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Inhibition de la bractée lors de la transition florale chez *Arabidopsis thaliana*

Sana Dieudonné

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Inhibition de la bractée
lors de la transition florale
chez *Arabidopsis thaliana*

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À Massy, ma capitale.

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Inhibition de la bractée lors de la transition florale chez *Arabidopsis thaliana*

Résumé de la thèse

Le phytomère constitue l'élément de base du développement continu des plantes. Il se compose d'un noeud constitué d'une feuille et d'un méristème à la base de la feuille, ainsi que d'un entrenoeud, segment de tige séparant deux noeuds. Le passage du stade végétatif au stade reproductif se traduit par un remodelage du phytomère. Chez les Brassicaceæ, comprenant la plante modèle *Arabidopsis thaliana*, cela s'illustre notamment par la perte de la feuille, appelée bractée. Cependant, de précédentes études ont rapporté la présence de bractées à l'aisselle des premières fleurs de certaines accessions naturelles de *A. thaliana*, lorsqu'elles sont cultivées dans des conditions environnementales spécifiques. Ces fleurs dites "chimeriques" résulteraient d'une conversion de branche en fleur. Au cours de ma thèse, nous montrons que la présence de bractées au niveau des premières fleurs est fréquente chez certaines accessions naturelles d'*A. thaliana*, mais pas chez l'accession de référence *Col-0*. Chez *Tsu-0*, notamment, la présence de ces bractées ne dépend pas des conditions environnementales. De plus, elles ne résultent pas d'une conversion de branches en fleurs, contrairement à ce qui avait été proposé par les études précédentes. Afin d'établir les bases moléculaires à l'origine du développement de ces bractées, nous avons procédé à l'analyse transcriptomique de méristèmes apicaux caulinaires au cours de la transition florale chez les deux accessions naturelles. Ces transcriptomes révèlent des hétérochronies dans la dynamique d'expression des gènes. Leur lien avec la présence de bractées reste à établir. Notre étude décrit pour la première fois les différences de dynamique d'expression de gènes au cours de la transition florale à l'échelle de la population d'*A. thaliana*.

Nous avons pu montrer que la différence d'expression des gènes entre ces deux accessions culmine au moment précis de la transition florale, faisant echo à ce qui avait été montré à l'échelle de l'espèce. De plus, nous avons cartographié les variants génétiques responsables de la présence de bractées chez *Tsu-0* en utilisant deux approches indépendantes, l'analyse génétique d'individus F2 ségrégants en masse et l'utilisation de lignées recombinantes ségrégantes. Nous avons ainsi pu identifier deux régions situées dans le chromosome 1 et associées au phénotype. Nous avons donc recoupé les éléments des analyses génétiques et ceux des analyses transcriptomiques afin de proposer de potentiels gènes candidats. Ainsi, cette thèse revisite l'étude des mécanismes qui contrôlent l'inhibition de la bractée au moment de la transition florale chez *A. thaliana*, en se basant sur une comparaison entre deux populations naturelles. Par conséquent, ces résultats peuvent également nous permettre de mieux comprendre l'origine évolutive de la perte des bractées chez les Brassicaceæ.

Abstract of the thesis

Continuously during their development, flowering plants produce a basic developmental unit: the phytomer. Canonical phytomers are composed of an internode and an axillary meristem subtended by a leaf. The abrupt transition from vegetative to reproductive state remodels the composition of the phytomers. In most Brassicaceae, including the model plant *Arabidopsis thaliana*, the reproductive phytomer loses its leaf, also called bract, producing a bractless lateral floral meristem. However, production of flowers subtended by bracts have been reported in specific environmental conditions, but only on the basal flowers of the inflorescence, produced during floral transition. We show that in some natural accessions of *Arabidopsis thaliana*, such basal bract-flowers are common, yet not in the reference *Col-0*. Notably, in *Tsu-0*, bract-flowers are frequent regardless of environmental conditions. Moreover, contrary to what have been historically proposed, bract-flowers in *Tsu-0* do not result from a conversion of branches into flowers. A transcriptomic analysis of shoot apices over floral transition in *Tsu-0* and *Col-0* reveals interesting heterochronies in the dynamic of gene expressions, although their potential link with bract production are still under investigation. To our knowledge, this study first exposes the natural variations of transcriptomes from vegetative to flowering stages in *A. thaliana*. Notably, we show that gene expressions diverge the most precisely at the time of floral transition, recapitulating what have been previously described at an inter-species level. We also tried to map the genetic variations controlling bracts in *Tsu-0* with both bulked F2 segregant populations and Recombinants Inbred Lines. The genetic determinism of this trait is complex and we identified two major loci located in chromosome 1. We then crossed evidence from our genomic and transcriptomic data (including bract mutants) to propose some plausible candidate genes. From a particular case of natural variation, this study revisits the mechanisms that control bract formation in *A. thaliana* and their links with floral transition. By extension, our results could also enlighten the evolutionary origin of bract loss in Brassicaceae.

List of abbreviations used

AGL24 : *AGAMOUS LIKE 24*
AHP6 : *ARABIDOPSIS HISTIDINE PHOSPHOTRANSFER PROTEIN 6*
aM : *Axillary Meristem*
ANT : *AINTEGUMENTA*
AP1 : *APETALA1*
ARF : *Auxin Response factor*
AS1 : *ASYMETRIC LEAVES 1*
A. thaliana : *Arabidopsis thaliana*
ATH1 : *ARABIDOPSIS THALIANA HOMEOBAX GENE1*
BOP : *BLADE-ON-PETIOLE*
BRC1 : *BRANCHED1*
CAL : *CAULIFLOWER*
CB : Cryptic Bract
CC : Creative Common
CLV3 : *CLAVATA 3*
CO : *CONSTANS*
Col-0 : Columbia-0 ecotype
CUC : *CUP-SHAPED COTYLEDON*
CZ : Central Zone
DRNL : *DORNRÖSCHEN-LIKE*
FIL : *FILAMENTOUS FLOWER*
FMs: Floral meristem
FPP : Flower preceding prophyll, equivalent to bract fpp.
FSB : Flower Subtending Bract, equivalent to bract stricto sensu
FT: *FLOWERING LOCUS T*
GRN : Gene Regulatory Network
GA : Gibberellic Acid GWAS: Genome Wide Association Study
HIF: Heterogeneous Inbred Family
IM: Inflorescence shoot apical Meristem
KAN1 : *KANADI 1*
KNAT1 : *Knotted-like from Arabidopsis thaliana 1*
KNOX : *Knotted-like homeobox*
LAS : *LATERAL SUPPRESSOR*
LFY : *LEAFY*
LOF : *LATERAL ORGAN FUSION*
MIR : Micro RNA
MP : *MONOPTEROS*
NL1 : *NECK LEAF 1*
OE : Over Expressor
PNY : *PENNYWISE*
PZ : Peripheral Zone
QTL : Quantitative Trait Locus
RA2 : *RAMOSA 2*

RAX : *REGULATOR OF AXILLARY MERISTEM*
REV : *REVOLUTA*
RIL: Recombinant Inbred Line
RZ : Rib Zone
SAM : Shoot Apical Meristem
SNP : Single Nucleotide Polymorphism
SOC1 : *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1*
SPL : *SQUAMOSA PROMOTER BINDING PROTEIN*
STM : *SHOOT MERISTEMLESS*
SVP : *SHORT VEGETATIVE PHASE*
T. hassleriana : *Tarenaya hassleriana*
TFL1 : *TERMINAL FLOWER 1*
TSF : *TWIN SISTER OF FT*
TSH4 : *TASSELSHEATH4*
Tsu-0 : Tsushima-0 ecotype *WUS* : *WUSCHEL*
YAB : *YABBY*

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Preamble

Many organisms develop through several phases in which they respond to different environmental conditions and stimuli. The switch from one phase to the other often involves various changes. For instance, the juvenile-to-adult transition generally comprises behavioural and physiological modifications, as well as a morphological remodelling. Insect metamorphosis well illustrates the abrupt transition from a juvenile larva to an adult imago. In fact, insect metamorphosis usually includes intense morphological changes (apparition of new organs, exoskeleton, etc.), physiological remodelling (change in the respiratory and hormonal system, etc.), and behavioural adaptation (mating, migration, predation, etc.). Although this developmental switch can be influenced by external stimuli, it is an irreversible and robust toggle: once the adult phase is reached, the organism does not return to its juvenile phase, whatever the environmental conditions. How developmental switches are controlled is an intriguing question in biology.

Modifications in the control of developmental switches can explain the diversification of species. In insects, the timing of ecdyzosteroids peak, the metamorphosis hormone, is responsible for different types of life cycle (Truman & Riddiford 1999). When the ecdyzosteroids peak happens before hatching, juvenile insects are born with a similar-to-adult shape: they are hemimetabolous. When the ecdyzosteroids peak occurs after hatching, juvenile insects are born with a larval form, and will metamorphose afterwards into their adult imago shape: they are holometabolous. Hence, the regulation of reproductive transition controls essential aspects of post-embryonic development, and its variations can participate to phenotypic diversification between species. How robust and irreversible developmental switches can also accommodate modifications is an emerging question.

Flowering plants provide a striking example of morphological remodelling at reproductive transition. In angiosperms, reproductive transition leads, in the first place, to flower production. In some species, other changes appear with floral transition. In the model plant *Arabidopsis thaliana*, bracts, the leaves that subtend flowers, disappear. However, a natural accession of *A. thaliana*, *Tsushima-0*, presents bracts under the first flowers, but not above. Hence, bract inhibition is still functional in *Tsu-0*, but temporarily disturbed at floral transition. What is the nature of such "bract-flower"? How does floral transition induce different developmental

trajectories at a population scale? What kind of genetic determinant(s) in *Tsu-0* can explain the temporal release of bract inhibition at floral transition?

In my thesis research, I use bract-flowers in *Tsu-0* to explore how floral transition can display morphological differences at a population scale, while remaining robust and functional. In this document, I first describe the presence of bract-flowers in some Brassicaceae species and in different natural populations of *A. thaliana*, including *Tsu-0* (Chapter 1). I then focused on *Tsu-0*'s bract-flowers and highlighted essential distinctions with previously described bracts (Chapter 2). In Chapter 2, I also investigated what characterizes the floral transition in *Tsu-0* from the reference *Col-0*, and identified a climax of gene expression divergence at the time of bract emergence. Finally, I assessed the complex genetic regulation of bract emergence in *Tsu-0*, and mapped its genetic determinants into several main Quantitative Trait Locus (QTLs) in chromosome 1, suitable for further research (Chapter 3). This work allows a better understanding of bract inhibition in *A. thaliana*, and brings insights into the mechanisms of regulation and evolution of developmental switches.

I will introduce my research by describing flowering plant architecture and its remodelling during floral transition, and then review our current knowledge on bract inhibition.

INTRODUCTION

1 Phytomers govern plant shoot architecture throughout life time

1.1 Phytomers are the basic units of plant growth

1.1.1 Conservation of the phytomer general structure among angiosperms

Contrary to most animals, plants final shape and form do not entirely rely on the embryonic establishment of a predictive body plan. Instead, plant embryogenesis only results in the formation of the essential features of the mature plant. This comprises the apical-basal axis, with at each tips an apical meristem (the shoot apical meristem - SAM - and the root apical meristem), the cotyledons, and the main tissue types. This "body plan" become more complex after germination, when the SAM start operating by producing phytomers. Phytomers are the basic unit of plant shoot development (Barlow 1989; Sussex 1989; White 1979). Phytomers are composed of a leaf, subtending one to several axillary meristems (aM, or buds), and attached together to the stem at the node. The phytomer also includes the stem portion between two consecutive nodes, called internode. Each aM can reiterate this basic developmental unit, by becoming a SAM of a new growth axis and producing phytomers (Fig.1A).

The structure of the phytomer is highly conserved among plant kingdom (McSteen & Leyser 2005). Hence, in mosses, like in *A. thaliana* or poplar, the aM develops at the axil of a leaf, and nodes are separated by a small stem portion (fig.1B). However, each of the phytomers' components has undergone structural and functional modifications along with plant diversification. For example, 480 million years of architecture divergence between moss and seed plant groups can be mainly explained by small changes at the phytomer level. These changes concern

the identity of the aM (that can be flowers in angiosperms), and the length and width of the internode (can be rigid). But the general structure of the phytomer have been maintained (fig.1).

