accounts for a large proportion of the P in the grain, it is noted that the accumulation of external P is greatest between stem elongation and anthesis which coincides with the period of maximum leaf area (Waldren and Flowerday, 1979). The uptake of P occurs beyond anthesis and continues during the grain filling period while the accumulation of dry matter continues until physiological maturity (Batten et al., 1986; Calderini et al., 2000).

Although there have been several investigations on the uptake and transport of P in wheat (Aziz et al., 2014; Masoni et al., 2007; Römer and Schilling, 1986), only a few studies examined in detail the implication of post-anthesis P uptake and its contribution to grain P (Masoni et al., 2007; Peng and Li, 2005). Many studies have focused on the vegetative stage because P deficiency strongly affects the growth of shoot and root during seedling development. Julia et al. (2016) investigated the partitioning of P and its remobilization during grain filling in rice using the ^{33}P radio-tracer. The authors found that P is mainly remobilised from leaves and that root P uptake during grain filling represented 40 and 50% of the aerial-P. They concluded that post-anthesis P uptake is a critical contributor to the accumulated P in rice grains (Julia et al., 2016). In contrast, it is difficult to make generalizations on the importance of post-anthesis P uptake for wheat because inconsistent results were reported (Batten, 1986b; Rose and Wissuwa, 2012). For example, Rose et al. (2007) reported little accumulation of P during the post-anthesis period whereas Pampana et al. (2007) showed that post-anthesis accumulation of P was the main source of grain P in durum wheat. Moreover, Manske et al. (2001) reported that post-anthesis P accumulation accounted for 31-42% of the total P uptake depending on P fertilization. The contribution of post-anthesis P uptake to grain P is shown to be greater under low P supply (Julia et al., 2016; Manske et al., 2001; Masoni et al., 2007).

The post-anthesis P supply is suggested to have little effect on wheat grain yield because of the high remobilization efficiency of P. However, this was not demonstrated experimentally, and the role of post-anthesis P uptake in crop nutrition is still unclear. It can be hypothesized that post-anthesis P uptake may contribute to maintaining higher P concentrations in leaves and therefore maintaining a higher rate of photosynthesis during grain filling, especially in stay-green genotypes that keeps photosynthesis for a more extended period. Batten et al. (1986) reported that maintaining post-anthesis P supply to wheat plants increased grain P content and tiller biomass but did not increase grain yield. Moreover, in one-tiller wheat, Peng and Li (2005) showed that removing P from the nutrient solution at the flag-leaf expansion stage did not influence grain growth and its final P content despite a decrease in the whole plant biomass. Under field conditions, post-anthesis P uptake from the soil can be restricted due to lower P availability and low diffusion rate caused by soil drying.

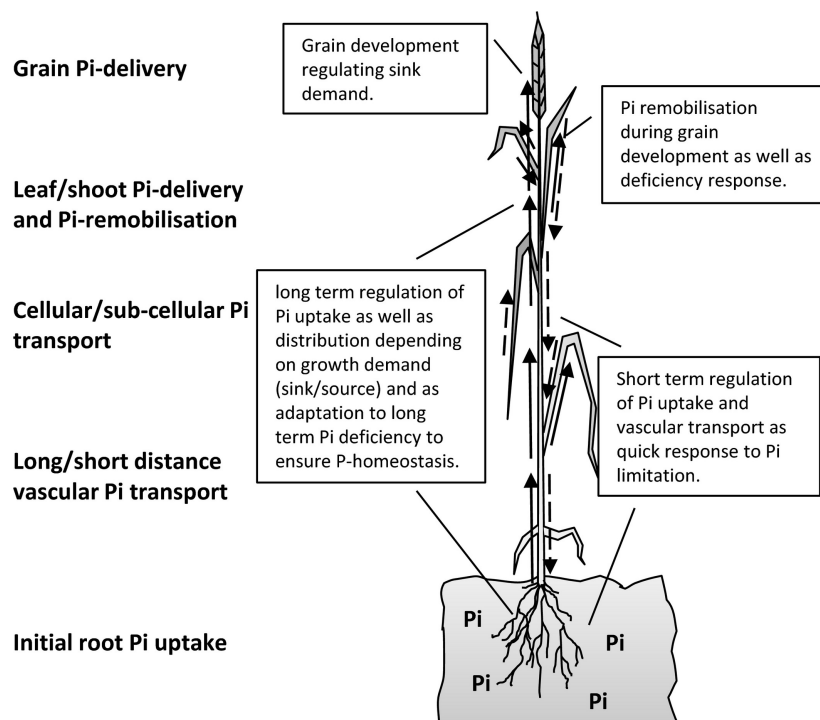


Figure 1.16 – Overview of phosphorus transport processes involved in delivering P to grains in wheat and responses to P limitation (From Grün et al., 2018)

Long-distance transport of P can occur in both xylem and phloem (Marschner, 2011; Schachtman et al., 1998), but the export of P from older organs to other growing sinks is largely phloem dependent (Figure 1.16) (Hill, 1980; Mimura, 1995). However, the transport pathway of P and to grains and signalling molecules are not fully known (Wang

et al., 2016). In the phloem, P is transported as phosphate, although some studies have found significant amounts of organic P (Schachtman et al., 1998). The phloem is assumed to be the main routes of P supply to grains because of the xylem discontinuity between the rachilla and the grain (Zee and O'Brien, 1970). Thus, direct transfer of P from root to grain is unlikely to occur. This implies that P transfer from xylem to phloem must take place before reaching the grain. Despite the importance of this routes in delivering P to growing grains, the dynamics P during the post-anthesis period and of xylem-to-phloem transfer are unknown (Wang et al., 2016).

1.5.3 Methodologies to study P fluxes at the whole plant level

Two methods can be used to evaluate the remobilization and the post-anthesis P uptake at the whole plant level (P fluxes). The most commonly used method consists in the measurement of the total amount of P present in different plant organs at anthesis and at maturity. The difference between the two harvests gives an estimation of the amount of remobilized or taken up P after anthesis. This method is called apparent remobilization or budget method. It is very useful in comparative studies but it provides only a rough estimate of the quantity of P absorbed (Havé et al., 2017). For example, the amount of P accumulated over time is a function of influx and efflux of P, net remobilization from an organ will only be detected when efflux exceeds influx. Thus, between anthesis and maturity, a given amount of P could pass through an organ with no detectable change in its content, resulting in no detectable net remobilization (Havé et al., 2017; Waters et al., 2009). Also, since the measurements are made on different plants, there is a risk of under or over-estimating the value of the post-anthesis P uptake or P remobilization.

Fortunately, the evaluation of P uptake and partitioning by isotope tracing gives accurate estimates. More importantly, it informs about the origin of P in each plant compartments. This kind of study is regularly performed for several plant species for mineral elements with stable isotopes such as nitrogen and sulphur (Dubousset et al., 2010; Maillard et al., 2015; Schiltz et al., 2005). Contrary to these elements, P has only ^{31}P -phosphorus as a stable isotope (IAEA, 2001; Morel and Fardeau, 1990). For this reason, radioactive isotopes of P are used as a tracer in plant nutrition and agronomic studies (Morel and Fardeau, 1990; Frossard et al., 2011). The choice of P tracer is generally made based on half-life (and cost), the most widely used are ^{32}P and ^{33}P (IAEA, 2001). Nevertheless, studies on the uptake and the allocation using P tracer, especially after anthesis, are much less abundant in literature than for N and S (Salon et al., 2017). Consequently, the mechanisms that govern the allocation of P to grains are still far from being elucidated (Rose and Wissuwa, 2012; Wang et al., 2016). Depending on the objectives, two labelling

strategies can be used: short-term labelling or pulse-chase allows to track the dynamics of the P recently absorbed in the plant and to determine its final allocation. The long-term labelling allows to accurately estimate the contribution of P fluxes to the accumulated P P in a given compartment. It should be noted that both methods are based on the assumption that there is no fractionation during root P uptake and transport between plant organs.

Conclusion and thesis objectives

The literature review showed that phosphorus nutrition and the mechanisms involved in the uptake and utilization of P as well as its role in the metabolism of cereals are well understood during the vegetative stages. On the other hand, the allocation of P and transport pathways within the plant and especially towards the grains are less well-identified, and the methods available to study it are more limited.

The carbohydrates, proteins and nutrients accumulated during grain development are essential reserves to support the germination and growth of young plants. They also serve as a vital source of food for humans and animals. Therefore, grain quality is a central food safety issue. A better understanding of the mechanisms implicated in nutrient allocation to grains would have a predominant role in improving the sustainability of cropping systems while increasing crop nutritional quality and reducing environmental damage. For phosphorus, the ultimate goal is to reduce the accumulation of P in grains as a means to limit the repeated export of P and its environmental and nutrition implications. As it was demonstrated in the section 1.4.3, minimising P export with harvested grains is thus possible without compromising yield and quality parameters. Therefore, a better understanding of the mechanisms implicated in delivering P to grains would help achieve these goals.

As illustrated in Figure 1.17, P fluxes to grains come from two sources: post-anthesis P uptake and P remobilization. However, the exact contributions of each source to the P accumulated in grains, and the impact of P supply on these contributions remain unclear. Therefore, this thesis aims to identify the determinants of P accumulation in grains through the investigation of P uptake and remobilization throughout the post-anthesis period and their impact on grain P concentrations in durum wheat. The specific objectives are to 1) investigate the importance of post-anthesis P uptake and the remobilization of endogenous P sources and 2) estimate their respective contribution to grain P under different conditions of P supply. To quantify these fluxes, experiments were conducted under greenhouse conditions to control environmental factors, including P supply to plants. In the first part of this thesis, we studied the response of two durum wheat cultivars with contrasting root biomass allocations to P deprivation throughout the post-anthesis period. A whole-plant P budget assessment was performed to estimate the potential net contribution of P remobilization to the accumulated P in the grains. In the second part of this thesis, we characterised the contribution of P remobilisation and post-anthesis P uptake to grain P with the use of a P tracer. Firstly, we performed a long-term ^{32}P -labelling experiment (from anthesis to maturity), which allowed for the identification of source

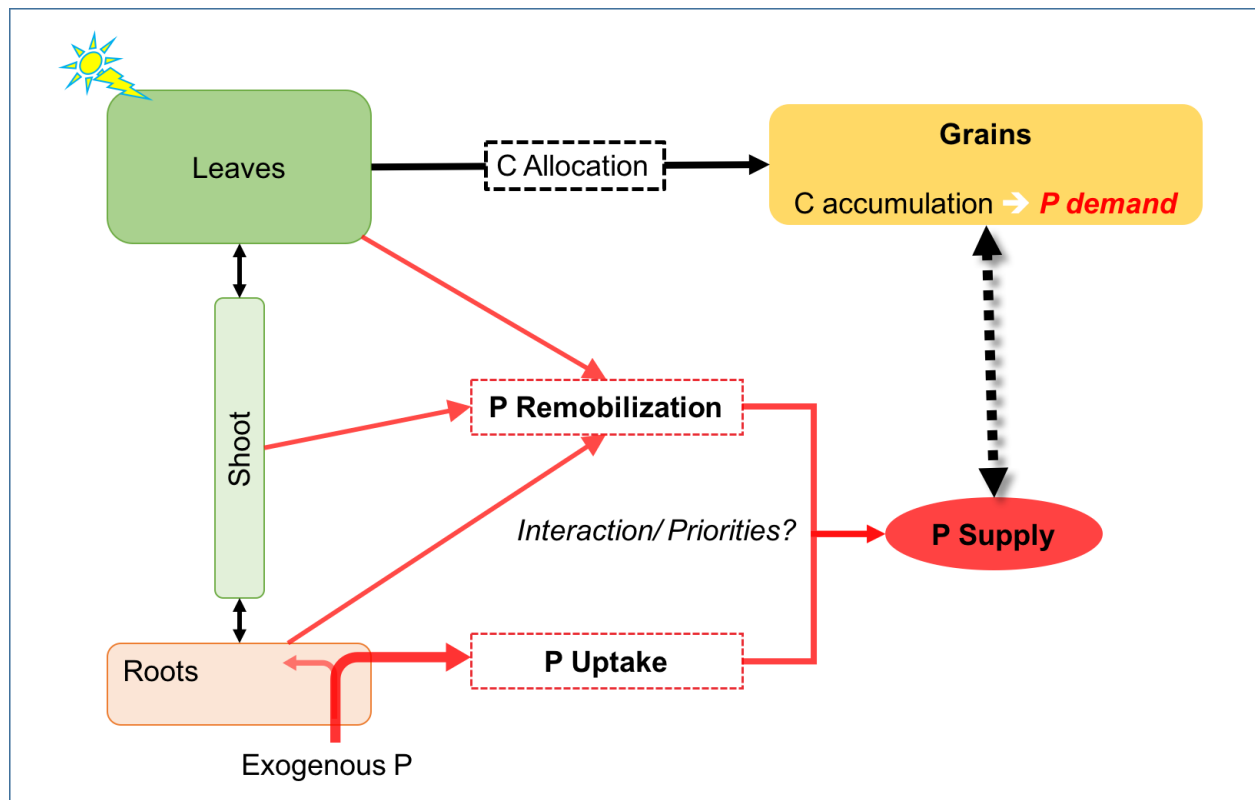


Figure 1.17 – Simplified schema of the processes involved in P allocation to grains

organs and the contribution of the remobilisation to the P accumulated in the grains. Secondly, we followed the fate of recently acquired P and its allocation to the grains by two short-term (24h) targeted ^{32}P -labelling experiments during the development of the grains. The effect of P supply on P fluxes at the whole-plant level was also determined by growing the plant in two contrasted P supply. The next chapter will present the material and method used in this thesis.

Chapitre 2

Matériels et méthodes généraux

Trois expérimentations en serres ont été réalisées au cours de la thèse à l'INRAE-Bordeaux à Villenave d'Ornon. Cette partie présente le dispositif expérimental, le matériel végétal et les techniques analytiques mis en place. Le matériel et méthode propre à chaque expérience est détaillé dans les chapitres résultats correspondants.

2.1 Matériel végétal et conditions de culture

2.1.1 Système de culture et matériel végétal

L'ensemble des trois expériences a été réalisé en hydroponie et non en présence de sol. Ce choix est motivé par la nécessité de contrôler l'offre en phosphore (P) pour les plantes à n'importe quel stade de développement et d'avoir un accès direct aux racines pour tester nos hypothèses. Ceci est particulièrement important lors des expériences de marquage isotopique où le changement de la solution nutritive et le rinçage du système racinaire sont nécessaires. La culture hydroponique permet également d'assurer le renouvellement de la solution en éléments nutritifs et l'absence de stress hydrique. La solution est aérée et le pH tamponné.

Dans la première expérience, nous avons utilisé deux cultivars de blé dur, *Dakter* et *Sculptur*. Leurs caractéristiques agronomiques sont décrites dans le Tableau 2.1. Ces deux cultivars ont été choisis pour leurs ratios root:shoot contrastés (rapport biomasse souterraine/biomasse aérienne) susceptibles d'affecter le prélèvement de P et sa répartition entre les puits. Ils ont également fait l'objet de nombreux travaux antérieurs dans le laboratoire ISPA et leur culture en hydroponie est maîtrisée (Perrier et al., 2016). Deux niveaux d'offre en P pendant la période post-floraison ont été appliqués pour étudier l'effet de la disponibilité en P pendant cette période sur l'accumulation et la remobilisation de P à l'échelle de plante entière. La sevrage en P (absence de P externe) pendant la

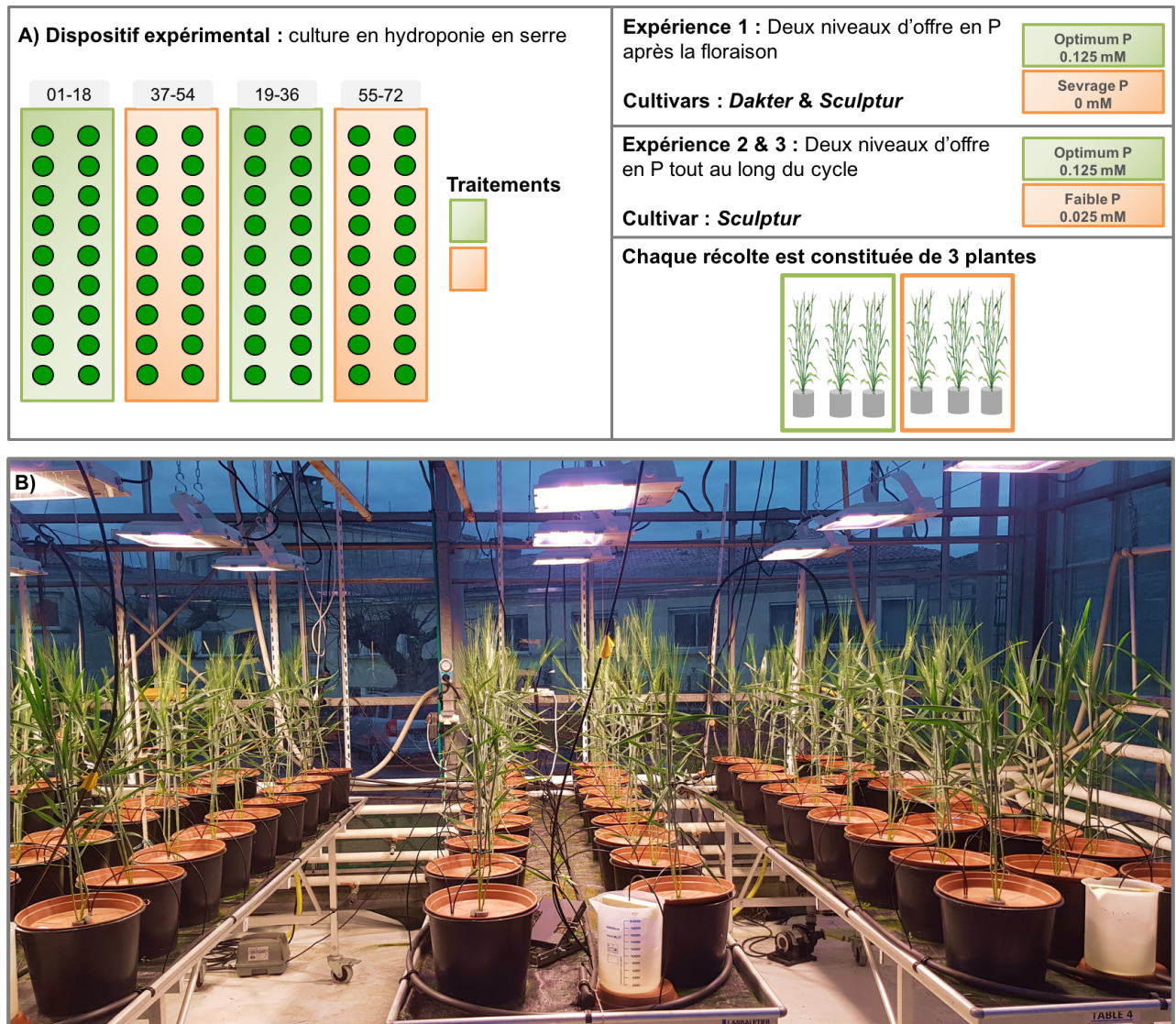


FIGURE 2.1 – Dispositif expérimental (A) et photographie (B) du système de culture du blé dur en hydroponie mis en place au cours de la thèse. Le système de culture est composé de 4 tables contenant chacune 18 plantes (Indiqué en haut de chaque table, 72 au total) reliés à deux réservoirs qui permettent d'alimenter les pots en solution nutritive.

période post-floraison permet de mettre en évidence les différents processus/sources impliqués dans l'utilisation interne du P.

Par la suite, seul le cultivar *Sculptur* est utilisé pour les expérimentations portant sur la quantification des flux de P pendant la période post-floraison à l'aide du marquage isotopique. Dans les deux expériences de marquage au ^{32}P , deux niveaux d'offre en P ont été appliqués pour avoir des plantes contrastées dans leur statut P. Les conditions de germination et de pré-culture décrites ci-dessous sont similaires pour les trois expérimentations (Figure 2.1)

TABLE 2.1 – Caractéristiques agronomiques des cultivars de blé dur étudiés

Cultivar	Fournisseur	Précocité	Taille des grains	Teneur en protéines*	Expérience
Dakter	Limagrain	Demi-précoce à demi-tardive	Grand	Moyen à élevé	1
Sculptur	RAGT Semences	Demi-précoce	Moyen	Faible à moyen	1,2,3

*La moyenne nationale en 2019 est à 13,9 % (FranceAgriMer / ARVALIS, 2019)

2.1.2 Conditions de culture et dispositif expérimental

Avant utilisation, les graines du blé dur (*Triticum turgidum* L. subsp. *durum*) de masse homogène (50 ± 5 mg) ont été stérilisées avec 6 % (v/v) d'hypochlorite de sodium pendant 10 min, puis rincées trois fois avec l'eau ultra-pure. Ces graines ont été mises à germer à l'obscurité à 25 °C pendant 3 jours. Ensuite, les graines germées de taille uniforme (e.g. taille racinaire d'une longueur de plus de 2 cm) ont été transférées dans des tubes Falcon® de 50 ml contenant de la solution nutritive de Hoagland modifiée. La composition de la solution nutritive est présentée dans le Tableau 2.2. Juste après l'émergence de la 3^{ème} feuille (après ~2 semaines de pré-culture), les plantules de taille homogène ont été sélectionnées et transplantées dans des pots en plastique (1 plante par pot) contenant 5,5 L de solution nutritive Hoagland modifiée. Nous avons également ajouté du SiO_2 (50 mg L^{-1}) qui est connu pour être bénéfique pour les céréales et le MES (Acide 2-morpholin-4-yléthanesulfonique) pour tamponner les variations du pH. Le pH initial de la solution nutritive a été ajusté à $6,0 \pm 0,5$ en utilisant du KOH. Le pH et la conductivité électrique ont été suivis 2 fois par semaine tout au long du cycle de la culture à l'aide d'un pH / conductimètre (HI 9814 GroLine, France). L'aération et l'oxygénation ont été maintenues dans les pots à l'aide d'une pompe électrique (compresseur d'air).

Trois niveaux de concentration en P ont été utilisés dans l'ensemble des expérimentations. 'Optimum P' correspond à la concentration du P dans la solution nutritive adaptée aux besoins nutritionnels pour la culture du blé dur en hydroponie. Cette concentration a été fixée à 0.125 mM de P à partir des expériences précédentes au sein de l'unité ISPA (Perrier et al., 2016; Yan et al., 2018). 'Faible P' correspond à une dilution de 5 fois de la concentration optimale. Ces deux traitements ont été appliqués dans l'objectif d'avoir des plantes contrastées dans leurs statut P. Pour éviter le développement massif des talles liés à la culture des plantes isolées en hydroponie, seuls les 4 premiers talles ont été conservés (5 pour la première expérience). Les autres talles végétatifs ont été supprimés au fur et à mesure de leurs apparitions.

Les trois expériences ont été réalisées selon le même dispositif expérimental (Figure 2.1). Pour limiter les variations dues aux conditions de culture, les pots ont été répartis de façon aléatoire sur les 4 tables contenant chacune 18 plantes. À partir de la floraison et pour chaque date d'échantillonnage, les plantes sont récoltées pour les différentes expériences par lot de 3 plantes par traitement et par cultivar. Ainsi, 44 plantes ont été récolté dans l'expérience 1, 12 dans l'expérience 2 et 60 dans l'expérience 3.

Toutes les cultures de blé dur ont été conduites en serre avec 16 heures de photopériode. L'éclairage naturel est supplémenté avec des lampes LED (Ledlyt, France) délivrant un PAR (Photosynthetically active radiation) moyen entre 350 et 450 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ à la hauteur de la canopée des plantes. Le PAR, la température et l'humidité ont été enregistrés quotidiennement toutes les 30 min à l'aide des capteurs répartis sur les tables dans la serre (Figure 2.2). Dans les expérimentations 2 et 3, la serre a été chauffée pour éviter les températures trop basses pendant l'hiver.

2.2 Stades de prélèvement et mesures réalisées

Le suivie des stades de développement végétatifs et reproducteurs du blé dur a été réalisé hebdomadairement suivant la notation de Zadoks (Zadoks et al., 1974). Après l'émergence de l'épi, les plantes ont été suivies individuellement pour déterminer la date de floraison qui correspond à la sortie de 50% des anthères (Zadoks 65). Tous les prélèvements comprennent trois répétitions par traitement. Les prélèvements ont été réalisés comme montré sur la figure 2.3 :

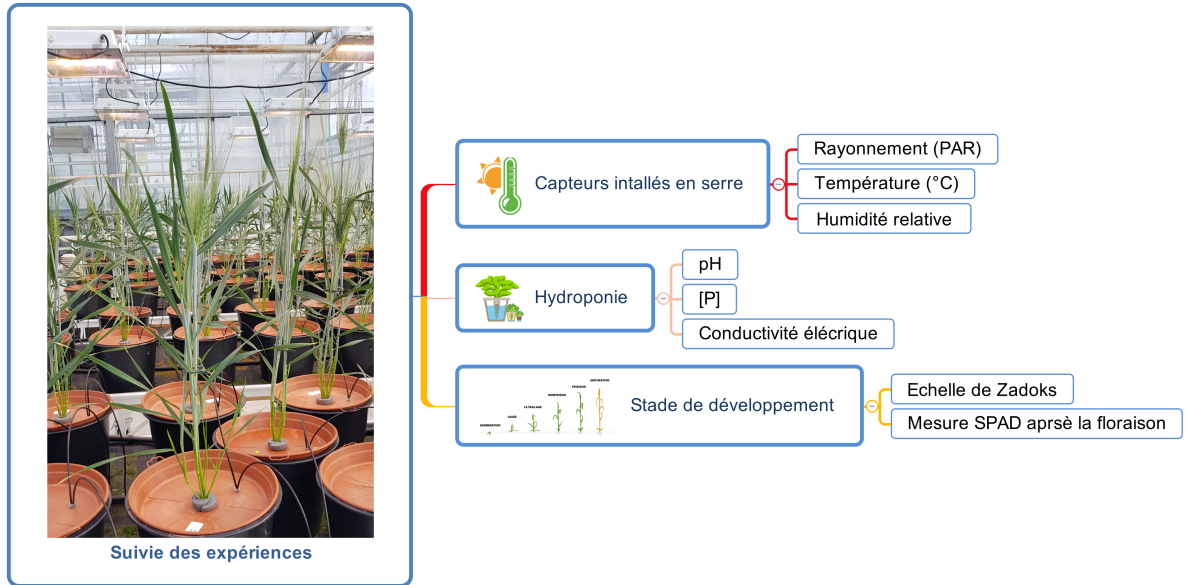


FIGURE 2.2 – Suivi réalisé au cours de la culture de blé dur en hydroponie en serre. Ce suivi permet de caractériser les conditions environnementales et de culture

TABLE 2.2 – Composition de la solution nutritive utilisée pour la culture de blé dur en hydroponie

Macroéléments	Hoagland 1/4 conc. (mM)	Microéléments	Hoagland 1/1 conc. (µM)
KH ₂ PO ₄	0.125 (Optimum P) / 0.025 (Faible P)	H ₃ BO ₃	46.25
KNO ₃	0.625	MnCl ₂ ·2H ₂ O	1
KCl	0.850	ZnSO ₄ ·7H ₂ O	10
Ca(NO ₃) ₂ ·4H ₂ O	1.250	CuSO ₄ ·5H ₂ O	2
MgSO ₄ ·7H ₂ O	0.500	(NH ₄) ₆ MO ₇ O ₂₄	0.03
		KCl	100
		FeNa EDTA·3H ₂ O	100
		HEDTA	25

- Quatre prélèvements pour l'expérience 1 : floraison, 10 et 20 jours après floraison et à maturité
- Deux prélèvements pour l'expérience 2 : floraison et maturité
- Dix prélèvements pour l'expérience 3 : floraison, 5, 6, 7, 9, 14, 15, 16, 18 jours après floraison et à maturité

La teneur en chlorophylle des feuilles est estimée de manière non destructive sur un nombre limité de plantes en utilisant le SPAD (Soil Plant Analysis Development, Minolta, SPAD-502 model, Tokyo, Japon). Ces mesures permettent également de suivre la sénescence des plantes.

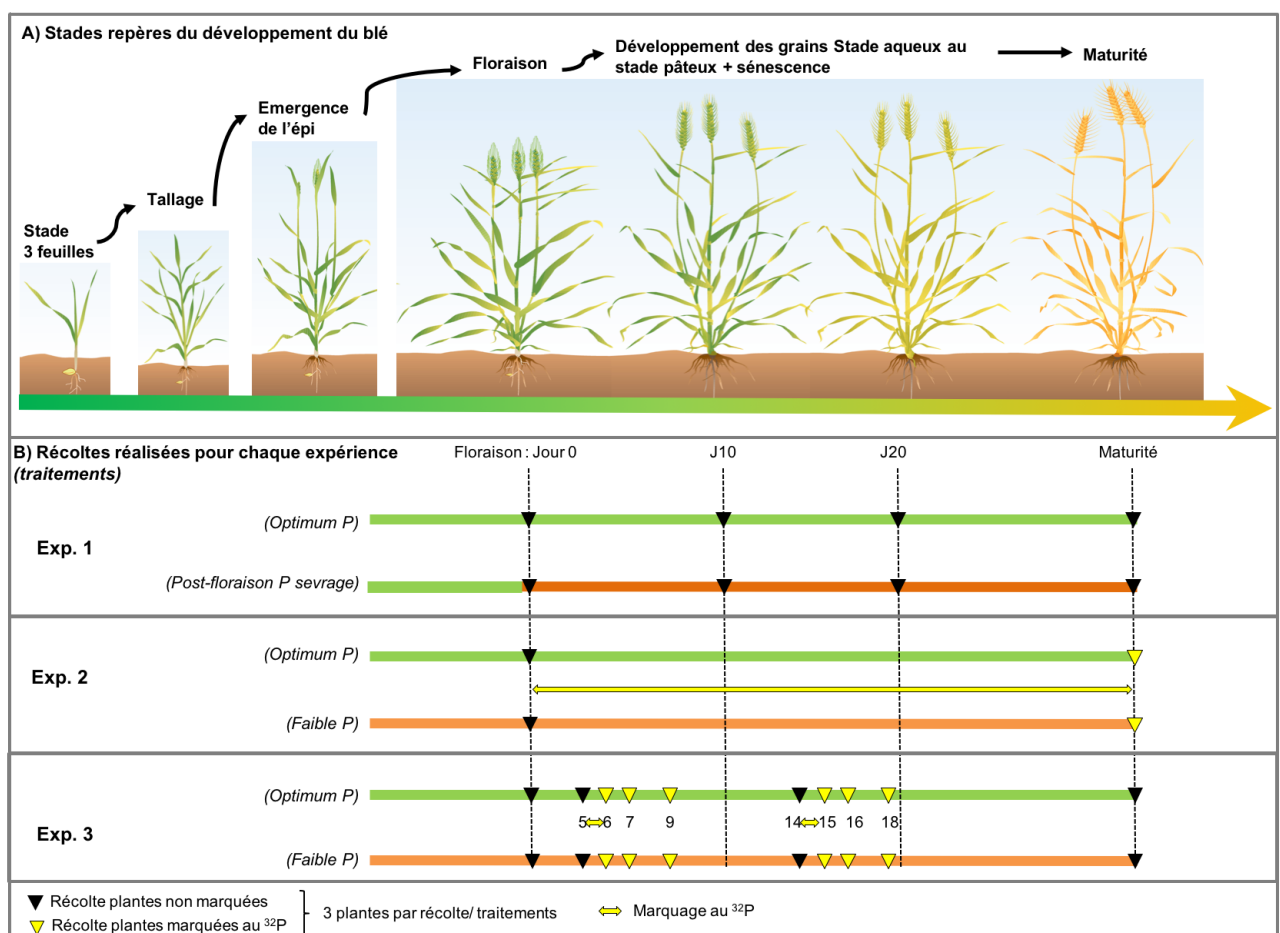


FIGURE 2.3 – Représentation schématique du cycle de développement du blé dur (A) et des récoltes réalisées pour chaque expérience (B). Trois plantes par traitement sont prélevées à chaque récolte.

A chaque prélèvement, le système racinaire est rincé trois fois dans un bac d'eau froide afin d'éliminer la solution nutritive adhérente à la surface des racines et arrêter le prélèvement. Les plantes récoltées sont divisées en sept compartiments (Figure 2.4) : racines,

tiges, feuilles du bas, feuilles étendards, rachis et pédoncules, épis et grains. Les compartiments des 4 (ou 5) talles sont regroupés par plante. À maturité, les composantes du rendement (le nombre de grains et le poids de mille de grains) ont été également mesurées.

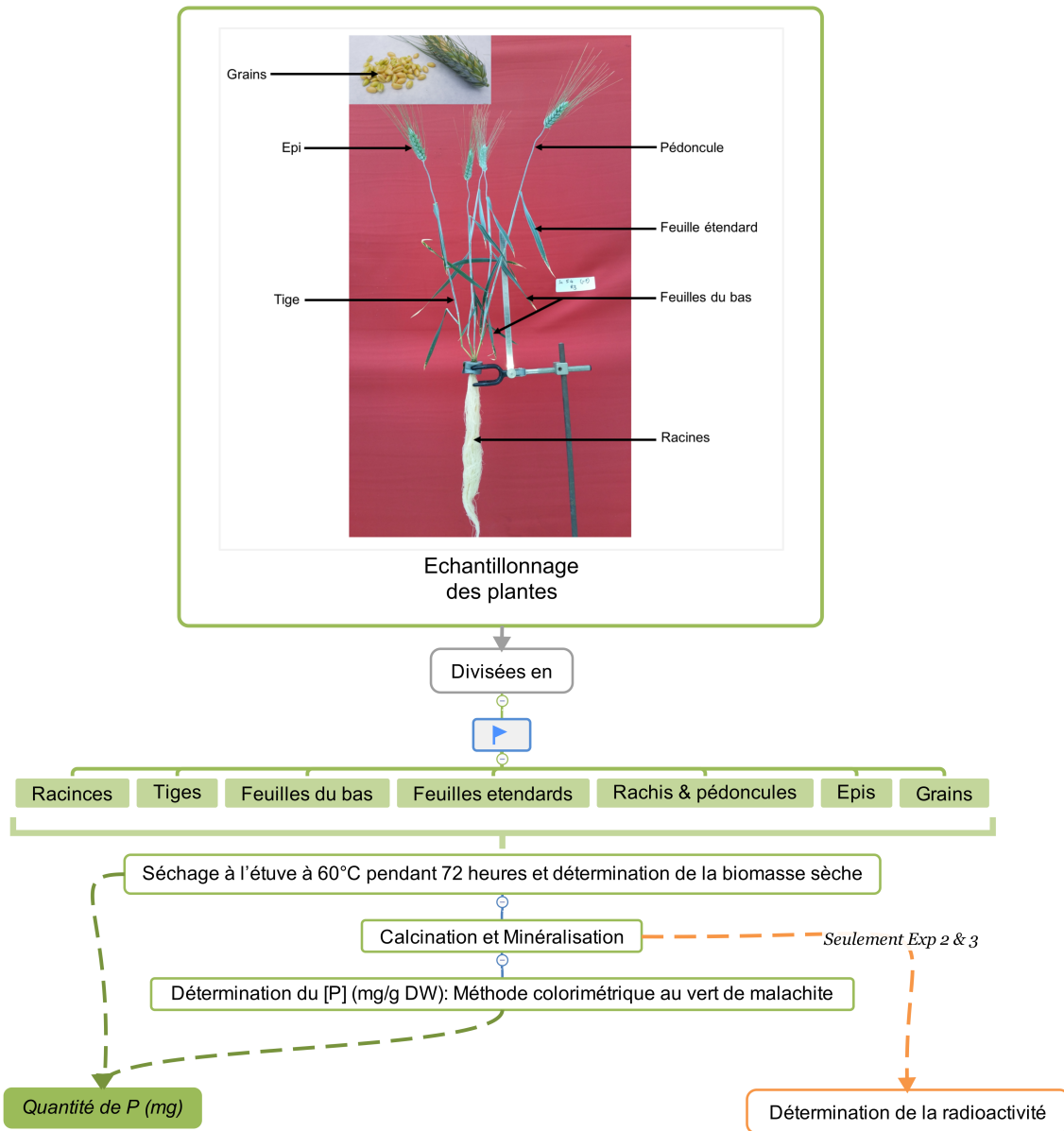


FIGURE 2.4 – Échantillonnage et aperçu des mesures effectuées après la récolte des plantes

2.3 Marquage isotopique au ^{32}P

2.3.1 Utilisation du P radioactif comme traceur

Contrairement aux éléments tels que le C, N, et S, le P ne possède qu'un seul isotope stable, le ^{31}P . C'est la raison pour laquelle on utilise les isotopes radioactifs du P comme traceurs dont les plus utilisés sont les radioisotopes ^{32}P et ^{33}P . Ils ont les mêmes propriétés chimiques mais diffèrent dans leur propriétés physiques comme la masse. Le ^{32}P est caractérisé par une demi-vie de 14,3 jours et se désintègre en émettant des particules β^- (énergie maximale de 1,71 MeV). Cette demi-vie est relativement courte en comparaison avec celle du ^{33}P qui a une demi-vie de 25,3 jours. Cependant, l'énergie maximale émise lors de la désintégration est plus faible ($E_{\text{max}} = 0,25 \text{ MeV}$). Par conséquent, nous avons choisi d'utiliser l'isotope ^{32}P dans les expériences 2 et 3 pour pouvoir quantifier précisément les flux du P exogène absorbé par les plantes au cours de la période post-floraison, et du P endogène remobilisé à partir des différents organes végétatifs vers les grains.