Plant general architecture results then from the geometrical interrelationships between phytomer elements, and from phytomer organisation along each stem (Barlow 1989; Barthélémy & Caraglio 2007). In fact, as described in Barthélémy *et al.* the entire diversity of plant architecture relies on three major "fundamental morphogenetic processes" affecting the phytomer: growth, branching, and reiteration (Barthélémy & Caraglio 2007). According to Barthélémy *et al.*, the three morphogenetic processes concern different part of the phytomer. The "growth" concerns the internode and will determine the length and thickness of the plant axis. "Branching" allows to establish new growth axis from the meristems, and thus determines the general plant architecture. In fact, the "branching" of a determinate meristems, the flower, will stop the growth axis, while the branching of an indeterminate meristems will reiterate the growth axis. Hence, the third morphogenetic process, the reiteration, is specific to aM. Because phytomers are produced continuously, one individual plant architecture varies constantly through a modulation of these three morphogenetic phenomena. This explains how a young vegetative plant displays distinct phenotype from its adult hundred-year-old version. Moreover, phytomer production in plants is highly variable depending on environmental conditions. Understanding the huge diversity of plant architecture thus requires to consider the formation and the plasticity of its basic developmental brick, the phytomer.

Before addressing how changes of phytomer elements participate to the diversity of plant architecture, I will briefly review how phytomers are continuously produced by the SAM. To avoid confusion, the rest will only consider angiosperms, and is based on the knowledge mainly obtained using the model plant *A. thaliana*.

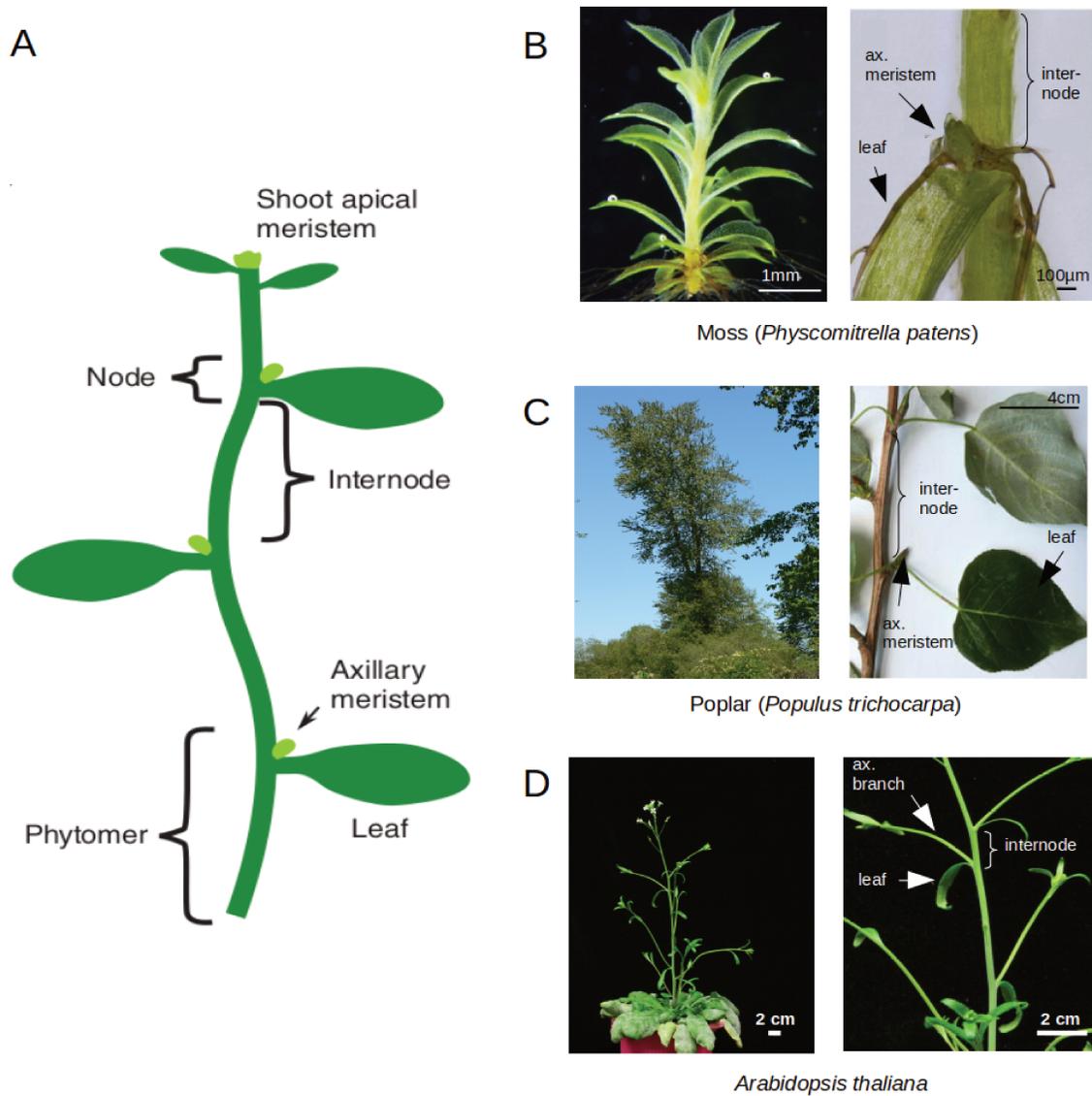


Figure 1: **Structure of the phytomer and its conservation among plant kingdom.** A) Schematic representation of a shoot. The shoot is composed of the accumulation of phytomers, produced by the shoot apical meristem. Each phytomer is composed of (at least) an axillary meristem, subtended by a leaf, and an internode. The axillary meristem and the leaf together form the node. From Bennett *et al.* 2016. B-D) Example of three different plant architectures, and close-up view showing their modular phytomer composition. B) *Physcomitrella patens*. Left picture: entire view of a leafy shoot in a gametophyte (source: Koshimizu *et al.* 2018). Right picture: close-up of one phytomer (adapted from Coudert *et al.* 2015). C) *Populus trichocarpa*. Left picture: architecture of a tree (credit: Walter Siegmund, under Creative Common (CC) license). Right picture: close-up of one phytomer (adapted from <https://visual-flora.org.uk/salicaceae/populus>). D) *A. thaliana*. Left picture: general architecture of an adult plant. Right picture: close-up showing the phytomer elements. When known, scale bars are represented on one corner of the picture.

1.1.2 Production of phytomers is ensured by the Shoot Apical Meristems

Plant development is characterized by a continuous production of new organs and tissues by the meristems. Apical meristems are a group of stem cells located in the two apices of the plant. The SAM produces all the aerial part of the plant by ensuring both the maintenance of the stem cell niche and the differentiation of cells into organs. The capacity to divide and self-renew can be conserved within the meristems during the entire plant life, and this characterizes their continuous development. Meristems have been described and studied for centuries. The SAM can be divided into several functional regions: the pool of meristematic cells is maintained within the central zone (CZ), the differentiation of organs happens in the peripheral zone (PZ), while the rib zone (RZ) contains the cells forming the inner part of the stem. In addition, the meristem can be divided into 3 main layers of cells with specific cell division plane (Carles & J. C. Fletcher 2003, fig.2). In the first two layers (called L1 and L2), cells only undergo division anticline to the surface plane, which establishes layer-specific cell lineages. In fact, the L1 layer mostly contributes to the epidermis establishment, while the L2 layer derives into the germinal cells and the ground tissue. The L3 layer undergoes division in all planes, and generates the most internal plant tissues, including the vascular system. The balance between stem cell pool maintenance and cell differentiation is tightly regulated and well understood.

The pool of undifferentiated meristematic cells is located in the central part of the meristem, into the CZ (fig.2A). Cells have a slow rate of division that is tightly controlled, which ensures the constant renewal of stem cells and controls meristem size (Grandjean *et al.* 2004). The control of the stem cell niche maintenance within the central zone is ensured by a genetic feedback loop relying on both cell-cell interactions and mobile protein signaling (fig.2C). *CLAVATA 3* (*CLV3*), *WUSCHEL* (*WUS*) and *KANADI 1* (*KAN1*) are the major regulators of the maintenance of the CZ (Yadav *et al.* 2014; Truskina & Vernoux 2018; Carles & J. C. Fletcher 2003). *CLV3* and *WUS* constitute a negative feedback loop that maintains the size of the *WUS* expression zone. *WUS* encodes a transcription factor that regulates gene expression in the SAM (see fig.2C), notably by activating cytokinin pathway, that maintains undifferentiated cells. *WUS* can also move through plasmodesmata into apical neighbouring cells, where it activates *CLV3*, a stem cell fate gene, in a dosage dependant manner (J. Fletcher 2018). *CLV3* gene encodes a peptide that binds, in part, to the CLV1 receptor, and transduces a *WUS* inhibition

signal. Hence, by restricting *WUS* expression domain, the *WUS-CLV* signaling dynamically regulates the pool of stem cells. Besides, *WUS* inhibits *KAN1*, that promotes differentiation in the PZ, ensuring the undifferentiated state of the pool of stem cells.

KAN1 is however expressed in the PZ, where cells divide more rapidly and differentiate. Cell differentiation in this PZ leads to primordia initiation at a specific position and timing. The arrangement of organs around the stem is called phyllotaxy and is usually a characteristic of the species. In *A. thaliana*, organs initiate one after the other at a $137,5^\circ$ angle on average, allowing us to visually trace back the sequence of organ initiation from a meristem top view (fig.2B). The timing of organ initiation is also regulated, and the time between the initiation of two consecutive organs is called the plastochron. The regulation of the position and the timing of primordia initiation mainly rely on hormonal signaling, including auxin and cytokinin (Truskina & Vernoux 2018; Galvan-Ampudia, Chaumeret, *et al.* 2016; Bhatia & Heisler 2018).

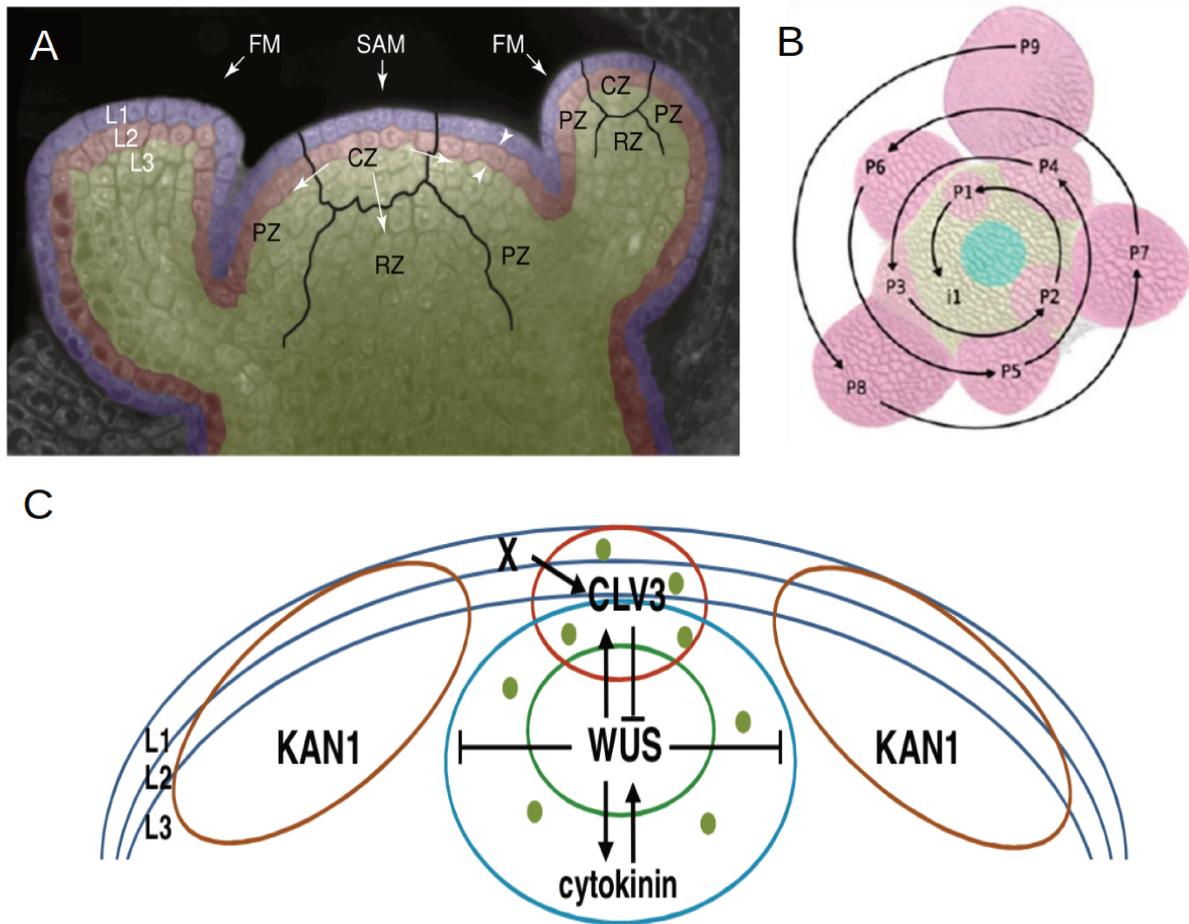


Figure 2: **Structure of the Shoot Apical Meristem (SAM)** A) Confocal laser-scanning micrograph through an *A. thaliana* inflorescence SAM and its adjacent floral meristems (FMs). Plant continuous development is ensured by the activity of meristems, plant stem cells niches, that ensure its self renewal as well as cell differentiation. Histological structure of the meristem have been well described, and are superimposed on the micrograph. The colored domains depict the different cell layers: in *A. thaliana*, the L1 layer produces the epidermal tissues and the L2 layer the subepidermal tissues, both of which divide into one division plane anticlinal to the surface. The L3 layer produces the inner plant tissues, and can divide into all planes. The two arrowheads point at an anticlinal cell division occurring in L2. The black outlines schematize the different meristematic zones: the peripheral zone (PZ) contributes cells to the formation of lateral organs, the rib zone (RZ) contributes cells to stem growth and the central zone (CZ) ensures the renewal of the meristematic stem cells. Taken from Carles & J. C. Fletcher 2003. B) Production of organs from the oldest P9 to the youngest P1. In *A. thaliana*, two consecutive organs are separated from each other by a $137,5^\circ$ angle. Hence, the site of initiation of the next organ (i1) can be easily predicted. Taken from Murray *et al.* 2012. C) The maintenance of the SAM is controlled by a complex Gene Regulatory Network (GRN), which is extremely simplified here down to a few major components of its self-regulation. Circles mark the expression domains of the genes *CLV3* (red), *WUS* (green), *KAN1* (brown) and delineate a region with high cytokinin-signaling activity (blue). Green dots show localization of *WUS* protein. X stands for a hypothetical L1-derived signal which would activate *CLV3* expression. Taken from Truskina & Vernoux 2018.

1.1.3 Control of phytomer production by the Shoot Apical Meristem

Leaf organogenesis Leaf organogenesis starts with a local and transient auxin maximum. Cells at the flank of the meristem then divide more rapidly than their neighbour and form a dome called the leaf primordium: the first constituent of the phytomer is specified. Development of the leaf primordium is not dissociable from the genetic specification of the leaf axis: the adaxial-abaxial (upper-lower), apical-basal, and lateral axis. The adaxial-abaxial axis is genetically established before any morphological distinction of such axis (F. Zhao & Traas 2021). Hence, leaf identity markers such as *ASYMETRIC LEAVES 1 (AS1)* is inseparable from its role in establishing the adaxial-abaxial axis (Machida *et al.* 2015). In fact, the adaxial *AS1-AS2* complex promotes leaf development and inhibits the SAM Class 1 KNOX genes such as *KNOTTED-LIKE FROM ARABIDOPSIS THALIANA (KNAT1)* (Guo *et al.* 2008, see fig.3). This allows the separation between the SAM and the organ. This complex also restricts abaxial markers *YABBY* genes, while promoting the adaxial *REVOLUTA (REV)* gene (Machida *et al.* 2015), and thus allows the separation between the two leaf faces: abaxial and adaxial. In addition to leaf-meristem separation, the early leaf partitioning guides cells differentiation within the leaf. For instance, the adaxial (upper) part of the leaf becomes specialized in light absorption, while the abaxial (lower) part becomes specialized in gas exchanges (Manuela & Xu 2020b). Disturbing the positional information leads to early malformation of the leaf with a radial symmetry (Machida *et al.* 2015). Hence, the leaf is the first element of a phytomer to be specified at the SAM and its identity is indiscernible from its polarity markers.

The establishment of the adaxial-abaxial axis is critical for the proper localisation of the axillary meristem initiation, as it appears at the axil (adaxial part) of the leaf (fig.3).

Axillary meristem organogenesis Axillary meristems (aM) emerge at the flank of the SAM, at the junction between the adaxial part of the leaf and the growth axis. Position of the aM is ensured by the promotion of *WUS* activity by adaxial markers such as *REV*, while abaxial markers like *YABBY* inhibit *WUS* (Sarojam *et al.* 2010). The aM is similar to a SAM, as it will reiterate plant growth in the same fashion (Xin *et al.* 2017). However, aMs are formed post-embryonically from meristematic cells originating from the SAM, that will later acquire similar SAM features (C. Zhang *et al.* 2018). The development of axillary branches includes

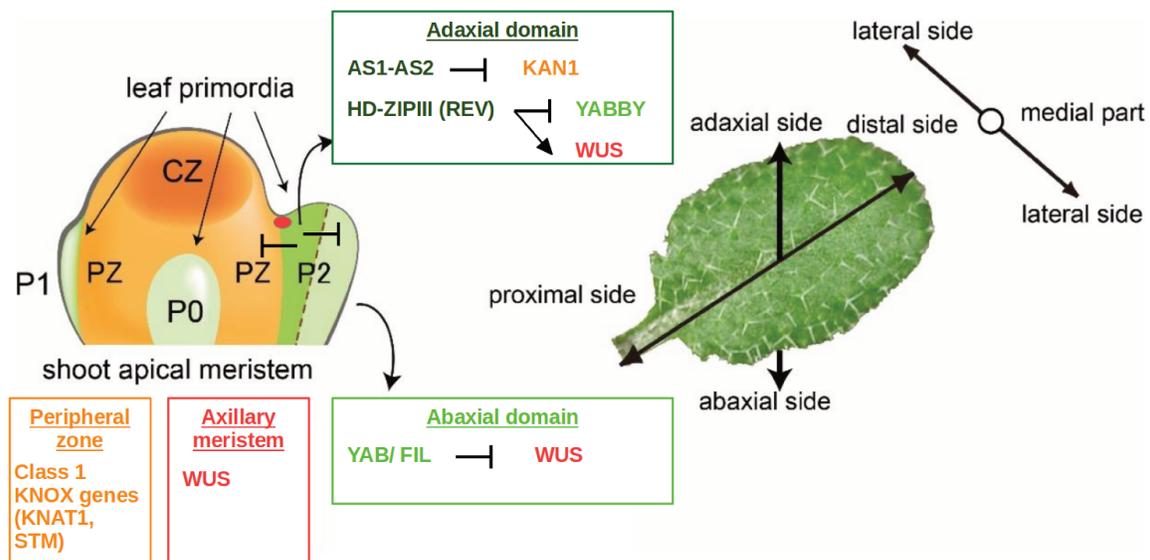


Figure 3: **Development of the leaf.** Leaf development occurs around three main axis as presented in the left picture. Early leaf primordium establishment relies mainly on the adaxial-abaxial axis. The adaxial domain allows both the separation of the organ from the SAM through Class1 KNOX genes inhibition, and also the distinction between adaxial and abaxial face through abaxial markers inhibition. aM usually emerges at the leaf axil thanks to the activation of the stem-cell positive regulator WUS by the adaxial identity promoter *REVOLUTA* (*REV*). See the text for deeper explanations. Adapted from Machida *et al.* 2015

two major steps: initiation and outgrowth (Y. Wang & Jiao 2018). These two steps can be eventually separated in time by a period of dormancy.

The localisation of the axillary stem cell niche is tightly regulated and depends on leaf boundary and polarity markers (McConnell & Barton 1998). aM only initiates at the adaxial part of the leaf, and at the boundary region: both *REV* (adaxial gene) and *BLADE-ON-PETIOLE* (*BOP*, boundary gene) regulate aM positioning (Hepworth & Pautot 2015; Q. Wang *et al.* 2016, see fig.4). Interestingly, ectopic meristems can develop in the leaf margin of compound leaves, that gather boundary and adaxial markers (Hagemann & Gleissberg 1996). Plus, disturbing leaf polarity in *Medicago truncatula* as well as in *A. thaliana* can lead to ectopic organ formation on the abaxial (and not adaxial) part of the leaf (McConnell & Barton 1998; Zhou *et al.* 2019). Hence, the spatial control of axillary meristem initiation relies on polarity and boundary information given by the previously formed leaf (fig.4).

At the leaf axil, the initiation of aM involves a complex gene regulatory network to establish meristematic features. Cells originating from the SAM continue to express a low level of *SHOOT MERISTEMLESS* (*STM*), that maintains the competence for meristem identity thanks to a *STM - ARABIDOPSIS THALIANA HOMEODOMAIN GENE1* (*ATH1*) feed-forward loop (Cao *et al.* 2020, fig.4). Expression of *REV* at the adaxial part of the leaf (around p10 stages) boosts *STM* expression, that reaches higher levels and induces the formation of the axillary meristem dome (Shi *et al.* 2016). *WUS* and *CLV3*, are expressed *de novo* following axillary meristem initiation (Xin *et al.* 2017) triggered by cytokinin signaling (J. Wang *et al.* 2017) in a pattern that recapitulates the one found in the SAM (fig.4). However, the aM are regulated by other transcription factors that do not participate to SAM regulation, like *LATERAL SUPPRESSOR* (*LAS*) and *REGULATOR OF AXILLARY MERISTEM 1* (*RAX1*) genes (Xue *et al.* 2020). The first is expressed in the leaf adaxial part (Yadav *et al.* 2014), and regulates *REV*; while *RAX1* is expressed in the boundary regions and is necessary for aM formation (Müller *et al.* 2006). Interestingly, *RAX1* is activated by LEAFY (LFY) transcription factor, a major determinant of floral meristem identity (Chahtane *et al.* 2013). First, this highlights a common feature between flowers and vegetative aMs, but also explains why aMs grow following floral initiation. Hence, aM initiation strongly relies on the presence of a leaf which provides essential landmarks for the subsequent development of a stem cell niche (fig.4).

The outgrowth of aM mainly relies on hormonal signaling (Y. Wang & J. Li 2008) and sugar content (Mason *et al.* 2014; Rameau *et al.* 2015), but also on environmental signals. Auxin has been for long associated with bud outgrowth in a process called apical dominance: cutting the plant apex would remove auxin basipetal transport from the SAM, and would therefore induce aM growth. It is not clear how differences in auxin concentrations and timing of actions can lead both to inhibition or the activation of axillary buds (Xue *et al.* 2020). Nevertheless, auxin acts with different hormones, and through different signal integrators. Auxin regulates cytokinin and strigolactone levels, which respectively activates and inhibits aM outgrowth (Dun *et al.* 2012). Integration of all hormonal information allows a precise regulation of bud outgrowth (Xue *et al.* 2020). In addition, sugar content and signaling have been shown to strongly contribute to aM development. Apex decapitation induces rapid redistribution of sugar towards new sinks - the aMs -, and promotes their growth (Barbier *et al.* 2015). Sugar metabolism with light signaling, are also key players of aM development (Wahl *et al.* 2013; Leduc *et al.* 2014). Hence, like flower development which I will discuss later, aM development involves a plethora of pathways, but also an important balance between source-sink status.

AMs are thus the second phytomer element to be produced. It requires the former production of a leaf and is regulated by environmental, hormonal, metabolic and genetic cues (fig.4).