Deux expériences de marquage ont été réalisées :

1. Marquage continu tout au long de la période post-floraison pour quantifier la contribution du P absorbé et remobilisé au remplissage des grains en P
2. Marquage ponctuel pendant 24h à deux stades clés pour suivre et quantifier le P qui a été récemment absorbé. Nous avons également mesuré les flux grâce aux prélèvements successifs de plantes après marquage.

2.3.2 Marquages des plantes au ^{32}P et prélèvement

Les expériences de marquage au $\text{H}_3^{32}\text{PO}_4$ ont été réalisées dans une serre dédiée à la manipulation des radioéléments avec le matériel de protection adapté (délimitation des locaux, contrôles avec dosimètre et sonde β). Les conditions de culture et la composition la solution nutritive étaient similaires à celles décrites ci-dessus (2.1). Une quantité R connue d'ions phosphates radioactifs ($\text{H}_3^{32}\text{PO}_4$) a été ajoutée à chaque solution nutritive en tenant compte de la durée de marquage et de la limite de détection de la radioactivité par le compteur à scintillation β (c.f. chapitre résultats 4 et 5). La solution marquée est soigneusement mélangée. Avant de mettre les plantes dans cette solution, les racines ont été rincées 3 fois avec de l'eau déminéralisée. À la fin de chaque expérience de marquage, les plantes sont échantillonnées et séparées en différents compartiment comme décrit ci-dessus puis soumis aux analyses.

2.4 Analyses et calculs

2.4.1 Préparation des échantillons, dosage de P et mesure de la radioactivité

Les échantillons sont séchés à l'étuve à 60 °C pendant 3 jours puis pesés pour déterminer la biomasse sèche avant d'être minéralisés par voie sèche. Les échantillons séchés (entier ou sous échantillon d'environ 0.5 g) sont mis dans des creusets à incinération et calcinés au four à 500°C pendant 5 heures. Les cendres obtenues après calcination et refroidissement sont traitées avec 2.5 ml d'acide nitrique concentré (14N). Les minéralisats sont ensuite évaporés à sec sur une plaque chauffante et puis repris à l'eau avant d'être filtrés (papier filtre Whatman, 110 mm) et dilués dans une fiole de 50 ml avec de l'eau ultra-pure. La concentration en P dans les solutions minéralisées est mesurée par colorimétrie (méthode au vert de malachite) à la longueur d'onde de 610 nm (Van Veldhoven and Mannaerts, 1987). La quantité de P dans chaque compartiment est obtenue en multipliant la concentration en P et la biomasse sèche. La somme des quantités de P dans tous les compartiments permet de déterminer la quantité totale de P contenue dans chaque plante. Les concentrations de C et de N dans les grains ont été déterminées avec un analyseur élémentaire (Flash EA1112, ThermoFisher) selon la méthode Dumas. Pour chaque série analytique, des blancs et des échantillons de contrôle (Poudre de maïs INRA-33) ont été analysés pour vérifier la qualité des résultats .

Pour les expériences avec utilisation du ^{32}P comme traceur des flux de P, la radioactivité dans les minéralisats filtrés (1 ml) est mesurée à l'aide d'un compteur à scintillation liquide (TriCarb 2100, Packard) après l'ajout de 3 mL de liquide scintillant (Insta-gel Plus Packard, Perkin-Elmer, Waltham, Massachusetts, USA). La durée de comptage est d'environ 2 minutes par échantillon. Les comptages de la radioactivité ont été corrigé pour tenir compte de la décroissance radioactive du ^{32}P durant l'expérience.

En considérant que le traceur (^{32}P) et l'élément tracé (^{31}P) ont le même comportement dans le système solution nutritive/plante et en absence de fractionnement isotopique ($^{32}\text{P}/^{31}\text{P}$) au cours de l'absorption racinaire, la quantité du P exogène absorbé (P_{exo} , mg P plant $^{-1}$) peut être calculée selon le principe de dilution isotopique en utilisant l'équation suivante (Morel and Fardeau, 1990) :

$$\frac{R}{P_{sn}} = \frac{r}{P_{exo}} \Rightarrow P_{exo} = \frac{r}{\left(\frac{R}{P_{sn}}\right)}$$

Où : R est la radioactivité totale introduite dans la solution nutritive, r est la radioactivité mesurée dans les échantillons filtrés, et P_{sn} est la quantité totale de P dans la

solution nutritive.

2.4.2 Calcul des indices d'accumulation de biomasse et de P

Plusieurs indices ont été calculés pour caractériser l'allocation de la biomasse et l'efficacité d'utilisation du P à l'échelle de plante (Bovill et al., 2013) :

- shoot :root ratio : rapport entre la biomasse aérienne et la biomasse des racines
- Indice de récolte (Harvest Index, HI) : rapport entre la biomasse des grains et la biomasse aérienne à maturité
- P Harvest Index : rapport entre la quantité de P dans les grains et la quantité de P dans la partie aérienne à maturité
- Efficacité d'utilisation interne du P : efficacité d'utilisation de P de la plante (parties aériennes) pour l'élaboration du rendement en grain (g DW mg P^{-1})
- Efficacité d'absorption du P : quantité de P absorbé par unité de biomasse sèche de racines ($\text{mg P g racine DW}^{-1}$)

2.5 Analyses statistiques

Les analyses statistiques des résultats ont été effectuées à l'aide du logiciel R version 3.4.4 (Team, 2018) en utilisant le package tidyverse (Wickham et al., 2019). Pour chaque variable mesurée (*e.g.* biomasse, rendements, concentration en P), des analyses de variance (ANOVA) à un ou deux facteurs ont été réalisées en prenant en compte le stade de récolte, le traitement en P et le cultivar (seulement pour l'expérience 1). Les hypothèses de normalité des résidus et d'homogénéité des variances ont été vérifiées par le test de Shapiro et de Bartlett. Lorsque des différences significatives sont observées ($P < 0,05$), un test de comparaisons multiples des moyennes entre les traitements et les cultivars à chaque récolte a été réalisé à l'aide du test Différence Significative Minimale (LSD).

Chapitre 3

Accumulation et remobilisation du phosphore chez deux cultivars de blé dur

Ce chapitre correspond à un article publié dans la revue *Plant and Soil* :

El Mazlouzi, M., Morel, C., Robert, T., Yan, B., Mollier, A. (2020). Phosphorus uptake and partitioning in two durum wheat cultivars with contrasting biomass allocation as affected by different P supply during grain filling. *Plant and Soil* 449, 179–192. [doi:10.1007/s11104-020-04444-0](https://doi.org/10.1007/s11104-020-04444-0)

Résumé

Contexte et objectifs

- ❑ L'exportation du phosphore (P) avec les grains est une étape clé dans le cycle du P dans les agrosystèmes. Chez le blé, l'accumulation de P dans les grains provient de l'absorption post-floraison et de la remobilisation du P à partir des réserves préexistantes avant la floraison. La remobilisation permet le recyclage du P lorsque l'absorption racinaire est limitée par la disponibilité en P. L'objectif de cette expérience était d'évaluer les effets de la privation en P, appliqués à partir de la floraison, sur la remobilisation et les teneurs en P des grains chez deux cultivars ayant une capacité d'accumulation de P contrastées. Pour cela, les plantes de blé dur ont été cultivées en hydroponie avec une solution nutritive complète (0.125 mM KH_2PO_4) jusqu'à la floraison. Par la suite, la moitié des plantes a été privée de P (0 mM KH_2PO_4) et l'autre moitié a été maintenue dans la solution nutritive complète pendant toute la période post-floraison.

Principaux résultats

- ❑ En conditions de culture optimales en P, les deux cultivars, *Dakter* et *Sculptur*, diffèrent dans leur capacité d'absorption du P pendant la période de remplissage du grain. *Dakter* maintient l'absorption post-floraison du P jusqu'à des stades tardifs alors que *Sculptur* cesse son absorption plus tôt (très peu d'absorption 20 jours après la floraison). Par contre, en conditions de privation en P, les deux cultivars ont des comportements similaires. Les principaux résultats mettent en évidence l'aptitude du blé dur à maintenir son rendement même si les plantes sont privées de P pendant la période de développement des grains grâce à la remobilisation du P à partir de tous les organes de la plante. Autrement dit, la remobilisation de P peut couvrir les besoins des grains si les plantes sont bien pourvues en P pendant la phase végétative. Cela s'est traduit par une augmentation dans l'indice de récolte du P en réponse à la privation en P. De plus, la privation en P semble provoquer une accélération de la sénescence foliaire chez les deux cultivars. Le calcul de la contribution moyenne de chaque organe au P accumulé dans les grains révèle l'importance de la remobilisation en provenance des feuilles (35%), racines (28%) et tiges (17%). Ces résultats ont permis de mieux appréhender le rôle de la remobilisation dans la nutrition phosphatée du blé dur pendant la période post-floraison.

Phosphorus uptake and partitioning in two durum wheat cultivars with contrasting biomass allocation as affected by different P supply during grain filling

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Abstract

- **Aims:** Phosphorus (P) export with harvested grains is a key step of the P cycle in agroecosystems. In wheat, the accumulation of P in grains originates from both exogenous and endogenous P sources. We investigated the effects of different post-anthesis P supply on P partitioning and P remobilization in two durum wheat cultivars with contrasting biomass allocation.
- **Methods:** Wheat plants were grown on a complete nutrient solution with sufficient P until anthesis. Thereafter, half of the plants were deprived of P and the other half was maintained on the complete nutrient solution. P uptake, allocation, remobilization, and traits related to yield and grain P were determined.
- **Results:** Modifications of post-anthesis P supply had no effect on grain yield. Grain P concentrations at maturity for deprived P supply ranged from 2.2 to 3.4 mg P g DW⁻¹. Without P, net P fluxes to grains essentially came from leaves (35%), roots (28%) and stems (17%). With P, net P fluxes came mainly from post-anthesis P uptake.
- **Conclusions:** Our results suggest that when the P nutrition of durum wheat is limited after anthesis, endogenous P remobilization can sustain grain growth with minor yield penalties if the plants are well supplied during vegetative growth.

Keywords: Phosphorus loading into grains, P remobilization, grain filling, durum wheat, post-anthesis P uptake

3.1 Introduction

Phosphorus (P) is an essential element for plant growth because of its pivotal role in many metabolic processes (Vance et al., 2003). The use of P fertilizers in agriculture is a widespread practice to maintain soil fertility and to ensure the sustainability of crop production (Tilman et al., 2002). However, given the non-renewable nature of this resource, P supply to agriculture will be limited by mineable reserves in the future (Cordell and White, 2014). Thus, reducing the use of mineral P fertilizers and improving P recycling while sustaining crop productivity are crucial to secure future food demand and to preserve our environment from eutrophication problems (Cordell and White, 2014). As it is the case for nitrogen, improving the P efficiency of farming systems can be achieved through the use of P-efficient cultivars and the optimization of crop P management (Veneklaas et al., 2012). The traits associated with P use efficiency (PUE) can be divided into two categories: the traits associated with the P acquisition efficiency and those associated with the internal use efficiency of the acquired P (Rose and Wissuwa, 2012). Another emerging solution is the use of low P grain genotypes that export less P from the field without impairing grain yield and consequently contributing to the sustainability of the farming system (Raboy, 2001; Rose et al., 2013; Wang et al., 2016). Targeting this trait in crops could be part of the solution to minimize P exportation and its associated environmental problems. Therefore, all these approaches require a deep understanding of the processes that determine the relationship between grain yield and grain P content (Veneklaas et al., 2012; Wang et al., 2016).

The demand for P changes over the annual cycle of the wheat plant (Römer and Schilling, 1986). During the vegetative stage, P is required for shoot and root growth (Engels et al., 2012). After the onset of anthesis, the grain becomes the main sink for P (Grant et al. 2001; Gregersen et al. 2008). Thus, the P demand of developing grain can be sustained from continuous post-anthesis P uptake and/or from the remobilization and translocation of endogenous P (Wang et al., 2016). Wheat grain development can be divided into three main phases (Shewry et al., 2012). In the first 14 days following anthesis, the grain grows very rapidly as a result of high cell division rate. During this period, P is required for energy transfer, nucleic acid synthesis and other metabolic and cellular processes (Iwai et al., 2012). Subsequently, grain filling is then initiated and consists of progressive storage of starch, proteins and nutrients (Shewry et al., 2012). This stage lasts between 14 and 20 days. Finally, the last phase is characterized by rapid water loss and a decrease in grain metabolic activity (Ferreira et al., 2012).

Plant vegetative organs play an important role in the whole plant P budget. They could

act as transition storage of P before its subsequent translocation to the growing grains (Wang et al., 2016). Depending on P availability, the proportion of total plant P that is found in grains has been reported to vary from 30% to 90% at maturity (Batten, 1992). In cereals, about 50 to 90% of grain P was estimated to be derived from re-mobilized P from vegetative organs depending on genotypes and growth conditions (Batten et al., 1986; Peng and Li, 2005; Veneklaas et al., 2012). Prior to its translocation to the grain, P must first be remobilized. Several studies showed that senescence in vegetative organs such as leaves induces nutrient remobilization (Engels et al., 2012; Etienne et al., 2018). However, P remobilization may occur also during vegetative growth when plant face P deficiency (Grant et al., 2001). Given its mobility in the phloem and xylem, the P becomes readily available to be transported to sink organs (Engels et al., 2012; Peng and Li, 2005). While extensive studies focused on the effect of early P limitation on plant growth, less effort has been devoted to the understanding of the effect of late P limitation and its consequences on yield and PUE, especially in wheat (Wang et al., 2016; White and Veneklaas, 2012).

In addition, up to 70% of grain carbon (C) is originated from C assimilated during grain filling (Masoni et al., 2007). During the vegetative stage, an insufficient P supply to plants can lead to a reduction in plant growth and to a greater root to shoot ratio (Plénet et al., 2000; Vance et al., 2003). Such a change in biomass allocation pattern is one of the strategies that plants use to increase access to a larger P sources in the soil. Decrease in grain yield and grain number are also reported in cereals (Colomb et al., 2007; Plénet et al., 2000). However, late season P limitation is known to have less impact on grain yield (Batten et al., 1986; Peng and Li, 2005). Batten and Wardlaw (1987) have shown that foliar or root applications of phosphate fertilizers during grain development delay leaf senescence without a significant increase in grain yield in wheat. Accordingly, it can be assumed that grain growth will not be limited by P supply in the post-anthesis period and that P remobilization would be enough to sustain the growing grains. One approach to test this hypothesis is to force the plant to rely on endogenous P source during grain filling. This can be achieved by removing the exogenous P supply during the post-anthesis period and then assessing the potential of P remobilization from wheat organs and their contribution to final grain P. To date, many studies have been done with rice but little information has been reported regarding P remobilization and P partitioning among different organs in wheat under different P supply over the post-anthesis period. For example, Julia et al. (2016) studied P partitioning and remobilization in rice plants and found that post-anthesis P uptake is a critical contributor to grain P content since it represented 40 to 70% of the above-ground P accumulated at maturity.

Durum wheat is one of the most important cereal crops in the Mediterranean area (Villegas et al., 2001). Although genotypic variation for P efficiency has been documented to occur in wheat (Deng et al., 2018; Ozturk et al., 2005; Wang et al., 2010b), there are little insights concerning the possible mechanisms that may underlie these variations, especially during the post-anthesis period. A plant's ability to cope with P limitation does not only depend on its capacity to take up and store P but also involves mechanisms of P transport and P remobilization to sink organs (Hammond et al., 2004; Veneklaas et al., 2012). If large root systems are important for efficient P acquisition, P remobilization is decisive, especially in the case of P limitation during grain development (Wang et al., 2010b). In our experiment, we used one cultivar with high shoot to root ratio and another one with low shoot to root ratio. We hypothesized that the two cultivars with different shoot to root ratio would have different P remobilization profiles during the grain filling period. We further hypothesized that P accumulation during the vegetative stage would be sufficient to supply the durum wheat grain production. Therefore, this study aimed i) to assess the effects of different post-anthesis P supply on the dynamics of P uptake, P partitioning and P remobilization in two durum wheat cultivars with contrasting shoot to root ratio, ii) test whether these processes are driven by biomass allocation, iii) to evaluate to which extent P remobilization could sustain grain growth.

3.2 Materials and methods

3.2.1 Experimental design

Two locally adapted French spring durum wheat cultivars (*Triticum turgidum* L. subsp. *durum*) cv. *Dakter* and cv. *Sculptur* were used in this experiment. *Sculptur* has previously been identified as having higher shoot to root ratio than *Dakter* (Perrier et al., 2016). The two cultivars were grown to the stage of anthesis in complete nutrient solution. After anthesis, half of the plants were P deprived until grain maturity while the other half were kept to grow on a complete nutrient solution (Online Resource 1). Phosphorus content and dry biomass of wheat organs (grains, spikelets, rachis & peduncles, stems, flag leaves and lower leaves, and roots) were determined at four stages during grain filling to quantify the net transfer of P from organs that play the role of P source to organs that play the role of P sink.

3.2.2 Plant growth conditions

Uniformly weighed seeds were surface sterilized in 6% (v/v) sodium hypochlorite (H₂O₂) solution for 10 min and rinsed thoroughly with ultra-pure water. The seeds were then pre-

germinated on moist paper kept in darkness for 3 days at 25 °C. The germinated seeds were then transferred to 50 mL Falcon[®] tubes filled with modified Hoagland nutrient solution ($\frac{1}{4}$ strength for macronutrients and full strength micronutrients). After third leaf emergence, 48 seedlings were transferred to 7.5 L plastic pots (1 plant per pot) containing 5.5 L of modified Hoagland's nutrient solution: 0.125 mM KH₂PO₄, 0.625 mM KNO₃, 0.85 mM KCl, 1.25 mM Ca(NO₃)₂, 0.5 mM MgSO₄, 46.25 μM H₃BO₃, 1 μM MnCl₂, 10 μM ZnSO₄, 2 μM CuSO₄, 0.03 μM (NH₄)₆Mo₇O₂₄, 100 μM NaFe EDTA, 50 mg L⁻¹ SiO₂, 25 μM HEDTA and 2 mM MES buffer. The pH was adjusted to 6 ± 0.5 with KOH. The nutrient solution was aerated and refreshed everyday using an overflow-type system. The pot was arranged in randomized complete block design. To limit the high rate of tillering associated to hydroponic condition (Perrier et al., 2016), only the first five tiller were allowed to grow while the late emerging tillers were removed every 2-3 days. At the onset of flowering, half of the wheat plants (24 plants) were maintained in P sufficient nutrition (0.125 mM KH₂PO₄) and the other half were transferred to a P deprivation treatment (0 mM KH₂PO₄) until maturity (Annexe A1).

Wheat plants were grown under greenhouse conditions, with a photo-period of 16h/8h day/night. Photosynthetically active radiation (PAR), air temperature and relative humidity were recorded every 30 min using a data logger (CR32X sensor, Campbell Scientific). Natural light was supplemented with high-pressure sodium lamps supplying an average photosynthetic photon flux density of 353.5 μmol photons m⁻² s⁻¹ to limit spatial differences in light intensity. The average of air temperature and relative humidity were 22 °C and 58%, respectively. Details on environmental conditions are given in Annexe A2.

3.2.3 Plant sampling and measurements

Phenological stages were closely recorded on main stems for each single plant after head emergence. Durum wheat plants are considered at anthesis stage when 50% of flowers are visible. Leaf senescence was monitored from anthesis until maturity by a non-destructive method, using a hand-held chlorophyll meter (SPAD-502, Minolta, Milton Keynes, Japan).

Harvest days were chosen to cover main grain development phases in wheat (Shewry et al., 2012). Wheat plants were harvested four times: Anthesis, 10, 20, and 42-49 (maturity) days after anthesis according to P supply and cultivar. Harvested plants (44 plants, n=3 at each harvest except at maturity for *Sculptur* where n=4) were cut with scissors and separated into seven parts: grains, spikelets, rachis & peduncles, stems, flag leaves

(including leaf sheaths), lower leaves, and roots (Annexe A1). Stems includes nodes and inter-nodes. Lower leaves are the remaining leaves. Roots were washed 3 times with distilled water to remove the adhered nutrient solution.

Plants organs were oven dried for 3 days at 60 °C, and dry biomass was assessed. At maturity, fresh and dry weights of grains were measured to determine the grain moisture content (GMC). Yield components including grain number (GN) and thousand grain weight (TGW) were measured.

Plant samples were calcined at 550 °C for 5 h. After cooling, ashes were dissolved in 2.5 mL of concentrated nitric acid and slowly heated on a hotplate to evaporate. Then, 5 ml of ultra-pure water was added until only a few drops were left. Mineralized solutions were filtered through ash-free filter paper and diluted to a final volume of 50 ml with ultra-pure water. Phosphorus concentrations in diluted digests (mg P g DW^{-1}) were determined using a malachite green colorimetric method (Van Veldhoven and Mannaerts, 1987). For each given plant compartment, the amount of P content was calculated by multiplying the dry biomass and its measured P concentration. The C and N concentration in the grains were assayed with an elemental analyzer (Flash EA112, ThermoFisher), according the Dumas method.

Whole plant P content (mg plant^{-1}) was determined by summing the P content of grains, spikelets, rachis & peduncles, stems, flag and lower leaves, and roots. Post-anthesis P uptake (mg plant^{-1}) was calculated as the difference between whole plant P at maturity and whole plant P at anthesis. Harvest index (HI) and P harvest index (PHI) were calculated as the ratio between grain weight or grain P content to above-ground biomass or above-ground P content respectively. The PHI is a measure of the efficiency of the allocation of P to the grains.

To characterize the dynamics of P in every organ during the post-anthesis period, net P remobilization from different organs was calculated as the difference between P contents in each organ at maturity and at anthesis. P utilization efficiency (PUtE, g DW mg P^{-1}) was estimated as grain yield per unit of P in the above-ground parts. P uptake efficiency (PUptE, $\text{mg P g root DW}^{-1}$) was calculated as post-anthesis P uptake per unit of root dry biomass.

3.2.4 Statistical analyses

Reported values are mean \pm SE of 3-4 replicates. To determine the statistical significance of the effect of P treatment or cultivar, the means of the different parameters were compared by conducting a two-way analysis of variance (ANOVA). When significant differences are observed ($P < 0.05$), multiple comparisons between treatment and cultivar at each harvest were conducted using Least Significant Difference (LSD) test. All statistical analyses were performed using $\text{\textcircled{R}}$ 3.4.4 statistical software (Team, 2018).

3.3 Results

3.3.1 Plant growth and biomass partitioning between organs during grain filling period

Durum wheat plants reached anthesis on 53 and 46 days after germination for *Dakter* and *Sculptur*, respectively. The most notable visual difference between the two treatments was the delayed senescence in the plant grown in P sufficient supply. This was confirmed by SPAD measurements on flag leaves. Both cultivars had similar chlorophyll contents during the first two weeks. Thereafter, chlorophyll content decreased more rapidly in P deprivation treatment, especially for *Sculptur* (Annexe A3). As a result, wheat plants grown under P deprivation treatment reached maturity earlier (42 days after anthesis for both cultivars) than plants under sufficient P supply (48 and 49 days after anthesis for *Dakter* and *Sculptur*, respectively). At maturity, grain moisture content was below 20 % for all plants, indicating that plants were harvested after grain filling ended, as no further grain filling occurs below 45% (Annexe A4).

The two cultivars differed significantly in total biomass accumulation with the smallest values in *Sculptur*. At maturity, total biomass averaged 26 g for *Dakter* and 22 g for *Sculptur* (Table 3.1). During grain filling, *Dakter* accumulated more biomass than *Sculptur* ($P < 0.05$). In contrast, no significant differences in total biomass were observed between P treatments for each cultivar except for *Dakter* at maturity where total biomass increased by 14 % in P sufficient plants ($P < 0.05$).

Dakter and *Sculptur* have similar patterns of grain development (Fig. 3.1a). Grain weight increased slowly during the first 10 days after anthesis. Thereafter, grain weight increased until maturity. Except leaves, all other organs showed a small increase in weight. In general, *Dakter* had a higher organ biomass than *Sculptur*. Regardless of P treatment, no differences in biomass accumulation among organs were observed except for lower

leaves in *Dakter* ($P < 0.05$).

The two cultivars had different biomass partitioning patterns as shown in Fig. 3.2a. Post-anthesis P supply did not influence the partitioning of biomass between organs ($P < 0.05$). As expected, *Dakter* allocated more biomass to the root than *Sculptur* (22.3% and 10.9 % respectively). The opposite pattern was observed for grain where it accounts for more than 53 % in *Sculptur* and less than 38 % in *Dakter*. The biomass partitioning within organs was not affected by P treatments although the total biomass was reduced by post-anthesis P deprivation for *Dakter*.

Table 3.1 – The impact of different post-anthesis P supply on total plant biomass and total plant P content of the two durum wheat cultivars *Dakter* and *Sculptur*. 0, 10, 20, and 42-49 refer to days after anthesis.

Cultivar	Treatment	Plant biomass (g plant ⁻¹)				Plant P content (mg plant ⁻¹)			
		0	10	20	42-49	0	10	20	42-49
<i>Dakter</i>	P sufficient	11.8 a	17.3 a	21.7 a	28.1 a	50.9 a	89.0 a	119.7 a	162.2 a
	P deprivation	-	16.2 a	22.2 a	24.1 b	-	48.6 b	46.3 c	39.0 c
<i>Sculptur</i>	P sufficient	7.1 b	10.4 b	14.7 b	22.1 b	35.9 b	48.5 b	67.0 b	77.7 b
	P deprivation	-	9.8 b	13.7 b	21.6 b	-	29.3 c	25.3 d	31.5 c
ANOVA	P treatment	-	ns	ns	*	-	***	***	***
	cultivar	***	***	***	***	***	***	***	***
	P x cultivar	-	ns	ns	ns	ns	*	***	***

Different letters within the same column indicate significant differences at $P < 0.05$ between treatments and cultivars. Two-way ANOVA was performed to assess the effects of P treatment and cultivar on total biomass and P content at each harvest time: *, **, *** significant at 0.05, 0.01 and 0.001 level of probability, ns: not significant. Values are means of 3-4 replicates

3.3.2 P concentration in durum wheat organs

Starting from 10 days after anthesis (DAA), grain P concentration decreased in both cultivars immediately in the P deprivation treatment and about 20 DAA for the P sufficient treatment (Fig. 3.1b). At maturity, grain P concentration was 3.4 to 4.4 mg P g⁻¹ DW for *Dakter* and 2.2 to 3.1 mg P g⁻¹ DW for *Sculptur* in the deprivation and sufficient P treatment, respectively.

For *Sculptur*, P concentrations decreased slightly throughout the post-anthesis period in rachis and peduncle, flag and lower leaves, and roots. In stems, P concentrations decreased rapidly only after 20 DAA. Roots P concentration showed a small decrease 10

DAA and then remained constant until maturity. For *Dakter*, flag leaves and spikelets P concentrations decreased in the first 20 DAA and then increased significantly. Lower leaves P increased throughout the post-anthesis period. P concentration in stems showed the same pattern as in *Sculptur*. Under P deprivation treatment, P concentration decreased strongly in all organs during the post-anthesis period for both cultivars (Fig. 3.1b).

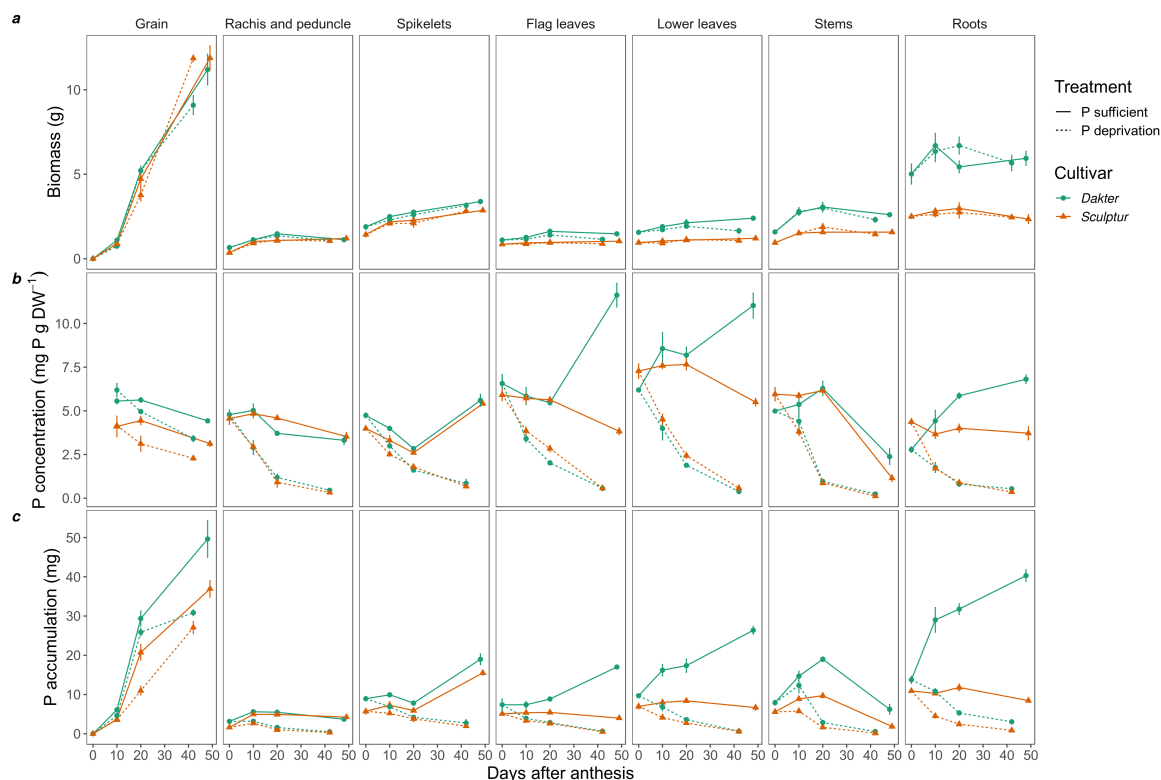


Figure 3.1 – Biomass (a), P concentration (b) and P accumulation (c) among organs of durum wheat cultivars *Dakter* and *Sculptur* grown under P sufficient and P deprivation treatments throughout the post-anthesis period. Each point is the mean of 3-4 replicates \pm SE

3.3.3 Post-anthesis P remobilization and redistribution

Under P sufficient supply, whole P contents increased from 50.9 to 162.2 mg P plant⁻¹ and from 35.9 mg P plant⁻¹ to 77.7 mg P plant⁻¹ from anthesis to maturity for *Dakter* and *Sculptur*, respectively (Table 3.1). Without P in the nutrient solution during grain filling, whole P contents decreased over the post-anthesis period as a consequence of the lack of P in the nutrient solution. This indicates that no P was taken up during this period. The small decrease might be due to P loss and efflux. Higher P contents in

Dakter compared to *Sculptur* at the P sufficient treatment was due to higher P uptake during the post-anthesis period (111.4 vs 41.7 mg P plant⁻¹, respectively). The large part of accumulated P in *Sculptur* was absorbed between anthesis and 20 days after anthesis, whereas *Dakter* accumulated P during the whole post-anthesis period.

Grain P contents increased as grains matured in both treatments (Fig. 3.1c). It was higher under P sufficient than under P deprivation treatment. Stem P contents increased during the first 10 and 20 days after anthesis for P deprivation and P sufficient treatment respectively before a huge decrease towards maturity. For *Dakter* under post-anthesis P sufficient treatment, P contents in all organs increased. Contrariwise for *Sculptur*, P contents in flag and lower leaves and roots were constant between anthesis and maturity.

As expected, P deprivation treatment led to a strong decrease in P content in all organs except grains indicating that there was a net export of P from these organs to the grain (Fig. 3.1c). Spikelets was a sink for P at maturity for both cultivars at sufficient P supply. Also for *Dakter*, flag and lower leaves were still accumulating P throughout the post-anthesis period for this treatment.

The two cultivars had different P partitioning patterns as shown in figure Fig. 3.2b. Under P sufficient supply, *Sculptur* allocated more than 47% of total P to the grain whereas *Dakter* allocated only 30%. The P partitioning during the grain filling period between the different organs is significantly affected by the lack of P in the nutrient solution. The difference between cultivars was smaller under P deprivation treatment where 86 and 79 % of P were allocated to grain for *Dakter* and *Sculptur*, respectively, which indicates that all organs acted as source of P for grain during this period. This enhanced P remobilization indicates that P could be remobilized from all organs.

3.3.4 Grain yield, its components and the indicator of P use efficiency

The yield and yield components varied among treatments and cultivars (Table 3.2). Post-anthesis P treatment had no impact on grain yield and grain number ($P < 0.05$). Grain yield averaged 10.2 and 11.8 g per plant for *Dakter* and *Sculptur*, respectively. Thousand grain weight was reduced significantly only for *Sculptur*. *Dakter* had larger grain but less grain per head in comparison to *Sculptur* with smaller grain but more grain per head. The P utilization efficiency (PUtE) differed between cultivars and P supply.

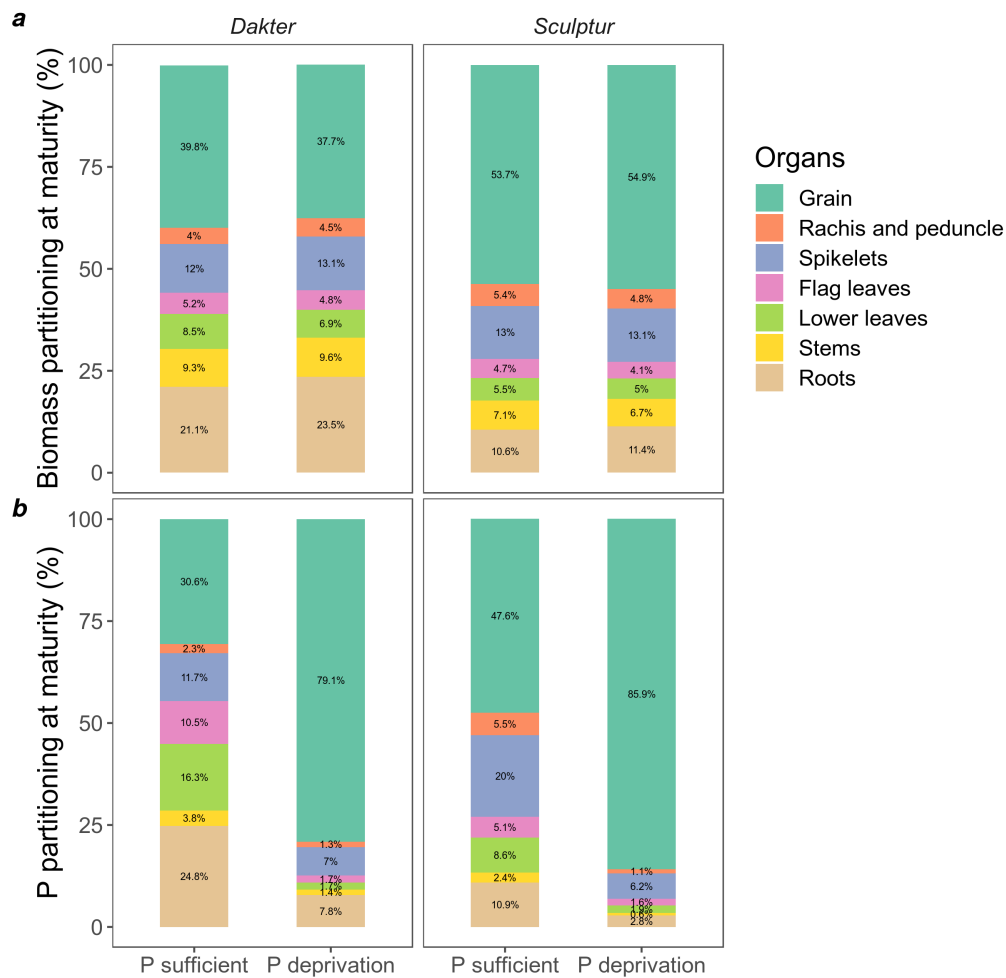


Figure 3.2 – Biomass (a) and P partitioning (b) to organs of durum wheat cultivars *Dakter* and *Sculptur* grown under P sufficient and P deprivation treatments throughout the post-anthesis period

Regardless of the post-anthesis P supply, *Sculptur* had a higher PUE compared to *Dakter*, 0.17 and 0.28 g DW mg⁻¹ respectively, indicating that *Sculptur* is more efficient in using the acquired P to produce grain yield than *Dakter*. In contrast, P uptake efficiency (PUptE) did not differ significantly among cultivars (18 mg P g root DW⁻¹) indicating that they have the same capacity to take up P during the post-anthesis period. Thus, in-between cultivar differences are largely explained by the greater root system size for *Dakter* in comparison with *Sculptur*.