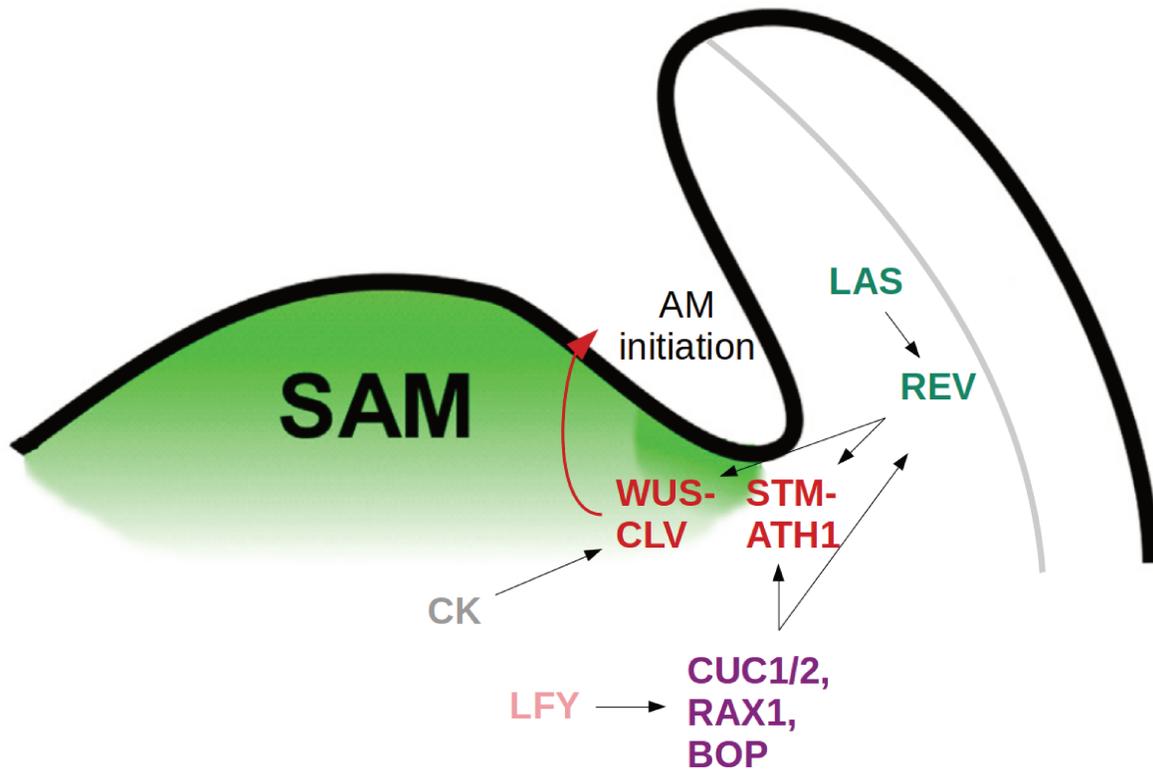


Figure 4: **Development of the axillary meristem (aM) relies on information provided by the leaf boundary and adaxial domains.** Cells at the origin of aM maintained their meristematic competence thanks to a *ATH1-STM* feed-forward loop. An increase in *STM* expression is induced by adaxial genes such as *REV*, that also promotes a *de novo* expression of *WUS*. Cytokinin signaling (CK) plays also a major role in *WUS* activation. *WUS* and *STM* expression together allow the acquisition of meristematic features (self renewal and undifferentiation). *STM* expression is also positively regulated by boundary genes such as *CUC1/2*, *BOP*, or *RAX1*. Interestingly, *RAX1* is found to be promoted by *LFY*, explaining axillary meristem developmental burst after floral transition. Arrows represent a genetic activation. Adaxial markers are in green, boundary markers in purple, floral transition markers in pink, cytokinin in grey, and the meristematic feature of the AM in red. Adapted from Cao *et al.* 2020.

Internode growth Internode is the last phytomer element that becomes visible in *A. thaliana*. Unlike aM or leaves, very little is known about internode development. Again, boundary genes are essential for the formation of the rib meristem, which ensures internode growth. The boundary between the differentiating meristematic cells and the elongating stem cells is built by *PENNYWISE* (*PNY*) genes (Bencivenga *et al.* 2016), which are essential for proper internode elongation. Before flowering, *ATH1* acts at the boundary region between stem and meristem and inhibits stem growth probably by promoting Gibberellin (GA) catabolism (Gómez-Mena & Sablowski 2008; L. Zhang, Sun, *et al.* 2018). We will see later that flowering triggers intense remodelling of the phytomers, including the internodes.

In *A. thaliana*, internodes elongate from the rib zone, under the control of both hormonal and light signals that trigger floral transition (fig.5). GA plays an important role in internode elongation, by triggering cells growth and division. GA level is tightly controlled by photo signaling through photo-receptors (Peng & Harberd 1997; Mazzella *et al.* 2000). Moreover, flowering signals may inhibit *ATH1* locally but also through mobile signals (McKim 2020), depressing stem growth. In fact, transport of the florigen FLOWERING LOCUS T (FT) has been shown to promote stem elongation (Shalit-Kaneh *et al.* 2019). Contrary to monocotyledon plants, in dicotyledons like *A. thaliana*, internode growth follows a basipetal gradient: the highest internodes elongate first. Nevertheless, very little is known about the establishment, maintenance and elongation of the internode, it is noteworthy that internode elongation highly depends on flowering signals (fig.5).

Shoot meristems are the place of a continuous organ production: leaf, aM and internode, which together form the phytomer. This production relies on positional and hormonal information. Moreover, modifications of the shape, size, or growth of the phytomer elements can arise both at an individual and at a species level. I will describe how changes in the relationship between each element can impact the general plant architecture.

1.1.4 Modifications of the phytomer structure impact the diversity of plant architectures

Despite the high conservation of phytomer among plant kingdom, its structure can diverge, explaining the diversity of plant architectures. The macroscopic divergence in the overall archi-

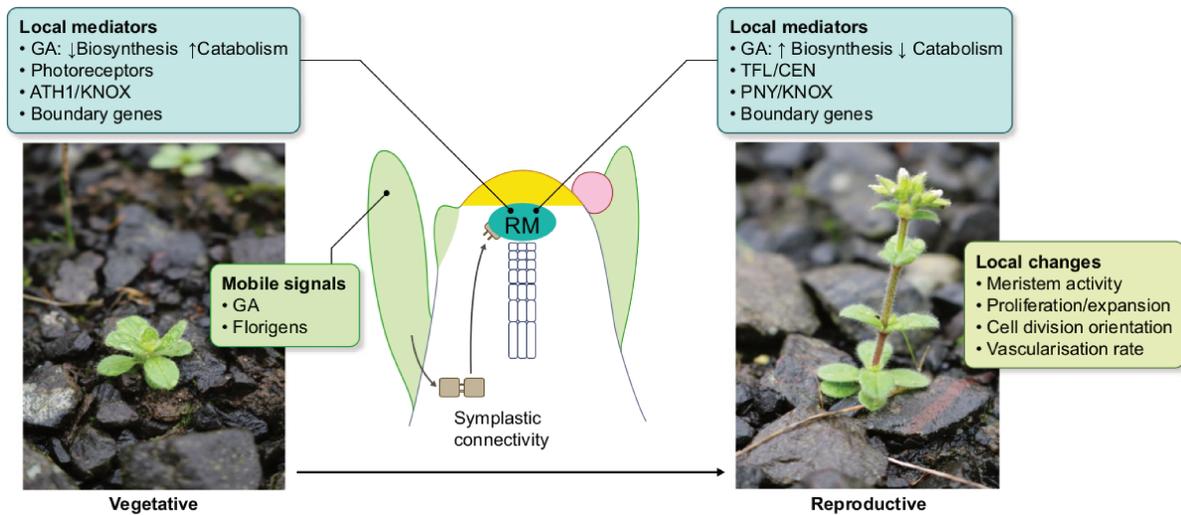


Figure 5: **The development of the internode strongly relies on GA and flowering signals, and is orchestrated locally by boundary genes.** Schematic representation of the major players of internode elongation. Before flowering, GA is maintained low thanks to its catabolism induced by ATH1. After flowering, increased GA allows stem elongation and cell proliferation. source: McKim 2020

texture of plants roots at modifications of the shape or relative growth of each of the phytomer elements.

Modification of the shape The shape and size of each phytomer elements can undergo intense modifications, that often accompany new functions. Leaf size and shape can vary from the simple shape of a few mm like in *Asparagus* to a compound 10m-long leaf like in the *Raphia* palm (fig.6). If the dorso-ventral symmetry of leaves is conserved in the entire plant kingdom, at least during their initial development, some mature leaves can adopt radial symmetry, participating to modification of leaf shapes (fig.6). Modifications of plant shoot also account for diversification of their architecture. Plants can have radial secondary growth of their shoot, that can range from few mm-diameter to several meters. Shoot heights are also highly variable among species.

Change in the relative growth between phytomer elements In addition to shape modifications, the relative growth of each element of the phytomer explains a large range of architecture diversity. The shoot branching pattern depends on the activation/growth of each

axillary meristem. Most palm trees (*Areaceae*) rarely produce branches (but they still can!), while in *Solanaceae* species (potato and nightshade family), almost every aM develops (fig. 6). In addition to its relative growth, the meristem itself has undergone intense modifications in seed plants, as they can become a determinate meristem: the flower.

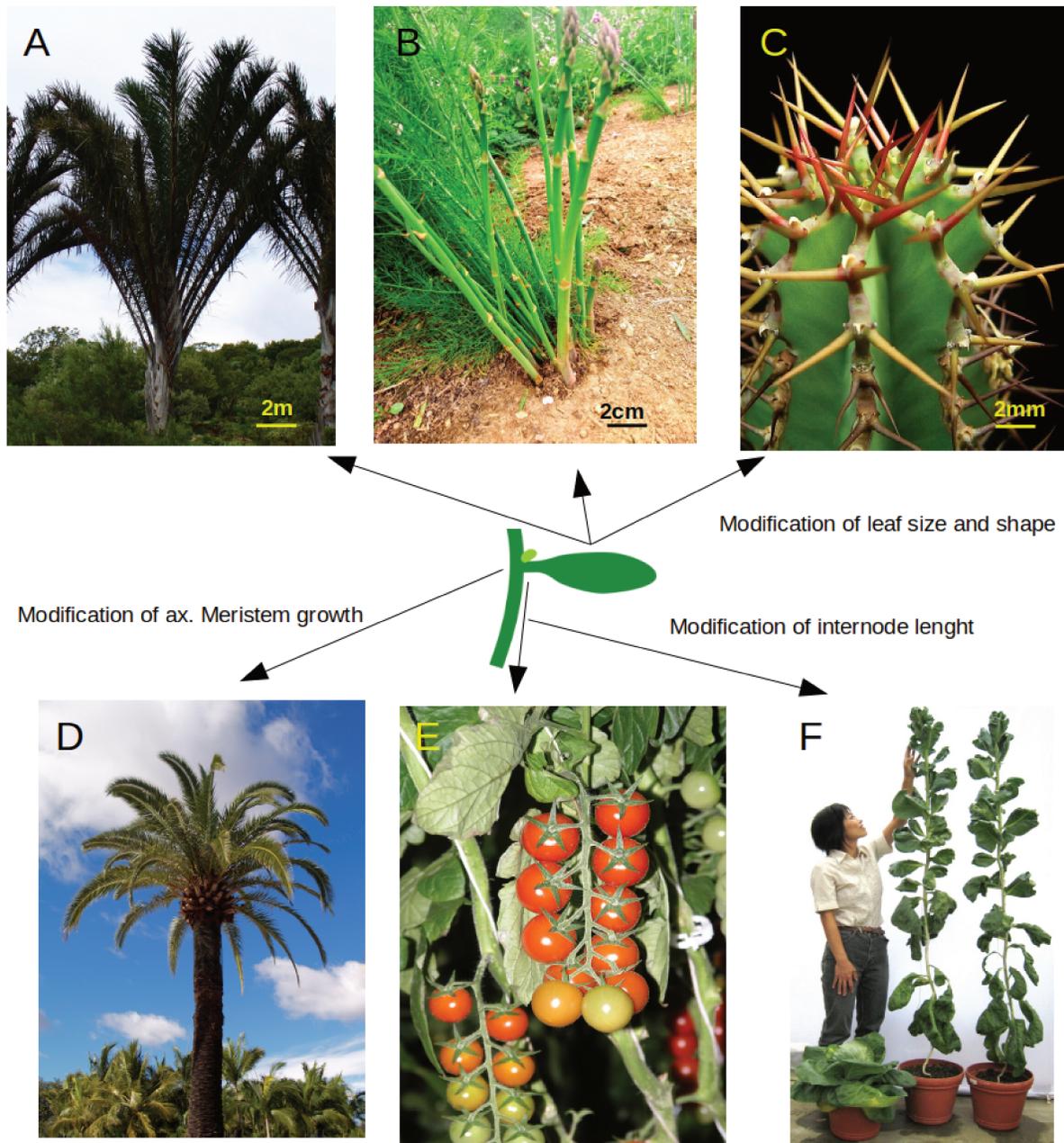


Figure 6: **Variations of the basic structure of the phytomer account for variations of the global plant architecture.** A) In *Rachia australis* (picture credit: Andrew Massyn), leaf can reach 25m long. B) In asparagus (*Asparagus officinalis*), leaves of the turion (a particular bud grown underground) do not exceed 1cm long (B, picture credit: Idéalités). C) In *Euphorbia virosa* (picture credit: Frank Vincentz), grown leaves have a radial rather than dorso-ventral symmetry. D-E) Modifications of branching: no branching in a palmae (D, photo credit: Cryon Ray Macey), to multiple branching in a cherry tomato truss (E, picture credit: Goldlocki) (A-E): pictures are under CC license. F) Variation of internode length in a cabbage (*Brassica oleracea* ssp): the two right cabbages have been exposed to GA treatment for 5 months (source: Tanimoto 2012)

The flower: a determinate meristem Flowering plants are characterized by the presence of a specialized meristem, that can be in axillary or apical position that compacts reproductive system: the flower. Hence, in addition to a vegetative indeterminate axillary meristem, angiosperms can produce a determinate meristem: the floral meristem. The first allows to reiterate plant growth through the development of a new shoot, while the second produces the flower and thus stops its own growth axis, impacting plant architecture (Prenner *et al.* 2009). Flower has undergone intense and diverse structural changes, allowing various reproductive strategies. Many examples can illustrate the diversity of flowers (fig.7), in term of shape, colors, size, and floral organ number.

Floral meristems produce a determinate number of floral organs, whose number and category vary among species (Endress 1990). Simultaneous mutations of all floral organ identity genes lead to homeotic conversion of all flower organs into leaf-like organs (Bowman, Alvarez, *et al.* 1993). It is now well accepted that floral organs are derived from leaves. Floral organs are then considered as "modules" (Klingenberg 2014), produced by the flower meristem, like phytomers. Their internodes and aM would then remain dormant. Still, in some mutants, the meristematic features at the axil of flower organs can re-emerge (Xu, Hu, McKim, *et al.* 2010).

Therefore flowers in angiosperms illustrate well how changes in the shape/size of the leaf, the axillary meristem and the internode, can lead to a complete novel phenotype.

The bract: a special leaf Most probably because of the particular status of flower, the leaf subtending it received a specific botanic term: bract. In some species, bracts have also undergone intense structural changes allowing distinct functions, from attracting pollinator with smells and colors (*Araceae*), to protecting the fruit (chestnut bur), or the flowers (artichoke) (fig.7).

So far, I very quickly illustrated how the tremendous variety of plant architecture across species can be in part explained by countless modifications of the phytomer. Nevertheless, phytomers can undergo modifications during plant life thus changing plant architecture through different developmental stages. Next, I will focus on phytomer modifications following floral transition, showcasing the knowledge acquired from the model plant *A. thaliana*.

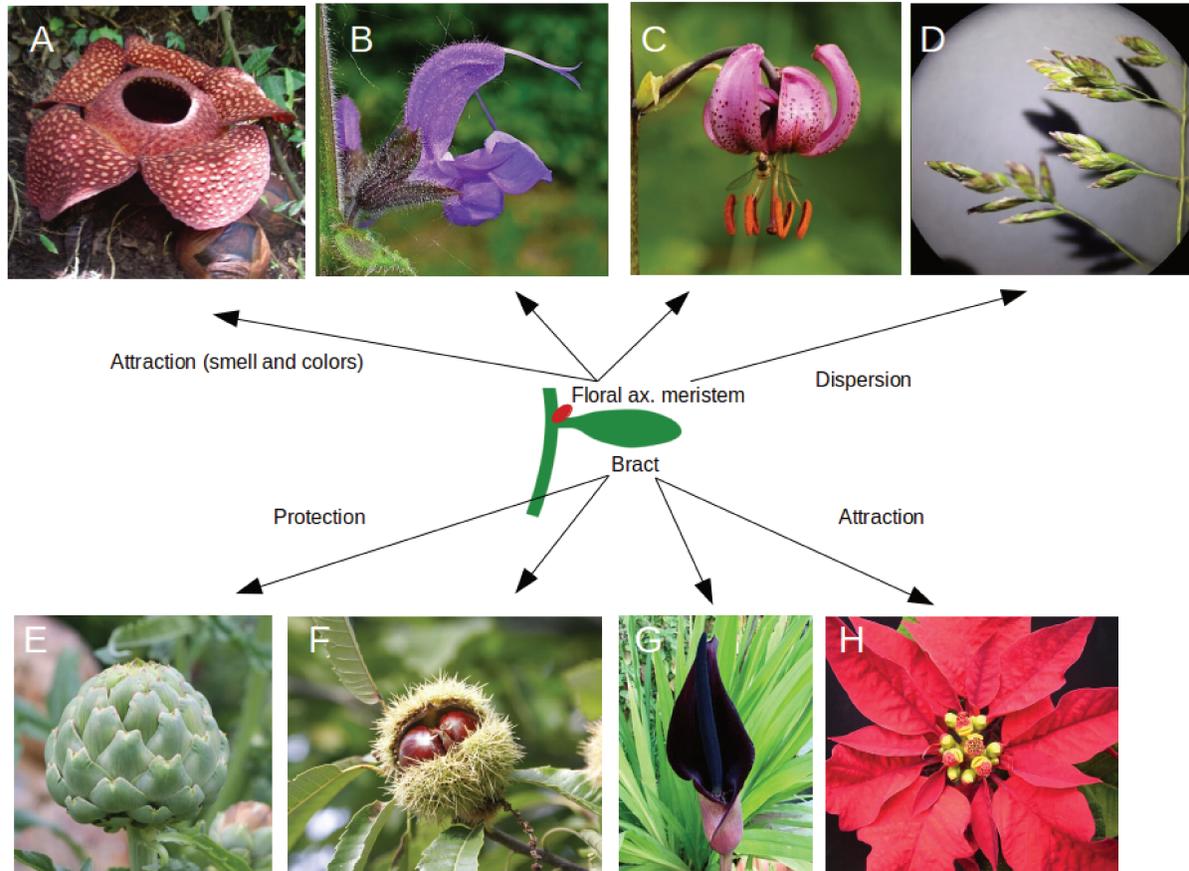


Figure 7: **Morphological variations of flowers and bracts associated to different functions in various angiosperm species.** A) *Rafflesia* flower (picture credit: masuska). B) *Salvia pratensis* (picture credit: H. Zell). C) *Lilium martagon* (picture credit: User Böhringer). D) *Poa pratensis* (picture credit: Stefan Lefnaer), E) artichoke (picture credit: Jamain), F) chestnut (picture credit: Fir002) G) *Arum palaestinum* (picture credit: Sean A. O’Hara). H) *Euphorbia pulcherrima* (picture credit: Franck Vincentz). While some variations are made by elements of the flowers itself (petals, fig. A-D), the bract (the leaf associated to flowers and inflorescence) can also play an important role in phenotypic diversification of reproductive axis. (All pictures are under CC license)

1.2 Floral transition, a developmental switch that remodels phytomers

If the SAM generates all the phytomer components, their respective growth is separated in time, making phytomers a spatial more than a temporal developmental unit. For instance, in *A. thaliana*, leaf cells divide, differentiate and grow in a determinate manner during a certain time after their initiation in the SAM, thanks to the activity of the leaf meristem (Ichihashi & Tsukaya 2015). Internode development occurs in another temporality. When they initiate, internodes consist of a thin layer of cells produced by the rib zone; their elongation occurs after a dormant stage and is triggered by flowering signals, that cause plant “bolting” (Serrano-Mislata & Sablowski 2018). The dynamics of axillary meristems depend on their nature. Floral meristem develops during a certain time just after its initiation at the SAM, while the indeterminate axillary meristem will develop after a dormant stage, sparked by flowering signals (Bencivenga *et al.* 2016). Therefore, phytomer structure depends on the plant species but can also be remodeled across time within the same species.

I will first rapidly describe the different developmental stages of *A. thaliana*, and then focus on the floral transition.

1.2.1 Developmental phase changes and architecture remodelling

Like many organisms, plant development also goes through different stages, in which they can have distinct architectures that serve specific functions. The phytomers are often remodelled during the switch from one phase to another. Several developmental stages have been defined for *A. thaliana*’s life cycle. The seed germination is considered as a developmental phase change because of the important remodelling it triggers. Like other developmental transition, seed germination is tightly controlled by both environmental, and genetic signals, as well as by the carbon content and hormonal signaling (Carrera-Castaño *et al.* 2020). The transition from a dormant state to a germinating plant marks the separation between the embryogenesis and the continuous development of the plant. After germination, *A. thaliana* plants will enter a vegetative stage, itself divided into juvenile and adult stage. Vegetative stage is characterized by an important growth of the leaf, while the internode and the axillary meristem remains dormant (Bencivenga *et al.* 2016). This allows the formation of the rosette, a condensed set of leaves arranged in a circle from the neck of the plant, that characterizes some *Brassicaceae* species.

Vegetative stage thus allows plants to optimize energy storage. During the juvenile stage, plants produce leaves thanks to the activity of the meristems, but they are not competent to flowering signals. The transition to an adult phase is marked by the acquisition of flowering competence, physiological modifications and leaf morphological changes (Yang *et al.* 2013; Périlleux *et al.* 2019). In fact, juvenile leaves have a rounded shape, that becomes bigger and more serrated in the adult phase. In addition, the distribution of trichome changes across the switch to the adult phase, in which trichomes become produced in both adaxial and abaxial sides of the leaf (Telfer *et al.* 1997). The juvenile-to-adult switch is mainly regulated by the *MIR156/SQUAMOSA PROMOTER BINDING PROTEIN LIKE (SPL)* age pathway, hormonal signaling, light and sugar content (Manuela & Xu 2020a). Finally, the annual plant *A. thaliana* undergoes a senescent phase in which plants lose their leaves, and store the energy into developing seeds, that will initiate a new life cycle (J. Kim *et al.* 2018).

Hence, biologists have distinguished several developmental stages that describe *A. thaliana*'s life cycle. Each transition is marked by major changes affecting morphological and physiological features, and are tightly regulated by multiple factors. I have merely provided a brief overview of these developmental switches, and I will now go deeper into the description of the floral transition.

1.2.2 Floral transition remodels both new and old phytomers

Following floral transition, plant architecture is drastically transformed. The flat ground-level rosette will become the pedestal of an aerial branched plant with flower-bearing axis, that optimizes fertilization and seed dispersion. This major transformation involves both alterations of pre-existing vegetative phytomers and a new layout for the *de novo* reproductive phytomers (fig. 9).

From vegetative to inflorescence SAM At floral transition, the shape of the SAM first changes by "doming", a typical morphology that allows to distinguish a vegetative SAM from an inflorescence SAM at the tissue scale. This phenomenon occurs early during the floral transition. It is under the control of the same key genes that regulate the global process of floral transition in a complex gene regulatory network (Kinoshita *et al.* 2020). Also, regulation

of local GA signaling was recently shown to be essential (Kinoshita *et al.* 2020). The doming is the result of an increase in both cell number and cell size.

Basipetal modification of pre-existing phytomers Following the reproductive switch, the inflorescence SAM starts to produce a novel meristem, the flower. The switch from vegetative meristem to floral meristem is abrupt: no intermediate branch-flower are typically produced at floral transition. Since flowers have a determinate growth, lateral branching is no longer possible, which draws typical linear inflorescence units (also called "a raceme"). Furthermore, in *A. thaliana*, the floral transition also induces modifications of pre-existing phytomers, that were produced during the vegetative stage, before the floral switch. Indeed, when plant flowers, the aMs initiated at the vegetative stage in the axil of leaves quit their dormancy state and initiate branches. Besides, internode elongates, driving plant "bolting" (Bencivenga *et al.* 2016).

Flowering-induced phytomer modifications are not synchronous: in *A. thaliana*, the development of the aM can be observed at the time the first flowers are produced. Internode elongation, and thus plant "bolting", is observed later (around 1-2 weeks later) (personal observations, see results section for description and timing of aM emergence). Plus, these modifications appear in a basipetal manner: the youngest phytomers are modified before the oldest, so that highest aM develops before lowest (Hempel & Feldman 1994). Interestingly, some of the oldest phytomers will never undergo internode elongation but develop buds at flowering. This results in lateral branches emerging from leaf rosette. This phenomena may be the result of a different level of sensibility to flowering signal (Périlleux *et al.* 2019).