C:N ratio was 11 and 15 for *Dakter* and *Sculptur*, respectively, indicating that *Dakter* has a higher grain protein content than *Sculptur*. There were no significant impacts of post-anthesis P deprivation on grain C:N ratio. The HI was 50% and 61 % for *Dakter* and *Sculptur*, respectively (Table 3.2). The phosphorus harvest index (PHI) was 86 and

41% for *Dakter* under P deprivation and P sufficient treatment, respectively, and from 89 and 53% for *Sculptur*. The shoot to root ratio was 3.5 in *Dakter* and 8.2 in *Sculptur*.

3.3.5 P budget at whole plant level during grain filling

The pattern of P uptake and remobilization differed between cultivars and post-anthesis P supply (Fig. 3.3). Post-anthesis sufficient supply lowered P remobilization while it was enhanced in the P deprivation treatment. Under sufficient post-anthesis P supply, P uptake was important for both cultivars accounting for 68% and 51% of total P for *Dakter* and *Sculptur*, respectively. *Dakter* grain P mainly originated from post-anthesis P uptake while more than 20% originated from remobilized P from stems, roots and leaves for *Sculptur*. The P taken up by *Dakter* were mainly allocated to roots, leaves and spikelets (Fig. 3.3a).

In contrast, under post-anthesis P deprivation, no exogenous P uptake was possible. Thus, all P accumulated in the grain came from P remobilization of plant organs (Fig. 3.3b). One important result is that all vegetative organs and roots acted as a net source for P. Organs contribution to grain P followed this ranking: leaves > roots > stems > spikelets and finally rachis and peduncle. Phosphorus efflux accounted for 28% and 14% of total P remobilized at maturity, for *Dakter* and *Sculptur*, respectively.

Table 3.2 – The impact of different post-anthesis P supply on grain yield, grain P content, thousand grain weight (TGW), grain number per plant (GN), harvest index (HI), P harvest index (PHI), shoot to root ratio (SR ratio), carbon to nitrogen ratio (C:N ratio), P utilization efficiency (PUtE) and P uptake efficiency (PUptE) in two durum wheat cultivars *Dakter* and *Sculptur*.

Cultivar	Treatment	Grain yield (g)	Grain P content (mg)	TGW (g)	GN	HI	PHI	SR ratio	C:N ratio	PUtE (g DW mg P ⁻¹)	PUptE (mg P g root DW ⁻¹)
<i>Dakter</i>	P sufficient	11.2 ab	49.6 a	55.3 a	202.3 bc	0.50 b	0.41 c	3.8 b	10.9 b	0.09 d	18.5
	P deprivation	9.1 b	30.8 bc	52.9 ab	172.7 c	0.49 b	0.86 a	3.3 b	11.3 b	0.25 b	-
<i>Sculptur</i>	P sufficient	11.9 a	36.9 b	55.2 a	214.8 ab	0.60 a	0.53 b	8.6 a	15.6 a	0.17 c	17.9
	P deprivation	11.9 a	27.1 c	48.8 b	243.3 a	0.62 a	0.89 a	7.8 a	15.1 a	0.39 a	-
ANOVA	P treatment	ns	***	**	ns	ns	***	ns	ns	***	-
	cultivar	*	**	ns	**	***	**	***	***	***	ns
	P x cultivar	ns	ns	ns	*	ns	*	ns	ns	ns	-

Different letters within the same column indicate significant differences at $P < 0.05$ between treatments and cultivars. Two-way ANOVA was performed to assess the statistical significance of P treatments and cultivars on each parameter: *, **, *** significant at 0.05, 0.01 and 0.001 level of probability, ns: not significant. Values are means of 3-4 replicates

3.4 Discussion

We assessed the effect of different post-anthesis P supply on the dynamics of P uptake, P partitioning and P remobilization in two durum wheat cultivars with contrasting biomass allocation. This study attempts to answer the question to what extent endogenous P could sustain grain growth. Phosphorus deprivation treatments were imposed during the grain filling period which allowed us to calculate the net P fluxes from vegetative organs towards the grains. The quantification of these net P fluxes is a key element for developing more efficient P management in cropping systems.

3.4.1 Impact of different post-anthesis P supply on biomass accumulation and grain yield

At anthesis, P concentrations in the plant organs were above the critical value of 4 mg P g DW⁻¹ reported for wheat (Römer and Schilling, 1986; Veneklaas et al., 2012), indicating that plants were well supplied with P during vegetative stages. *Dakter* continued to take up P during the whole post-anthesis period while *Sculptur* maintained P uptake only between anthesis and 20 days after anthesis (Table 3.1). Different post-anthesis P treatments had no effect on grain yield. This result is a direct demonstration that P remobilization could be sufficient to sustain grain growth if plants are well supplied with P during the vegetative stage. Since the number of tillers is determined before anthesis, the exogenous sufficient P supply is more critical during the vegetative stage than during the grain filling stage when the potential yield is already determined.

This finding is consistent with that of Peng and Li (2005) who found that withdrawing P from the nutrient solution during flag leaf expansion did not influence grain growth even if there was a reduction in total biomass accumulation. In addition, late foliar applications of P delayed leaf senescence without a significant increase in grain yield (Batten and Wardlaw, 1987). These studies and our results support the idea that P absorbed throughout the post-anthesis period does not contribute significantly to increase grain yield (Batten et al., 1986; Grant et al., 2001; Peng and Li, 2005). In fact, previous studies have reported that P uptake is more critical during vegetative stages than during grain filling in wheat (Grant et al., 2001; Rose et al., 2007). Indeed, since P treatments started after flowering, yield potential for both treatments is assumed to be the same. Hence, grain number is already set up whereas grain weight will rely on post-anthesis carbon (C) supply (González et al., 2011). To maintain C supply to grains as long as possible, plants might remobilize less essential P compounds. This hypothesis is consistent with the results of Jeong et al. (2017b) who showed that rice plants first remobilize less essential

P pools such as vacuolar phosphate and phospholipids. In that way P can be recycled within the plant organs without affecting grain yield.

In addition, the P deprivation treatment has no effect on grain yield. Consequently, the requirements of P during early grain development is fulfilled through P remobilization. At later stage, P is stored in the form of phytic acid in grains (Iwai et al., 2012; Raboy, 1997). This would imply that the reduction of grain P content found under P deprivation treatment is due to a decrease in phytic acid content. This is found to be the case for bread and durum wheat (Batten et al., 1986), barley, maize and soybean where grain P and phytic acid P are found to be highly correlated (Raboy, 1997, 2001). Thus, the variation in grain P found in our study is likely due to the variation in phytic acid P content.

3.4.2 Net P fluxes at whole plant level

Under post-anthesis P sufficient supply, grain P was largely fulfilled by continuous P uptake and to a lesser extent by remobilization of endogenous P (Fig. 3.3a). This finding was confirmed by remarkable increases in P concentration found in some organs over the post-anthesis period, especially for *Dakter*, indicating that P is preferentially distributed to the apical organs (Fig. 3.1c). Similarly, the increase of stem P contents during the first 20 days after anthesis and also the increase of P content in spikelets at maturity may suggest that stems and spikelets act as a temporary storage organs of P before its translocation to the grain. In line with this hypothesis, Julia et al. (2016) reported little direct P allocation to grain throughout the post-anthesis period in rice grown under hydroponic condition. In the present study, the wheat plants received ample P during the vegetative stage. Thus, high P remobilization would be the major processes determining grain P under P deprivation treatment. In the fields, this may be related to the limitation of post-anthesis P uptake due to lower P availability (e.g. drier soil, depletion of P around the roots) or lower demand because plants can efficiently remobilize the acquired P before anthesis. However, this is not the case for all species because other studies conducted with canola showed that post-anthesis P supply can be critical if the plants have not accumulated enough P during the vegetative stage (Rose et al., 2007).

As mentioned earlier, post-anthesis P uptake was not associated with any increase in grain yield. P is a mobile element in both phloem and xylem and its transport within the plant organs depend on developmental stage and P supply (Engels et al., 2012). In our study, the cultivar with low shoot to root ratio continued P uptake until a later stage while the other cultivar slowed its uptake earlier. This finding is in contrast with some

field studies that showed that P uptake does not always occur in wheat at later stages after anthesis (Batten, 1992; Masoni et al., 2007; Rose et al., 2007). One explanation could be that environmental conditions, especially under field conditions, are generally not favorable for post-anthesis P uptake rather than the plant's ability itself. Furthermore, the grain filling period often coincides with drought period which limit water and nutrient availability, including P (Villegas et al., 2001). Another explanation could be that under hydroponic conditions, the decline of root activity is less marked than in field conditions which may lead to an overestimation of the contribution of post-anthesis root P uptake. However, the regulation of root activity and root nutrient remobilization during this period is not fully understood (Wang et al., 2016).

On the other hand, under post-anthesis P deprivation treatment, P grain requirements are mainly coming from remobilization and re-translocation of pre-anthesis P in different organs (Fig. 3.3b). As a result, P distribution at the whole plant level was strongly affected, leading to a high P remobilization efficiency. The main contributors to grain P were in decreasing order leaves > roots > stems and then spikelets, indicating that P remobilization can occur in all organs for both cultivars. The optimization of the internal P use is a common response of plants to P limitations (Hammond et al., 2004). As P deprivation sets in, plants start to remobilize P from non metabolic P compounds such as vacuolar P. The replacement of membrane phospholipids by sulfolipids and galactolipids is also an important mechanism in recycling P within the plant to supply growing tissues (Jeong et al., 2017b; Veneklaas et al., 2012). In addition, considerable amounts of P between anthesis and maturity was lost for both cultivars. This P loss was attributed to efflux of P from the roots. P efflux in wheat is reported to changes in the presence of different levels of P supply (Cogliatti and María, 1990). These authors found that P efflux from wheat roots has increased from 28% of influx at 50 μM to 90% at 5 mM.

The differences in post-anthesis P uptake between the two cultivars resulted in differences in grain P content at maturity ($P < 0.05$). The cultivar with high shoot to root ratio remobilized more than 7 mg of P while the other cultivar remobilized only 1.6 mg of P per plant. This means that with a high shoot to root ratio, the reduced P uptake due to small root system was compensated by a greater P remobilization of P from vegetative organs and roots. This difference for post-anthesis root P uptake suggests that root activity may be regulated independently from remobilization which has major implications for crop breeding. These results indicate that an improved P remobilization during the post-anthesis period could be an important lever to maximize the whole plant P use efficiency.

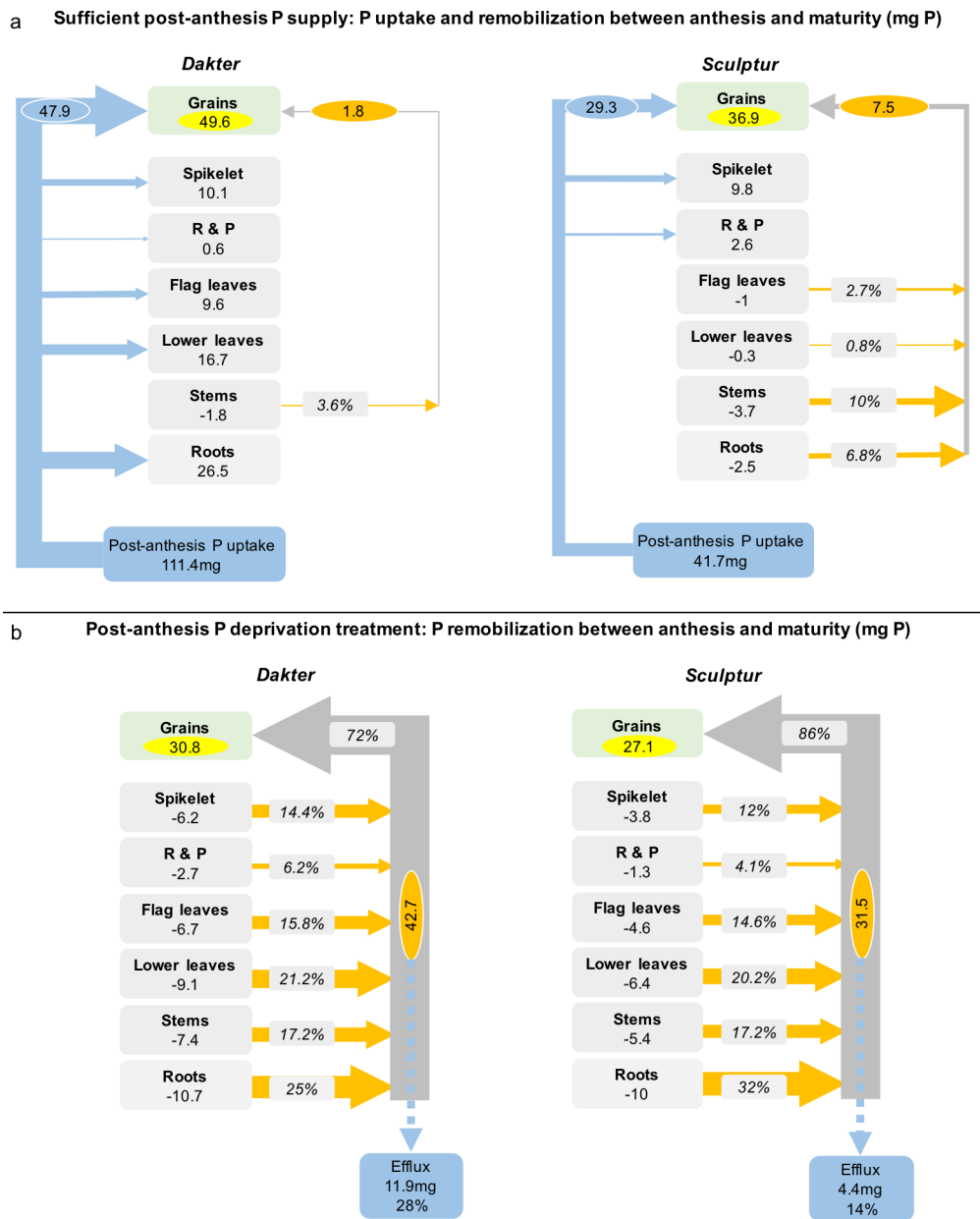


Figure 3.3 – Net P fluxes (mg P plant⁻¹) from anthesis to maturity among organs of durum wheat cultivars *Dakter* and *Sculptur* grown under P sufficient (a) and P deprivation (b) treatments throughout the post-anthesis period. Net fluxes of P from remobilization (-) or post-anthesis P uptake (+) in the different organs are expressed as mg P organs⁻¹. The percentage (%) are the contribution of P remobilization in each organs to grain P contents. Efflux refer to the loss of P by the roots. R & P are rachis and peduncle. Data are means of 3-4 replicates

3.4.3 Biomass and P allocation relationship

To answer the question whether the difference in biomass allocation has an impact on P remobilization, we looked at the net fluxes of P at whole plant level. We found no difference between the two cultivars regarding the contribution of the different organs to the remobilization of P even if the allocation patterns of biomass were different. In addition, the P harvest index greatly increased under P deprivation treatment without any increase in harvest index.

Overall, these results suggest an impairment between C and P allocation to the grain. The reason behind this impairment may be that the allocation of C and P to grain is regulated independently in the P deprivation treatment. First, P and C flux into grain is largely provided by different sources (Masoni et al., 2007). In wheat, grain C is mainly supplied via photosynthesis and stem C remobilization while grain P is provided via P remobilization from other plant tissues (Masoni et al., 2007; Peng and Li, 2005). Secondly, although the transport via xylem is possible, the exportation of P to the grain is mostly covered through the phloem unlike C which is only transported via phloem (Engels et al., 2012; Maillard et al., 2015; Peng and Li, 2005).

In the present study, post-anthesis P supply had no effect on grain C:N ratio (Table 3.2). Wheat grain N is known to be mainly supplied by the remobilization of leaf N providing up to 90% of grain N (Distelfeld et al., 2014; Masclaux-Daubresse et al., 2008). In our study, P remobilization has occurred from all organs. Therefore, this suggests that P remobilization is not necessarily associated with N remobilization, contrary to what we initially expected, or that N remobilization from the leaves was enough to meet grain N demand. In addition, root P remobilization contributed up to 28% of the remobilized P under P deprivation treatment. Nevertheless, under field conditions, the contribution of root N remobilization to grain N is found to be very low (Allard et al., 2013). This variation could be useful in modulating the accumulation of P in wheat grain.

We found also that P deprivation accelerated leaf senescence and shortened the period of grain filling. Our results are in accordance with Maillard et al. (2015), who showed that nutrient deficiency, including P, modulate nutrient remobilization during leaf senescence. In wheat and monocarpic crops, upon the onset of senescence, vegetative organs shift from being a sink to be a source for phloem mobile nutrients (Etienne et al., 2018; Gregersen et al., 2008). Previous studies with bean plants also reported that P remobilization in leaves occurred earlier under low P supply in comparison to high P supply (Snapp and Lynch, 1996).

3.4.4 Grain P and its implications for P management

Without exogenous post-anthesis P supply, the range of grain P concentrations found in the two cultivars was 2.2 to 3.4 mg P g⁻¹ DW. These concentrations are similar to those found by Batten (1992) in tetraploid wheats grown under low P conditions. In fact, grain is a net sink for P after anthesis. Therefore, P and other nutrients are allocated in priority to sustain grain growth (Veneklaas et al., 2012). These concentrations are above those required for normal functioning for a cereal crop. Recent studies have shown that grain P concentrations as low as 0.9 mg P g DW⁻¹ were sufficient for normal functioning during seedling growth of rice (Pariasca-Tanaka et al., 2015). In addition, with almost equal yield, wheat plants grown under continuous post-anthesis P supply would export more P than those grown under P deprivation conditions. Assuming that mean durum wheat yield in France is around 5.7 t ha⁻¹ (AGRESTE, 2019), P export would be equivalent to 22 and 16 kg P ha⁻¹ respectively, which mean an exportation of P 25% higher under continuous P supply. Lowering grain phosphorus concentration is particularly important when one considers that 75% of total P in grain is in the form of phytate (Raboy, 2001). This storage form of P, which is mainly found in cereal aleurone layer, is indigestible by human and monogastric animals. Furthermore, it is also an anti-nutrient due to its capacity to chelate zinc, iron and other micronutrients that are essential to human nutrition (Iwai et al., 2012; Raboy, 2001). This finding suggests that an improved P remobilization while reducing post-anthesis P uptake could be an important way to maximize the whole plant P efficiency and contribute to the optimization of field P balance. In addition to this, recently Yamaji et al. (2017), described a P transporter, SULTR-like phosphorus distribution transporter (SPDT), located in the rice nodes that is involved in P allocation to the grain. Loss of function of this transporter reduced total P content by 20% without yield penalty. This is an interesting aspect because even under high P availability, efficient P remobilization is required to minimize soil P depletion, without neglecting that roots are of great importance for uptake of water and other nutrients. Screening for P transporters responsible for P allocation to the grain is a promising approach to reduce P exportation from the field.

3.5 Conclusion

Our study demonstrated that even under a situation of total limitation of external P supply during durum wheat grains filling, grain P nutrition can be fully ensured by the internal transfer of P from leaves, roots and stems. Our results showed also that all vegetative organs can act as a reserve of P for optimal grain production. Thus, an enhancement of P remobilization during the grain filling while reducing post-anthesis

P uptake could be an important lever to maximize the whole plant P use efficiency. Further research will concentrate on determining the relative contribution of exogenous and endogenous P source to grain P using isotope tracer technique to quantify the gross P fluxes between the different plant organs during grain filling.

Acknowledgements

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Conflict of Interest

The authors declare that they have no conflict of interest.

Chapitre 4

Contribution de la remobilisation et de l'absorption post-floraison au phosphore accumulé dans les grains

Ce chapitre correspond à un article publié dans la revue *Frontiers in Plant Science (Plant Nutrition section)* :

El Mazlouzi M., Morel C., Chesseron C., Robert T., Mollier A. (2020). Contribution of External and Internal Phosphorus Sources to Grain P Loading in Durum Wheat (*Triticum durum* L.) Grown Under Contrasting P Levels. *Front. Plant Sci.* 11:870. doi: [10.3389/fpls.2020.00870](https://doi.org/10.3389/fpls.2020.00870)

Résumé

Contexte et objectifs

- ❑ Les résultats obtenus dans le chapitre précédent ont démontré que la remobilisation du P à partir des organes végétatifs est suffisante pour alimenter les grains en P sans effet majeur sur le rendement et ses composantes lorsque les plantes sont bien alimentées en P avant la floraison. Néanmoins, la remobilisation était faible voire nulle dans le traitement avec une offre continue en P pendant la période post-floraison. L'objectif de cette expérience est d'analyser l'effet de l'accumulation de P pendant les phases végétatives et le prélèvement post-floraison sur l'accumulation de P dans les grains. La quantification de la contribution du P exogène (absorbé pendant la période post-floraison) et de la remobilisation ainsi que l'identification des organes sollicités nécessite l'utilisation du traçage isotopique. Ainsi, les flux de P ont été quantifiés entre la floraison et la maturité dans les différents organes du blé dur (cv. *Sculptur*). Dans cette expérience, les plantes de blé dur ont été cultivées jusqu'à la floraison avec une solution nutritive non marquée à forte et faible concentration en P (0.125 mM vs 0.025 mM). À partir de la floraison, le P de la solution nutritive a été marqué avec du ^{32}P jusqu'à maturité.

Principaux résultats

- ❑ Les résultats montrent que la remobilisation du P endogène a contribué à 81 % du P accumulé dans les grains chez plantes cultivées avec un faible niveau de P tandis qu'elle a contribué à 65 % pour celles cultivées avec un niveau élevé de P. Les organes qui ont été sollicités le plus chez les plantes cultivées avec un faible niveau en P sont les épis (43%) et les feuilles (35%). De plus, les plantes cultivées avec un faible niveau de P ont alloué plus (72 %) du P absorbé au cours de la période post-floraison grains, alors que les plantes cultivées avec un niveau élevé de P (56 %). Nos résultats montrent que, excepté les grains, tous les organes du blé dur peuvent être des sources de P au cours de la période post-floraison. Toutefois, leur contribution au P accumulé dans les grains diffère selon le niveau de P dans le milieu.

Contribution of external and internal phosphorus sources to grain P loading in durum wheat (*Triticum durum* L.) grown under contrasting P levels

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Abstract

— Phosphorus (P) in durum wheat grains after anthesis originates from either the external P source or the internal remobilization of P from different plant organs. The supply of P and its use by the plant are important factors that can affect the contribution of each source to grain P nutrition. Thus, this experiment aimed to quantify the origin of P in grains of durum wheat plants with different P nutritional status. Wheat plants were grown from juvenile stages to maturity in complete nutrient solutions with either high (0.125 mM) or low (0.025 mM) P concentrations in greenhouse conditions. Phosphorus in nutrient solutions was spiked by introducing ³²P after anthesis to quantify the external P uptake and its partitioning within plant organs (spikelets, leaves, stems, roots and post-anthesis tillers) and grains. P use efficiency in durum wheat plants was also determined. The low and high P supply resulted in two highly different nutritional P status. Plants with low P status remobilized most of their stored P in all organs and allocated more than 72% of post-anthesis P uptake to grain P nutrition, whereas in the high P plants this was only 56%. Enhanced remobilization of P and the efficient allocation of newly acquired P to grains were crucial for durum wheat grain P nutrition grown under low P supply. The remobilization of P represented 81% of grain P in low P plants while it represented 65% for high P plants. Organs that contributed the most to P remobilization in low P plants were spikelets (43%) and leaves (35%). The post-anthesis tiller development was reduced in low P plants suggesting a preferential allocation of P to grains under this treatment. We concluded that P loading into grains in durum wheat is mainly derived from the remobilization of internal P sources stored before anthesis, even at high external P supply during grain filling.

Keywords: Durum wheat, Phosphorus, ³²P tracer, grain P concentrations, Post-anthesis P uptake, P remobilization, hydroponic conditions

4.1 Introduction

Phosphorus (P) in soils is one of the most limiting factors for plant growth (Raghothama, 1999). In the conventional farming system, the application of P fertilizers is required to achieve optimal crop yield (Tilman et al., 2002). Phosphorus fertilizer management is mainly driven by the export of P in harvested products (Lott et al., 2000; Rose et al., 2013). Furthermore, P fertilizers are relatively inefficient, as only 20% of the applied P is taken by crops in the first year (Vance et al., 2003). This low efficiency is linked to a substantial negative impact associated with the transfer of P to the aquatic ecosystems (Brinch-Pedersen et al., 2002). In addition, unlike nitrogen, rock phosphate is a non-renewable resource that is subject to fluctuations in supply, which could be problematic to ensure future food security (Cordell and White, 2014; Vance et al., 2003). Thereby, improving P efficiency in cropping systems is of major interest given all these current challenges. Achieving higher P efficiency is possible through a better understanding of the coordination of P uptake, transport, and remobilization in crops (Brinch-Pedersen et al., 2002; Rose et al., 2013; Wang et al., 2016).

Plants can adjust their metabolism in response to P availability (Hawkesford et al., 2012; White and Hammond, 2008b). Although limited P supply might affect many physiological functions such as photosynthesis and energy transfer, it does not immediately lead to deficiency symptoms, except in cases of severe P limitation (Grant et al., 2001). This is because the internal remobilization of P from plant tissues could provide adequate amounts of P to ensure the growth of new sinks (Veneklaas et al., 2012). For cereals such as wheat, limiting P conditions reduce grain yield by limiting the number of productive tillers before anthesis (Horst et al., 1993). This strategy lowers the plant P requirement for biomass and grain production (Horst et al., 1993). Furthermore, low P requirement to produce biomass is associated with P utilization efficiency (PUE, defined here as biomass increase per unit of accumulated P) that was identified as an important target for crop improvement (Manske et al., 2002; Rose and Wissuwa, 2012). Crops with high PUE have typically lower tissue P concentrations and an optimized recycling and redistribution of P within the plant (Veneklaas et al., 2012; White and Hammond, 2008b).

Phosphorus in grains is mainly stored in the form of phytate and play a crucial role in supplying P for seedling before the root development (Nadeem et al., 2011; White and Veneklaas, 2012; Raboy, 2009). However, the concentrations of P in grains are often over the need for germination and plant establishment (Pariasca-Tanaka et al., 2015; Rose et al., 2012; Wang et al., 2017). For example, Julia et al. (2018) reported that rice growth and development was unaffected by seed P concentration at seedling stage because root

P uptake commences at the very earliest stages of plant development (two days after germination). Thus, lowering P concentration in grains would reduce the P export and thus contribute to the long-term sustainability of cropping systems (Julia et al., 2018; Wang et al., 2016). Prior to anthesis, wheat plants can accumulate large amount of P in their organs (Grant et al., 2001; Veneklaas et al., 2012). After satisfying P demand created by different processes, the remaining P might be stored in vacuole which could contain up to 90% of cellular P at adequate P supply (Raghothama, 1999). Its role is to maintain P homeostasis in cytoplasm by adjusting the magnitude of P concentration fluctuations caused by different metabolic activities (Hawkesford et al., 2012; Raghothama, 1999). However, the growing grains became the main sink of P during the post-anthesis period. Plant organs could serve as a reservoir for P that can be remobilized to the developing grains (Dissanayaka et al., 2018; El Mazlouzi et al., 2020b). Thus, the main sources of grain P are the internal P remobilized from different senescing plant organs and post-anthesis P uptake (Grant et al., 2001). Wheat grain P is subject to genetic variability and depends on environmental conditions such as P and water availability (Batten, 1992; Manske et al., 2002). For instance, several studies have shown that low P supply increases the proportion of remobilized P to grains (Batten, 1992; El Mazlouzi et al., 2020b). Until now, the mechanisms of P uptake and transport during early plant growth are well characterized (Aziz et al., 2014; Nadeem et al., 2011). However, studies on the post-anthesis P fluxes in wheat are scarce. Although considerable progress has been made in understanding the processes controlling P fluxes from vegetative to reproductive organs, the mechanisms involved in P loading into grains are, however, not fully understood (Dissanayaka et al., 2018; Wang et al., 2016).

The objective of this study was therefore to quantify the contribution of external and internal P sources to the grain P nutrition in durum wheat plants with contrasted P supply. Our working hypothesis was that plants grown with high P supply are expected to have a large proportion of P stored in different plant organs and would rely on these internal sources to supply the grain with P. In contrast, plants grown under low P supply are expected to maintain P uptake and optimize P remobilization to fulfil the grain P requirement. Therefore, plants of the latter group would depend on both external post-anthesis P uptake and a high P remobilization efficiency. The contributions of external P and internally remobilized P as sources for grain P nutrition can be quantified precisely using P tracer. Thus, we performed a ^{32}P -labelling experiment from anthesis to maturity to evaluate the contribution of both sources to grain P nutrition in durum wheat.

4.2 Materials and methods

4.2.1 Experimental set-up, plant growth and P treatments

Hydroponic culture conditions were similar to those previously described by El Mazlouzi et al. (2020b). Briefly, seeds of *Triticum durum* cv. *Sculptur*, a spring durum wheat cultivar widely cultivated in France, were disinfected in 6% (v/v) sodium hypochlorite (H_2O_2) solution and then germinated on moist paper and kept in darkness for 3 days at 25 °C. After germination, seedling were transferred to 50 mL Falcon[®] tubes filled with modified Hoagland nutrient solution. Subsequently, fifteen days old plants of similar size were selected and transplanted to black plastic pots (1 plant per pot) containing 5.5 L of modified Hoagland nutrient solution. The nutrient solution had the following initial composition: 0.625 mM KNO_3 , 0.85 mM KCl , 1.25 mM $\text{Ca}(\text{NO}_3)_2$, 0.5 mM MgSO_4 , 46.25 μM H_3BO_3 , 1 μM MnCl_2 , 10 μM ZnSO_4 , 2 μM CuSO_4 , 0.03 μM $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$, 100 μM NaFe EDTA , 25 μM HEDTA , 2 mM MES buffer and 50 mg L^{-1} SiO_2 . Phosphorus was supplied in nutrient solution from juvenile stages (three-leaf stage) to maturity as KH_2PO_4 at concentrations of 0.025 or 0.125 mM, designated hereafter in text as low and high P supply, respectively. These two levels of P supply were set to induce a differential plant P nutritional status (Figure 4.1). KCl was added to the low P supply to ensure the same potassium concentration for both treatment. The initial pH was adjusted to 6.0 ± 0.5 using solid KOH . The nutrient solution was mixed by air-bubbling and refreshed continuously by an overflow-type system. Since high rate of pre-anthesis tillers are common in hydroponic culture, only the first four tillers were allowed to grow. Tillers developed after anthesis were allowed to grow during the labelling period (see next section). The pots were arranged in a randomised block design with three replicates of each treatment.

Plants were grown in greenhouse under a day/night cycle of 16/8 h. The average air temperature and relative humidity were 17 °C and 64%, respectively. Natural light was supplemented with LED lamps (model LED, Ledlyt, France) providing an average of photosynthetic photon flux density of 450 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$. Air temperature and relative humidity during the growing period are presented in the Annexe B. Three replicates of each P treatment with synchronized plant development were harvested at anthesis to determine their P status. Three other replicates were selected for the ^{32}P -labelling experiment and harvested at maturity.

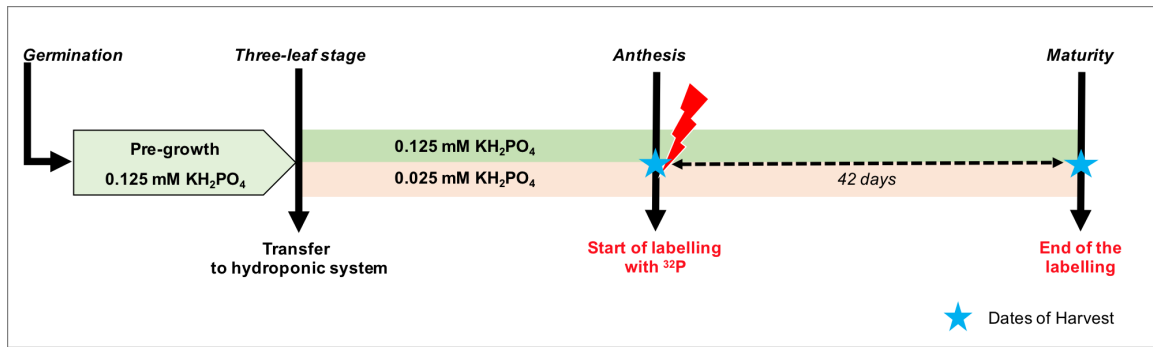


Figure 4.1 – Schematic overview of the experimental design used in the experiment. After third leaf emergence, durum wheat plants were grown in nutrient solution with two levels of P supply. Immediately after anthesis three plants of each treatments were labelled with ³²P until maturity. The double-sense arrow indicates the labelling period and stars indicate the date of harvests. Growth conditions (temperature and relative humidity) are presented in the Annexe B

4.2.2 ³²P labelling of phosphate ions in nutrient solutions

The labelling experiment was carried out in a greenhouse dedicated to handle radioisotopes. The environmental conditions were similar to those described above. To prepare the labelled nutrient solution on the day of anthesis, 24 L of the nutrient solution with the same composition was prepared for each treatment as mentioned earlier. Then, the low and high P nutrient solutions were spiked with about 8 and 4 MBq of H₃³²PO₄ with specific radioactivity of 325 TBq/mmol (NEX054010MC, Perkin Elmer). Three replicates of one wheat plant were transferred to pots containing 5 L of each labelled nutrient solution. Before changing the nutrient solution, the root systems of the wheat plants were carefully washed three times. Thereafter, because of experimental constraints and to limit exposure to radiation, nutrient solutions were not refreshed continuously but resupplied twice a week with of the same labelled nutrient solution until harvest. The nutrient solution was aerated continuously. Before each resupply, the ³²P-labelled nutrient solution was stirred thoroughly using a small water pump. The labelling period lasted for 42 days (Figure 4.1).

4.2.3 Plant sampling and measurements

To ensure plant sampling homogeneity, heads that reached anthesis on the same day were tagged. At anthesis and 42 days after anthesis, plants (3 replicates) were harvested and divided into the following organs: roots, stems, lower leaves, flag leaves, spikelets (including rachis) and grains. The tillers developed after anthesis were pooled. Roots were washed with deionized water three times to remove the adhered nutrient solution. Plant organs were then oven-dried at 60°C for 72h and their dry weights were determined.

The fresh weight of grain at maturity were determined to calculate the grain moisture content (GMC). In addition to grain yield, grain number per plant and thousand-grain weight were also determined.

4.2.4 Phosphorus concentration in plant organs

The P concentration (mg g^{-1}) in roots, stems, lower leaves, flag leaves, spikelets, grains and pooled tillers was determined using the same procedures as described in El Mazlouzi et al. (2020b). Briefly, sub-samples of plant material were ashed at 550°C for 5 h. The ashes were dissolved in 2.5 mL nitric acid and placed on a hotplate to evaporate. After cooling, the mineralized samples were filtered and diluted to 50 mL in ultra-pure water. Phosphorus concentration (mg g^{-1}) was then determined using the malachite green colorimetric method (Van Veldhoven and Mannaerts, 1987). The P amount in plant organs was calculated by multiplying their respective P concentrations by dry weight. The P amount in the whole plant (mg P plant^{-1}) was calculated as the sum of P amount measured in each organs.

4.2.5 Determination of ^{32}P activity and parameters of biomass and P partitioning

A sub-sample of 1 mL of each mineralized and filtered samples and 3 mL of scintillation cocktail for aqueous solution (Insta-gel Plus Packard, Perkin-Elmer, Waltham, Massachusetts, USA) were placed in a glass scintillation vial (SimportTM Scientific S207). Thereafter, the ^{32}P -activity in filtered samples were measured using a liquid scintillation counter (TriCarb 2100, Packard). All ^{32}P countings were corrected for the radioactivity decay.

External P uptake from the nutrient solution P was quantified by applying isotopic dilution principles and assuming that no $^{32}\text{P}/^{31}\text{P}$ -fractionation occurred during external P uptake by roots and P transport within the plant. Considering that R/P_{ns} is the specific activity measured in the nutrient solution, and r is ^{32}P -activity measured in the mineralized samples, then the following calculations can be used to calculate the amount of P in each organ that derives from the external nutrient solution (P_{ext} , mg P plant^{-1}) (Morel and Fardeau, 1990):

$$\frac{R}{P_{ns}} = \frac{r}{P_{ext}} \Rightarrow P_{ext} = \frac{r}{\left(\frac{R}{P_{ns}}\right)}$$

where R and P_{ns} are the amount of ^{32}P and ^{31}P measured in the nutrient solution, respectively. Thus, post-anthesis P uptake is the sum of external P measured in all plant

organs.

The total amount of remobilized P was calculated by subtracting the amount of P originating from post-anthesis P uptake in net sink organs (grains and post-anthesis tillers) from whole plant P amount. However, the amount of remobilized P from each plant organs that acted as source of P (spikelets, leaves, stems and roots) was calculated as the difference in P amount between anthesis and maturity. All these fluxes were expressed as the percentage of P at whole plant level. The efficiency of P remobilization (%) was calculated as $(\text{total P remobilization})/(\text{total P anthesis}) \times 100$. The efficiency of P utilization (%) was calculated as the ratio of grain yield to the amount of P in whole plant.

Shoot:root ratio was calculated as the ratio of total biomass in the aboveground parts and root biomass. Harvest index (HI, %) and P harvest index (PHI,%) were calculated as grain dry biomass or amount of P in grain as a percentage of the aboveground biomass or aboveground P, respectively. Although the post-anthesis tillers have produced spikes, these did not contain viable grains as they did not reach maturity and are therefore not included in the harvest index calculations.

4.2.6 Statistical analysis

Statistical analyses were undertaken using R statistical software version 3.4.4 (Team, 2018). Data were expressed as mean \pm standard error (SE) of three replicates. Significant differences between P treatment and harvest stages (anthesis vs maturity) were determined separately using Student's t test ($P < 0.05$).

4.3 Results

4.3.1 Plant growth and grain characteristics

Durum wheat (cv. *Sculptur*) plant biomass increased from anthesis to maturity in both P supply. Nevertheless, plants grown under low P supply produced fewer post-anthesis tillers than those grown at high P supply ($P < 0.05$), which led to significantly lower total biomass in these plants at maturity (Table 4.1). The biomass of spikelets, stems and roots were also increased while biomass of leaves remained relatively constant throughout the post-anthesis period. At maturity, tillers biomass represented 28% of total biomass at high P supply while it represents only 4% at low P supply.

Unlike the expectation, there was no significant effect of the low P supply on grain

yield and grain number, but thousand grain weight was significantly decreased ($P < 0.05$). The high variability in grain number obtained by each plant under low P supply might explain this result (Table 4.2). Low P plants invested more biomass into roots which resulted in lower shoot to root ratio in comparison to high P plants (5.9 ± 0.2 and 7.4 ± 0.1 , respectively). The harvest index of low P plants were higher than those of high P plants. Accelerated leaf yellowing was observed at maturity for the plants grown under low P supply in comparison to high P supply plants (Figure 4.2). Consistent with the accelerated senescence, grain moisture content was slightly lower in low P plants (high P: 40% , low P: 36%).

Table 4.1 – Biomass (g), P concentration (mg g^{-1}) and P amount (mg) at anthesis and maturity of different durum wheat organs grown under high P (0.125 mM) and low P (0.025 mM) supply.

		Biomass (g)							
Stage	P supply	Grain	Spikelets	Flag leaves	Lower leaves	Stems	Roots	Post-anthesis tillers	Whole plant
Anthesis	High P	-	1.6 ± 0.1	0.88 ± 0.08	1.2 ± 0.07	1.8 ± 0.1	2.4 ± 0.3	-	7.9 ± 0.7
	Low P	-	1.6 ± 0.02	0.97 ± 0.02	1.3 ± 0.04	1.8 ± 0.06	2.1 ± 0.1	-	7.8 ± 0.2
Maturity	High P	11.3 ± 0.3	2.8 ± 0.2	0.99 ± 0.02	1.1 ± 0.06	3.5 ± 0.1	4.0 ± 0.1	9.5 ± 0.5	33.2 ± 1.1
	Low P	9.7 ± 0.5	3.1 ± 0.2	0.95 ± 0.02	1.1 ± 0.07	3.6 ± 0.1	$3.3 \pm 0.1^*$	$0.9 \pm 0.1^{***}$	$22.7 \pm 0.9^{**}$
		P concentration (mg g^{-1})							
Anthesis	High P	-	5.4 ± 0.1	5.8 ± 0.3	6.4 ± 0.3	6.3 ± 0.5	4.1 ± 0.4	-	-
	Low P	-	$3.6 \pm 0.3^*$	$2.7 \pm 0.1^{**}$	$1.7 \pm 0.1^{**}$	$1.3 \pm 0.2^{**}$	$1.5 \pm 0.4^*$	-	-
Maturity	High P	3.8 ± 0.1	1.6 ± 0.3	2.1 ± 0.2	2.6 ± 0.2	1.2 ± 0.3	1.3 ± 0.2	1.4 ± 0.1	-
	Low P	$1.8 \pm 0.1^{***}$	$0.3 \pm 0.1^*$	$0.4 \pm 0.1^*$	$0.3 \pm 0.1^{**}$	0.2 ± 0.3	$0.6 \pm 0.1^*$	0.6 ± 0.1	-
		P amount (mg)							
Anthesis	High P	-	8.9 ± 0.9	5.2 ± 0.7	7.4 ± 0.7	11.4 ± 1.5	9.8 ± 1.6	-	42.7 ± 5.4
	Low P	-	5.9 ± 0.4	2.6 ± 0.2	$2.1 \pm 0.1^{***}$	$2.2 \pm 0.3^{***}$	$3.0 \pm 0.1^*$	-	$15.8 \pm 0.9^*$
Maturity	High P	42.9 ± 0.7	4.7 ± 1	2.1 ± 0.2	2.9 ± 0.1	4.2 ± 0.9	5.1 ± 0.7	13.6 ± 1	75.5 ± 2.6
	Low P	$17.3 \pm 0.9^{**}$	$0.9 \pm 0.2^{**}$	$0.4 \pm 0.04^*$	$0.4 \pm 0.1^{**}$	$0.7 \pm 0.04^*$	2 ± 0.5	$0.5 \pm 0.1^{***}$	$22.2 \pm 0.9^{***}$

Values are means \pm standard error of 3 replicates. For each stage, means followed by asterisk indicate significant difference between P treatment (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$).

4.3.2 Phosphorus concentrations and partitioning among plant organs

At both anthesis and maturity, P concentration in all durum wheat organs were significantly lower in low P plants compared with the high P plants ($P < 0.05$) (Table 4.1). From anthesis to maturity and regardless of P supply, P concentration decreased in all plant organs. At anthesis, the highest P concentration in plant organs was observed in leaves ($6.4 \pm 0.3 \text{ mg g}^{-1}$) for high P supply plants whereas it was observed in spikelets for

low P supply plants ($3.6 \pm 0.3 \text{ mg g}^{-1}$). The average P concentration in the grains was two fold higher under high P supply than under low P supply (high P: $3.8 \pm 0.1 \text{ mg g}^{-1}$, low P: $1.8 \pm 0.1 \text{ mg g}^{-1}$) (Table 4.1). The extent of change observed in P concentrations between anthesis and maturity in low P plants was different from that observed for high P plants. This extent was more pronounced for low P plants reaching concentration as low as $0.2 \pm 0.3 \text{ mg g}^{-1}$ in stems for example suggesting an enhanced P remobilisation in those plants.

As expected, the high P plants accumulated a significantly greater amount of P than the low P plants ($P < 0.05$). Whole plant P amount increased from $42.7 \pm 5.4 \text{ mg}$ to $75.5 \pm 2.6 \text{ mg}$ and from 15.8 ± 0.9 to 22.2 ± 0.9 in high P and low P plants between anthesis and maturity, respectively (Table 4.1). The amount of P in grain and tiller increased during grain filling in high P plants while the grain was the only sink of P in low P plants. The amount of in roots, stems, leaves and spikelets decreased in both P treatment during grain filling indicating net P remobilization from these organs. However, P remobilization in low P plants was more pronounced than in high P plants leading to P amount as low as 0.4 mg P in flag leaves for example.

At maturity, P partitioning between different plant organs was influenced by P supply. Under high P supply, grain P represented 57% of total P at whole plant levels while post-anthesis tillers represented 17%. In contrast, grain P represented 78% of total P under low P supply indicating a high rate of P allocation to grains. The P harvest index calculated as P amount in grain to the aboveground P was significantly higher in low P plants in comparison to high P plants. Efficiency of P utilization (defined as the ratio of grain yield to whole plant P amount) was significantly lower in high P plants ($15 \pm 0.9\%$) compared to low P plants ($43.7 \pm 0.6\%$). The efficiency of P remobilization was slightly higher in low P plants but was not significantly different between high and low P supply.

4.3.3 Post-anthesis P uptake and remobilization of P from organs

The calculations undertaken using ^{32}P as tracer showed that durum wheat plants had absorbed about $4.5 \pm 0.5 \text{ mg P}$ and $26.6 \pm 2.1 \text{ mg P}$ for low and high P supply from anthesis to maturity, respectively (Figure 4.3). This is equivalent to, respectively, 20% and 35% of total P uptake. In high P plants, 56% is recovered in grain and 22% in tillers. The remaining external P is allocated to roots, spikelets and leaves. In contrast, grains in low P

Table 4.2 – Grain number, thousand grain weight (TGW), grain moisture content (GMC), shoot:root ratio, harvest index (HI), P harvest index (PHI), efficiency of P remobilization, and efficiency of P utilization in high P and low P durum wheat plants.

	High P plants	Low P plants
Grain number (plant ⁻¹)	245 ± 4	237 ± 11
TGW (g)	46.1 ± 1.03	40.9 ± 0.4*
GMC (%)	40 ± 2	36 ± 2
Shoot:root ratio	7.4 ± 0.1	5.9 ± 0.2**
HI (%)	39 ± 1	50 ± 1***
PHI (%)	62 ± 3	86 ± 1**
Efficiency of P remobilization (%)	73.3 ± 2.8	82.1 ± 2.3
Efficiency of P utilization (%)	15 ± 0.9	43.7 ± 0.6**

Values are means ± standard error of 3 replicates. Significant difference between high P and low P plants are indicated by asterisks (*P < 0.05; ** P < 0.01; *** P < 0.001).

plant recovered 72% of external P and the remainder is mainly distributed to roots (16%) and other organs, suggesting a tight regulation of P transport and translocation to grains.

All plants organs present at anthesis acted as a source of P as shown by the decline in their P amount at maturity. The total net P remobilized from these organs P was 14.4 ± 0.9 mg P and 35.7 ± 0.4 mg P for low and high P plants, respectively. Stems, leaves and roots have contributed to more than 82% of remobilized P in high P plants (Figure 4.3A). Net remobilization of P was observed in all plant organs. In low P plants, spikelets and leaves contributed to more than 78% of remobilized P (Figure 4.3B). The enhanced in P remobilization contributed to fulfilling grain P requirement in both P treatments. In low P plants, P remobilization from different plant organs represented 81% of grain P while its represent 65% for high P plants.

4.4 Discussion

Phosphorus loading into grains results from post-anthesis P uptake and the remobilization of internal P sources. Both sources contribute to the whole plant P use efficiency. Thus, understanding the relationship between these two components is valuable for engineering crops with improved P nutrition strategies. Tracer methods offer some advantages compared to the budget method that has been widely used to study nutrient fluxes at whole plant level (Masclaux-Daubresse et al., 2008). They provide better estim-



Figure 4.2 – The phenotype of durum wheat plants (cv. *Sculptur*) at maturity. Plants were grown in nutrient solution with high or low P supply from third-leaf stage to maturity.

ation of external P uptake and reduce uncertainties associated with inter-plant variability (Masclaux-Daubresse et al., 2008). In the present study, the use of ^{32}P -tracer enabled us to determine the partitioning of the newly acquired P during the post-anthesis period. It also allowed us to quantify the net contribution of internal P sources to the P allocated to grains at maturity in durum wheat (*Triticum durum* L.).

4.4.1 Effects of P supply on plant growth and P accumulation

The lack of response to P supply in term of biomass production at the anthesis or in grain yield at maturity indicates that the levels of P supply to plants were sufficient for durum wheat growth under our experimental conditions. However, high P plants had accumulated large amounts of P in comparison to low P plants, which resulted in plants with highly different P nutritional status. Phosphorus concentrations were significantly higher in the plants grown at high P supply than in those of plants grown at low P supply (Table 4.1). Leaf P concentration in low P plants at anthesis was slightly below the critical reported value for maximum yield of 3 mg P g^{-1} for wheat (Veneklaas et al., 2012). Nevertheless, this critical value could vary depending on species, genotype and the supply of other nutrients (White and Hammond, 2008b). It also appeared that high P plants have accumulated much more P than what they require to produce optimum yield.

Concentration as high as 6.4 mg P g⁻¹ in lower leaves suggests the occurrence of P luxury consumption in high P plants. In addition, the production of high post-anthesis tiller number, which is uncommon under field conditions, could potentially have influenced the amount of carbon and P allocated to grains in high P plants as they represented 28% of total biomass at maturity. Our results show that these additional sink have competed with grains for P and potentially for other nutrients. Bollons and Barraclough (1997) found that the main effect of low P supply on wheat growth was the decrease in the number and weight of tillers. In our study, since tillers were removed before anthesis, the total biomass was not different between treatment at this stage. However, after anthesis where tillers were allowed to grow, differences in biomass accumulation were related to the capacity of the plants to produce post-anthesis tillers. The P concentration in the grain of durum wheat (3.8 mg P g⁻¹ for high P plants vs. 1.8 mg P g⁻¹ for low P plants) was in the range of P concentrations previously reported under various field conditions in wheat (Batten, 1986a; Manske et al., 2002).

4.4.2 Contribution of external and internal P to grain P nutrition

The amount of P in grains of low P plants was 2.4-fold lower than in high P plants. This difference was not due to a grain dilution effect since there was no significant difference in grain yield between high and low P plants (Table 4.1). Our previous research with the same cultivar have shown that durum wheat plants can rely on P remobilization to sustain grain growth if they were well supplied with P during vegetative growth (El Mazlouzi et al., 2020b). In addition, P can be remobilized from all plant organs including roots, it can also be stored in both inorganic and organic form (Veneklaas et al., 2012). When the growth requirements are satisfied, plants can store and accumulate further nutrient in their organs for later use (Chapin et al., 1990). Lauer et al. (1989) showed that storage P was remobilized to a greater extent than metabolic P in hydroponically grown soybeans. In other species (e.g Proteaceae), the remobilization of P can reach 85% of total P in senescent leaves (Lambers et al., 2015; Veneklaas et al., 2012). In the present study, the high P plants had a higher P pool to be remobilized to sustain grain growth and filling whereas low P plants should remobilize metabolic P from different plant organs. This may explain the visual accelerated senescence in this treatment (Figure 2). The up-regulation of enzymes such as RNases and acid phosphatases that are involved in the breakdown of organic P (e.g P-esters) has been also shown to increase during leaf senescence in many species (Jeong et al., 2018; Veneklaas et al., 2012). Grains are the last sink accumulating

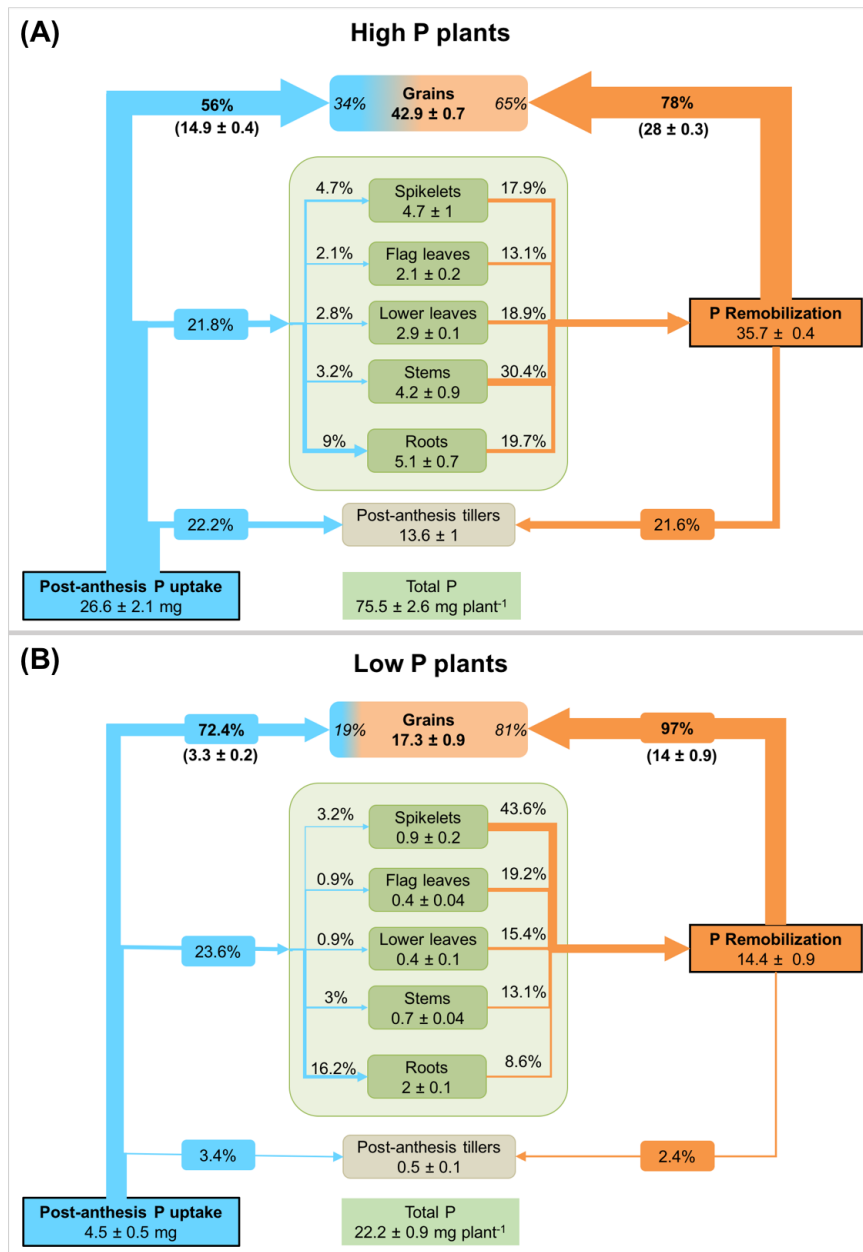


Figure 4.3 – Net P fluxes in durum wheat plants grown under high P (A) or low P supply (B). P fluxes are presented as a percentage of post anthesis P uptake (blue arrows, determined from ³²P-labelling) and remobilized P (orange arrows, calculated from the difference between P amount at anthesis and maturity) in different durum wheat plants organs. Values inside plant organs represent P amount (mg P) at maturity. Percentage values inside the grain compartment represent the contribution of post-anthesis P uptake and P remobilization to grain P. Values are means \pm standard error of 3 replicates.

P in wheat. Processes involved in the accumulation of P in grains include P remobilization from senescing organs, source-sink relationship and P transporters (Wang et al., 2016). Rae et al. (2003) have reported that the P transporters Pht1.1 was root specific but Pht1.6 was expressed in flag leaves and played a role in P remobilization into grains

in barley. Furthermore, the barley *lpa1-1* gene was identified as a member of the sulphate transporter family that probably is a P transporter involved in P allocation to grains Ye et al. (2011). Targeting an orthologue of this gene was used to engineer low grain P rice (Yamaji et al., 2017). In this recent study, Yamaji et al. (2017) have demonstrated that a node specific transporter, sulphate transporter-like phosphorus distribution (SPDT) was implicated in P distribution through nodes in rice. Knocking out this transporter resulted in a 20% reduction of total P in the grain without any decrease in grain yield.

Although this experiment was carried out in hydroponic conditions, the results obtained indicate that the accumulation of P into grains was essentially via remobilization and this is hardly influenced by post-anthesis P uptake. Low P supply altered the partitioning of the new acquired P within the plant. In high P plants, 56% of P taken up during the post-anthesis had been translocated to grains, but 22% remained in tillers and the remainder was partitioned between root and leaves. In contrast, low P plants translocated more than 72% of post-anthesis P uptake to grains whereas only small proportions were found in roots and tillers. Nevertheless, some of this difference in redistribution might be due to the excess levels of P in the high P plants. Our results suggest that durum wheat plants with low P nutritional status regulate tightly the allocation of the newly acquired P to grains. Whether the new acquired P arrives directly in wheat grains or not is not known. The fine characterization of the processes implicated into P loading to grains would require a short-term labelling experiment. Nevertheless, using ^{33}P -tracer in a hydroponic study, Julia et al. (2016) have reported that P loading into rice grains involves indirect transfer of P originating from P previously acquired P and stored in different vegetative organs.

4.4.3 Lowering grain P: Implications for P use and recycling

To fulfil grain P requirement, the depletion of tissues of P from spikelets, leaves and stems resulted in higher P remobilization efficiency in low P plant (Table 4.2). In contrast, a large proportion of the P remained in the vegetative tissues of the high P plants (eg. post-anthesis tillers, roots and leaves). These findings suggest that reducing P remobilization or the allocation of remobilized P to grains could lower durum wheat grain P concentrations. Retaining more P in the leaves and other tissues means that less P is available to be transferred into grains. It has been also shown that retaining P in leaves maintains higher photosynthetic capacity in rice (Jeong et al., 2017b).

In addition, P nutrition does not only affect plant P metabolism, but also could influence the uptake, transport and storage of other nutrient, especially micro-nutrient such as

zinc (Zn) and iron (Fe) (Briat et al., 2015). In the particular case of cereals intended for human and feed consumption, lowering P loading into grains is beneficial because the bioavailability of iron and zinc uptake might be also improved in grains with low P concentrations (Brinch-Pedersen et al., 2002). Phytate, the storage form of P in grains have a strong capacity in chelating these elements (Raboy, 2009). Low phytate crops are considered a promising strategy to increase grain quality and optimize P balance in farming systems (Raboy, 2009). One successful example of low phytate crop that reached the field is the barley *low phytic acid 1-1* (Bregitzer and Raboy, 2006). This genotype has a 10 to 15% reduction in grain total P and 50% of phytic acid but no yield reduction as compared with wild type (Bregitzer and Raboy, 2006; Raboy et al., 2015). A similar result was also obtained for soybean where large reductions in seed phytic acid content had no detectable effect on seed germination and early seedling growth (Raboy et al., 1985). Lott et al. (2000) estimated that the amount of P in phytate is equal to nearly 65% of the global annual P applied as fertilizer. Consequently, grain P has value as a target in P management at regional and global scale. How these nutrient are transported to grains and mechanism of regulation are not yet fully characterized. One major knowledge gap remains to know whether the P allocated to grains comes from direct fluxes (root to grain via xylem transport) or indirect P fluxes (via phloem loading). A better understanding of these relationships is important to determine which trait to prioritize in genetic improvement programs and could help improve the food quality and optimize crop P uptake and use.

4.5 Conclusion

We investigated the effect of P supply on the partitioning of post-anthesis P uptake and the contribution of internal P sources to grain P nutrition in durum wheat cv. *Sculptur*. Our findings indicate that a large proportion of grain P originates from the remobilization of internal P even at high external P supply during grain filling. The enhanced remobilization of P and the efficient allocation of newly acquired P to grains were crucial to provide the grains with P under low P supply. Nevertheless, the relative increase in the remobilization of pre-anthesis P stores when the plant P status was low indicates that these fluxes are inter-dependent with the post-anthesis P uptake. For a fine characterization of the processes implicated into P loading to grains, experiments in our laboratory are underway to determine the fate of the post-anthesis P before reaching the grain using a short-term ^{32}P tracer approach. Understanding the mechanism that regulate P loading into grains might help prioritize routes for the improvement of more P-efficient crops.

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Author Contributions

ME and AM conceived and designed the experiments. ME, CC, TR, and AM performed experiments and collected the data. ME, CM, and AM analyzed the data. ME wrote the original manuscript. CM and AM revised the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Chapitre 5

Dynamique d'allocation du phosphore prélevé et de sa remobilisation pendant le développement des grains

Les résultats rassemblés dans ce chapitre sont présentés sous forme d'un article en préparation pour soumission à une revue internationale :

El Mazlouzi et al., The dynamics of phosphorus uptake and utilization throughout the grain development period in durum wheat plants grown under different external P levels (en préparation).

Résumé

Contexte et objectifs

- ❑ Les études réalisées jusqu'à présent ont permis d'estimer la contribution de la remobilisation et de l'absorption post-floraison au P accumulé dans les grains chez le blé dur. Bien que la contribution de l'absorption post-floraison au P des grains soit faible, aucune étude à ce jour n'a caractérisé la dynamique du P pendant la phase développement des grains. Ainsi, l'objectif de cette 3ème expérience est d'étudier la dynamique courte du P récemment absorbé pendant la période post-floraison et apporter des éléments de compréhension sur les voies de transport du P vers les grains. Les plantes de blé dur ont été cultivées en hydroponie avec deux niveaux d'offre en P comme décrit dans le chapitre précédent. Deux marquages ponctuels au ^{32}P de 24h ont été réalisés au cours du développement des grains : le premier pendant la phase de division cellulaire (5 jours après la floraison) et le second pendant la phase du remplissage (14 jours après la floraison). Ensuite, les plantes ont été prélevées à différentes dates après la période de marquage (après 24h, 48h et 96h).

Principaux résultats

- ❑ Les résultats montrent que la répartition du P récemment absorbé au début du développement des grains (5 jours après la floraison) se fait au profit de tous les organes végétatifs, plus particulièrement de ceux qui se situent vers le haut de la tige tels que les feuilles et les épis.. Les plantes cultivées avec un faible niveau de P ont alloué plus de 75 % du P récemment absorbé aux organes aériens, tandis que les plantes cultivées avec un niveau élevé de P ont conservé 45% dans les racines. Dans le deuxième marquage (14 jours après la floraison), une plus grande quantité de P a été allouée aux grains. Au bout de 96 heures après marquage, plus de 50 % du P récemment absorbé se trouve dans les grains des plantes cultivées avec un faible niveau de P . La dynamique de l'allocation de P aux grains semble être plus rapide dans les plantes cultivées avec un faible niveau de P que dans celles cultivées avec un niveau élevé. Dans les deux cas, le P final des grains est issu principalement de la remobilisation du P accumulé avant la floraison. Nos résultats mettent en évidence que le transfert direct de la racine aux grains est très faible.

The dynamics of phosphorus uptake and utilization throughout the grain development period in durum wheat plants grown under different external P levels

5.1 Abstract

- Post-anthesis phosphorus (P) uptake and the remobilization of previously acquired P are the principal sources of grain P nutrition in wheat. However, how the absorbed P reaches the grains and short-term dynamics are not known. In the present study, the dynamics of P uptake and allocation to grains throughout the post-anthesis period in durum wheat were examined using pulse-chase ^{32}P -labelling experiments at two key stages of grain development. Plants were harvested 24h, 48h and 96h following each labelling experiment.
- Durum wheat plants (*Triticum turgidum* L. subsp. durum) were grown hydroponically under low and high P supply (0.025 mM vs 0.125 mM). Two short-term (24h) ^{32}P -labelling experiments were performed, at 5 and 14 days after anthesis to follow P fluxes within durum wheat organs. Phosphorus supply affected the allocation of the newly acquired P at the whole plant level. In the first labelling, little newly P is allocated to grains following the 24h after labelling.
- The plants grown under low P supply allocated more than 75% of the newly acquired P to the aboveground organs while the plants grown under high P supply retained 45% in roots. In the second labelling, more P was partitioned to grains under low P supply. Following 96h after labelling, more than 50% of the newly acquired P was found in grains in the low P supply. The dynamics of P allocation to grain was faster in the plants grown under low P than that those grown under high P supply. In both P supply, final grain P was mainly dependent on P remobilization from other organs. Our results show that the origin of phosphorus accumulated in grains is mostly from pre-anthesis P with little contribution of post-anthesis P uptake. Direct root to grains transfer is found to be very low.
- Our study presents evidence for the indirect transfer of P taken up during the post-anthesis period to the grain of durum wheat through the phloem pathways. It emphasizes the role of vegetative plant organs as temporary storage of P taken up during the grain filling period before its final translocation into grains.

Keywords: Phosphorus, ^{32}P tracer, Grain development, Post-anthesis P uptake, P remobilization, P fluxes, Durum wheat,

5.2 Introduction

Phosphorus is an essential nutrient for all living organisms. In plants, it is required for energy transfer (ATP) and signal transduction as well as for the structure of nucleic acids and phospholipids (Marschner, 2011). Plants absorb P through the root cell transporters as phosphate ions (Pi) present in the soil solution (Raghothama and Karthikeyan, 2005; Schachtman et al., 1998). In cereals crops, most of the absorbed P is allocated to the grains at maturity (Batten, 1992; Rose et al., 2010). Exported P through harvest is regularly replaced by P fertilizers to maintain high crop productivity (Rose et al., 2013; Raboy, 2009). However, only 15-30% of applied fertilizer is taken up by crop roots (Cordell and White, 2014; Schachtman et al., 1998). This low efficiency is due to the high fixation capacity of many soils and the low diffusion of P (Schachtman et al., 1998). Moreover, mineral P fertilizers are extracted from phosphate rock, which is a finite resource (Cordell and White, 2014). The demand for high quality P fertilizer is expected to increase in the upcoming year as a result of the increasing population (Cordell and White, 2014). One strategy to lower the amount of P application is the reduction in the exportation of P from the field by reducing grain P concentration in the grains (Cong et al., 2020; Rose et al., 2013). According to Lott et al. (2009) estimates, the annual quantities of P exported with the harvested product (dry seed and grain) are equivalent to 85% of applied P fertilizers on a global scale. Therefore, improving P use efficiency in cropping systems will require a better understanding of the mechanisms implicated in P loading into grains of cereal crops (Cong et al., 2020; Wang et al., 2016).

Plants have developed several mechanisms to acquire P and increase its use efficiency (Veneklaas et al., 2012; White and Hammond, 2008b). This mechanism includes modification of root architecture and physiology to increase root surface area and to mobilize more P from the soil (Schachtman et al., 1998; Shen et al., 2011). These processes have been well characterized during the early stage of plant development because the impact of P deficiency has a more notable negative consequence on crop productivity than P deficiency during later stages (Aziz et al., 2014; Grant et al., 2001). Nevertheless, a better understanding of the processes implicated in delivering P to grains is necessary to reduce the accumulation of P in the grains (Wang et al., 2016). In cereals crops, anthesis indicates the end of the vegetative stage and the beginning of the reproductive stage (Shewry et al., 2012). After anthesis, developing grains are the terminal sink for carbon (C), proteins and nutrients. The developing grain goes through three main phases: a cell division and expansion phase, a grain filling phase, and a maturation phase (Shewry et al., 2012). The first two phases are the most important for grain weight and final grain yield (Sabelli and Larkins, 2009). Carbon supply to grains is mainly derived from assimilates during this

period and from stem reserve that could provide up to 70% of total carbon (Masoni et al., 2007). Grain P demand is fulfilled by two processes: the remobilization of P from different plant organs and current P uptake (Batten et al., 1986; Veneklaas et al., 2012). In durum wheat, up to 80% of P in grains can be attributed to the remobilization of pre-anthesis P stored in vegetative tissues. However, the contribution of P remobilization to final grain P varies depending on pre-anthesis and post-anthesis conditions, especially P and water availability (El Mazlouzi et al., 2020b; Manske et al., 2001; Römer and Schilling, 1986). The remobilization of P is reported to increase in response to P deficiency or during the grain filling period as a result of plant tissue senescence (Batten and Wardlaw, 1987; Maillard et al., 2015). Given its mobility in xylem and phloem, P in older tissues can be easily remobilized and distributed within plant parts under P limited conditions and during senescence (Marschner, 2011).

Past studies of P dynamics using P-tracer have generally been carried out at early growth stages (Greenway and Gunn, 1966; Julia et al., 2018; Nadeem et al., 2011). For example, thanks to the labelling of external nutrient solution with ^{32}P , Nadeem et al. (2011) were able to estimate the relative contribution of root P uptake and seed P reserves to P nutrition of maize during seedling establishment. They found that seedling roots started to take up external P five days after sowing and they concluded that seed P reserves is required to supply maize seedling before root emergence (Nadeem et al., 2011). In contrast, little information is available on the dynamics of P during grain filling in wheat. Tracer experiments using stable or radioactive isotopes are powerful tools to investigate nutrient fluxes at whole plant level (Havé et al., 2017; Salon et al., 2017). They have been used extensively to study nutrient dynamics during grain filling, especially for elements with stable isotopes such as nitrogen and sulphur (Dubousset et al., 2010; Schiltz et al., 2005). Nevertheless, Julia et al. (2016) used the ^{33}P -tracer to study P fluxes in rice plants grown in hydroponic and found that P loading into grains involves mainly indirect P fluxes originating from previously acquired P and stored in the vegetative tissues.

Grain reserves are the primary source of minerals and energy to seedling during the heterotrophic stage before root emergence (Julia et al., 2018; Nadeem et al., 2011; White and Veneklaas, 2012). Despite its great importance for germination and early plant development, grain P with concentration ranging from 3 to 5 mg g⁻¹ are often considered over the need for seedling development (Pariasca-Tanaka et al., 2015; Rose et al., 2012; White and Veneklaas, 2012). It is possible to both exploit the genotypic variation in grain P concentrations observed in many crops such wheat (Guttieri et al., 2004), rice (Vandamme et al., 2016b) and maize (Lorenz et al., 2008) or to engineer crop with reduced

grain P concentrations by manipulating the processes involved in the transport and the translocation of P to grains (Dissanayaka et al., 2018; Wang et al., 2016). While the first strategy is long and traditional breeding selection might lead to undesirable traits (e.g. low seedling vigour) and would be environment-dependent (Raboy, 2020; Vandamme et al., 2016b). The second strategy offers a better perspective and might be used for many crop species (Wang et al., 2016). For example, the knowledge gained concerning the mechanisms of P transport and loading into rice grains has led to the identification of low grain P rice (Yamaji et al., 2017). The identified rice mutants obtained by the knockout of SULTR-like phosphorus distribution transporter (SPDT) involved in the translocation of P showed a 20% reduction in grain P without negative impact on agronomic parameters (Yamaji et al., 2017). Despite these advances in our understanding on the dynamics of P in rice (Julia et al., 2016; Yamaji et al., 2017), many uncertainties persist on how the newly absorbed P is partitioned to different plant organs and transported to the grain. Particularly, the regulation and the partitioning of post-anthesis P uptake and its loading into wheat grains remain unclear.

The growing grains are the dominant sink for carbon and nutrient including P during the post-anthesis period (Etienne et al., 2018; Hawkesford et al., 2018). Before reaching the grains, the P goes through several events including exogenous P uptake, transport and remobilization before the eventual delivery to the grains (Dissanayaka et al., 2018). However, the dynamics of these events and the importance of post-anthesis P uptake in supplying the grain with P is unclear (Dissanayaka et al., 2018; El Mazlouzi et al., 2020a; Wang et al., 2016). A better understanding of the mechanism involved in P loading to grains could help identify key processes and potential means to improve crop P nutrition (Dissanayaka et al., 2018; Wang et al., 2016). Thus, the aims of this study were to determine the short-term dynamics of P uptake and transport to grains during the post-anthesis period in durum wheat. We hypothesized that during early grain development, little P would be allocated to the grain as a result of the low sink strength (e.g. cell division phase). In contrast, during the grain filling period, the remobilization would contribute more to the allocated P to the grains as a result in the progress of plant senescence. We assumed that the intensity of P allocation to grains would be dependent on the plant P status. To test these hypotheses and to distinguish between the endogenous and exogenous P fluxes, two short-term (24 hours) ^{32}P labelling experiments were performed, at 5 and 14 days after anthesis, to follow P fluxes within durum organs and its allocation to grains. The dynamics of P loading into grains were also compared between durum wheat plants with different P nutritional status obtained by growing the plant with high and low P supply.

5.3 Materials and methods

5.3.1 Plant growth conditions

Durum wheat plants were grown hydroponically as previously described in El Mazlouzi et al. (2020a). Uniform seeds of *Triticum durum* cv. *Sculptur* (RAGT Semences, France) were disinfected using 6% (v/v) sodium hypochlorite (H_2O_2) and germinated in moist paper for 3 days at 25°C. Thereafter, germinated seeds were placed in 50 mL Falcon[®] tubes filled with modified Hoagland nutrient solution (25% strength for macro-nutrients and full strength micro-nutrients). Two weeks after germination, 72 uniform wheat seedlings were then transferred to individual pots containing 5.5 L of modified Hoagland's nutrient solution consisting of: 0.125 mM KH_2PO_4 , 0.625 mM KNO_3 , 0.85 mM KCl , 1.25 mM $\text{Ca}(\text{NO}_3)_2$, 0.5 mM MgSO_4 , 46.25 μM H_3BO_3 , 1 μM MnCl_2 , 10 μM ZnSO_4 , 2 μM CuSO_4 , 0.03 μM $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$, 100 μM NaFe EDTA , 25 μM HEDTA , 2 mM MES buffer and 50 mg L^{-1} SiO_2 . To obtain plants of different P nutritional status, two levels of P supply were applied: 0.025 mM KH_2PO_4 (Low P supply) and 0.125 mM KH_2PO_4 (High P supply). To compensate for the difference in the K supplied, KCl was added to the lowest P treatment. The initial pH was adjusted to 6 ± 0.5 with KOH . Aeration and renewal of the nutrient solution was maintained by overflow-type system. The pots were arranged in a randomized block design (18 plants per block). To prevent high tillering rate associated with hydroponic culture, only the first four tiller were allowed to grow.

Plants were grown under greenhouse conditions, with a photo-period of 16h/8h day/night and an average relative air humidity of 64%. Air temperature and relative humidity were recorded every 30 min using a data logger (CR32X sensor, Campbell Scientific). The plants were illuminated with an average of 450 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ provided by natural light supplemented with LED lamps (model LED, Ledlyt, France).