Because of its huge agronomic relevance, floral transition and flower development have been extensively studied, and reviewed. The various effects of the environment as well as many flowering time genes have been identified. A comprehensive knowledge has been built about the genetic regulatory network (GRN) accompanying floral transition in *A. thaliana* (Wils & Kaufmann 2017). Because this biological process just precedes the phytomer remodeling, I will briefly summarize some of its important points, focusing on the GRN, to sketch a relevant context in which the developmental switch that affects phytomer occurs.

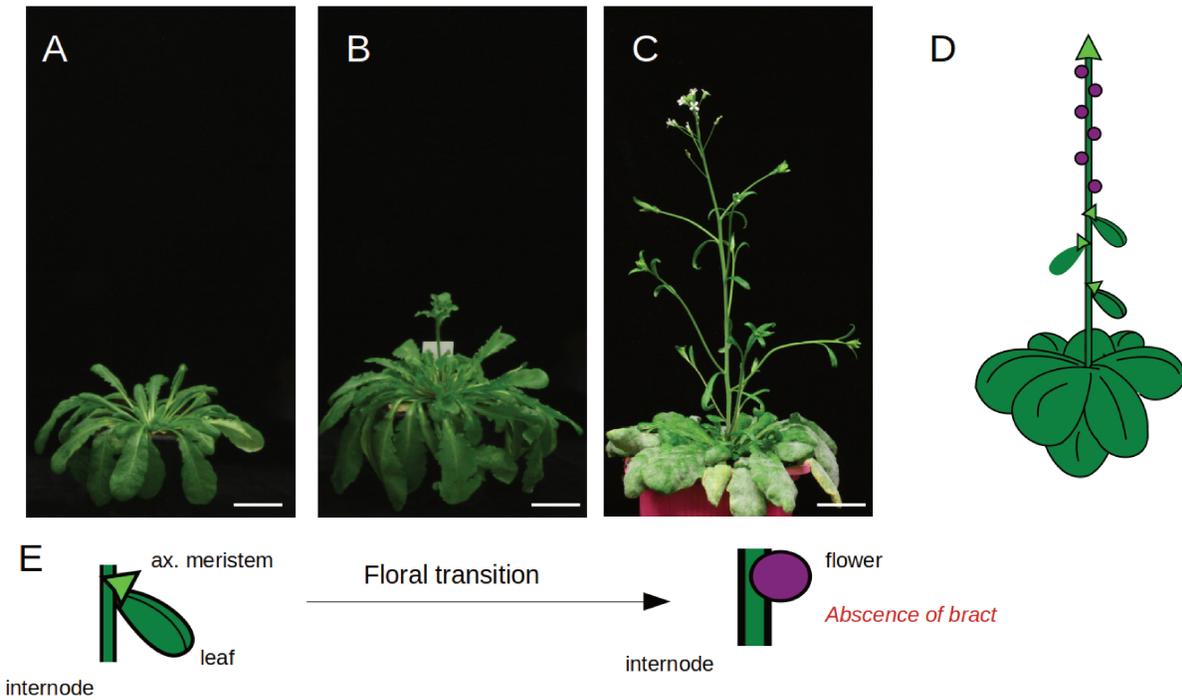


Figure 8: **Floral transition and remodelling of phytomer in *A. thaliana*.** A-C: *A. thaliana* plants at different developmental stages: the rosette stage (A, flat ground-level cluster of leaves with almost no internode elongation); the bolting stage (B, internodes elongate on the main stem and become visible); reproductive stage (C, flowers becomes visible and aM develop into branches). D) Schematic representation of the reproductive *A. thaliana* stage. E) Schematic representation of the structure of phytomers that are produced before and after floral transition. Vegetative phytomers are composed of an internode, a leaf and a vegetative aM. The reproductive phytomer are composed of an internode and a floral axillary meristem. Leaves (bracts) are absent from reproductive phytomers in *A. thaliana*. Note: Dissections show that flowers are produced at the rosette stage, more than a week before bolting. (scheme credit: Fabrice Besnard)

1.2.3 Regulation of Floral Transition

Flowering is controlled by numerous external and internal factors, including photoperiod, ambient temperature, vernalization, sugars and hormones content, and age (Adrian *et al.* 2009; Conti 2017; Johansson & Staiger 2015; L. Li *et al.* 2016). Because different plant species respond differently to those signals, we will only focus on *A. thaliana*.

Floral transition results in the activation of the inflorescence identity within the SAM, and the expression of floral meristem genes in the lateral zone. This is controlled by a complex but well known GRN that integrates the different flowering signals. All signaling pathways lead to the essential integrator *FLOWERING LOCUS T (FT)* (Koornneef *et al.* 1991) and *TWEEN SISTER OF FT (TSF)* (A. Yamaguchi *et al.* 2005), that moves from the leaves to the shoot apex (Corbesier *et al.* 2007) where it combines with *FD* (Abe *et al.* 2005) to activate the floral meristem genes together with *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1 (SOC1)* (fig.9). Mutants of *FT*, *FD* or *SOC1* are late flowering (Koornneef *et al.* 1991; Abe *et al.* 2005; Samach *et al.* 2000), but can still produce flowers thanks to genetic redundancy. Indeed, several mutations are needed to completely inhibit flowering (Reeves & Coupland 2001), suggesting the evolutionary importance of flowering control.

The key integrators of flowering are activated by different partially redundant pathways depending on the flowering signals. Photoperiod, *i.e.* the period of lighting time within a 24-h period, is captured by both phytochromes and circadian clocks genes. *CONSTANS (CO)* is expressed on a rhythmical fashion regardless of light input (Yanovsky & Kay 2002). Under dark, CO protein are degraded, while it is stabilized in long days afternoon by light signalling (Soltis *et al.* 2006). Hence, photoperiod is sensed by the coincidence between light exposure and afternoon, that stabilized CO protein. CO is a transcription factor that activates *FT* expression (Yoo *et al.* 2005, fig.9).

Even when exposed to inductive photoperiod, young plants will not flower. Indeed, the competence to flower also depends on plant age as well as sugar resources. Plant senses its age thanks to the *MIR156/SPL* pathway. *MIR156* is a micro RNA expressed during juvenile phase, that inhibit *SPLs* genes, activators of floral transition (Schmid 2003). *SPL9* and *SPL10*, especially, induce *MIR172* expression that remove *SCHLAFMUTZE (SMZ) - TARGET OF EARLY ACTIVATION TAGGED 1 (TOE1)* mediated floral transition inhibition (Aukerman

& Sakai 2003). *MIR156* level is inversely proportional to sucrose content (Yang *et al.* 2013), suggesting that plants competence to flower is conditioned by an adequate energy storage.

In addition to age, sugar and photoperiod, plant flowering is regulated by other factors such as by ambient temperature or cold exposure (vernalization). Although we are not going to detail those pathways, it is noteworthy that their understanding have been greatly enlightened by studies on natural populations of *A. thaliana* (Lempe *et al.* 2005; Brachi *et al.* 2010; L. Zhang & Jiménez-Gómez 2020; Weigel 2012).

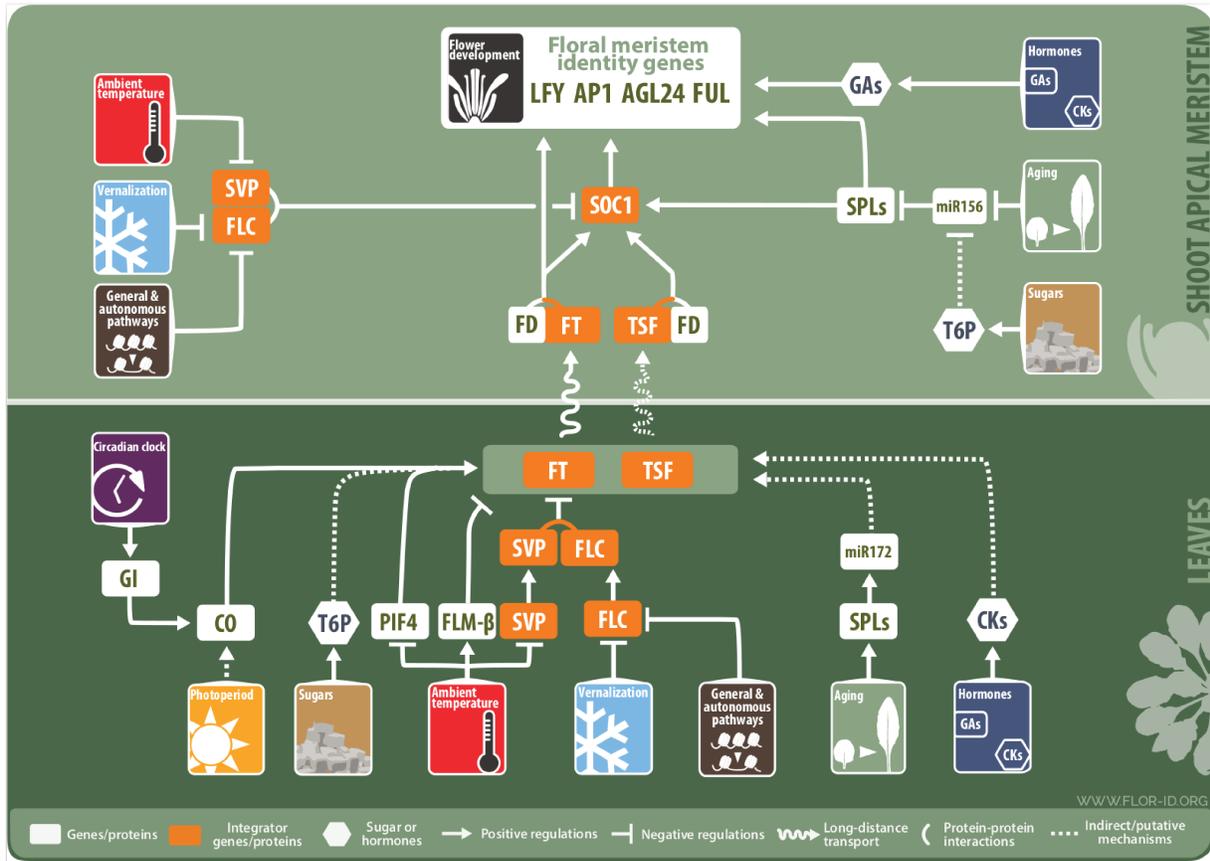


Figure 9: **Schematic overview of the main regulators of floral transition in *A. thaliana*.** Floral transition relies on external (light, temperature, photoperiod, cold exposure) and internal (sugar, hormonal, age, circadian clock) pathways. Signals are transduced from the leaves (bottom part of the figure) to the central meristem (upper part of the figure) in which they are integrated. This scheme represents the main integrators of these pathways. The relationship between each actors is specified on the bottom part of the scheme. source: Bouché *et al.* 2016, Flor-ID: (<http://www.phytosystems.ulg.ac.be/florid/>)

I resumed how environmental signal can be integrated and can rewire plant GRN, that will trigger the formation of a reproductive phytomer. I will now address the genetic pathways that ensure the proper development of a reproductive phytomer. Because very little is known about internode elongation, I will focus only on the control of the flower meristem. I will later address the leaf part of the reproductive phytomer.

1.3 The genetic control of flower development

Flower production involves a complex GRN that have been extensively studied. This process can be divided into three key developmental steps: floral meristem specification, floral organs patterning and development, and floral meristem termination. We will briefly describe the main regulators of the two first steps in *A. thaliana*. Specification of floral meristem is the first step that mark the divergence between vegetative and reproductive phytomer. Floral organ patterning and termination ensure a proper development of the flower. When disrupted, flowers often show sign of vegetative phenotypes.

1.3.1 Specification of a new type of meristem: the flower

The floral meristem initiates in the PZ of the inflorescence shoot apical meristem. This step is extremely studied because flower emergence can be accessible after plant bolting, that makes the dissection easier. Like for leaves during the vegetative phase, the position of the floral meristem is dictated by phyllotaxis that emerges from the dynamical auxin transport from cell to cell (Smith *et al.* 2006; de Reuille *et al.* 2006). A local auxin maxima at $137,5^\circ$ from the *i* organ precedes the emergence of the *i*+1 floral primordium. This auxin maximum is transduced to an intracellular signal notably by the action of *MONOPTEROS/AUXIN RESPONSE FACTOR 5* (*MP/ARF5*), a transcription factor that will modify gene expression (Aida *et al.* 2002; Reinhardt *et al.* 2003; Przemeck *et al.* 1996). Among others, *MP* up-regulates its own expression and reinforces local convergence of auxin fluxes, creating a positive feed-back loop driving organogenesis (Bhatia & Heisler 2018). *MP* also stabilizes the plastochron by activating *ARABIDOPSIS HISTIDINE PHOSPHOTRANSFER PROTEIN 6* (*AHP6*), a negative regulator of cytokinin signaling. This small protein moves between cells through plasmodesmata, generating an inhibitory field that slows down the activation of neighbouring flower initia, whose sites

are already getting specified by auxin accumulation (Besnard *et al.* 2014; Galvan-Ampudia, Cerutti, *et al.* 2020).

Besides, within the anlagen, *MP* will also activate both *LEAFY* (*LFY*) and *AINTEGUMENTA* (*ANT*) genes family, essential for the acquisition of floral meristem identity (N. Yamaguchi, Wu, *et al.* 2013). Because of its predominant role in establishing flower meristem identity, *LFY* have been intensively studied. Its main upstream and downstream regulators have been identified (Winter *et al.* 2015).

In addition to *MP*, *LFY* is activated by other pathways, involving the *DORNRÖSCHEN-LIKE* (*DRN/DRNL*), the *PUCHI/BLADE-ON-PETIOLE* (*BOP*) genes (Chandler & Werr 2017) and by genes involved in the floral transition pathway (Abe *et al.* 2005).

LFY regulates hormonal status within the floral meristem, notably cytokinin, auxin and GA, resulting in a local maximum of cytokinin and auxin, and a local minimum of GA (Denay *et al.* 2017). Cytokinin and auxin maxima will drive the meristematic identity of the cells, while the minimum of GA will activate DELLA proteins, that together with *SPL* activate many MADS-box transcription factors (N. Yamaguchi, Winter, *et al.* 2014). The activation of *APETALA 1* (*AP1*), a key MADS-box transcription factor, is also ensured directly by *LFY* (Wagner *et al.* 1999). The combined activity of *AP1* and its closely related *CAULIFLOWER* (*CAL*), and *LFY*, with other essential MADS-box genes such as *AGAMOUS-LIKE 24* (*AGL24*) and *SHORT VEGETATIVE PHASE* (*SVP*), will trigger the acquisition of floral meristem identity (Denay *et al.* 2017). This is achieved by the inhibition of *TERMINAL FLOWER 1* (*TFL1*) that ensures the inflorescence state (Bradley 1997), and the activation of floral identity genes (Kaufmann *et al.* 2010) (fig. 10). *TFL1* inhibition does not affect the SAM, that stays indeterminate, in part thanks to its activation by *AGL14* (Pérez-Ruiz *et al.* 2015). However, some divergence in the activity of these transcription factors is noteworthy. Notably, it has been suggested that *LFY* and *AP1/CAL* have antagonistic consequences on *TFL1* expression: *LFY* activates *TFL1*, while *AP1* inhibits it (Goslin *et al.* 2017; Serrano-Mislata, Goslin, *et al.* 2017). It is hypothesized that this antagonistic interplay ensures that the SAM remains indeterminate after floral transition (Serrano-Mislata, Goslin, *et al.* 2017). The aM, on the other hand, are prevented from the flowering signals thanks to the activity of *BRANCHED1* (*BRC1*), that will delay floral transition in the buds (Niwa *et al.* 2013) (fig.10).

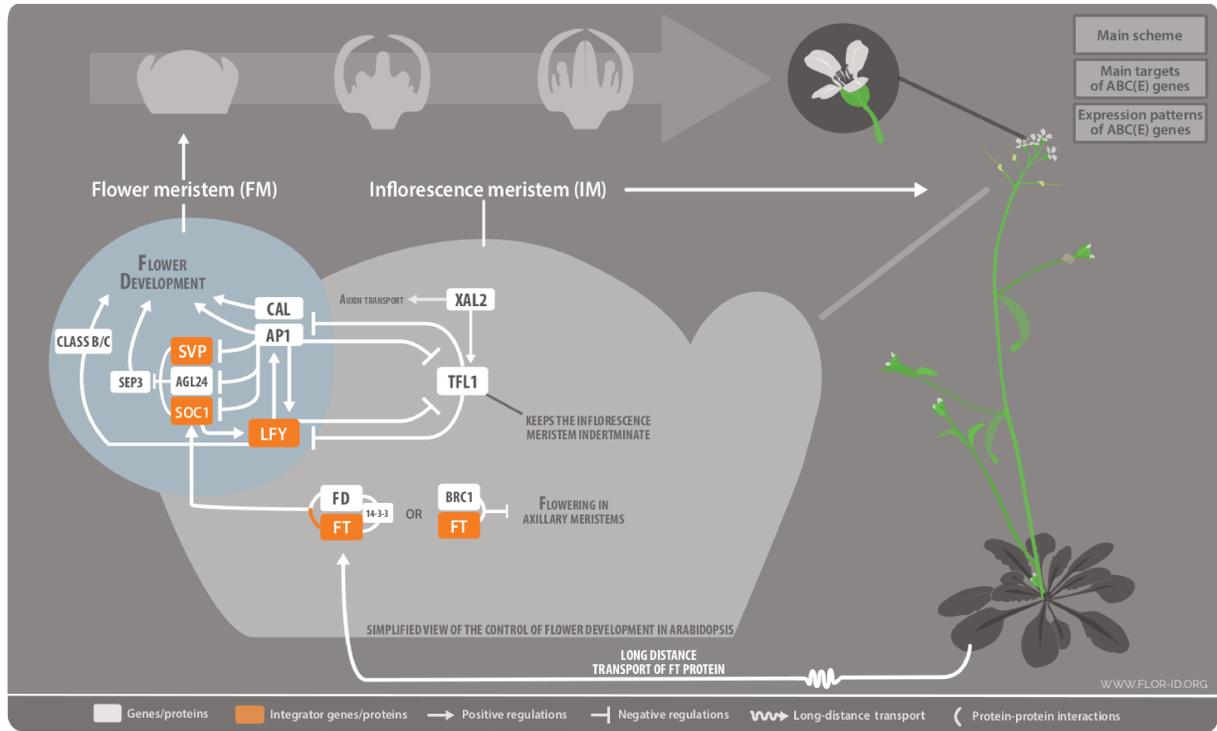


Figure 10: **Schematic overview of the main regulators of floral meristem specification and development in *A. thaliana*.** The relationship between each actors is specified on the bottom part of the scheme. Taken from Bouché *et al.* 2016, Flor-ID (<http://www.phytosystems.ulg.ac.be/florid/>).

1.3.2 Proper patterning and termination of flowers are necessary to avoid shoot features in flowers

Once the floral meristem is initiated, a combination of transcription factors ensure the expression of each floral organ (sepals, petals, stamens and carpel) in a sequential mode referred to as the ABC model (Coen & Meyerowitz 1991). Since then, increasing research on *A. thaliana* but also other species have precised the original ABC model, that can slightly diverge across angiosperms. Briefly, in the ABC model, for each floral organ corresponds a combination of MADS-box transcription factors that give the organ identity (fig.11). The spatial and temporal regulation of each MADS-box gene is however complex and involve a lot of actors (Krizek & J. C. Fletcher 2005). The establishment of boundary between each floral organ is essential for the proper whorls patterning, similarly to leaf and organ development. This is ensured by general "boundary genes" that also act in the leaf boundary establishment such as *CUP-SHAPED COTYLEDON 3 (CUC3)* (Vroemen *et al.* 2003), but also genes that are specific to the separation between two whorls. For instance, *SUPERMAN* ensures the boundary between carpel and stamens and thus the proper number and position of both whorls (Prunet *et al.* 2017). Hence, production of floral whorls by the floral meristem can remind the production of organs by the SAM or the aM. The overlapping functions of genes in leaves and flowers organs suggest their homology and is in line with the modular/ phytomer-based plant growth. Hence, leaf and floral organs developmental program share similarities, and a misregulation of flower patterning often leads to vegetative branch-like features (fig.11).

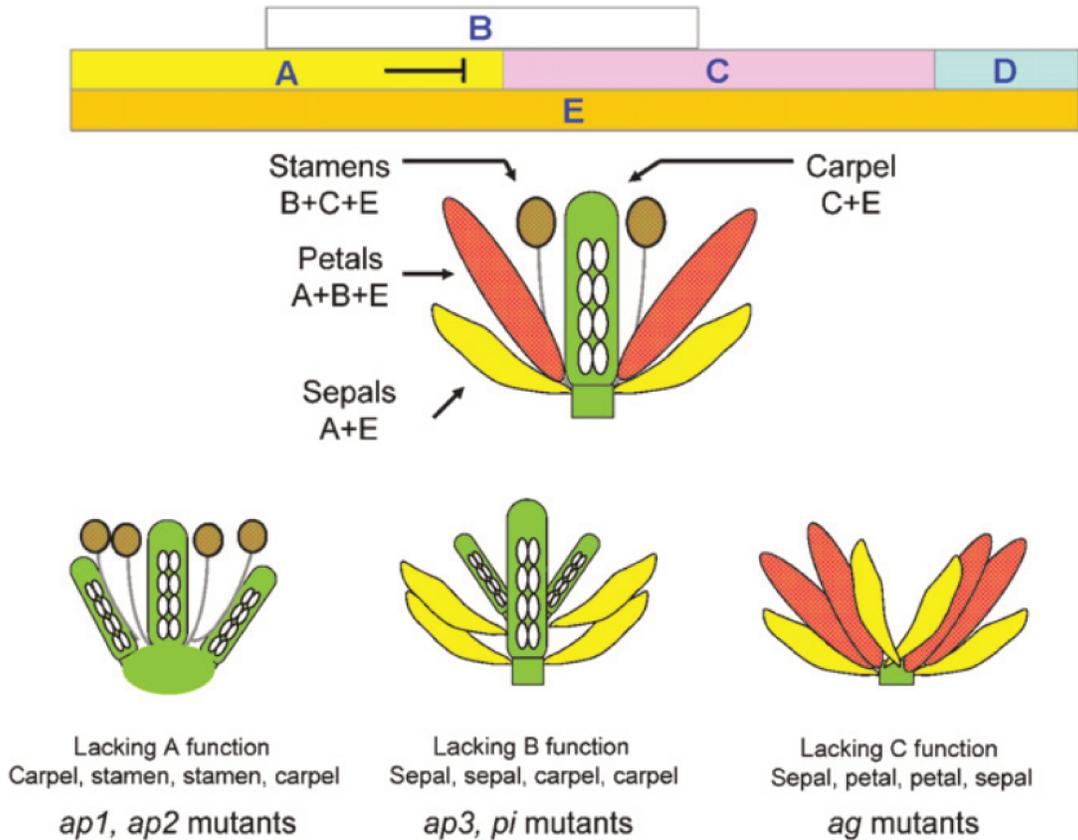


Figure 11: Schematic expression pattern of the keys ABC genes that specify floral organs development, and phenotypes of some mutants. A lack of proper flower development can induce phenotypes that remind vegetative branching. From Soltis *et al.* 2006

In annual plant like *A. thaliana*, reproductive stage specializes in fertilization and seeds dispersion, and leads to phytomer remodelling. The function of hundreds of genes in the regulation of the key developmental steps of flower formation have been uncovered, for which we have merely provided a brief overview. Contrary to flower, the development of its subtending leaf, the bract, have not received much attention. However, in the model plant *A. thaliana*, like most Brassicaceae, floral transition is also characterized by bract inhibition. Absence of bract on floral phytomers is common to several clades, but the mechanism underlying its inhibition remains unclear. This next part will be dedicated to our current knowledge about bract development and inhibition, and will consider not only *A. thaliana*, but also several Poaceae species.

2 Developmental regulation of the bract in the reproductive phytomer

Bracts are very common among Angiosperms. In many cases, the term bract is used in its broad sense and refers to either leaf subtending the flower or the inflorescence. Here, we will define bract *stricto sensu* as the leaf composing the reproductive phytomer of Angiosperms. Hence, bracts are leaves *subtending* the flower within the phytomer unit (Flower Subtending Bract, FSB in fig.14). Therefore, bracts are not produced by the floral meristem but rather by the inflorescence meristem. They are thus distinct from leaf-like sepals that *precede* the other floral organs (Flower Preceding Prophyll, FPP fig.14). In the literature on Poaceae's, bracts often refer to leaves subtending an inflorescence (bracts *lato sensu*, see fig.14). Still, these studies can give insights on the mechanisms behind leaf suppression. Hence, bracts can be used to describe structure from different developmental origin. In this manuscript, we will precise three different terms depending on the developmental origin: bract *stricto sensu* (*s.s.*) defines a leaf subtending a flower (FSB in Prenner *et al.* 2009), bract *lato sensu* (*l.s.*) defines a leaf subtending an inflorescence, and bract *fpp* defines modified perianth preceding the flower (see fig.14). When the developmental origin is not known, we will refer as to "bract" or bract-like structure.