5.3.2 Plant sampling and measurements

The time-line of plant sampling and labelling is provided in Figure 5.1. After head emergence, the plants were individually monitored to determine the anthesis date. For both treatment, three plant replicates ($n=3$) were harvested at anthesis (D0) and at 5, 6, 7, 9, 14, 15, 16, and 18 days after anthesis (DAA). At each harvest date, plants were separated into grains, spikelets (including rachis), flag leaves, lower leaves, stems (including peduncles) and roots. Root systems were rinsed with deionised water and blotted with paper towels to remove surface-bound nutrient solution. For each plant, organs from the four tillers were pooled and then oven-dried at 60 °C for 72h and their

dry weights were determined.

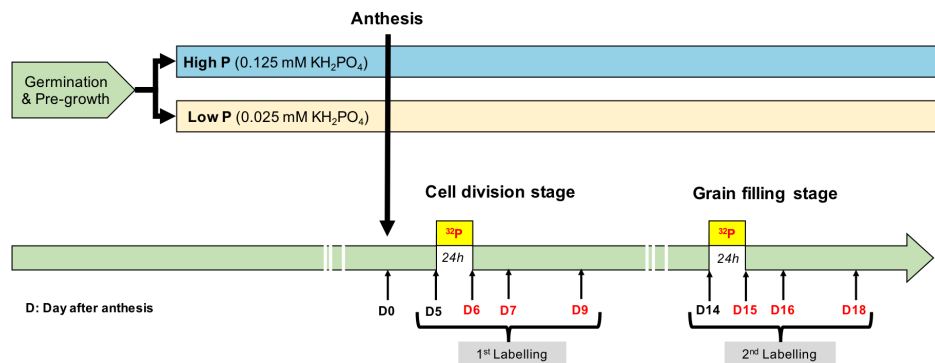


Figure 5.1 – The experimental design used for plant growth and ^{32}P labelling. Durum wheat plants were grown hydroponically under high and low P supply under greenhouse conditions. The ^{32}P labelling experiments were performed at 5 and 14 days after anthesis and lasted for 24h. For each labelling, plants were harvested after 24, 48 and 96h after labelling. Plant were also harvested at anthesis and at physiological maturity. Three plant replicates were sampled at each harvest date.

5.3.3 Plant labelling with ^{32}P

The labelling experiments with ^{32}P (half-life 14,3 days) were performed at two key stages of grain development: the first at 5 DAA (cell division phase) and the second at 14 DAA (grain filling) (Shewry et al., 2012) (Figure 5.1). On the day of the first labelling, stock nutrient solution (54L for each treatment) with the same composition as previously described was prepared and spiked with a known quantity of ^{32}P of approximately 6 MBq and 13 MBq for low and high P supply, respectively. Thereafter, 18 plants (9 per treatment) were transferred to adjacent greenhouse dedicated to handling radioisotopes using the same conditions specified above. Labelling experiments were initiated 8h after the start of photoperiod. The plants remained in the labelling solution for 24h. Immediately after the end of the labelling period, 6 labelled plants (3 per treatment) were harvested and separated as detailed above. The remaining 12 plants were placed in a fresh unlabelled nutrient solution to continue their development under the same conditions until harvest. Before transferring plants on the unlabelled solution, roots were bathed and carefully rinsed three times with deionised water to eliminate adhered ^{32}P from root surface. The 12 remaining plants were harvested at 6 and 9 days after anthesis (*i.e.* 48h and 96h after labelling). The second ^{32}P labelling at 14 DAA was carried out following the same procedure as the first one (Figure 5.1).

5.3.4 Phosphorus concentration, ^{32}P analysis and partitioning indices

Phosphorus concentration (mg g^{-1}) in plant organs was determined using the malachite green colorimetric method at 610 nm (Van Veldhoven and Mannaerts, 1987). Briefly, sub-samples of each plant organs were ashed in a muffle at 550 °C for 5h. Ashes were digested in 2.5 mL of HNO_3 and then washed with 5 mL of ultra-pure water. Digests were evaporated until only a few drops were left. Following evaporation, the sample was filtered and the volume was made up to 50 mL with ultra-pure water and used to determine the P concentration and ^{32}P activity. For a given plant organ, the amount of P was calculated by multiplying the dry weight and its measured P concentration. The total amount of per plants was calculated as the sum of the amount of P in all plant organs.

Radioactivity in plant samples and in the nutrient solution were measured using a liquid scintillation counter (TriCarb 2100, Packard). For each measurement, 1 mL of filtered samples was mixed with 3 mL of scintillation cocktail (Insta-gel Plus Packard, Perkin-Elmer, Waltham, Massachusetts, USA) and measured for two minutes. All radioactivity measurement was corrected for the radioactive decay to a single time point. Assuming that no fractionation between ^{31}P and ^{32}P occurred during root P uptake and P transport within the plant, the exogenous P (P_{exo}) in each plant organs derived from the nutrient solution during the labelling period was calculated according to the following formula as described in Morel and Fardeau (1990):

$$\frac{R_t}{P_{ns}} = \frac{r_t}{P_{exo}} \Rightarrow P_{exo} = \frac{r_t}{\left(\frac{R_t}{P_{ns}}\right)}$$

where R_t/P_{ns} is the specific activity measured at time t in the nutrient solution, r_t is ^{32}P activity measured in the plant samples at harvest time t. The total amount of P derived from exogenous P uptake during the 24h of ^{32}P labelling was calculated as the sum of exogenous P found in all plant organs. In the following sections, we will refer to the exogenous P taken up during the 24h of labelling (estimated from ^{32}P measurements) as the newly acquired P.

5.3.5 Calculation of P partitioning and remobilization indices

Two indicators were calculated to follow the partitioning and the allocation of the newly acquired P absorbed during grain development period: PHI and ^{32}PHI . The phosphorus harvest index (expressed in %, PHI) was calculated as the amount of P in the grain divided by the total amount of P in the plant (including the roots). The ^{32}PHI refers to the ratio of the newly accumulated P in the grain to the total newly acquired P in the whole plant. The contribution of the newly acquired P to the accumulated P in the

grain at each harvest (in %) was calculated as the amount of newly P in the grain divided by the total amount of P in the grain. This last indicator can be used to estimate the contribution of P remobilization to grain P, which can be determined as grain total P minus the newly P in the grain.

5.3.6 Statistical analyses

All statistical analyses were conducted with R statistical software version 3.4.4 (Team, 2018). Data were reported as mean \pm standard error (SE) of three replicates (n=3). Significant difference between measured parameters (e.g. biomass, P amount) were tested across harvest days for each P treatment using a two-way ANOVA ($P < 0.05$). When ANOVA indicated a significant difference among harvest days or treatment, a multiple comparison test was carried out using Least Significant Difference test ($P < 0.05$). Prior to analyses of variance (ANOVA), normality and homogeneity of variance were checked.

5.4 Results

5.4.1 Plant growth and phosphorus accumulation

Durum wheat plants (cv. *Sculptur*) were grown with two levels of P supply to obtain plants with different P status. The plants were harvested at anthesis to determine the P status at this stage. Thereafter, two groups of plants were selected for the two labelling experiments as explained above. Total plant biomass was similar for both P supply and increased significantly during the post-anthesis period, although total P amount was substantially greater under high P supply. Between anthesis and 18 days after anthesis (DAA), it increased from 7.9 ± 0.7 to 22.5 ± 0.6 g for high P plants and from 7.7 ± 0.2 to 21.4 ± 0.5 g for low P plants (Figure 5.2A). We observed a remarkable increase in total biomass in high P plants starting from 14 DAA, which is probably due to a larger plant group in the second labelling compared with the first one. A significant difference in total biomass was only observed at 14 DAA. Dry weight accumulation in grains did not vary between P supply. It started to increase from 5 DAA and continued during the grain filling period. Between 5 and 18 DAA, grain dry weight increased from 0.05 to 4.9 g in high P plants and from 0.04 to 5.5 g in low P plants. The partitioning of biomass among plant organs was slightly different between high and low P supply. Plant grown in low P supply allocated more biomass to grains (26%) than those grown under high P supply (22%). The proportion of total biomass allocated to roots and stems were lower in low P plants compared to high P plants, particularly between 14 and 18 DAA.

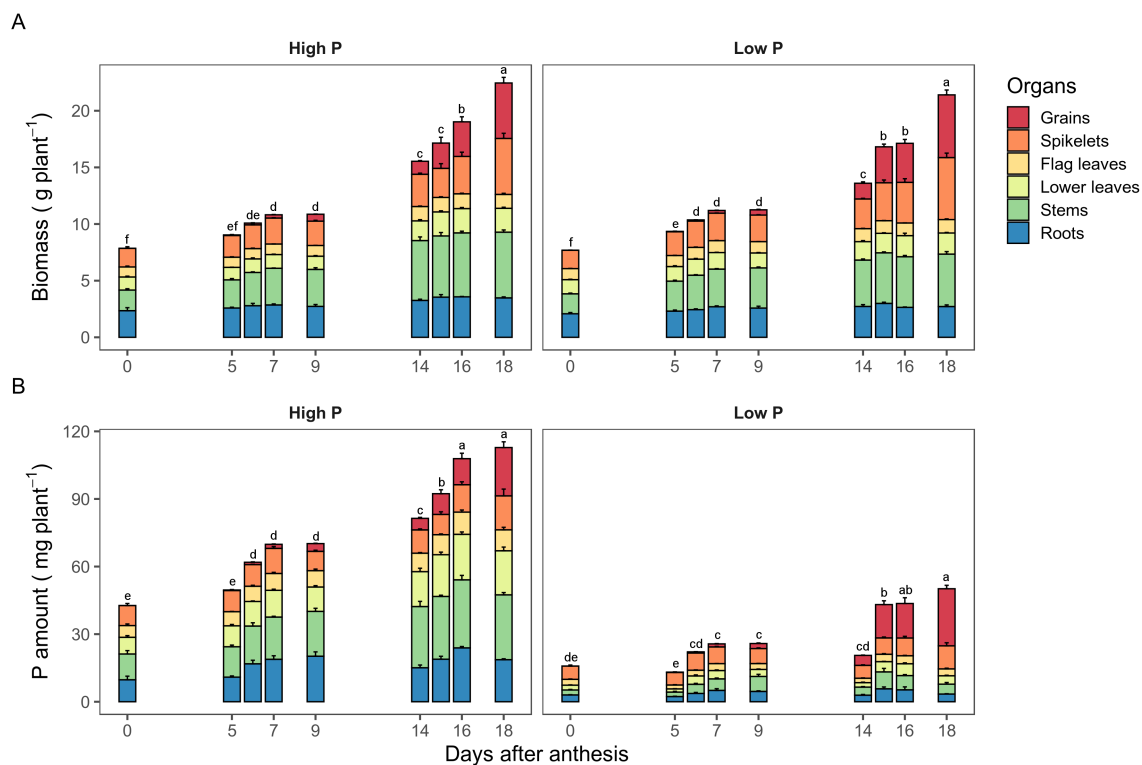


Figure 5.2 – Biomass (A) and P amount (B) in different organs of durum wheat grown under high and low P supply. Values are means \pm SE of three replicates. Different letters indicate significant differences between days for total biomass or P amount according to the LSD test at $P < 0.05$.

Unlike biomass, durum wheat plants showed a contrasting pattern of P accumulation in response to P supply. Plants grown in high P supply accumulated significantly higher P amount than those grown with a low P supply across harvest days (Figure 5.2B). Despite the non significant decrease in the amount of P in low P plants at 14 DAA, the accumulation of P continued progressively during this period for both P supply. The total amount of P in high P plant increased from 42.7 ± 5.4 to 112 ± 4 mg at between anthesis and 18 DAA whereas for low P plants, the P amount increased from 15.8 ± 1 to 50.2 ± 4 mg during the same period. The amount of P accumulated during this period represents 62% and 68% of total P for high and low P supply respectively. The increase in the total amount of P in high P plants was mainly associated with the increase of P amount in grains and existing organs such as roots, stems and lower leaves. However, grains and spikelets were responsible for the increased total P in low P plants.

The partitioning of P was also strongly affected by P supply. For example, the proportion of P allocated to spikelets was higher under low P supply (37%) compared to high

P supply (21%) at anthesis. At 18 DAA, low P plant allocated a much higher proportion of P to grain (50%) and spikelets (20%) than high P plants, which allocated only 19% to grain and 13% to spikelets. However, the amount of P in grain was not significantly different between P supply (21.4 and 25.3 mg for high and low P supply, respectively).

In all plants organs except grains and regardless of harvest days, high P plants had significantly greater P concentration than low P plants indicating that plants had different P status (Figure 5.3). Grain P concentration had similar trend for both treatment and was only significantly different at 9 and 14 DAA. For high P plants, it increased from 5 ± 0.3 at 5 DAA to 6.7 ± 0.3 mg g⁻¹ at 6 DAA and then decreased to 4.3 ± 0.2 mg g⁻¹ at 18 DAA. For low P plants, it start from 4 ± 0.6 at 5 DAA to reach 5.2 ± 0.7 mg g⁻¹ at 18 DAA. The concentration of P in spikelets ranged from 5.4 ± 0.1 to 3 ± 0.6 and from 3.6 ± 0.2 to 1.9 ± 0.1 mg g⁻¹ in high and low P supply respectively. Plants grown under low P supply had largely lower concentration than those grown under high P supply (2.6 ± 0.1 vs. 5.8 ± 0.3 mg g⁻¹ at anthesis, respectively). The highest P concentration was found in lower leaves of high P plants and in spikelets and grains for low P plants. The P concentration in other organs was two to three time greater in high P plants in comparison to low P plants. The concentrations of P in leaves and roots were stable whereas it decreased slightly in spikelets and stems across P treatment.

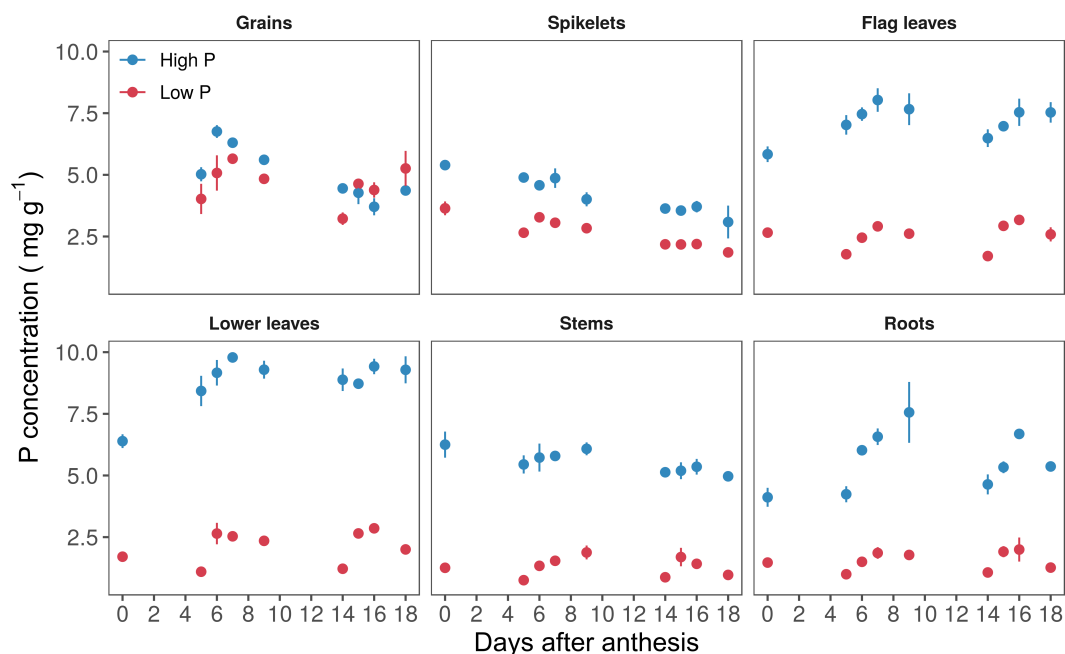


Figure 5.3 – Phosphorus concentration in durum wheat organs between anthesis and 18 days after anthesis. Plants were grown in hydroponics with high or low P supply. Values are means \pm SE of three replicates.

5.4.2 Time course of the newly acquired P during the post-anthesis period

The isotopic labelling of exogenous P was carried out to determine the dynamics of the newly acquired P and its partitioning and remobilization among plants organs during grain development. The first labelling started at 5 DAA (cell division stage) and the second at 14 DAA (grain filling stage) and lasted for 24h. The amount of exogenous P uptake during the 24h of labelling was higher in the plants grown under high P supply in comparison to those grown under low P supply in both labellings (Table 5.1). While low P plants acquired the totality of P present in the nutrient solution (In average 3.2 mg in the 1st labelling and 4.6 mg in the 2nd labelling, respectively), we observed more variability between individual plants in the high P plants as it can be seen in table 5.1. Subsequently, the results are expressed as a percentage (%) relative to total exogenous P uptake (Figure 5.4).

Table 5.1 – Total amount of P (mg plant⁻¹) derived from exogenous P uptake during the two labelling experiments. Each labelling experiment lasted for 24h

Hour after labelling	1st labelling (5 DAA)			2nd labelling (14 DAA)		
	24	48	96	24	48	96
High P	11.3 ± 2.5	11.5 ± 3.5	4.4 ± 0.8	8 ± 2.7	11.2 ± 2.5	7.6 ± 1.2
Low P	3.2 ± 0.1	3.2 ± 0.1	3.3 ± 0.1	4.2 ± 0.1	4.3 ± 0.2	5.3 ± 0.4

Values are means ± SE of three replicates.

5.4.2.1 Partitioning of the newly acquired P during the cell division stage (5 DAA)

In the first labelling, 100% of the newly acquired P is equivalent in average to 9.1 ± 2.3 mg and 3.3 ± 0.03 mg for high and low P, respectively (Table 5.1). The partitioning of the newly acquired P among plants organs varied between P supply and across days after anthesis (Figure 5.4). In high P plants twenty-four-hour after the first labelling (6 DAA), about 47% of newly acquired P was recovered in the aboveground organs mainly in leaves (20%), stems (14%) and spikelets (11%). By 96h after labelling, 60% of the newly P was found in aboveground organs where stems (21%) and leaves (23%) being the primary sink for P. The grain accumulated only 5% of the newly acquired P. In contrast,

following 24h after labelling, the majority of the newly acquired P in low P plants (80%) was transported to the aboveground organs where 36% found in leaves, 23% in stems and 20% in spikelets, whereas only 20% remained in the roots. After 96h, the newly acquired P increased in stems (31%) and in grains (8%) indicating that in-between organs P transfer has occurred.

5.4.2.2 Partitioning of the newly acquired P during the grain filling stage (14 DAA)

Durum wheat plants continued to take up exogenous P from the nutrient solution after 14 DAA in both P supply. In the second labelling 100% of the newly acquired P is equivalent in average to 8.9 ± 1.1 and 4.6 ± 0.4 mg for high and low P supply, respectively (Table 5.1). In high P plants, half of newly acquired P was allocated to the above-ground organs whereas the other half was retained in roots 24h after labelling at 14 DAA (Figure 5.4). The proportion of newly P in roots decreased progressively between 48 and 96h after labelling with a concurrent increase in above-ground organs, indicating root to above-ground organs P transfer. The main sink organs for newly P was stems, lower leaves, spikelets and grains. In low P plants, 66% of the newly acquired P after 24h of labelling was allocated the aboveground organs. This proportion increased to 92% by 96h after labelling. From 24h to 96h after labelling, the proportion of newly acquired P in vegetative organs declined while it continued to increase in grains and spikelets. It accounted for 48% in grains, followed by leaves (19%) and spikelets (14%) at 96h after labelling. The relative reduction in the partitioning of the newly acquired P in roots and vegetative organs indicate that these organs serve as a transit for the post-anthesis P uptake before its transport to grains that represent the final sink of P in the plant.

The result of the two labellings underlines the importance of vegetative organs as temporary storage of P before its subsequent transfer to the grains. This transfer seem to be regulated by plant P status and sink strength. Higher proportion of newly acquired P was allocated to the grains in the plants grown under low P supply than in the plants grown under high P supply.

5.4.3 Phosphorus fluxes into the developing grains

Despite the lower total amount of P accumulated in the low P plants, the amount of P in grains was not significantly different between the two P supply in all dates. The amount of newly acquired P allocated to grains increased progressively during grain development

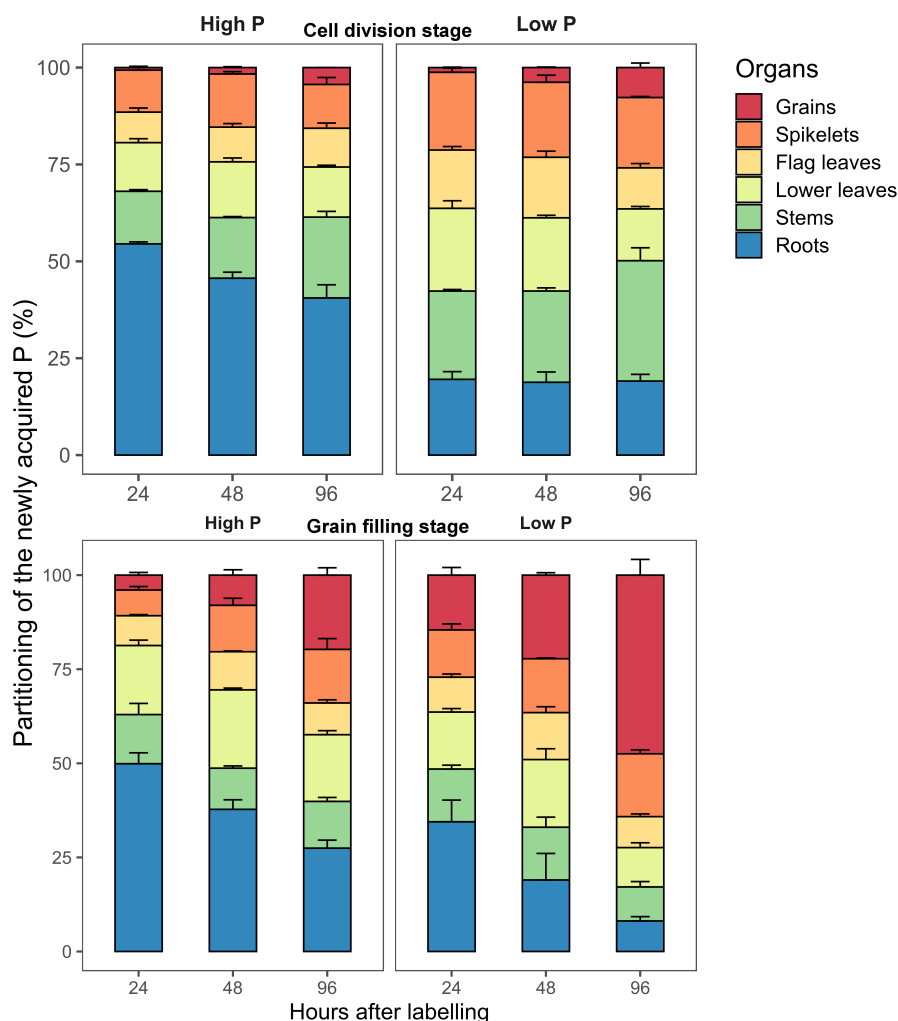


Figure 5.4 – Short-term partitioning of the newly taken acquired P in different durum wheat organs for high and low P plants in the first (5DAA) and in the second (14 DAA) ^{32}P -labelling.

in both labelling as shown in Figure 5.5. Between the first and the second labelling, it increased from 0.07 ± 0.01 to 0.3 ± 0.06 and from 0.04 ± 0.003 to 0.6 ± 0.1 mg after 24h of labelling for high and low P supply, respectively. The only significant difference in the allocation of newly P to grain was observed 24h after the 2nd labelling.

Although the newly P uptake for high P plants was consistently greater than low P plants, its allocation to grains was significantly lower, especially in the 2nd labelling, as it can be seen from the ^{32}PHI values (Tableau 5.2). Between the first and the second labelling, it increased from 0.62 to 3.6 and from 1.2 to 14.6 after 24h of labelling for high and low P supply, respectively. At 96h after the second labelling, about 48% of the newly acquired P was found in grains in the low P plants while only 19% in high P plants. Despite the significant allocation of newly acquired P to grains, more than 90% of total P

Table 5.2 – Phosphorus harvest index (PHI), ^{32}P PHI and the contribution of newly acquired P to the accumulated P in the grains of durum wheat grown under high and low P supply.

1 st labelling (5DAA)				
Treatment	DAA (HAL)	PHI (%)	^{32}P PHI (%)	Contribution of newly acquired P to total grain P (%)
High P	6 (24h)	1.7	0.62	6.65
	7 (48h)	2.6	1.5	9.66
	9 (96h)	4.9	4.3	5.57
Low P	6 (24h)	2.4	1.2	7.46
	7 (48h)	5.3	3.7	9.11
	9 (96h)	8.7	7.9	11.37
2 nd labelling (14 DAA)				
High P	15 (24h)	10.0	3.6	3.17
	16 (48h)	10.7	7.6	7.37
	18 (96h)	19.0	19.2	6.80
Low P	15 (24h)	34.3	14.6	4.13
	16 (48h)	35.1	22.2	6.29
	18 (96h)	50.5	48.0	10.01

in grains comes from the remobilization since recently taken up P does only represent a small proportion of the total P in grains (Table 5.2). However, it seem that post-anthesis P uptake contribute more to grain P in low P plant than in high P plants.

5.5 Discussion

The two systems providing the developing grains with P are the post-anthesis P uptake and the remobilization of P from endogenous sources taken up before anthesis. A better understanding of the origin and the fluxes of P during grain filling is essential to identify potential targets to engineer crop plants with higher P use efficiency and reduce the unnecessary P export from the field. The dynamics of post-anthesis P uptake and its allocation to grains was examined by short-term (24h) ^{32}P -labelling experiments at 5 and 14 days after anthesis. Our findings provide new insights into the short-term dynamics of P during grain development and the possible routes implicated in the transport and loading of P into the grain of durum wheat (*Triticum durum* cv. *Sculptur*).

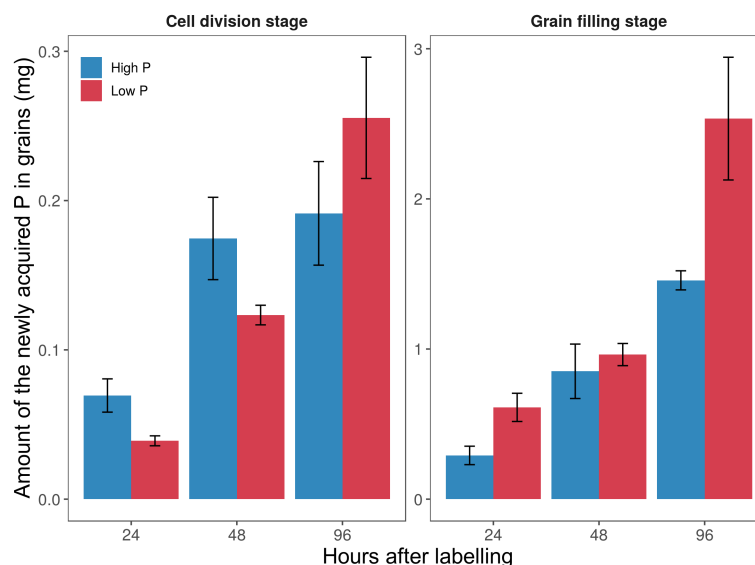


Figure 5.5 – The amount of newly acquired P allocated to grains after the two labelling experiments in high and low P durum wheat plants. 24h, 48h and 96h represent hours after ^{32}P labelling. Values are means \pm SE of three replicates. The asterisk indicates statistical significance between P supply ($P < 0.05$).

5.5.1 Effects of P status on the partitioning of the newly acquired P within plant organs during grain development

In the current study, the P supply had no significant impacts on biomass accumulation, although we observed differences in biomass partitioning among plant organs. This is possibly due to continuous removing of vegetative tillers for both P treatments. In contrast, durum wheat plants had different P status because the accumulation of P was higher in high P supply than in low P supply (Figure 5.2). Moreover, the allocation of the newly acquired P among different plant organs was affected by the plant P status. Regardless of the labelling dates, low P plants exported higher proportion of the newly acquired P to the aboveground organs than high P plants (Figure 5.4). During early grains development, phosphorus is needed to maintain metabolic activity in photosynthetic organs (e.g. leaves, spikelets) and to support grain growth (Jeong et al., 2017a; Sanchez-Bragado et al., 2016; Römer and Schilling, 1986). The competition between these two processes can lead to different P partitioning pattern in high and low P plants. While high P plants had ample P suggesting the absence or low competition between vegetative organs and developing grains, this hypothesis can not be excluded for low P plants. Phosphorus demand created by growing rice (*Oryza sativa* L.) grains can impair photosynthesis under limited P supply (Jeong et al., 2017a). However, it should be noted that P demand of growing grains is low during early period of grain development. Most accumulated P in durum wheat grains is loaded starting from 10 days after anthesis (El Mazlouzi et al., 2020b). In contrast,

during the grain filling stage, the competition between vegetative organs and developing grains becomes lower as a result of the progression of plant senescence (Distelfeld et al., 2014; Hawkesford et al., 2018). Thus biomass increase occurring during this stage can be attributed to grain filling.

Our result suggest that the proportion of newly acquired P allocated to grains is controlled by the plant P status since low P plants allocated more newly P to grains than high P plants following 96h after labelling in both labelling dates. The difference between the two treatments may result from the rapid allocation of P to the grains in low P plants and/or from the limited redirection of the newly acquired P toward grains by the high P plants. The remobilized P seem to be satisfactory to meet the demand of the growing grains in high P plants. Modification of P distribution is one of the common responses to P limitations in cereals crops (Lyu et al., 2016; Marschner et al., 1996). During early plant growth, this modification lead generally to higher allocation of carbon and P to root resulting in higher root to shoot ration in low P plants (Marschner et al., 1996; Wissuwa et al., 2005). Also, a lower proportion of newly P remained in root following 96h after the 2nd labelling in comparison to the first labelling where higher proportion of newly P was retained in roots, particularly in high P plants (40%). This indicate that roots can act as an active sink for P during grain filling. In fact, roots continue to take up nutrients after anthesis despite the progressive decrease of their activity due to plant senescence (Hawkesford et al., 2018). During grain filling, the grains represent the strongest sink for nutrient and consequently P fluxes are governed by the demand created by grains. In a short-term (6h) P uptake experiment using ^{32}P -tracer, Snapp and Lynch (1996) showed that bean roots retained over 80% of the acquired P under low P conditions while only 20% were retained at high P conditions. They suggested that bean roots retain P when exogenous P levels are low to sustain nutrient and water uptake as long as possible (Snapp and Lynch, 1996).

5.5.2 The fate of the newly acquired P and the mechanisms implicated in P transport and loading into the grain

The short-term ^{32}P -labelling used in this experiment allowed to trace the fate of the newly acquired P during grain development among durum wheat organs. It revealed that the post-anthesis root P uptake is rapidly translocated to the aboveground organs. However, immediately after the end of the first labelling period (5 days after anthesis (DAA)), the grains received a little proportion ($< 2\%$) of the newly acquired P, although newly P was present in spikelets and flag leaves (Figure 5.4). However, this proportion was much higher in the 24h following the second labelling, particularly for the low P plants (16%).

This proportion increased over time for both P supply, but it was more pronounced in low P plants than in high P plants. In the second labelling, the proportion of the newly P found in the grains could be attributed to the redistribution from other organs, which may have already started during the 24h of labelling time. These findings demonstrate that P taken up by roots during grain development is not directly partitioned to grains, but transit by vegetative organs first before reaching final sinks. Taken together, these data imply that P taken up during the post-anthesis period is loaded into grains through phloem pathways. Accordingly, Marshall and Wardlaw (1973) estimated the velocity of P following application of ^{32}P to the flag leaf blade to be 51 cm hour⁻¹ in wheat. It is generally admitted that the acquired P by roots moves rapidly in the form of inorganic phosphorus to aboveground parts via xylem whereas its translocation within the plants occurs essentially via the phloem (Raghothama and Karthikeyan, 2005; Smith et al., 2003). Consistent with our finding, wheat grains are characterized by the xylem discontinuity at the base of the grain (Zee and O'Brien, 1970), which mean that xylem-to-phloem transfer of P is required before entering the grain. Furthermore, this discontinuity in the xylem may limit the transport of P with transpiration stream, which is the principal driver of P transport in the xylem (Bieleski, 1973; Hüve et al., 2007; Rausch and Bucher, 2002). The results obtained in our study reinforces previous findings showing that the loading of P into rice grains is taken part mostly via the indirect transfer of the newly taken up P stored in vegetative organs (Julia et al., 2016). While rice and wheat may have common mechanisms regarding P remobilization and P transport within the plants, the mechanism of P loading into grains may be different because the grain histology is different (Stomph et al., 2009; Zee and O'Brien, 1970). For example, rice has no discontinuity in the xylem, suggesting that P transfer can take place directly from roots to grains through the xylem (Stomph et al., 2009).

The faster allocation of P to grains in low P plants may also be associated with an earlier sink formation (grains) in low P plants in comparison to high P plants. Nutrient stress including P is shown to accelerate senescence and the development of grains in wheat and thus the remobilization of nutrients (Batten and Wardlaw, 1987; Maillard et al., 2015; Peng and Li, 2005). This is in line with the findings of Snapp and Lynch (1996), who found that leaf remobilization occurred earlier in low P bean plants in comparison to high P plants. Generally monocarpic senescence start after anthesis and progresses during grain development (Distelfeld et al., 2014). This is consistent with the dynamics of P observed in roots, stems or lowers leaves, which turned from sink to source for the newly acquired P following 96h after the 2nd labelling. This is also in agreement with the observed accumulation of newly P in flag leaves and spikelets, which are the organs with

late senescence (Distelfeld et al., 2014). When the senescence is advanced, the remobilization of P stored in the vegetative organs dominates P fluxes at the whole plant level (Distelfeld et al., 2014; Maillard et al., 2015).

In our study, grain P was mainly provided through the remobilization of endogenous P sources since the P taken up during the post-anthesis period provided only between 7% and 4% in the first and second labelling, respectively (Table 5.2). The contribution of newly P seems to be lower during the late stage of grain filling than at the beginning. This lower contribution may be associated with the progression of plant senescence and the remobilization which could provide adequate P to supply the grains. Post-anthesis root P uptake capacity in wheat reported in the literature is considerably different and vary between genotypes and environmental conditions (Batten and Wardlaw, 1987; El Mazlouzi et al., 2020b; Manske et al., 2001; Römer and Schilling, 1986; Rose et al., 2007). For example, Rose et al. (2007) showed that wheat plant ceases P uptake after anthesis while Manske et al. (2001) found that post-anthesis P uptake represented 48% of total P uptake at maturity. In contrast, many finding showed that wheat plants are heavily dependant on P remobilization from vegetative organs to fulfil grain P requirements (Maillard et al., 2015; El Mazlouzi et al., 2020b; Peng and Li, 2005). By removing the post-anthesis P supply to durum wheat plants, El Mazlouzi et al. (2020b) was able to demonstrate that P remobilization from senescing tissue was sufficient to supply the grain with P without affecting grain yield. In our recent study with the same cultivar, the remobilization of P reserves from spikelets and leaves to grains was estimated to represent more than 78% of the grain P at maturity in low P plants (El Mazlouzi et al., 2020a). Similarly, withdrawing P from the nutrient solution during flag-leaf expansion did not influence grain P and yield in one-tiller wheat (Peng and Li, 2005). At low P supply or when the demand for P is high, the stored P can be easily remobilized and transported to sink organs (Veneklaas et al., 2012; Yang et al., 2017). The high efficiency of P remobilization in wheat plants has been suggested to be an important player in P fluxes to grains (Dissanayaka et al., 2018; El Mazlouzi et al., 2020a; Maillard et al., 2015). Around 50 to 90% of grain P can be attributed to P remobilization of endogenous P sources in wheat (Batten et al., 1986; Maillard et al., 2015). This was consistent with the decrease of P retention in roots and vegetative organs in low P plants. In addition to its high mobility in both phloem and xylem, P can be easily remobilized either from vacuoles, which is the main storage compartment in plant cells or following the breakdown on macromolecules such as nucleic acid-P and phospholipids (Kisko et al., 2018; Stigter and Plaxton, 2015). The ribonucleases (RNases) and acid phosphatases are shown to have a key role in liberating P from macromolecules during leaf senescence (Stigter and Plaxton, 2015; Jeong et al.,

2017a).

5.5.3 Routes for lowering grain P accumulation in durum wheat

Although the values of post-anthesis P uptake could be overestimated due the fresh nutrient solution and the immediate availability of nutrients for root uptake (Table 5.1), our ^{32}P -tracer study gives a clear overview on how the absorbed P is partitioned at the whole-plant level during grain development. It also demonstrates that when P supply is not limited, P uptake can occur during grain filling in durum wheat. Moreover, the remobilization of endogenous P sources was the main contributor of P accumulated in durum wheat grains. Theoretically, a reduction in grain P seems to be achievable by altering the allocation of the newly taken up P to grains or by decreasing P remobilization and transport to grains. The modification of the remobilization is not likely because it provides the majority of the elements accumulated in the grains. Mobile elements such as zinc (Zn) and iron (Fe) originate from remobilization of endogenous resources (Iwai et al., 2012; Kutman et al., 2012). Additionally, about 66 to 82% of nitrogen (N) originates from remobilization of pre-anthesis stored N in winter wheat (Kichey et al., 2007). Any change in P remobilization through delaying senescence or by depressing the expression of enzymes implicated in P-containing molecules breakdown could negatively affect the mineral composition of the grains (Etienne et al., 2018; Wang et al., 2016). In fact, the mechanisms involved in senescence are not specific to P, and their alteration may impact other elements (Wang et al., 2016). For example, the reduction in the expression NAM-B1 transcription factor, which play a crucial role in the remobilization of nutrients to grains during leaf senescence, lead to a decrease in P concentration and also decrease the concentration of N, Zn and Fe (Waters et al., 2009). Additionally, the downregulation of a transcription factor that reduces leaf senescence (e.g. GPC) resulted in the reductions in grain Zn and Fe content in tetraploid wheat (Pearce et al., 2014). Any change the senescence-related mechanism should take into account grain N because the grain protein content is the primary quality indicator that determines the end-use of wheat. The study of plant and grain ionome could provide interesting insight giving the complexity of interaction that exists among nutrient in plant.

Although the post-anthesis P uptake was not the major contributor to durum wheat grain P, reducing the allocation of newly taken up P to grains could be the most accessible way to reduce grain P content. Accordingly, by knocking out the rice SULTR gene, Yamaji et al. (2017) demonstrated that it is possible to lower grain P concentrated up 20% through reducing the translocation of P taken up during the post-anthesis period toward the developing rice grains. More importantly, the reduction of grain P was accompanied

by a decrease of 30% of phytate in the grains. The authors also reported an increase in the bioavailability of zinc and iron (Yamaji et al., 2017). In fact, phytic acid is considered as an anti-nutrient agent because of its capacity to bound micro-nutrient such as zinc and iron and make them non-available to monogastric animals (Raboy, 2009).

5.6 Conclusion

Our study revealed three main findings. Firstly, the P taken up by roots during the post-anthesis period is not directly partitioned to grains. It was stored temporarily in vegetative organs (leaves, spikelets and stems) before being re-translocated to the developing grains. Secondly, post-anthesis uptake transfer to grains seem to be regulated by plant P status and sink strength as low P plants allocated more newly acquired P to grains than high P plants. Finally, the remobilization of P from endogenous P sources is the main supplier of P to grains in durum wheat. Small reduction in grain P concentrations can be achieved through limiting the allocation of newly taken up P to grains rather than reducing P remobilization.

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Chapitre 6

Discussion générale, conclusion et perspective

6.1 Éléments de contexte et rappel des objectifs

En agronomie, réduire la dépendance vis-à-vis des engrais de synthèse tout en maximisant le rendement et en préservant l'environnement est l'un des enjeux majeurs de la recherche sur la nutrition minérale des plantes. Le phosphore (P) est un élément nutritif essentiel pour tous les êtres vivants. Chez les plantes, le P est un constituant non substituable impliqué dans plusieurs fonctions telles que la structure membranaire, le métabolisme énergétique et la réplication du matériel génétique (Maathuis, 2009; Veneklaas et al., 2012). La gestion de la nutrition phosphatée des plantes est une composante majeure dans la conception des systèmes de culture plus performants à bas niveau d'intrant (Richardson et al., 2011; Tilman et al., 2002). Chez les céréales, environ 65 % du P apporté par les engrais phosphatés est retiré du champ avec les produits récoltés chaque année (Lott et al., 2009). Le phosphore exporté est principalement stocké sous forme de phytate dans les grains (Raboy, 2009; Rose et al., 2013). Le phytate est indigestible par l'homme et les animaux monogastriques et finit par se retrouver dans l'environnement où il contribue aux phénomènes d'eutrophisation (Brinch-Pedersen et al., 2002; Raboy, 2009). De plus, l'efficacité d'utilisation des engrais est généralement faible, car seuls 20 à 30 % des engrais appliqués sont récupérés par les cultures (Cordell and White, 2014; Schachtman et al., 1998). L'un des moyens pour diminuer le recours à la fertilisation minérale ou organique en P serait d'augmenter l'efficacité d'acquisition du P du sol et d'utilisation du P absorbé par les cultures (Rose and Wissuwa, 2012; Wang et al., 2010b). La première stratégie couplée à l'optimisation des pratiques de fertilisation plus adaptée aux besoins des cultures ou des apports localisés par exemple ont permis de diminuer la quantité de P à apporter (Pellerin and Nesme, 2012). Elle a également amélioré l'efficacité d'acquisition du P par les plantes. Néanmoins, à l'échelle du cycle du P dans les agrosystèmes, le flux de P asso-

cié aux exportations avec les récoltes reste un flux important. De ce fait, la réduction de l'accumulation de P dans les grains tout en maintenant la production est un autre levier d'action pour améliorer l'efficacité d'utilisation du P à l'échelle du cycle biogéochimique du P dans les agrosystèmes. De plus, en réduisant la quantité de P stockée sous forme de phytate dans les grains, la quantité de P non assimilable introduite dans la chaîne alimentaire sera réduite et limitera les risques de transfert du P vers l'environnement. Malgré cette stratégie prometteuse, les travaux qui ont été faits dans ce sens restent limités et les connaissances acquises jusqu'à présent proviennent principalement d'études menées sur le riz.

Ainsi, les travaux de ma thèse visent à améliorer notre connaissance sur les déterminants écophysologiques de l'allocation du phosphore au cours du développement des grains chez le blé dur. Afin de mieux comprendre ces déterminants, les processus tels que l'absorption, le transport, le stockage et la remobilisation doivent d'être bien caractérisés. Dans ce contexte, les objectifs de ma thèse étaient d'une part d'évaluer l'effet de la disponibilité en P au stade post-floraison sur la remobilisation et les teneurs en P des grains, et d'autre part de déterminer la contribution de la remobilisation et de l'absorption post-floraison au P accumulé dans les grains ainsi que la dynamique et les voies de transport du P. Après une analyse de la littérature scientifique sur le sujet, dans la première partie de ce travail de thèse (Chapitre 3), nous avons analysé les effets d'une privation en P pendant la période post-floraison sur la remobilisation et les flux de P et ses conséquences sur le rendement et la teneur en P des grains. Avec l'application de ce traitement, l'objectif était d'évaluer la remobilisation « potentielle » du P accumulé pendant la phase végétative vers les grains en réalisant des bilans de P dans les compartiments de la plante. Deux cultivars de blé dur avec des capacités d'accumulation de P contrastées ont été étudiés. Dans la seconde partie (Chapitres 4 & 5), nous avons utilisé deux approches de marquage complémentaire au ^{32}P afin de caractériser la dynamique du P à l'échelle de la plante entière. D'abord un marquage continu de la floraison jusqu'à la maturité. Ce type de marquage a permis de quantifier la contribution de la remobilisation et de l'absorption post-floraison au P accumulé dans les grains. Ensuite, un marquage ponctuel de 24h à deux stades de développement des grains : le premier pendant la phase de division cellulaire (5 jours après la floraison) et le second pendant la phase du remplissage (14 jours après la floraison). Ce marquage a permis d'étudier la cinétique et la répartition du P récemment absorbé ainsi que les voies de transport impliquées dans la délivrance du P aux grains. L'effet de la disponibilité en P sur l'ensemble des processus identifiés a été étudié en manipulant l'offre en P dans le milieu.

6.2 Effet de la limitation en P pendant la phase post-floraison sur la remobilisation et les teneur en P des grains

Nos travaux ont montré que la privation en phosphore appliqué à partir de la floraison n'avait pas d'effet négatif sur le rendement et sur la qualité des grains récoltés (ratio C :N), malgré la diminution dans la biomasse totale des plantes (particulièrement chez *Dakter*). Ce résultat est une démonstration directe que la croissance des grains n'est pas limitée par l'offre en P pendant la période post-floraison si les plantes sont suffisamment alimentées en P durant la phase végétative. En effet, les données de la littérature suggéraient que la nutrition phosphatée du blé après la floraison aurait peu d'effet sur le rendement en raison de la grande efficacité de remobilisation du P, même si cela n'a pas été directement démontré. Les premiers travaux chez le blé rapportent qu'un apport tardif du P autour de la floraison ne se traduit pas par une augmentation des rendements (Batten et al., 1986; Batten and Wardlaw, 1987; Rose et al., 2007). En accord avec nos résultats, Batten et al. (1986) ont montré que l'apport du P au-delà de la floraison entraîne une augmentation de la biomasse totale et de la teneur en P des grains sans augmentation du rendement. En effet, l'impact d'une carence en P chez le blé se manifeste tôt pendant la phase végétative par une réduction du nombre de talles, diminuant ainsi le nombre des épis et des grains à maturité (Elliott et al., 1997; Grant et al., 2001; Rodríguez et al., 1999; Wang et al., 2010a). Étant donné que le nombre de talles est déterminé avant la floraison, la disponibilité en P est plus critique pendant la phase végétative que pendant la phase reproductive où seulement le nombre et le poids final des grains pourrait être affecté (Grant et al., 2001; Wang et al., 2010a).

La privation en P tout au long de la phase post-floraison a modifié la répartition du P entre les organes du blé dur sans affecter le rendement final. Ceci s'explique par la grande efficacité de remobilisation et de transport du P vers les grains. En effet, dans ces conditions expérimentales, la totalité du P accumulé dans les grains provient de la remobilisation du P prélevé avant la floraison. De ce fait, l'indice de récolte du phosphore (Phosphorus harvest index, PHI), qui est une mesure de l'efficacité de l'allocation du P aux grains, est plus élevé en condition de privation post-floraison qu'en conditions d'offre continue en P. Généralement chez les céréales, la proportion du P total qui est allouée aux grains est plus élevée que la proportion du carbone (masse sèche), témoignant de la translocation intense du P dans les graines pendant la période post-floraison (Veneklaas et al., 2012). En conditions de faible de disponibilité en P, un PHI allant de 0,6 à 0,9 est fréquemment évoqué dans la littérature (Batten, 1992; Rose et al., 2010; Sinclair and

Vadez, 2002). En effet, la sélection de variétés à fort rendement a permis d'augmenter la proportion de masse sèche allouée aux grains, ce qui a entraîné une diminution de la concentration en P par un effet de dilution (Batten, 1986b; Calderini et al., 1995; Wang et al., 2017). Cette augmentation du rendement est accompagnée par une augmentation, mais à moindre degré, de l'allocation de P (PHI) et d'autres éléments tel que l'azote vers les grains (Calderini et al., 1995; Wang et al., 2017).

En revanche, l'indice de récolte (Harvest index, HI) n'est pas différent entre les deux traitements, suggérant que la remobilisation du P n'est pas forcément dépendante de celle du carbone lorsque les plantes sont privées du P, bien que le remplissage des grains en carbone et en P soient étroitement liés. En effet, de fortes corrélations ont été observées entre l'accumulation de carbone et de P dans les grains en croissance, impliquant que l'allocation du P vers les grains est probablement déterminée par ses capacités à accumuler du carbone (force de puits) (Marshall and Wardlaw, 1973). Une des explications est que le métabolisme carboné n'était pas impacté par la limitation en P pendant le remplissage des grains, et par conséquent, l'allocation du carbone aux grains n'était pas affectée. Ceci pourrait être dû à la capacité des plantes à remobiliser les pools de P les moins essentiels pour le fonctionnement du métabolisme carboné tels que le P stocké dans les vacuoles ou celui des phospholipides qui peuvent être remplacés par des lipides ne contenant pas du P (e.g. galactolipides, sulfolipides). C'est le cas chez le riz où il a été démontré que la privation en P après la floraison n'avait pas d'effet sur la photosynthèse puisque le recyclage interne et la récupération du P présent dans les vacuoles et les membranes phospholipidiques permet de la maintenir (Jeong et al., 2017b). De plus, en conditions limitantes en P, la stimulation des voies métaboliques consommant moins de P et utilisant les pyrophosphates comme substrat énergétique permet de maintenir l'activité métabolique malgré le manque de P (Hammond et al., 2004; Stigter and Plaxton, 2015; Veneklaas et al., 2012). L'ensemble de ces ajustements est également observé dans la famille des *Protéacées*, qui se sont adaptées aux milieux très pauvres en P et qui sont caractérisées par une haute efficacité d'utilisation du P (Lambers et al., 2011, 2015).

L'interconnexion entre le métabolisme carboné et phosphaté résulte du fait que le P entre dans la composition des acides nucléiques et de l'ATP qui sont nécessaires pour la photosynthèse et la plupart des activités métaboliques (Marschner, 2011). Au niveau de la plante entière, une limitation en P entraîne le plus souvent une redirection des assimilats carbonée vers les racines, entraînant une augmentation dans le ratio de biomasse des parties racinaires sur celle des parties aériennes (Cakmak et al., 1994; Mollier and Pellerin, 1999). Cette réponse à la limitation en P est souvent plus prononcée aux stades jeunes

qu'aux stades plus tardifs (Elliott et al., 1997; Grant et al., 2001). Après la floraison, les racines ne représentent plus un puits majeur de P malgré l'absence de remobilisation en conditions d'offre continue en P.

6.3 Contribution de l'absorption post-floraison du phosphore et de la remobilisation au P accumulé dans les grains

Dans la première expérimentation (Chapitre 3), le phosphore accumulé dans les grains provient principalement de l'absorption post-floraison en condition d'offre continue en P (96 % pour *Dakter* Vs. 80% pour *Sculptur*). L'absorption post-floraison de P a représenté respectivement 68 % et 51 % du P totale pour le *Dakter* et le *Sculptur*. L'efficacité d'absorption du P par unité de biomasse racinaire n'est pas significativement différente entre les cultivars. Ainsi, les différences observées dans l'accumulation du P entre les deux cultivars s'expliquent en grande partie par la taille plus importante du système racinaire chez *Dakter* par rapport à celui de *Sculptur*. Ces résultats s'accordent en partie avec les données de littérature. En effet, comme souligné dans la synthèse bibliographique, les estimations de l'absorption post-floraison et de sa contribution au P des grains chez le blé sont très variables (Manske et al., 2001; Rose et al., 2007; Pampana et al., 2007). Bien que l'absorption post-floraison de P soit possible, sa contribution au P total accumulé par la plante est faible, ce qui contraste quelque peu avec nos résultats. L'explication la plus plausible serait que dans les conditions hydroponiques, la diminution de l'activité racinaire est moins prononcée que dans les conditions de champ, ce qui peut conduire à une surestimation de l'absorption post-floraison de P par les racines. De façon générale, l'absorption des nutriments baisse après la floraison en raison de la progression de la sénescence (Hawkesford et al., 2018; White and Hammond, 2008b). De plus, la faible absorption post-floraison du P évoquée dans la littérature pourrait être associée aux conditions environnementales de culture en fin de cycle de développement, qui ne sont souvent pas favorables à l'absorption du P plutôt qu'à l'altération des capacités d'absorption racinaire (Manske et al., 2002; Römer and Schilling, 1986; Rose et al., 2007). Parmi les paramètres impliqués, la disponibilité en eau semble jouer un rôle majeur dans l'absorption de P en conditions de culture au champ (Hinsinger, 2001; Shen et al., 2011). Les plantes absorbent le P sous forme d'ions phosphate présent dans la solution du sol. Pour cela, l'absorption de P dépend d'un niveau minimum d'humidité du sol. Il a été démontré que l'absorption de P diminue avec la baisse de la disponibilité de l'eau dans les conditions du champ (Clarke et al., 1990).

En condition non limitante en P pendant la période post-floraison, la remobilisation du P à partir des organes végétatifs a contribué respectivement à 20 et à 4 % du P accumulé dans les grains chez *Sculptur* et *Dakter*. La contribution de la remobilisation dans ces conditions pourrait être sous-estimée car son calcul est basé sur un bilan réalisé entre la floraison et la maturité. Étant donné que la variation de la quantité de P dans un organe se fait en fonction de l'influx (absorption post-floraison) et de l'efflux, la remobilisation nette ne sera détectée que lorsque l'efflux dépasse l'influx. Avec cette méthode de calcul, le P pourrait être remobilisé d'un organe tout en s'accumulant dans un autre ou sans changement dans la quantité totale sans être détecté. Afin d'estimer la contribution de l'absorption post-floraison et de la remobilisation au P accumulé dans les grains, un traçage isotopique de la floraison jusqu'à la maturité est nécessaire. C'est ce que la deuxième expérience (Chapitre 4), où seul le cultivar *Sculptur* a été utilisé, nous a permis de quantifier. Les résultats obtenus montrent que la remobilisation du P a contribué à 81 % du P des grains dans chez plantes cultivées avec un faible niveau en P tandis qu'elle a contribué à 65 % pour celles cultivées avec un niveau élevé en P. Ce résultat est en accord avec des travaux antérieurs montrant que la remobilisation du P augmente en conditions de limitation en P (Abbas et al., 2018; Jeong et al., 2017b; Julia et al., 2016; Maillard et al., 2015). À titre de comparaison, dans la première expérience, nous avons observé que la remobilisation du P à partir des racines se produisait à la fois chez *Dakter* (25 %) et *Sculptur* (28 %) pendant la privation du P. Cette valeur semble être surestimée puisque l'utilisation du traceur a révélé que seuls 19 et 9 % du P étaient remobilisés à partir des racines en situation de forte et de faible offre en P, respectivement.

La comparaison des deux cultivars dans le Chapitre 3 montre que le P peut être remobilisé à partir de tous les organes du blé dur quand ils sont soumis à une limitation totale en P pendant la période post-floraison. Néanmoins, les deux cultivars se distinguent quant à leur aptitude à remobiliser le P en condition non limitante en P. Dans ces conditions, nous avons observé de la remobilisation nette à partir des tiges (10 %) racines (7 %), et feuilles (3.5 %) chez *Sculptur* et seulement à partir des tiges (4 %) chez *Dakter*. Ceci peut être lié à leur capacité à maintenir l'absorption post-floraison. En effet, *Dakter* a continué d'absorber du P pendant toute la période post-floraison, tandis que *Sculptur* a maintenu l'absorption de P uniquement jusqu'à 20 jours après l'anthèse. Ce résultat diffère de celui obtenu par Rose et al. (2007) qui ont montré que les racines du blé n'agissent pas comme une source de P pour le grain de blé (*Triticum aestivum* L.). Néanmoins, les mêmes auteurs ont rapporté qu'une partie du P contenu dans les racines du colza a pu être transférée aux graines suggérant que la remobilisation peut avoir lieu dans les racines. Les données sur la remobilisation des nutriments dans les racines sont rares et variables

selon les conditions de culture. Par analogie avec le P, la quantité d'azote contenu dans les racines du blé à maturité, cultivé en conditions hydroponiques a été estimé de représenter 10 à 20 % de l'azote total de la plante alors qu'il ne représente que 4 % en conditions de culture au champ (Allard et al., 2013; Andersson and Johansson, 2006). Par conséquent, l'azote contenu dans les racines a un effet marginale sur les flux de l'azote dans la plante car sa remobilisation ne représente que 3 % de l'azote total des grains (Allard et al., 2013).

6.4 Importance de la remobilisation dans le remplissage des grains en phosphore

La remobilisation est un processus important qui permet le recyclage des nutriments en cas de carence ou au cours la sénescence (Distelfeld et al., 2014; Etienne et al., 2018; Maillard et al., 2015). Nos travaux ont montré que lorsque les plantes sont bien alimentées pendant la phase végétative, la remobilisation du P est suffisante pour couvrir les besoins des grains en croissance en P, même avec une privation totale de P exogène après la floraison (Chapitre 3). Le traçage isotopique à l'aide du ^{32}P (Chapitre 4) a permis de montrer que la remobilisation du P représente 81 % du P des grains pour les plantes cultivées avec un faible niveau en P tandis qu'elle représente 65 % pour les plantes cultivées avec un niveau élevé en P. Les organes les plus sollicités et qui contribuent le plus au P accumulé dans les grains des plantes cultivées avec un faible niveau en P sont les épis, les feuilles et les tiges. Leur contribution dépasse 91 % du P remobilisé à l'échelle de la plante entière. Ces résultats sont cohérents avec à ceux obtenus dans d'autres expériences chez le blé. Par exemple, Masoni et al. (2007) ont trouvé que selon le type du sol, la remobilisation du P représentait 56 à 63 % du P des grains du blé dur. Chez le blé déficient en P, environ 50 et 90 % du P des grains pouvait être attribué au P remobilisé à partir des réserves préalablement stockées dans les tissus végétatifs (Batten, 1986b). Dans la littérature, la remobilisation du P à partir des feuilles a reçu beaucoup plus d'attention que pour les autres organes de la plante (Etienne et al., 2018; Maillard et al., 2015). On estime qu'en condition de limitation en P, 90 % du P contenu dans les feuilles âgées du blé pourrait être remobilisé et transporté vers les tissus en croissance (Etienne et al., 2018). Les espèces à sénescence monocarpique sont caractérisées pour une remobilisation et une redistribution intense des nutriments au cours du développement des grains (Distelfeld et al., 2014; Gregersen et al., 2008; Hawkesford et al., 2018). En effet, pendant cette phase, les grains constituent le puits majeur du carbone et des nutriments dont le P (Dissanayaka et al., 2018; Veneklaas et al., 2012). C'est ce que nous avons pu également évaluer dans le Chapitre 4. Les plantes cultivées avec une offre faible en P ont produit très peu de talles post-floraison (4 % de la biomasse totale) que celles cultivées avec une offre élevée en P

(28 % de la biomasse totale). Ceci montre une allocation préférentielle du P et du carbone aux grains au détriment des talles tardives chez les plantes cultivées avec un faible niveau en P. Ce résultat confirme que les grains sont le principal puits de P pendant le développement des grains chez le blé dur et que la production tardive de talles n'est possible que lorsque l'offre en P dépasse la demande générée par les grains en croissance.

La sénescence s'accompagne par des changements macroscopiques (e.g. jaunissement des feuilles), qui sont le reflet d'événements biochimiques et moléculaires qui se mettent en place au cours de la période post-floraison. C'est ce que nous avons pu constater chez les plantes qui ont été cultivées avec un faible niveau en P ou celles privées de P durant la période post-floraison grâce à la mesure des teneurs en chlorophylle (mesures SPAD). Chez ces plantes, nous avons observé un jaunissement plus précoce témoignant de leur sénescence rapide en comparaison avec celles cultivées avec un niveau élevé en P. Au cours de la sénescence des feuilles, la chlorophylle et d'autres macromolécules telles que les protéines, les lipides et les acides nucléiques sont dégradées et les éléments, principalement l'azote, qu'elles contiennent sont remobilisés et transportés vers les grains en croissance. La remobilisation et le transport du P sont facilités par sa mobilité dans le xylème et le phloème (Marschner, 2011). De plus, le P stocké dans les vacuoles sous forme de d'ion phosphate, qui peut représenter jusqu'à 90 % du P totale dans la cellule lorsque les plantes sont bien pourvues en P (Raghothama and Karthikeyan, 2005; Yang et al., 2017), pourrait être plus facilement remobilisé en comparaison avec celui stocké sous forme organique. De ce fait, le P remobilisé à partir des vacuoles permet à la plante de maintenir la croissance des grains sans nécessairement réduire les capacités de photosynthèse. Cela atténue l'effet de la privation de P en fournissant la totalité du P accumulé dans les grains sans effet majeur sur le rendement et ses composantes.

6.5 La dynamique des flux du phosphore pendant la phase post-floraison et voies de transport vers les grains

Les deux marquages successifs au ^{32}P au cours du développement des grains du blé dur ont permis d'obtenir des résultats originaux concernant la dynamique du P récemment absorbé pendant la période post-floraison. À notre connaissance, c'est la première fois qu'un tel type de marquage est effectué chez le blé. Les deux niveaux d'offre en P appliquées tout au long du cycle (0.125 mM ou 0.025 mM de KH_2PO_4) ont permis d'obtenir des plantes avec des statuts P contrastés. D'abord, quel que soit le stade de développe-

ment des grains considéré, peu de P récemment absorbé est alloué directement aux grains, bien qu'il soit présent dans les organes végétatifs proches de celle-ci tels que les épis et les feuilles. Toutefois, cette proportion a augmenté avec le temps pour les deux niveaux d'offre en P. Elle est plus importante chez les plantes à faible statut P que chez les plantes à statut P élevé. Nos résultats révèlent donc que le P absorbé par les racines pendant la période post-floraison n'est pas directement alloué aux grains. Le P est stocké temporairement dans les organes végétatifs (épis et feuilles) avant d'être redistribué aux grains en croissance. Le transfert xylème-phloème est alors important dans ce processus. L'ensemble de ces résultats est concordant avec les observations de Zee and O'Brien (1970) qui ont démontré l'existence d'une discontinuité au niveau du xylème chez le blé. Cela implique que le P récemment absorbé par les racines ne pourrait pas être directement transféré aux grains via le xylème qui représente la principale voie du transport du P jusqu'aux parties aériennes. De ce fait, le P récemment absorbé devrait être déchargé dans le phloème avant d'être transféré vers les grains. Contrairement au blé, le riz ne possède pas de discontinuité au niveau du xylème, suggérant que le P peut être chargé directement aux grains. Bien que cela soit possible chez le riz, Julia et al. (2016) ont démontré que la grande majorité du prélevé pendant la période post-floraison n'est pas directement alloué dans les grains, ce qui implique l'existence de plusieurs points de régulation. Chez la plupart des céréales, il a été démontré que les nœuds jouent un rôle prépondérant dans le partitionnement des éléments absorbés et remobilisés à l'échelle de la plante entière (Huang et al., 2020; Kisko et al., 2018; Yamaji and Ma, 2014). Récemment, Yamaji et al. (2017) ont identifié un transporteur appelé SPDT impliqué dans l'allocation du P aux grains chez le riz. La perte de fonction de ce transporteur, fortement exprimé au niveau des nœuds, modifie la distribution du P avec une réduction de 20 % du P des grains sans affecter le rendement et la capacité germinative des grains produits. La réduction de l'allocation du P aux grains est accompagnée d'une augmentation dans la concentration du P dans les feuilles et une diminution de 20 à 30 % des phytates dans les grains (Yamaji et al., 2017). Ainsi, l'identification de ce type de transporteur chez le blé pourrait être un moyen efficace pour réduire l'accumulation du P dans les grains sans pénaliser le rendement.

6.6 Les leviers pour réduire l'accumulation du P dans les grains et améliorer l'efficience d'utilisation du P par les cultures

À partir des résultats obtenus et en s'appuyant sur l'analyse de la littérature, deux stratégies émergent pour réduire l'accumulation du P dans les grains, et en conséquence

diminuer les exportations du P avec les récoltes chez le blé. La première est d'identifier des variétés avec une faible capacité d'absorption post-floraison ou avec une remobilisation moins importante aux cours du développement des grains . En effet, nos résultats ont montré que P absorbé durant la période post-floraison ne contribue pas significativement à augmenter les rendements (El Mazlouzi et al., 2020a,b). De plus, la variabilité génotypique concernant la capacité de stockage et de remobilisation de P existe chez le blé comme chez la plupart des céréales (Abbas et al., 2018; Bovill et al., 2013; McDonald et al., 2015). La deuxième stratégie consiste à limiter l'allocation du P absorbé pendant la période post-floraison aux grains. En se situant dans un contexte plus large et en considérant le fonctionnement de la plante dans son intégralité, on s'aperçoit que la première stratégie n'est pas idéale pour plusieurs raisons. D'abord, l'absorption post-floraison est un processus important pour d'autres éléments comme l'azote, dont dépend la quantité de protéines dans les grains (Clarke et al., 1990; Kichey et al., 2007; Masoni et al., 2007). Cette dernière est l'un des critères de qualité les plus importants à prendre en compte chez le blé (Grant et al., 2012a). De même, la manipulation de la remobilisation de P à travers le ralentissement de la sénescence (e.g. knock-out des gènes régulant la sénescence) pourrait avoir des conséquences sur les flux des autres éléments dont la remobilisation est le contributeur majeur (en particulier les micro-éléments) (Etienne et al., 2018; Uauy et al., 2006).

En effet, des mutants de blé dur avec une senescence retardée ont été identifiés (Spano et al., 2003). Ces génotypes, qualifiés de « stay-green », conservent une activité photosynthétique sur une période prolongée en comparaison avec les génotypes non « stay-green ». Dans la littérature, ce caractère est associé avec des caractéristiques agronomiques intéressantes telles que l'augmentation du rendement, une meilleure utilisation de l'azote et une tolérance aux stress abiotique (e.g. stress thermique) (Gregersen et al., 2008; Kichey et al., 2007; Spano et al., 2003; Uauy et al., 2006). Néanmoins, aucune étude n'a pu mettre en relation l'impact de ce trait sur l'accumulation du P dans les grains. On peut émettre l'hypothèse que les plantes ayant un caractère « stay-green » peuvent retenir plus de P dans leurs tissus végétatifs, ce qui entraînerait une réduction de l'allocation du P aux grains. La caractérisation des transporteurs du P dans les tissus sources ainsi que ceux impliqués dans son transfert dans les grains est primordiale pour les études futures. L'étude des mutants déjà identifiés pourrait apporter quelques éléments de réponse (Spano et al., 2003). Nous avons contacté ces auteurs pour tester nos hypothèses, mais ces génotypes ne sont plus disponibles dans leur laboratoire.

Néanmoins, le caractère stay-green du blé peut également avoir des effets non-désirables. Ce trait pourrait être associé avec des altérations au niveau de la machinerie permettant

la dégradation ou le transport des éléments remobilisés (Gregersen et al., 2008). Chez certains géotypes « stay-green », l'indice de récolte est plus faible malgré que la teneur en chlorophylle, la photosynthèse, et la biomasse totale étaient toutes plus élevées en comparaison avec le géotype de la base (Gong et al., 2005). Ceci est dû à la faible efficacité de la remobilisation du carbone vers les grains (Gong et al., 2005; Gregersen et al., 2008). Par ailleurs, la réduction de l'expression du facteur de transcription NAM-B1, qui est impliqué dans la remobilisation des nutriments vers les grains pendant la sénescence des feuilles chez le blé, entraîne une diminution de la concentration de P et diminue également celle de l'azote, du zinc et du fer (Waters et al., 2009). Il a été également démontré que l'altération ou la dérégulation de l'autophagie (mécanisme cellulaire responsable de la dégradation et du recyclage des nutriments) affecte la remobilisation de l'azote et du fer vers les graines chez *Arabidopsis* (Masclaux-Daubresse et al., 2017; Pottier et al., 2018). Chez le blé, le manque de connaissances spécifiques concernant les acteurs moléculaires régulant la remobilisation du P pendant la sénescence reste un frein pour considérer cette stratégie. Par exemple, seules les phosphatases acides (e.g. Purple Acid Phosphatases, PAPs) et les RNases sont spécifiquement impliqués dans la remobilisation du P contenus dans les phospholipides et les acides nucléiques en réponse à la privation en P ou pendant le remplissage des grains chez riz (Jeong et al., 2017a; Lambers et al., 2015; Wang et al., 2016). Enfin, toute modification des processus liés à la sénescence devrait alors prendre en compte son effet sur la remobilisation et l'absorption post-floraison des autres nutriments, car ils sont essentiels pour l'élaboration de la qualité nutritionnelle des grains.

En revanche, réduire l'allocation du P absorbé pendant la période post-floraison aux grains semble réalisable. En effet, comme mentionné dans la partie 6.5, la manipulation des transporteurs de P peut être une stratégie très efficace pour diminuer l'allocation de P aux grains sans affecter les performances agronomiques et la qualité des grains comme l'ont démontré Yamaji et al. (2017). En effet, ces auteurs ont également rapporté que la biodisponibilité du Zn et du Fe est améliorée en raison de la réduction des phytates dans les grains. Chez le blé, les acteurs moléculaires impliqués dans la régulation du transport de P aux grains ne sont pas encore clairement identifiés. La caractérisation des systèmes de transports est une approche prometteuse pour réduire les exportations du P et améliorer notre connaissance sur les mécanismes contrôlant le transport du P aux grains.

6.7 Retour sur la démarche de thèse et perspectives

Comme évoqué précédemment, la réduction de l'accumulation du phosphore dans les grains représente un enjeu majeur à plusieurs niveaux. D'abord en terme de rendement

des cultures où la fertilisation phosphatée doit être maîtrisée pour limiter les fuites vers l'environnement et boucler le cycle de P au regard de l'avenir incertain de la disponibilité des engrais P de haute qualité. Ensuite, en terme de qualité si l'on considère les effets anti-nutritionnels des phytates. La stratégie de recherche adoptée au cours de ce travail de thèse repose essentiellement sur une démarche expérimentale en conditions contrôlées qui a permis d'améliorer notre connaissance sur la dynamique du P pendant le développement des grains chez le blé dur. Nous avons également fourni de nouveaux éléments de compréhension concernant les voies de transport impliquées dans le transfert du P aux grains. Sur la base de ces travaux, de multiples perspectives pour les recherches futures pourraient être envisagées.

La culture des plantes en hydroponie a été choisie parce qu'elle présente l'avantage de permettre un contrôle complet et précis des milieux nutritifs ainsi que la récupération du système racinaire intact qui est difficile à évaluer dans le sol. Contrairement aux résultats des études précédentes (Osborne and Rengel, 2002b; Rodríguez et al., 1998b), le niveau de P qualifié ici de faible (0.025 mM) n'a pas eu un effet significatif sur le rendement, bien que la biomasse totale et les teneurs en P ont été affectées. Il est probable que, dans nos conditions expérimentales, le niveau de P apporté dans la solution nutritive n'a pas été suffisamment faible pour induire une carence forte en P chez *Sculptur*. L'autre élément à prendre en compte est le nombre de talles par plantes. En effet, l'absence d'effet sur le rendement pourrait être due au fait que nous avons contrôlé le nombre de talles formés tout au long de la période de croissance des plantes, ce qui est un élément clé dans l'établissement du rendement chez le blé. En hydroponie, le contrôle du nombre des talles est important pour synchroniser le cycle de développement et pour éviter le développement d'un grand nombre de talles sur des plantes isolées. Afin de s'affranchir de ces deux limites dans les prochaines expérimentations, il est nécessaire de déterminer les concentrations en P dans la solution nutritive permettant d'affecter, dans ces conditions expérimentales, significativement la croissance du blé dur.

L'étude de l'implication des relations sources-puits dans la répartition du carbone et du P au cours du développement des grains est également un élément important à prendre en considération dans les prochaines études. Ceci permettra de tester l'hypothèse émise pour expliquer le lien entre la remobilisation du carbone et celle du phosphore au cours de la privation en P durant la période post-floraison. La manipulation de la force des sources (e.g. défoliation, gamme de concentrations en P contrastées) ou celle des puits (réduction dans le nombre de grains) pourrait aider à mettre en lumière les mécanismes de régulation des flux du P. Ceci pourrait également apporter des éléments de compré-

hension sur la compétition entre les organes pendant la période post-floraison. De plus, ces données sont essentielles pour modéliser l'accumulation de carbone et du P dans les grains en réponse à la disponibilité en P.

Outre les effets évidents sur la croissance et le rendement du blé, la nutrition phosphatée peut également influencer la qualité des grains. Des études antérieures sur la relation entre le niveau de nutrition en P et la composition en protéines et en microéléments ont montré l'existence des effets contrastés (Iwai et al., 2012; Pearce et al., 2014; Zhang et al., 2017; Wu et al., 2013). Au cours de la seconde expérimentation (Chapitre 4), les teneurs en microéléments incluant Zn, Fe, Cu, Mn, Mo ont été analysés par ICP-MS dans le cadre d'un projet interne annuel 2020 (Collaboration avec unité BIOGET, Jean-Yves CORNU et Christophe Nguyen). Le but de ces analyses était d'examiner l'effet de la baisse de l'offre en P sur les teneurs en microéléments dans les grains. Les premières analyses montrent que la réduction de la teneur en P dans les grains chez les plantes qui ont été cultivées avec une offre faible en P a été accompagnée par une diminution significative de la teneur en Zn et en Mn, en comparaison avec les grains des plantes cultivées avec une offre élevée en P. Les concentrations en Fe, Cu et Mo dans les grains ne sont pas significativement différents entre les deux niveaux d'offre P (cf. résultats préliminaires C). Ces analyses n'ont pas pu être finalisées pendant le temps imparti de la thèse. Par la suite nous voulons déterminer la spéciation et la localisation des microéléments, en particulier le lien entre le Zn, Fe et les phytates dans les grains chez le blé dur. Pour répondre à cet objectif, les teneurs en phytates seront dosées et les lieux de stockage des micronutriments dans les grains seront cartographiés par LA-ICP-MS. Ces analyses permettront de déterminer la co-localisation et le degré d'association des microéléments aux phytates dans les grains en fonction de l'offre en P.

Enfin, et comme on peut le déduire, l'amélioration de l'efficacité d'utilisation du P n'a pas le même objectif que celle de l'azote ou d'autres éléments. Cette dernière cherche à augmenter l'allocation de l'azote aux grains afin d'augmenter la teneur en protéines tandis que pour le phosphore, l'objectif est de diminuer l'allocation du P aux grains et plus particulièrement sa teneur en phytates (Rose et al., 2013; Uauy et al., 2006). L'essor de l'ionomic, qui a pour but d'étudier conjointement les processus impliqués dans l'acquisition, le transport et le stockage des éléments nutritifs, permettra d'intégrer les connaissances acquises sur les interactions entre éléments (Baxter, 2009; Gupta et al., 2015; Salon et al., 2017; Sparvoli and Cominelli, 2015). Ceci est une étape indispensable pour expliquer la variabilité génotypique et celle liée à l'environnement et afin d'améliorer à la fois le rendement et l'efficacité d'utilisation des éléments.

Annexes

Annexe A

Matériel supplémentaire pour le chapitre 3

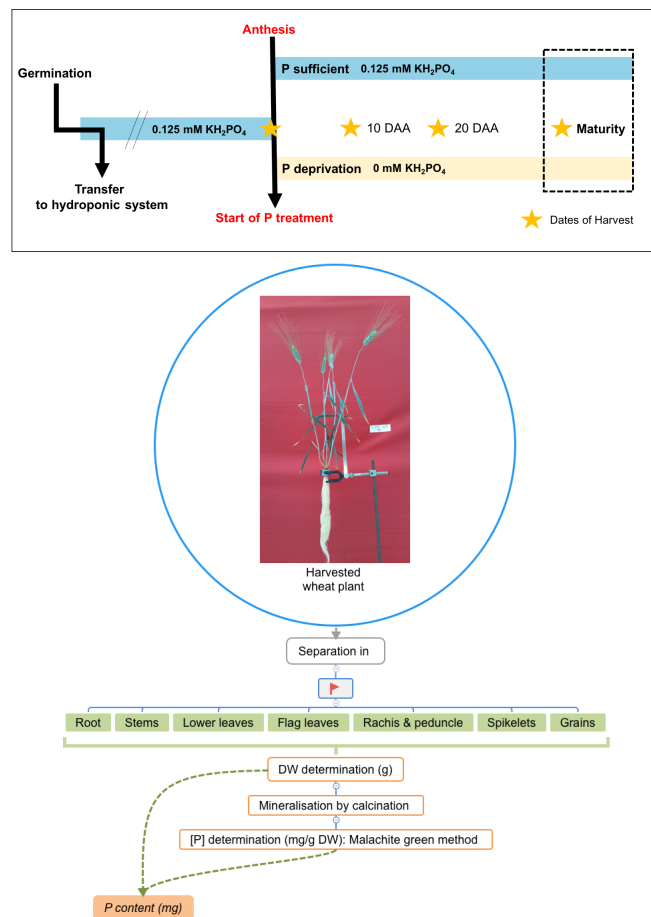


FIGURE A.1 – Schematic representation of hydroponic cultures of durum wheat supplied with or without P during the post-anthesis period : experimental design and the workflow. The two durum wheat cultivars were cultivated in greenhouse conditions. Plants were subjected to two P treatments after anthesis and harvested for analysis at anthesis, 10, 20 days after anthesis (DAA) and at maturity. The second illustration represent an overview of the measurements performed after plant harvest

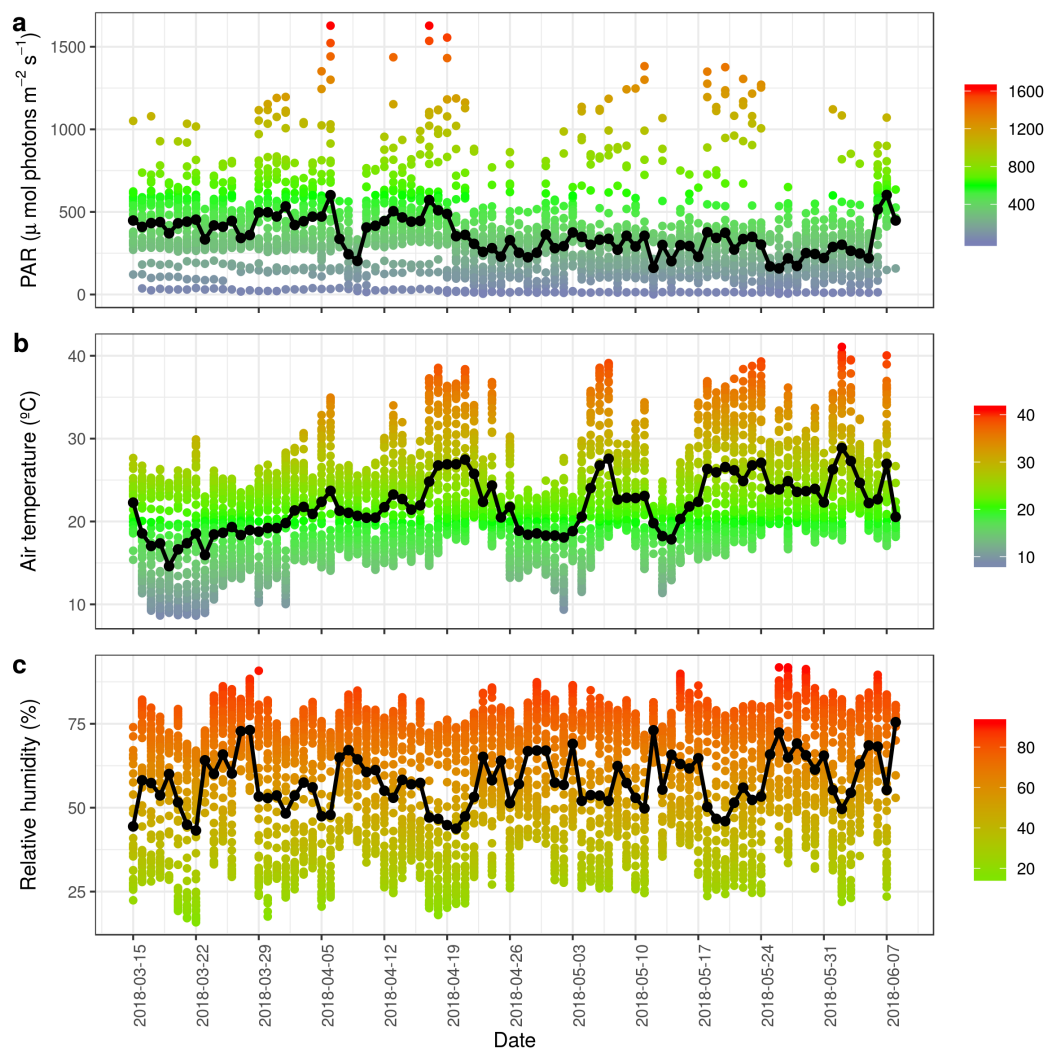


FIGURE A.2 – Greenhouse conditions of the two durum wheat cultivars Dakter and Sculpur from germination to maturity. Time course of a) photosynthetically active radiation during the photo-period (PAR, $\mu\text{mol photons m}^{-2} \text{s}^{-1}$), b) Air temperature ($^{\circ}\text{C}$), and c) Relative humidity (%). The black line shows the daily average of each measurement

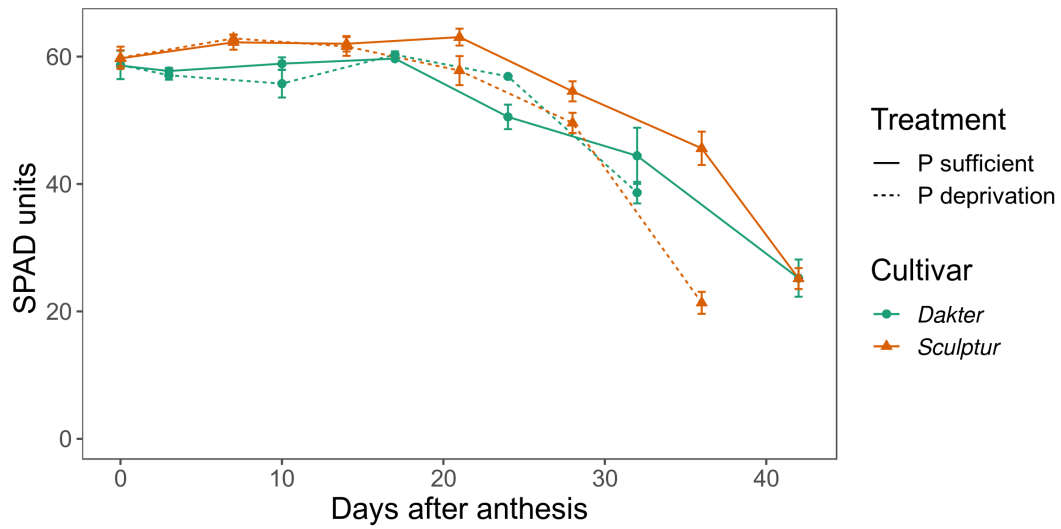


FIGURE A.3 – Chlorophyll content measured as SPAD value on flag leaf of *Dakter* and *Sculptur* grown under P sufficient and P deprivation treatments throughout the post-anthesis period. Each point is the mean of 9 SPAD measurements

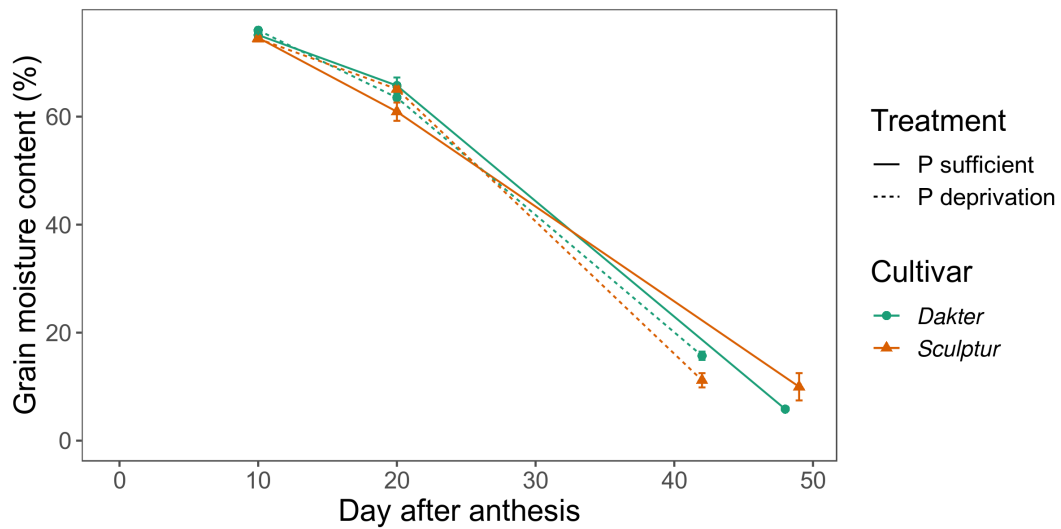


FIGURE A.4 – Grain moisture content of *Dakter* and *Sculptur* grown under P sufficient and P deprivation treatments throughout the post-anthesis period

Annexe B

Matériel supplémentaire pour le chapitre 4

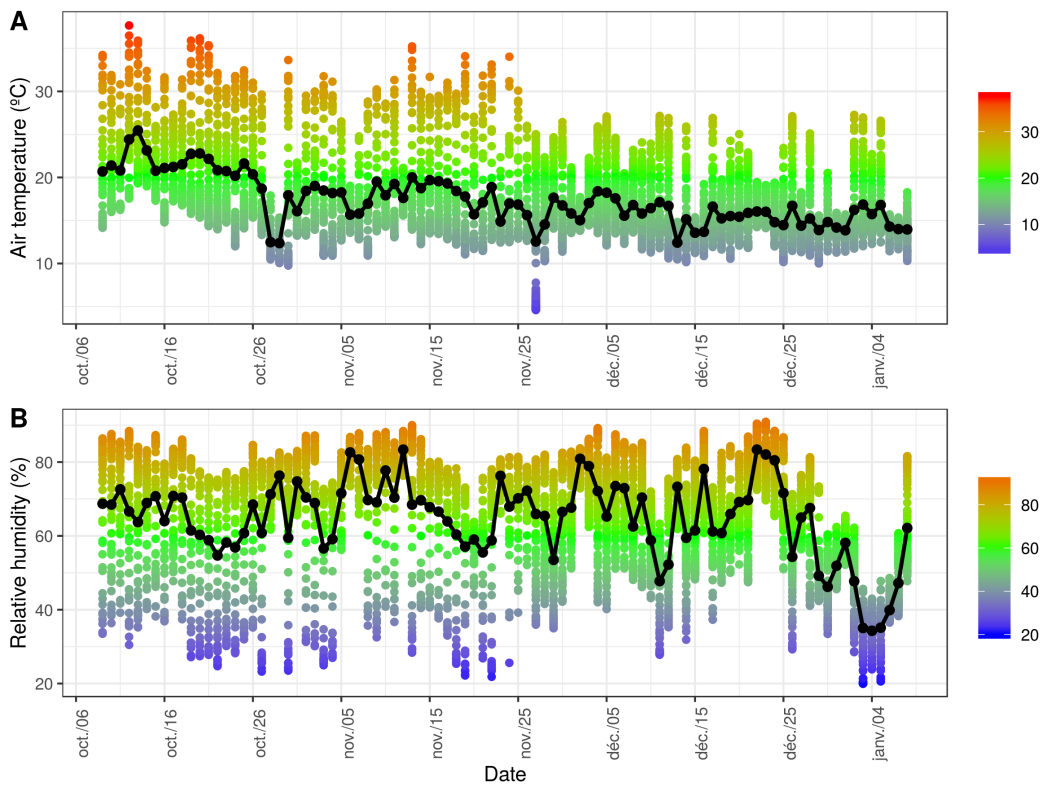


Figure B.1 – Plant growth conditions in the greenhouse. Air temperature (A) and relative humidity (B) during the experiment. For each measurement mean is shown in black line.

Annexe C

Résultats complémentaires le chapitre 6

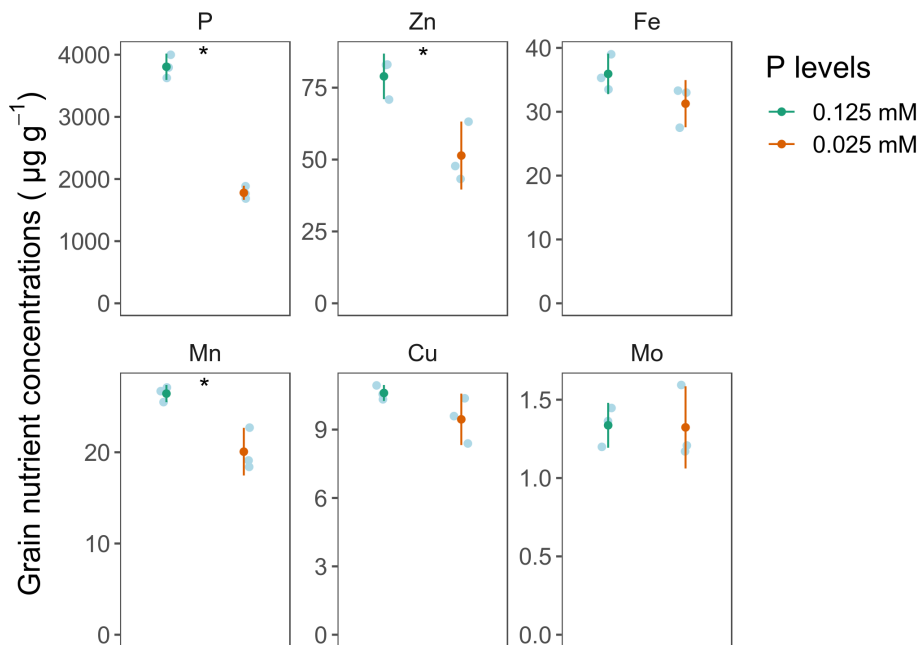


Figure C.1 – Concentrations of P, Zn, Fe, Mn, Cu, Mo in grain of durum wheat plants grown under high P (0.125 mM) and low P (0.025 mM) supply. Error bars are mean \pm standard error of 3 replicates

Références bibliographiques

- Abbas, M., Shah, J. A., Irfan, M. and Memon, M. Y. (2018). Remobilization and utilization of phosphorus in wheat cultivars under induced phosphorus deficiency. *J Plant Nutr* *41*, 1522–1533.
- Allard, V., Martre, P. and Le Gouis, J. (2013). Genetic variability in biomass allocation to roots in wheat is mainly related to crop tillering dynamics and nitrogen status. *Eur J Agron* *46*, 68–76.
- Andersson, A. and Johansson, E. (2006). Nitrogen Partitioning in Entire Plants of Different Spring Wheat Cultivars. *J Agron Crop Sci* *192*, 121–131.
- Andersson, M. X., Stridh, M. H., Larsson, K. E., Liljenberg, C. and Sandelius, A. S. (2003). Phosphate-deficient oat replaces a major portion of the plasma membrane phospholipids with the galactolipid digalactosyldiacylglycerol. *FEBS Lett* *537*, 128–132.
- Assuero, S. G., Mollier, A. and Pellerin, S. (2004). The decrease in growth of phosphorus-deficient maize leaves is related to a lower cell production. *Plant Cell Environ* *27*, 887–895.
- Azeke, M. A., Egielewa, S. J., Eigbogbo, M. U. and Ihimire, I. G. (2011). Effect of germination on the phytase activity, phytate and total phosphorus contents of rice (*Oryza sativa*), maize (*Zea mays*), millet (*Panicum miliaceum*), sorghum (*Sorghum bicolor*) and wheat (*Triticum aestivum*). *J Food Sci Technol* *48*, 724–729.
- Aziz, T., Finnegan, P. M., Lambers, H. and Jost, R. (2014). Organ-specific phosphorus-allocation patterns and transcript profiles linked to phosphorus efficiency in two contrasting wheat genotypes. *Plant Cell Environ* *37*, 943–960.
- Barber, S. A. (1995). *Soil Nutrient Bioavailability : A Mechanistic Approach*. John Wiley & Sons.
- Batten, G. D. (1986a). Phosphorus fractions in the grain of diploid, tetraploid, and hexaploid wheat grown with contrasting phosphorus supplies. *Cereal Chem* *63*, 384–387.
- Batten, G. D. (1986b). The Uptake and Utilization of Phosphorus and Nitrogen by Diploid, Tetraploid and Hexaploid Wheats (*Triticum* spp.). *Ann Bot* *58*, 49–59.
- Batten, G. D. (1992). A review of phosphorus efficiency in wheat. *Plant Soil* *146*, 163–168.

- Batten, G. D. and Wardlaw, I. F. (1987). Senescence of the flag leaf and grain yield following late foliar and root applications of phosphate on plants of differing phosphorus status. *J Plant Nutr* *10*, 735–748.
- Batten, G. D., Wardlaw, I. F. and Aston, M. J. (1986). Growth and the distribution of phosphorus in wheat developed under various phosphorus and temperature regimes. *Aust J Agric Res* *37*, 459–469.
- Battini, F., Grønlund, M., Agnolucci, M., Giovannetti, M. and Jakobsen, I. (2017). Facilitation of phosphorus uptake in maize plants by mycorrhizosphere bacteria. *Sci Rep* *7*, 4686.
- Baxter, I. (2009). Ionomics : studying the social network of mineral nutrients. *Current Opinion in Plant Biology* *12*, 381–386.
- Bieleski, R. L. (1973). Phosphate Pools, Phosphate Transport, and Phosphate Availability. *Annu Rev Plant Physiol* *24*, 225–252.
- Bollons, H. M. and Barraclough, P. B. (1997). Inorganic orthophosphate for diagnosing the phosphorus status of wheat plants. *J Plant Nutr* *20*, 641–655.
- Bonhomme, R. (2000). Bases and limits to using ‘degree.day’ units. *Eur J Agron* *13*, 1–10.
- Bovill, W. D., Huang, C. Y. and McDonald, G. K. (2013). Genetic approaches to enhancing phosphorus-use efficiency (PUE) in crops : challenges and directions. *Crop Pasture Sci* *64*, 179.
- Bregitzer, P. and Raboy, V. (2006). Effects of Four Independent Low-Phytate Mutations on Barley Agronomic Performance. *Crop Sci* *46*, 1318–1322.
- Briat, J.-F., Rouached, H., Tissot, N., Gaymard, F. and Dubos, C. (2015). Integration of P, S, Fe, and Zn nutrition signals in *Arabidopsis thaliana* : potential involvement of Phosphate Starvation Response 1 (PHR1). *Front Plant Sci* *6*.
- Brier, N. D., Gomand, S. V., Donner, E., Paterson, D., Smolders, E., Delcour, J. A. and Lombi, E. (2016). Element distribution and iron speciation in mature wheat grains (*Triticum aestivum* L.) using synchrotron X-ray fluorescence microscopy mapping and X-ray absorption near-edge structure (XANES) imaging. *Plant Cell Environ* *39*, 1835–1847.
- Brinch-Pedersen, H., Sørensen, L. D. and Holm, P. B. (2002). Engineering crop plants : getting a handle on phosphate. *Trends Plant Sci* *7*, 118–125.
- Broadley, M. R., Bowen, H. C., Cotterill, H. L., Hammond, J. P., Meacham, M. C., Mead, A. and White, P. J. (2004). Phylogenetic variation in the shoot mineral concentration of angiosperms. *J Exp Bot* *55*, 321–336.

- Brouns, F., Hemery, Y., Price, R. and Anson, N. M. (2012). Wheat Aleurone : Separation, Composition, Health Aspects, and Potential Food Use. *Crit Rev Food Sci Nutr* 52, 553–568.
- Cadot, S., Bélanger, G., Ziadi, N., Morel, C. and Sinaj, S. (2018). Critical plant and soil phosphorus for wheat, maize, and rapeseed after 44 years of P fertilization. *Nutr Cycl Agroecosyst* 112, 417–433.
- Cakmak, I., Hengeler, C. and Marschner, H. (1994). Partitioning of shoot and root dry matter and carbohydrates in bean plants suffering from phosphorus, potassium and magnesium deficiency. *J Exp Bot* 45, 1245–1250.
- Cakmak, I. and Marschner, H. (1986). Mechanism of phosphorus-induced zinc deficiency in cotton. I. Zinc deficiency-enhanced uptake rate of phosphorus. *Physiol Plant* 68, 483–490.
- Calderini, D. F., Abeledo, L. G. and Slafer, G. A. (2000). Physiological Maturity in Wheat Based on Kernel Water and Dry Matter. *Agron J* 92, 895–901.
- Calderini, D. F., Torres-León, S. and Slafer, G. A. (1995). Consequences of Wheat Breeding on Nitrogen and Phosphorus Yield, Grain Nitrogen and Phosphorus Concentration and Associated Traits. *Ann Bot* 76, 315–322.
- Chapin, F. S. and Bielecki, R. L. (1982). Mild phosphorus stress in barley and a related low-phosphorus-adapted barleygrass : Phosphorus fractions and phosphate absorption in relation to growth. *Physiol Plant* 54, 309–317.
- Chapin, F. S., Schulze, E. and Mooney, H. A. (1990). The ecology and economics of storage in plants. *Annu Rev Ecol Syst* 21, 423–447.
- Clarke, J. M., Campbell, C. A., Cutforth, H. W., DePauw, R. M. and Winkleman, G. E. (1990). Nitrogen and phosphorus uptake, translocation, and utilization efficiency of wheat in relation to environment and cultivar yield and protein levels. *Can J Plant Sci* 70, 965–977.
- Cogliatti, D. H. and María, G. E. S. (1990). Influx and efflux of phosphorus in roots of wheat plants in non-growth-limiting concentrations of phosphorus. *J Exp Bot* 41, 601–607.
- Colomb, B., Debaeke, P., Jouany, C. and Nolot, J. (2007). Phosphorus management in low input stockless cropping systems : Crop and soil responses to contrasting P regimes in a 36-year experiment in southern France. *Eur J Agron* 26, 154–165.
- COMIFER (2019). La fertilisation P-K-Mg : Les bases du raisonnement.
- Cong, W.-F., Suriyagoda, L. D. B. and Lambers, H. (2020). Tightening the Phosphorus Cycle through Phosphorus-Efficient Crop Genotypes. *Trends Plant Sci* 25, 967–975.
- Cordell, D., Drangert, J.-O. and White, S. (2009). The story of phosphorus : Global food security and food for thought. *Global Environ Change* 19, 292–305.

- Cordell, D. and White, S. (2014). Life's bottleneck : sustaining the world's phosphorus for a Food secure future. *Annu Rev Environ Resour* 39, 161–188.
- Crafts-Brandner, S. J. (1992a). Phosphorus Nutrition Influence on Leaf Senescence in Soybean. *Plant Physiol* 98, 1128–1132.
- Crafts-Brandner, S. J. (1992b). Significance of Leaf Phosphorus Remobilization in Yield Production in Soybean. *Crop Sci* 32, 420–424.
- Criado, M. V., Boem, F. H. G., Roberts, I. N. and Caputo, C. (2015). Post-anthesis N and P dynamics and its impact on grain yield and quality in mycorrhizal barley plants. *Mycorrhiza* 25, 229–235.
- Deng, Y., Teng, W., Tong, Y.-P., Chen, X.-P. and Zou, C.-Q. (2018). Phosphorus efficiency mechanisms of two wheat cultivars as affected by a range of phosphorus levels in the field. *Front Plant Sci* 9.
- Dissanayaka, D. M. S. B., Plaxton, W. C., Lambers, H., Siebers, M., Marambe, B. and Wasaki, J. (2018). Molecular mechanisms underpinning phosphorus-use efficiency in rice. *Plant Cell Environ* 41, 1483–1496.
- Distelfeld, A., Avni, R. and Fischer, A. M. (2014). Senescence, nutrient remobilization, and yield in wheat and barley. *J Exp Bot* 65, 3783–3798.
- Dubousset, L., Etienne, P. and Avice, J. C. (2010). Is the remobilization of S and N reserves for seed filling of winter oilseed rape modulated by sulphate restrictions occurring at different growth stages? *J Exp Bot* 61, 4313–4324.
- Egle, K., Manske, G., Römer, W. and Vlek, P. L. G. (1999). Improved phosphorus efficiency of three new wheat genotypes from CIMMYT in comparison with an older Mexican variety. *J Plant Nutr Soil Sci* 162, 353–358.
- El Mazlouzi, M., Morel, C., Chesseron, C., Robert, T. and Mollier, A. (2020a). Contribution of External and Internal Phosphorus Sources to Grain P Loading in Durum Wheat (*Triticum durum* L.) Grown Under Contrasting P Levels. *Front Plant Sci* 11, 870.
- El Mazlouzi, M., Morel, C., Robert, T., Yan, B. and Mollier, A. (2020b). Phosphorus uptake and partitioning in two durum wheat cultivars with contrasting biomass allocation as affected by different P supply during grain filling. *Plant Soil* 449, 179–192.
- Elliott, D. E., Reuter, D. J., Reddy, G. D. and Abbott, R. J. (1997). Phosphorus nutrition of spring wheat (*Triticum aestivum* L.). 1. Effects of phosphorus supply on plant symptoms, yield, components of yield, and plant phosphorus uptake. *Aust J Agric Res* 48, 855–868.

- Engels, C., Kirkby, E. and White, P. (2012). Chapter 5 - Mineral nutrition, yield and source–sink relationships. In Marschner’s Mineral Nutrition of Higher Plants (Third Edition) pp. 85–133. Academic Press San Diego.
- Etienne, P., Diquelou, S., Prudent, M., Salon, C., Maillard, A. and Ourry, A. (2018). Macro and micronutrient storage in plants and their remobilization when facing scarcity : the case of drought. *Agriculture* 8, 14.
- European-Commission (2017). List of Critical Raw Materials for the EU.
- Evers, T. and Millar, S. (2002). Cereal Grain Structure and Development : Some Implications for Quality. *J Cereal Sci* 36, 261–284.
- Ferreira, M. S. L., Martre, P., Mangavel, C., Girousse, C., Rosa, N. N., Samson, M.-F. and Morel, M.-H. (2012). Physicochemical control of durum wheat grain filling and glutenin polymer assembly under different temperature regimes. *J Cereal Sci* 56, 58–66.
- FranceAgriMer (2020). Qualité du blé tendre et du blé dur français : récolte 2019. FranceAgriMer : établissement national des produits de l’agriculture et de la mer.
- Frossard, E., Achat, D. L., Bernasconi, S. M., Bünemann, E. K., Fardeau, J.-C., Jansa, J., Morel, C., Rabeharisoa, L., Randriamanantsoa, L., Sinaj, S., Tamburini, F. and Oberson, A. (2011). The Use of Tracers to Investigate Phosphate Cycling in Soil–Plant Systems. In *Phosphorus in Action : Biological Processes in Soil Phosphorus Cycling Soil Biology* pp. 59–91. Springer Berlin, Heidelberg.
- Gahoonia, T., Care, D. and Nielsen, N. E. (1997). Root hairs and phosphorus acquisition of wheat and barley cultivars. *Plant Soil* 191, 181–188.
- Gahoonia, T. S., Asmar, F., Giese, H., Gissel-Nielsen, G. and Erik Nielsen, N. (2000). Root-released organic acids and phosphorus uptake of two barley cultivars in laboratory and field experiments. *Eur J Agron* 12, 281–289.
- Godfray, H. C. J., Beddington, J. R., Crute, I. R., Haddad, L., Lawrence, D., Muir, J. F., Pretty, J., Robinson, S., Thomas, S. M. and Toulmin, C. (2010). Food Security : The Challenge of Feeding 9 Billion People. *Science* 327, 812–818.
- Gong, Y.-H., Zhang, J., Gao, J.-F., Lu, J.-Y. and Wang, J.-R. (2005). Slow Export of Photoassimilate from Stay-green Leaves during Late Grain-Filling Stage in Hybrid Winter Wheat (*Triticum aestivum* L.). *Journal of Agronomy and Crop Science* 191, 292–299.
- González, F. G., Miralles, D. J. and Slafer, G. A. (2011). Wheat floret survival as related to pre-anthesis spike growth. *J Exp Bot* 62, 4889–4901.

- Grant, C., Cubadda, F., Carcea, M., Pogna, N. E. and Gazza, L. (2012a). CHAPTER 7 - Vitamins, minerals, and nutritional value of Durum wheat. In *Durum Wheat AACC* pp. 125–137. International Press.
- Grant, C., Di Fonzo, N. and Pisante, M. (2012b). CHAPTER 3 - Agronomy of Durum Wheat Production. In *Durum Wheat AACC* pp. 37–55. International Press.
- Grant, C. A., Flaten, D. N., Tomaszewicz, D. J. and Sheppard, S. C. (2001). The importance of early season phosphorus nutrition. *Can J Plant Sci* 81, 211–224.
- Greenway, H. and Gunn, A. (1966). Phosphorus retranslocation in *Hordeum vulgare* during early tillering. *Planta* 71, 43–67.
- Gregersen, P. L., Holm, P. B. and Krupinska, K. (2008). Leaf senescence and nutrient remobilisation in barley and wheat. *Plant Biol* 10, 37–49.
- Grün, A., Buchner, P., Broadley, M. R. and Hawkesford, M. J. (2018). Identification and expression profiling of Pht1 phosphate transporters in wheat in controlled environments and in the field. *Plant Biol* 20, 374–389.
- Gupta, R. K., Gangoliya, S. S. and Singh, N. K. (2015). Reduction of phytic acid and enhancement of bioavailable micronutrients in food grains. *J Food Sci Technol* 52, 676–684.
- Guttieri, M., Bowen, D., Dorsch, J. A., Raboy, V. and Souza, E. (2004). Identification and Characterization of a Low Phytic Acid Wheat. *Crop Science* 44, 418–424.
- Hammond, J. P., Broadley, M. R. and White, P. J. (2004). Genetic responses to phosphorus deficiency. *Ann Bot* 94, 323–332.
- Hammond, J. P., Broadley, M. R., White, P. J., King, G. J., Bowen, H. C., Hayden, R., Meacham, M. C., Mead, A., Overs, T., Spracklen, W. P. and Greenwood, D. J. (2009). Shoot yield drives phosphorus use efficiency in *Brassica oleracea* and correlates with root architecture traits. *J Exp Bot* 60, 1953–1968.
- Havé, M., Marmagne, A., Chardon, F. and Masclaux-Daubresse, C. (2017). Nitrogen remobilization during leaf senescence : lessons from *Arabidopsis* to crops. *J Exp Bot* 68, 2513–2529.
- Hawkesford, M., Horst, W., Kichey, T., Lambers, H., Schjoerring, J., Møller, I. S. and White, P. (2012). Chapter 6 - Functions of Macronutrients. In *Marschner's Mineral Nutrition of Higher Plants (Third Edition)*, (Marschner, P., ed.), pp. 135–189. Academic Press San Diego.
- Hawkesford, M. J., Buchner, P. and Riche, A. B. (2018). Nutrient Dynamics in Wheat. In *Annual Plant Reviews online* pp. 705–718. John Wiley & Sons, Ltd.
- Heffer, P., Gruère, A. and Roberts, T. (2017). Assessment of fertilizer use by crop at the global level. *Consumption Reports Focus on Plant Nutrition*, International Fertilizer Association.

- Heuer, S., Gaxiola, R., Schilling, R., Herrera-Estrella, L., López-Arredondo, D., Wissuwa, M., Delhaize, E. and Rouached, H. (2017). Improving phosphorus use efficiency : a complex trait with emerging opportunities. *Plant J* 90, 868–885.
- Hidaka, A. and Kitayama, K. (2013). Relationship between photosynthetic phosphorus-use efficiency and foliar phosphorus fractions in tropical tree species. *Ecol Evol* 3, 4872–4880.
- Hill, J. (1980). The remobilization of nutrients from leaves. *J Plant Nutr* 2, 407–444.
- Hinsinger, P. (2001). Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemical changes : a review. *Plant Soil* 237, 173–195.
- Hinsinger, P., Brauman, A., Devau, N., Gérard, F., Jourdan, C., Laclau, J.-P., Cadre, E. L., Jaillard, B. and Plassard, C. (2011). Acquisition of phosphorus and other poorly mobile nutrients by roots. Where do plant nutrition models fail? *Plant Soil* 348, 29.
- Hiradate, S., Ma, J. F. and Matsumoto, H. (2007). Strategies of Plants to Adapt to Mineral Stresses in Problem Soils. In *Advances in Agronomy* vol. 96, of *Advances in Agronomy* pp. 65–132. Academic Press.
- Hoppo, S. D., Elliott, D. E. and Reuter, D. J. (1999). Plant tests for diagnosing phosphorus deficiency in barley (*Hordeum vulgare* L.). *Aust J Exp Agric* 39, 857–872.
- Horst, W. J., Abdou, M. and Wiesler, F. (1993). Genotypic differences in phosphorus efficiency of wheat. *Plant Soil* 155-156, 293–296.
- Huang, S., Wang, P., Yamaji, N. and Ma, J. F. (2020). Plant Nutrition for Human Nutrition : Hints from Rice Research and Future Perspectives. *Molecular Plant* 13, 825–835.
- Hüve, K., Merbach, W., Remus, R., Lüttschwager, D., Wittenmayer, L., Hertel, K. and Schurr, U. (2007). Transport of phosphorus in leaf veins of *Vicia faba* L. *J Plant Nutr Soil Sci* 170, 14–23.
- IAEA (2001). *Use of Isotope and Radiation Methods in Soil and Water Management and Crop Nutrition*. International Atomic Energy Agency.
- Iwai, T., Takahashi, M., Oda, K., Terada, Y. and Yoshida, K. T. (2012). Dynamic changes in the distribution of minerals in relation to phytic acid accumulation during rice seed development. *Plant Physiol* 160, 2007–2014.
- Jeong, K., Baten, A., Waters, D. L. E., Pantoja, O., Julia, C. C., Wissuwa, M., Heuer, S., Kretzschmar, T. and Rose, T. J. (2017a). Phosphorus remobilization from rice flag leaves during grain filling : an RNA-seq study. *Plant Biotechnol J* 15, 15–26.

- Jeong, K., Julia, C. C., Waters, D. L. E., Pantoja, O., Wissuwa, M., Heuer, S., Liu, L. and Rose, T. J. (2017b). Remobilisation of phosphorus fractions in rice flag leaves during grain filling : Implications for photosynthesis and grain yields. *PLOS ONE* *12*, e0187521.
- Jeong, K., Pantoja, O., Baten, A., Waters, D., Kretzschmar, T., Wissuwa, M., Julia, C. C., Heuer, S. and Rose, T. J. (2018). Transcriptional response of rice flag leaves to restricted external phosphorus supply during grain filling in rice cv. IR64. *PLOS ONE* *13*, e0203654.
- Jones, G. P. D., Blair, G. J. and Jessop, R. S. (1989). Phosphorus efficiency in wheat — A useful selection criterion? *Field Crops Res* *21*, 257–264.
- Jordan-Meille, L., Rubæk, G. H., Ehlert, P. a. I., Genot, V., Hofman, G., Goulding, K., Recknagel, J., Provolò, G. and Barraclough, P. (2012). An overview of fertilizer-P recommendations in Europe : soil testing, calibration and fertilizer recommendations. *Soil Use Manage* *28*, 419–435.
- Julia, C., Wissuwa, M., Kretzschmar, T., Jeong, K. and Rose, T. (2016). Phosphorus uptake, partitioning and redistribution during grain filling in rice. *Ann Bot* *118*, 1151–1162.
- Julia, C. C., Rose, T. J., Pariasca-Tanaka, J., Jeong, K., Matsuda, T. and Wissuwa, M. (2018). Phosphorus uptake commences at the earliest stages of seedling development in rice. *J Exp Bot* *69*, 5233–5240.
- Karagiannidis, N. and Hadjisavva-Zinoviadi, S. (1998). The mycorrhizal fungus *Glomus mosseae* enhances growth, yield and chemical composition of a durum wheat variety in 10 different soils. *Nutr Cycl Agroecosyst* *52*, 1–7.
- Karandashov, V. and Bucher, M. (2005). Symbiotic phosphate transport in arbuscular mycorrhizas. *Trends Plant Sci* *10*, 22–29.
- Kichey, T., Hirel, B., Heumez, E., Dubois, F. and Le Gouis, J. (2007). In winter wheat (*Triticum aestivum* L.), post-anthesis nitrogen uptake and remobilisation to the grain correlates with agronomic traits and nitrogen physiological markers. *Field Crops Res* *102*, 22–32.
- Kirk, G. J. D., Santos, E. E. and Santos, M. B. (1999). Phosphate solubilization by organic anion excretion from rice growing in aerobic soil : rates of excretion and decomposition, effects on rhizosphere pH and effects on phosphate solubility and uptake. *New Phytol* *142*, 185–200.
- Kisko, M., Shukla, V., Kaur, M., Bouain, N., Chaiwong, N., Lacombe, B., Pandey, A. K. and Rouached, H. (2018). Phosphorus Transport in Arabidopsis and Wheat : Emerging Strategies to Improve P Pool in Seeds. *Agriculture* *8*, 27.
- Kutman, U. B., Kutman, B. Y., Ceylan, Y., Ova, E. A. and Cakmak, I. (2012). Contributions of root uptake and remobilization to grain zinc accumulation in wheat depending on post-anthesis zinc availability and nitrogen nutrition. *Plant Soil* *361*, 177–187.

- Lambers, H., Finnegan, P. M., Jost, R., Plaxton, W. C., Shane, M. W. and Stitt, M. (2015). Phosphorus nutrition in Proteaceae and beyond. *Nat Plants* 1.
- Lambers, H., Finnegan, P. M., Laliberté, E., Pearse, S. J., Ryan, M. H., Shane, M. W. and Veneklaas, E. J. (2011). Phosphorus Nutrition of Proteaceae in Severely Phosphorus-Impoverished Soils : Are There Lessons To Be Learned for Future Crops? *Plant Physiol* 156, 1058–1066.
- Lauer, M. J., Blevins, D. G. and Sierzputowska-Gracz, H. (1989). ³¹P-Nuclear Magnetic Resonance Determination of Phosphate Compartmentation in Leaves of Reproductive Soybeans (*Glycine max* L.) as Affected by Phosphate Nutrition. *Plant Physiol* 89, 1331–1336.
- Li, H., Mollier, A., Ziadi, N., Messiga, A. J., Shi, Y., Pellerin, S., Parent, L.-E. and Morel, C. (2019). Long-term modeling of phosphorus spatial distribution in the no-tilled soil profile. *Soil Tillage Res* 187, 119–134.
- Liao, M., Hocking, P. J., Dong, B., Delhaize, E., Richardson, A. E. and Ryan, P. R. (2008). Variation in early phosphorus-uptake efficiency among wheat genotypes grown on two contrasting Australian soils. *Aust J Agric Res* 59, 157–166.
- Liu, T.-Y., Huang, T.-K., Yang, S.-Y., Hong, Y.-T., Huang, S.-M., Wang, F.-N., Chiang, S.-F., Tsai, S.-Y., Lu, W.-C. and Chiou, T.-J. (2016). Identification of plant vacuolar transporters mediating phosphate storage. *Nat Commun* 7, 11095.
- Liu, X., Zhao, X., Zhang, L., Lu, W., Li, X. and Xiao, K. (2013). TaPht1;4, a high-affinity phosphate transporter gene in wheat (*Triticum aestivum*), plays an important role in plant phosphate acquisition under phosphorus deprivation. *Functional Plant Biol* 40, 329–341.
- Lorenz, A. J., Scott, M. P. and Lamkey, K. R. (2008). Genetic Variation and Breeding Potential of Phytate and Inorganic Phosphorus in a Maize Population. *Crop Sci* 48, 79–84.
- Lott, J. N., Bojarski, M., Kolasa, J., Batten, G. D. and Campbell, L. C. (2009). A review of the phosphorus content of dry cereal and legume crops of the world. *Int J Agric Resour Gov Ecol* 8, 351.
- Lott, J. N. A., Ockenden, I., Raboy, V. and Batten, G. D. (2000). Phytic acid and phosphorus in crop seeds and fruits : a global estimate. *Seed Sci Res* 10, 11–33.
- Luan, M., Tang, R.-j., Tang, Y., Tian, W., Hou, C., Zhao, F., Lan, W. and Luan, S. (2016). Transport and homeostasis of potassium and phosphate : limiting factors for sustainable crop production. *J Exp Bot* 68, 3091–3105.
- Lynch, J. P. (2011). Root Phenotypes for Enhanced Soil Exploration and Phosphorus Acquisition : Tools for Future Crops. *Plant Physiol* 156, 1041–1049.

- Lynch, J. P. and Brown, K. M. (2001). Topsoil foraging – an architectural adaptation of plants to low phosphorus availability. *Plant Soil* 237, 225–237.
- Lyu, Y., Tang, H., Li, H., Zhang, F., Rengel, Z., Whalley, W. R. and Shen, J. (2016). Major Crop Species Show Differential Balance between Root Morphological and Physiological Responses to Variable Phosphorus Supply. *Front Plant Sci* 7.
- Lázaro, L., Abbate, P. E., Cogliatti, D. H. and Andrade, F. H. (2010). Relationship between yield, growth and spike weight in wheat under phosphorus deficiency and shading. *J Agric Sci* 148, 83.
- López-Arredondo, D. L., Leyva-González, M. A., González-Morales, S. I., López-Bucio, J. and Herrera-Estrella, L. (2014). Phosphate Nutrition : Improving Low-Phosphate Tolerance in Crops. *Annu Rev Plant Biol* 65, 95–123.
- Maathuis, F. J. (2009). Physiological functions of mineral macronutrients. *Curr Opin Plant Biol* 12, 250–258.
- MacDonald, G. K., Bennett, E. M., Potter, P. A. and Ramankutty, N. (2011). Agronomic phosphorus imbalances across the world's croplands. *PNAS* 108, 3086–3091.
- Maillard, A., Diquélou, S., Billard, V., Lâiné, P., Garnica, M., Prudent, M., Garcia-Mina, J.-M., Yvin, J.-C. and Ourry, A. (2015). Leaf mineral nutrient remobilization during leaf senescence and modulation by nutrient deficiency. *Front Plant Sci* 6.
- Manske, G., Ortiz-Monasterio, J., Van Ginkel, M., González, R., Rajaram, S., Molina, E. and Vlek, P. (2000). Traits associated with improved P-uptake efficiency in CIMMYT's semidwarf spring bread wheat grown on an acid Andisol in Mexico. *Plant Soil* 221, 189–204.
- Manske, G., Ortiz-Monasterio, J., van Ginkel, R., Rajaram, S. and Vlek, P. (2002). Phosphorus use efficiency in tall, semi-dwarf and dwarf near-isogenic lines of spring wheat. *Euphytica* 125, 113–119.
- Manske, G. G. B., Ortiz-Monasterio, J. I., van Ginkel, M., González, R. M., Fischer, R. A., Rajaram, S. and Vlek, P. L. G. (2001). Importance of P uptake efficiency versus P utilization for wheat yield in acid and calcareous soils in Mexico. *Eur J Agron* 14, 261–274.
- Marschner, H. (2011). *Marschner's Mineral Nutrition of Higher Plants*. Academic Press.
- Marschner, H., Kirkby, E. A. and Cakmak, I. (1996). Effect of mineral nutritional status on shoot-root partitioning of photoassimilates and cycling of mineral nutrients. *J Exp Bot* 47, 1255–1263.
- Marshall, C. and Wardlaw, I. F. (1973). A Comparative Study of the Distribution and Speed of Movement of ¹⁴C Assimilates and Foliar-Applied ³²P-Labelled Phosphate in Wheat. *Aust. Jnl. Of Bio. Sci.* 26, 1–14.

- Masclaux-Daubresse, C., Chen, Q. and Havé, M. (2017). Regulation of nutrient recycling via autophagy. *Curr Opin Plant Biol* 39, 8–17.
- Masclaux-Daubresse, C., Reisdorf-Cren, M. and Orsel, M. (2008). Leaf nitrogen remobilisation for plant development and grain filling. *Plant Biol* 10, 23–36.
- Masoni, A., Ercoli, L., Mariotti, M. and Arduini, I. (2007). Post-anthesis accumulation and remobilization of dry matter, nitrogen and phosphorus in durum wheat as affected by soil type. *Eur J Agron* 26, 179–186.
- McDonald, G., Bovill, W., Taylor, J. and Wheeler, R. (2015). Responses to phosphorus among wheat genotypes. *Crop Pasture Sci* 66, 430–444.
- Mimura, T. (1995). Homeostasis and Transport of Inorganic Phosphate in Plants. *Plant Cell Physiol* 36, 1–7.
- Mimura, T., Sakano, K. and Shimmen, T. (1996). Studies on the distribution, re-translocation and homeostasis of inorganic phosphate in barley leaves. *Plant Cell Environ* 19, 311–320.
- Misson, J., Thibaud, M.-C., Bechtold, N., Raghothama, K. and Nussaume, L. (2004). Transcriptional regulation and functional properties of Arabidopsis Pht1;4, a high affinity transporter contributing greatly to phosphate uptake in phosphate deprived plants. *Plant Mol Biol* 55, 727–741.
- Mohamed, G. E. S. and Marshall, C. (1979). The Pattern of Distribution of Phosphorus and Dry Matter with Time in Spring Wheat. *Ann Bot* 44, 721–730.
- Mollier, A., De Willigen, P., Heinen, M., Morel, C., Schneider, A. and Pellerin, S. (2008). A two-dimensional simulation model of phosphorus uptake including crop growth and P-response. *Ecol Modell* 210, 453–464.
- Mollier, A. and Pellerin, S. (1999). Maize root system growth and development as influenced by phosphorus deficiency. *J Exp Bot* 50, 487–497.
- Morel, C. and Fardeau, J. C. (1990). Uptake of phosphate from soils and fertilizers as affected by soil P availability and solubility of phosphorus fertilizers. *Plant Soil* 121, 217–224.
- Mudge, S. R., Rae, A. L., Diatloff, E. and Smith, F. W. (2002). Expression analysis suggests novel roles for members of the Pht1 family of phosphate transporters in Arabidopsis. *Plant J* 31, 341–353.
- Mueller, N. D., Gerber, J. S., Johnston, M., Ray, D. K., Ramankutty, N. and Foley, J. A. (2012). Closing yield gaps through nutrient and water management. *Nature* 490, 254–257.

- Mukherjee, S., Liu, A., Deol, K. K., Kulichikhin, K., Stasolla, C., Brûlé-Babel, A. and Ayele, B. T. (2015). Transcriptional coordination and abscisic acid mediated regulation of sucrose transport and sucrose-to-starch metabolism related genes during grain filling in wheat (*Triticum aestivum* L.). *Plant Sci* 240, 143–160.
- Młodzińska, E. and Zboińska, M. (2016). Phosphate Uptake and Allocation – A Closer Look at *Arabidopsis thaliana* L. and *Oryza sativa* L. *Front Plant Sci* 7.
- Nadeem, M., Mollier, A., Morel, C., Vives, A., Prud'homme, L. and Pellerin, S. (2011). Relative contribution of seed phosphorus reserves and exogenous phosphorus uptake to maize (*Zea mays* L.) nutrition during early growth stages. *Plant Soil* 346, 231–244.
- Nagarajan, V. K., Jain, A., Poling, M. D., Lewis, A. J., Raghothama, K. G. and Smith, A. P. (2011). *Arabidopsis* Pht1;5 Mobilizes Phosphate between Source and Sink Organs and Influences the Interaction between Phosphate Homeostasis and Ethylene Signaling. *Plant Physiol* 156, 1149–1163.
- Nussaume, L., Kanno, S., Javot, H., Marin, E., Nakanishi, T. M. and Thibaud, M.-C. (2011). Phosphate Import in Plants : Focus on the PHT1 Transporters. *Front Plant Sci* 2.
- O'Dell, B. L., De Boland, A. R. and Koirtyohann, S. R. (1972). Distribution of phytate and nutritionally important elements among the morphological components of cereal grains. *J Agric Food Chem* 20, 718–723.
- Osborne, L. D. and Rengel, Z. (2002a). Genotypic differences in wheat for uptake and utilisation of P from iron phosphate. *Aust J Agric Res* 53, 837–844.
- Osborne, L. D. and Rengel, Z. (2002b). Screening cereals for genotypic variation in efficiency of phosphorus uptake and utilisation. *Aust J Agric Res* 53, 295–303.
- Ozturk, L., Eker, S., Torun, B. and Cakmak, I. (2005). Variation in phosphorus efficiency among 73 bread and durum wheat genotypes grown in a phosphorus-deficient calcareous soil. *Plant Soil* 269, 69–80.
- Pampana, S., Mariotti, M., Ercoli, L. and Masoni, A. (2007). Remobilization of Dry Matter, Nitrogen and Phosphorus in Durum Wheat as Affected by Genotype and Environment. *Ital J Agron* 2, 303–314.
- Pandey, R., Singh, B. and Nair, T. V. R. (2005). Impact of Arbuscular-Mycorrhizal Fungi on Phosphorus Efficiency of Wheat, Rye, and Triticale. *J Plant Nutr* 28, 1867–1876.
- Pariasca-Tanaka, J., Vandamme, E., Mori, A., Segda, Z., Saito, K., Rose, T. J. and Wissuwa, M. (2015). Does reducing seed-P concentrations affect seedling vigor and grain yield of rice? *Plant Soil* 392, 253–266.

- Pearce, S., Tabbita, F., Cantu, D., Buffalo, V., Avni, R., Vazquez-Gross, H., Zhao, R., Conley, C. J., Distelfeld, A. and Dubcovsky, J. (2014). Regulation of Zn and Fe transporters by the GPC1 gene during early wheat monocarpic senescence. *BMC Plant Biol* 14, 368.
- Pearse, S. J., Veneklaas, E. J., Cawthray, G. R., Bolland, M. D. A. and Lambers, H. (2006). Carboxylate release of wheat, canola and 11 grain legume species as affected by phosphorus status. *Plant Soil* 288, 127–139.
- Pellerin, S. and Nesme, T. (2012). La maîtrise de la fertilité phosphatée : du raisonnement de la fertilisation à la gestion durable d’une ressource qui se raréfie. *Innovations Agronomiques* 21, 71–83.
- Peng, Z. and Li, C. (2005). Transport and partitioning of phosphorus in wheat as affected by P withdrawal during flag-leaf expansion. *Plant Soil* 268, 1–11.
- Perrier, F., Yan, B., Candaudap, F., Pokrovsky, O. S., Gourdain, E., Meleard, B., Bussière, S., Coriou, C., Robert, T., Nguyen, C. and Cornu, J. Y. (2016). Variability in grain cadmium concentration among durum wheat cultivars : impact of aboveground biomass partitioning. *Plant Soil* 404, 307–320.
- Plénet, D., Mollier, A. and Pellerin, S. (2000). Growth analysis of maize field crops under phosphorus deficiency. II. Radiation-use efficiency, biomass accumulation and yield components. *Plant Soil* 224, 259–272.
- Pottier, M., Dumont, J., Masclaux-Daubresse, C. and Thomine, S. (2018). Autophagy is essential for optimal Fe translocation to seeds in Arabidopsis. *bioRxiv* 70, 859–869.
- Raboy, V. (1997). Accumulation and storage of phosphate and minerals. In *Cellular and Molecular Biology of Plant Seed Development Advances in Cellular and Molecular Biology of Plants* pp. 441–477. Springer, Dordrecht.
- Raboy, V. (2001). Seeds for a better future : ‘low phytate’ grains help to overcome malnutrition and reduce pollution. *Trends Plant Sci* 6, 458–462.
- Raboy, V. (2007). Seed phosphorus and the development of low-phytate crops. In *Inositol phosphates : linking agriculture and the environment* vol. 01, p. 111. CABI UK.
- Raboy, V. (2009). Approaches and challenges to engineering seed phytate and total phosphorus. *Plant Sci* 177, 281–296.
- Raboy, V. (2020). Low phytic acid Crops : Observations Based On Four Decades of Research. *Plants* 9, 140.
- Raboy, V., Gerbasi, P. F., Young, K. A., Stoneberg, S. D., Pickett, S. G., Bauman, A. T., Murthy, P. P., Sheridan, W. F. and Ertl, D. S. (2000). Origin and Seed Phenotype of Maize low phytic acid 1-1 and low phytic acid 2-1. *Plant Physiol* 124, 355–368.

- Raboy, V., Hudson, S. J. and Dickson, D. B. (1985). Reduced Phytic Acid Content Does Not Have an Adverse Effect on Germination of Soybean Seeds. *Plant Physiol* 79, 323–325.
- Raboy, V., Peterson, K., Jackson, C., Marshall, J. M., Hu, G., Saneoka, H. and Bregitzer, P. (2015). A Substantial Fraction of Barley (*Hordeum vulgare* L.) Low Phytic Acid Mutations Have Little or No Effect on Yield across Diverse Production Environments. *Plants* 4, 225–239.
- Rae, A. L., Cybinski, D. H., Jarmey, J. M. and Smith, F. W. (2003). Characterization of two phosphate transporters from barley; evidence for diverse function and kinetic properties among members of the Pht1 family. *Plant Mol Biol* 53, 27–36.
- Raghothama, K. G. (1999). Phosphate Acquisition. *Annu Rev Plant Physiol Plant Mol Biol* 50, 665–693.
- Raghothama, K. G. and Karthikeyan, A. S. (2005). Phosphate acquisition. *Plant Soil* 274, 37.
- Rao, I. M., Friesen, D. K. and Osaki, M. (1999). Plant adaptation to phosphorus : Limited tropical soils. In *Handbook of plant and crop stress*. Marcel Dekker.
- Rausch, C. and Bucher, M. (2002). Molecular mechanisms of phosphate transport in plants. *Planta* 216, 23–37.
- Raven, J. A. (2008). Phosphorus and the future. In *The Ecophysiology of Plant-Phosphorus Interactions*, (White, P. J. and Hammond, J. P., eds), *Plant Ecophysiology* pp. 271–283. Springer Netherlands Dordrecht.
- Rengel, Z. and Marschner, P. (2005). Nutrient availability and management in the rhizosphere : exploiting genotypic differences. *New Phytol* 168, 305–312.
- Richardson, A. E., Lynch, J. P., Ryan, P. R., Delhaize, E., Smith, F. A., Smith, S. E., Harvey, P. R., Ryan, M. H., Veneklaas, E. J., Lambers, H., Oberson, A., Culvenor, R. A. and Simpson, R. J. (2011). Plant and microbial strategies to improve the phosphorus efficiency of agriculture. *Plant Soil* 349, 121–156.
- Rodriguez, D. and Goudriaan, J. (1995). Effects of phosphorus and drought stresses on dry matter and phosphorus allocation in wheat. *J Plant Nutr* 18, 2501–2517.
- Rodríguez, D., Andrade, F. and Goudriaan, J. (1999). Effects of phosphorus nutrition on tiller emergence in wheat. *Plant Soil* 209, 283–295.
- Rodríguez, D., Keltjens, W. and Goudriaan, J. (1998a). Plant leaf area expansion and assimilate production in wheat (*Triticum aestivum* L.) growing under low phosphorus conditions. *Plant Soil* 200, 227–240.

- Rodríguez, D., Pomar, M. and Goudriaan, J. (1998b). Leaf primordia initiation, leaf emergence and tillering in wheat (*Triticum aestivum* L.) grown under low-phosphorus conditions. *Plant Soil* 202, 149–157.
- Rose, T., Liu, L. and Wissuwa, M. (2013). Improving phosphorus efficiency in cereal crops : Is breeding for reduced grain phosphorus concentration part of the solution ? *Front Plant Sci* 4.
- Rose, T. J., Pariasca-Tanaka, J., Rose, M. T., Fukuta, Y. and Wissuwa, M. (2010). Genotypic variation in grain phosphorus concentration, and opportunities to improve P-use efficiency in rice. *Field Crops Res* 119, 154–160.
- Rose, T. J., Pariasca-Tanaka, J., Rose, M. T., Mori, A. and Wissuwa, M. (2012). Seeds of doubt : Re-assessing the impact of grain P concentrations on seedling vigor. *J Plant Nutr Soil Sci* 175, 799–804.
- Rose, T. J., Rengel, Z., Ma, Q. and Bowden, J. W. (2007). Differential accumulation patterns of phosphorus and potassium by canola cultivars compared to wheat. *J Plant Nutr Soil Sci* 170, 404–411.
- Rose, T. J. and Wissuwa, M. (2012). Chapter five - Rethinking internal phosphorus utilization efficiency : A new approach is needed to improve PUE in grain crops. In *Advances in Agronomy* pp. 185–217. Academic Press.
- Royo, C., Dreisigacker, S., Ammar, K. and Villegas, D. (2020). Agronomic performance of durum wheat landraces and modern cultivars and its association with genotypic variation in vernalization response (*Vrn-1*) and photoperiod sensitivity (*Ppd-1*) genes. *Eur J Agron* 120, 126129.
- Römer, W. and Schilling, G. (1986). Phosphorus requirements of the wheat plant in various stages of its life cycle. *Plant Soil* 91, 221–229.
- Sabelli, P. A. and Larkins, B. A. (2009). The Development of Endosperm in Grasses. *Plant Physiol* 149, 14–26.
- Salon, C., Avice, J.-C., Colombié, S., Dieuaide-Noubhani, M., Gallardo, K., Jeudy, C., Ourry, A., Prudent, M., Voisin, A.-S. and Rolin, D. (2017). Fluxomics links cellular functional analyses to whole-plant phenotyping. *J Exp Bot* 68, 2083–2098.
- Sanchez-Bragado, R., Molero, G., Reynolds, M. P. and Araus, J. L. (2016). Photosynthetic contribution of the ear to grain filling in wheat : a comparison of different methodologies for evaluation. *J Exp Bot* 67, 2787–2798.
- Schachtman, D. P., Reid, R. J. and Ayling, S. M. (1998). Phosphorus Uptake by Plants : From Soil to Cell. *Plant Physiol* 116, 447–453.

- Schiltz, S., Munier-Jolain, N., Jeudy, C., Burstin, J. and Salon, C. (2005). Dynamics of Exogenous Nitrogen Partitioning and Nitrogen Remobilization from Vegetative Organs in Pea Revealed by ^{15}N in Vivo Labeling throughout Seed Filling. *Plant Physiol* 137, 1463–1473.
- Shane, M. W. and Lambers, H. (2005). Cluster Roots : A Curiosity in Context. *Plant Soil* 274, 101–125.
- Shen, J., Yuan, L., Zhang, J., Li, H., Bai, Z., Chen, X., Zhang, W. and Zhang, F. (2011). Phosphorus Dynamics : From Soil to Plant. *Plant Physiol* 156, 997–1005.
- Shen, Q., Wen, Z., Dong, Y., Li, H., Miao, Y. and Shen, J. (2018). The responses of root morphology and phosphorus-mobilizing exudations in wheat to increasing shoot phosphorus concentration. *AoB PLANTS* 10.
- Shewry, P. R. (2009). Wheat. *J Exp Bot* 60, 1537–1553.
- Shewry, P. R., Mitchell, R. A. C., Tosi, P., Wan, Y., Underwood, C., Lovegrove, A., Freeman, J., Toole, G. A., Mills, E. N. C. and Ward, J. L. (2012). An integrated study of grain development of wheat (cv. Hereward). *J Cereal Sci* 56, 21–30.
- Siebers, M., Dörmann, P. and Hölzl, G. (2015). Membrane remodelling in phosphorus-deficient plants. In *Annual Plant Reviews Volume 48* pp. 237–263. John Wiley & Sons, Ltd.
- Sinclair, T. R. and Vadez, V. (2002). Physiological traits for crop yield improvement in low N and P environments. *Plant and Soil* 245, 1–15.
- Smith, A. P., Fontenot, E. B., Zahraeifard, S. and DiTusa, S. F. (2015). Molecular Components that Drive Phosphorus-Remobilisation During Leaf Senescence. In *Annual Plant Reviews Volume 48 : Phosphorus Metabolism in Plants*, (Plaxton, W. C. and Lambers, H., eds), pp. 159–186. John Wiley & Sons, Inc. USA.
- Smith, F. W., Mudge, S. R., Rae, A. L. and Glassop, D. (2003). Phosphate transport in plants. *Plant Soil* 248, 71–83.
- Snapp, S. S. and Lynch, J. P. (1996). Phosphorus distribution and remobilization in bean plants as influenced by phosphorus nutrition. *Crop Sci* 36, 929–935.
- Spano, G., Di Fonzo, N., Perrotta, C., Platani, C., Ronga, G., Lawlor, D. W., Napier, J. A. and Shewry, P. R. (2003). Physiological characterization of 'stay green' mutants in durum wheat. *J. Exp. Bot.* 54, 1415–1420.
- Sparvoli, F. and Cominelli, E. (2015). Seed Biofortification and Phytic Acid Reduction : A Conflict of Interest for the Plant? *Plants* 4, 728–755.
- Sreenivasulu, N. and Schnurbusch, T. (2012). A genetic playground for enhancing grain number in cereals. *Trends Plant Sci* 17, 91–101.

- Statista (2020). Grain production worldwide by type, 2018/19.
- Stigter, K. A. and Plaxton, W. C. (2015). Molecular Mechanisms of Phosphorus Metabolism and Transport during Leaf Senescence. *Plants* 4, 773–798.
- Stomph, T., Jiang, W. and Struik, P. C. (2009). Zinc biofortification of cereals : rice differs from wheat and barley. *Trends Plant Sci* 14, 123–124.
- Su, J.-Y., Zheng, Q., Li, H.-W., Li, B., Jing, R.-L., Tong, Y.-P. and Li, Z.-S. (2009). Detection of QTLs for phosphorus use efficiency in relation to agronomic performance of wheat grown under phosphorus sufficient and limited conditions. *Plant Sci* 176, 824–836.
- Sutton, P. J., Peterseon, G. A. and Sander, D. H. (1983). Dry Matter Production in Tops and Roots of Winter Wheat as Affected by Phosphorus Availability During Various Growth Stages1. *Agron J* 75, 657–663.
- Syers, J. K., Johnston, A. E. and Curtin, D. (2008). Efficiency of soil and fertilizer phosphorus use : reconciling changing concepts of soil phosphorus behaviour with agronomic information. *FAO fertilizer and plant nutrition bulletin*, Food and Agriculture Organization, Rome. OCLC : ocn231748259.
- Tang, X., Ma, Y., Hao, X., Li, X., Li, J., Huang, S. and Yang, X. (2009). Determining critical values of soil Olsen-P for maize and winter wheat from long-term experiments in China. *Plant Soil* 323, 143–151.
- Team, R. C. (2018). R : A language and environment for statistical computing.
- Tiessen, H. (2008). Phosphorus in the global environment. In *The Ecophysiology of Plant-Phosphorus Interactions*, (White, P. J. and Hammond, J. P., eds), *Plant Ecophysiology* pp. 1–7. Springer Netherlands Dordrecht.
- Tilman, D., Cassman, K. G., Matson, P. A., Naylor, R. and Polasky, S. (2002). Agricultural sustainability and intensive production practices. *Nature* 418, 671.
- Uauy, C., Distelfeld, A., Fahima, T., Blechl, A. and Dubcovsky, J. (2006). A NAC Gene Regulating Senescence Improves Grain Protein, Zinc, and Iron Content in Wheat. *Science* 314, 1298–1301.
- USGS (2020). U.S. Geological Survey : Mineral Commodity Summaries.
- Van Veldhoven, P. P. and Mannaerts, G. P. (1987). Inorganic and organic phosphate measurements in the nanomolar range. *Anal Biochem* 161, 45–48.
- Vance, C. P., Uhde-Stone, C. and Allan, D. L. (2003). Phosphorus acquisition and use : critical adaptations by plants for securing a nonrenewable resource. *New Phytol* 157, 423–447.

- Vandamme, E., Rose, T., Saito, K., Jeong, K. and Wissuwa, M. (2016a). Integration of P acquisition efficiency, P utilization efficiency and low grain P concentrations into P-efficient rice genotypes for specific target environments. *Nutr Cycl Agroecosyst* 104, 413–427.
- Vandamme, E., Wissuwa, M., Rose, T., Dieng, I., Drame, K. N., Fofana, M., Senthilkumar, K., Venuprasad, R., Jallow, D., Segda, Z., Suriyagoda, L., Sirisena, D., Kato, Y. and Saito, K. (2016b). Genotypic Variation in Grain P Loading across Diverse Rice Growing Environments and Implications for Field P Balances. *Front Plant Sci* 7.
- Vashishth, A., Ram, S. and Beniwal, V. (2017). Cereal phytases and their importance in improvement of micronutrients bioavailability. *3 Biotech* 7, 42.
- Venegas, J. P., Graybosch, R. A., Baenziger, P. S., Bai, G. and Amand, P. S. (2018). Registration of Great Plains–Adapted Reduced Phytate Winter Wheat Germplasm. *J Plant Regist* 12, 405–410.
- Veneklaas, E. J., Lambers, H., Bragg, J., Finnegan, P. M., Lovelock, C. E., Plaxton, W. C., Price, C. A., Scheible, W.-R., Shane, M. W., White, P. J. and Raven, J. A. (2012). Opportunities for improving phosphorus-use efficiency in crop plants. *New Phytol* 195, 306–320.
- Vergauwen, D. and De Smet, I. (2017). From early farmers to Norman Borlaug — the making of modern wheat. *Curr Biol* 27, R858–R862.
- Villegas, D., Aparicio, N., Blanco, R. and Royo, C. (2001). Biomass accumulation and main stem elongation of durum wheat grown under mediterranean conditions. *Ann Bot* 88, 617–627.
- Waldren, R. P. and Flowerday, A. D. (1979). Growth Stages and Distribution of Dry Matter, N, P, and K in Winter Wheat1. *Agron J* 71, 391–397.
- Wang, F., King, J. D. M., Rose, T., Kretschmar, T. and Wissuwa, M. (2017). Can natural variation in grain P concentrations be exploited in rice breeding to lower fertilizer requirements? *PLOS ONE* 12.
- Wang, F., Rose, T., Jeong, K., Kretschmar, T. and Wissuwa, M. (2016). The knowns and unknowns of phosphorus loading into grains, and implications for phosphorus efficiency in cropping systems. *J Exp Bot* 67, 1221–1229.
- Wang, L., Chen, F., Zhang, F. and Mi, G. (2010a). Two strategies for achieving higher yield under phosphorus deficiency in winter wheat grown in field conditions. *Field Crops Res* 118, 36–42.
- Wang, X., Shen, J. and Liao, H. (2010b). Acquisition or utilization, which is more critical for enhancing phosphorus efficiency in modern crops? *Plant Sci* 179, 302–306.

- Wang, Z., Sadras, V. O., Hoogmoed, M., Yang, X., Huang, F., Han, X. and Zhang, S. (2017). Shifts in nitrogen and phosphorus uptake and allocation in response to selection for yield in Chinese winter wheat. *Crop Pasture Sci.* *68*, 807–816.
- Waters, B. M., Uauy, C., Dubcovsky, J. and Grusak, M. A. (2009). Wheat (*Triticum aestivum*) NAM proteins regulate the translocation of iron, zinc, and nitrogen compounds from vegetative tissues to grain. *J Exp Bot* *60*, 4263–4274.
- White, P. J. and Hammond, J. P., eds (2008a). *The Ecophysiology of Plant-Phosphorus Interactions*. Plant Ecophysiology, Springer Netherlands.
- White, P. J. and Hammond, J. P. (2008b). Phosphorus nutrition of terrestrial plants. In *The Ecophysiology of Plant-Phosphorus Interactions Plant Ecophysiology* pp. 51–81. Springer, Dordrecht.
- White, P. J. and Veneklaas, E. J. (2012). Nature and nurture : the importance of seed phosphorus content. *Plant Soil* *357*, 1–8.
- Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L. D., François, R., Golemund, G., Hayes, A., Henry, L., Hester, J., Kuhn, M., Pedersen, T. L., Miller, E., Bache, S. M., Müller, K., Ooms, J., Robinson, D., Seidel, D. P., Spinu, V., Takahashi, K., Vaughan, D., Wilke, C., Woo, K. and Yutani, H. (2019). Welcome to the Tidyverse. *J Open Source Softw* *4*, 1686.
- Wissuwa, M., Gamat, G. and Ismail, A. M. (2005). Is root growth under phosphorus deficiency affected by source or sink limitations? *J Exp Bot* *56*, 1943–1950.
- Wissuwa, M., Yano, M. and Ae, N. (1998). Mapping of QTLs for phosphorus-deficiency tolerance in rice (*Oryza sativa* L.). *Theor Appl Genet* *97*, 777–783.
- Wu, B., Andersch, F., Weschke, W., Weber, H. and Becker, J. S. (2013). Diverse accumulation and distribution of nutrient elements in developing wheat grain studied by laser ablation inductively coupled plasma mass spectrometry imaging. *Metallomics* *5*, 1276–1284.
- Xynias, I. N., Mylonas, I., Korpetis, E. G., Ninou, E., Tsaballa, A., Avdikos, I. D. and Mavromatis, A. G. (2020). Durum Wheat Breeding in the Mediterranean Region : Current Status and Future Prospects. *Agronomy* *10*, 432.
- Yamaji, N. and Ma, J. F. (2014). The node, a hub for mineral nutrient distribution in graminaceous plants. *Trends Plant Sci* *19*, 556–563.
- Yamaji, N., Takemoto, Y., Miyaji, T., Mitani-Ueno, N., Yoshida, K. T. and Ma, J. F. (2017). Reducing phosphorus accumulation in rice grains with an impaired transporter in the node. *Nature* *541*, 92–95.

- Yan, B.-F., Nguyen, C., Pokrovsky, O. S., Candaudap, F., Coriou, C., Bussière, S., Robert, T. and Cornu, J. Y. (2018). Contribution of remobilization to the loading of cadmium in durum wheat grains : impact of post-anthesis nitrogen supply. *Plant Soil* 424, 591–606.
- Yang, S.-Y., Huang, T.-K., Kuo, H.-F. and Chiou, T.-J. (2017). Role of vacuoles in phosphorus storage and remobilization. *J Exp Bot* 68, 3045–3055.
- Ye, H., Zhang, X.-Q., Broughton, S., Westcott, S., Wu, D., Lance, R. and Li, C. (2011). A nonsense mutation in a putative sulphate transporter gene results in low phytic acid in barley. *Funct Integr Genomics* 11, 103–110.
- Yuan, H. M., Blackwell, M., Mcgrath, S., George, T. S., Granger, S. H., Hawkins, J. M. B., Dunham, S. and Shen, J. B. (2016). Morphological responses of wheat (*Triticum aestivum* L.) roots to phosphorus supply in two contrasting soils. *J Agric Sci* 154, 98–108.
- Zadoks, J. C., Chang, T. T. and Konzak, C. F. (1974). A decimal code for the growth stages of cereals. *Weed Res* 14, 415–421.
- Zee, S. Y. and O'brien, T. P. (1970). A Special Type of Tracheary Element Associated with "Xylem Discontinuity" in the Floral Axis of Wheat. *Aust Jnl Of Bio Sci* 23, 783–792.
- Zehetner, F., Wuenscher, R., Peticzka, R. and Unterfrauner, H. (2018). Correlation of extractable soil phosphorus (P) with plant P uptake : 14 extraction methods applied to 50 agricultural soils from Central Europe. *Plant Soil Environ* 64 (2018), 192–201.
- Zhang, F., Ma, J. and Cao, Y. P. (1997). Phosphorus deficiency enhances root exudation of low-molecular weight organic acids and utilization of sparingly soluble inorganic phosphates by radish (*Raghanus satiuvs* L.) and rape (*Brassica napus* L.) plants. *Plant Soil* 196, 261–264.
- Zhang, W., Liu, D., Liu, Y., Chen, X. and Zou, C. (2017). Overuse of Phosphorus Fertilizer Reduces the Grain and Flour Protein Contents and Zinc Bioavailability of Winter Wheat (*Triticum aestivum* L.). *J Agric Food Chem* 65, 1473–1482.
- Zhu, Y.-G. and Smith, S. E. (2001). Seed phosphorus (P) content affects growth, and P uptake of wheat plants and their association with arbuscular mycorrhizal (AM) fungi. *Plant Soil* 231, 105–112.