2.1 Convergent evolution of bract loss

Bracts are not visible in several angiosperm clades, some of which include model species like in Brassicaceae or Poaceae. Despite the lack of precise functional knowledge, it seems that several independent genetic events occur to converge to bract loss in these different clades (Mach 2010). Bract loss is therefore an open question, both in term of their evolutionary and developmental origin.

Before addressing the developmental origins in the following, I would like to briefly address the possible evolutionary forces that could have favored such a recurrent scenario of bract loss.

Well developed bracts can achieve various functions, as shown in fig.7: colored bracts can attract pollinators, while a spiny cupule protects young floral buds. However in grasses, like maize, rice, or wheat, and also in Brassicaceae, bract loss seems to follow a flower size reduction. Besides, as most Poaceae and Brassicaceae are annual plants, floral production marks the end of the individual plant life cycle. Bract loss could have resulted from a different partitioning of cell fate within the nascent primordium. It could then be the results of a different source-sink energy distribution in favor of the reproductive organ. Such mechanism that favors flower growth could have been selected in plants with an annual life cycle. Thus, one evolutionary explanation for bract loss is its hypothetical way to optimize energy distribution in favor of a rapid and numerous flower organs initiation (Chuck *et al.* 2010; Hodgson *et al.* 2017; Midgley & Bond 1989). While this remains very hypothetical, understanding the mechanism of bract inhibition could help address the evolutionary significance of bract loss.

2.2 Cryptic bract in bractless species

As already mentioned, *A. thaliana* flowers usually do not bear bract, at least when observing the plant with the naked eye. However, microscopic observations of the first steps of flower development suggest the presence of a cryptic bract, that is rapidly inhibited.

2.2.1 Morphological evidence in *A. thaliana*

Monitoring of variations of the SAM surface growth during flower development using the sequential replica method (Kwiatkowska 2008) shows that the first steps of flower development are

marked by the presence of a shallow crease associated to the first lateral bulge of organogenesis. The spherical flower meristem develops later just at the position of this crease (Kwiatkowska 2006). This crease is proposed to be the axil of a cryptic bract, whose growth stops just when floral meristem emerges (fig.12). In this article, the authors speculate that the inhibition of bract growth is triggered by the floral meristem itself.

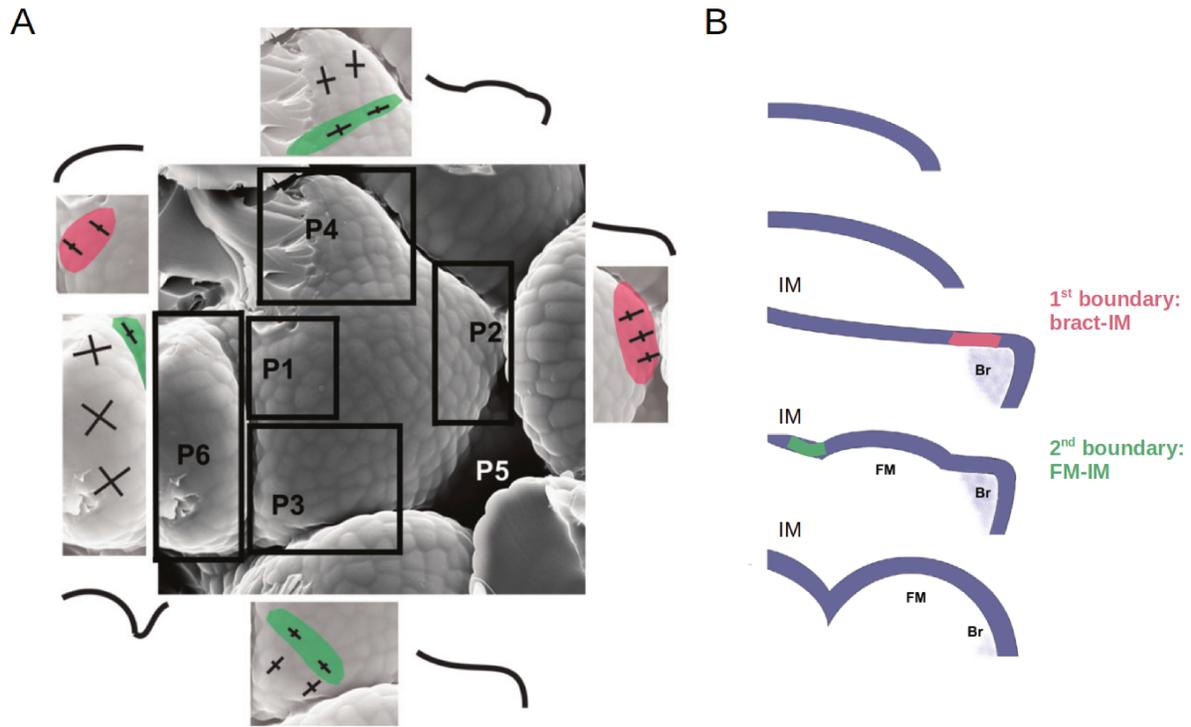


Figure 12: **Schematic representation of growth pattern in early stages of lateral flower development in *A. thaliana*.** A) Scanning electron micrograph of the inflorescence SAM replicate in the top view, is shown in the centre. Primordia are labelled as P1–6 with a number increasing with the primordium age. By using sequential views of the same SAM, growth patterns on the apex surface can be computed. They are shown for the outlined regions as crosses with the arms pointing to the principal growth directions. Arm lengths are proportional to the corresponding principal growth rates. Areas shadowed in red point to the regions where the axils of putative rudimentary bracts (boundaries between the bracts and the SAM) are formed, green areas point to boundaries between the SAM and flower primordia proper. For each primordium, the outline of the corresponding median longitudinal apex section is shown. P1 and P2 are in the stage of lateral bulging, leading to the shallow crease formation (P3). In P3 and P4, the upward bulging at the bottom of the shallow crease, takes place. P6 is in the fast growing bulge stage. From Kwiatkowska 2008. B) Geometry of the inflorescence shoot apical meristem (IM) and flower meristem (FM) during the first stages of development of the latter. On the flank of the IM, a first bulge that corresponds to the rudimentary bract (Br) appears. On its axil, a second bulge forms and it continues to grow engulfing the first one and forming the FM proper. These stages of FM development correspond to P2 and P3 according to A). The geometrical formation of the Bract - IM and the FM - IM boundaries are reported from A). Adapted from Alvarez-Buylla *et al.* 2010

2.2.2 Molecular evidence in *A. thaliana*

The cryptic bract domain is also associated with several molecular markers. On its first steps, the primordium anlagen of an inflorescence SAM loses *STM* expression, which specifies meristematic cells (fig.13A-B). *STM* expression re-appears later when floral meristem reaches a certain size, emphasizing Kwiatkowska's morphological observations (Long & Barton 2000). In addition to a lack of *STM* (fig.13B), the abaxial region of the primordia shows expression of lateral organ identity genes such as *ANT* (Long & Barton 2000; fig.13C-D). Also, the abaxial region of the primordium shows specific expression patterns of known polarity genes such as *YAB3* and *FILAMENTOUS FLOWER (FIL)* (Goldshmidt *et al.* 2008, fig.13E-F). This cryptic bract domain also shows expression of genes involved in boundary establishment like *CUC3* (Hibara *et al.* 2006), which marks a first boundary between the cryptic bract and the floral meristem, and a second boundary between the floral meristem and the SAM (fig.13G). Besides, genes involved in developmental transition such as *SPL9* are also expressed in the cryptic bract domain (Hyun, Richter & Coupland 2017). Altogether, these data support the existence of a specific region in the abaxial part of the emerging flower, with morphological and gene expression parameters that resemble the leaf. This cryptic bract is transient during flower emergence, and its function (if any) remains unaddressed. Nonetheless, the cryptic bract can develop into a full bract in particular conditions that I will discuss below.

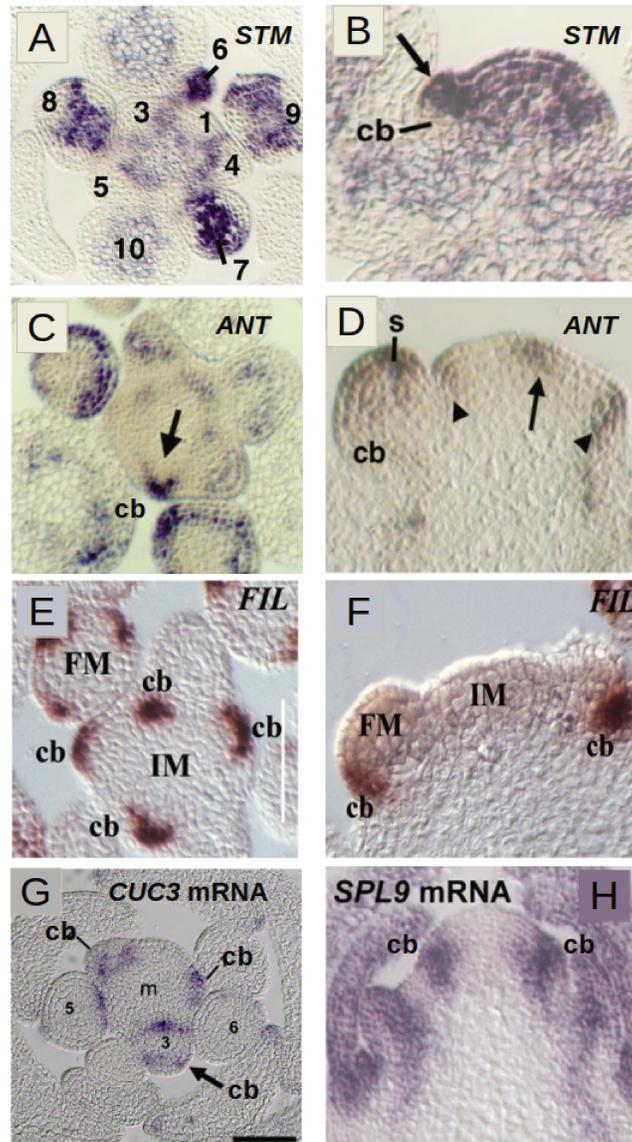


Figure 13: **Cryptic bract domain is associated with numerous molecular features.** *In situ* hybridization of WT inflorescence SAM (A-H) of *A. thaliana* in apical (left) or lateral (right) view, with *STM* (A-B), *ANT* (C-D), *FIL* (E-F), *CUC3* (G), *SPL9* (H) probes. A-B: Cryptic bract domain (cb) lacks *STM* expression. Absence of *STM* mRNA in young floral primordia before P5 (A), and absence of *STM* on the abaxial side of a stage 2 flower, under a *STM*-positive bulge (B) (Adapted from Long & Barton 2000). C-D: *ANT* is expressed in the cryptic bract domain, in a complementary pattern than *STM*. *ANT* expression on the abaxial part of flower primordia (arrowhead) and on P1 stage (arrow). (Adapted from Long & Barton 2000) E-F: *FIL* is expressed in the cryptic bract domain (cb), as well as the sepals of floral meristem (FM). (Adapted from Goldshmidt *et al.* 2008) G: *CUC3* is expressed on the boundaries between the SAM (m) and the flower primordia. But also marks the cryptic bract domain (cb), highlighting the boundary between the bract and the flower. (Adapted from Hibara *et al.* 2006) H: *SPL9* expression in inflorescence SAM of 6 weeks old plants cultivated in short day. *SPL9* is expressed in the leaves (bottom), but also in the cryptic bract domain of emerging flower primordia (Adapted from Hyun, Richter, Vincent, *et al.* 2016).

2.2.3 Cryptic bracts in other species

Apart from Brassicaceae, bract loss appears in different group, which the most studied are grasses (Poaceae), and especially the model plant maize. In maize, inflorescence meristems (IM) are divided into two different types: the tassel IM, located in the terminal position at the top of the plant and producing male flowers, while the ear IM, are axillary axis subtended by a leaf and producing female flowers. Both IM produce spikelet pair meristem (SPM), which in turn will produce spikelet meristems (SM). The spikelet meristem starts by producing a pair of leaf-like structures called the glumes and can then produce several florets in the spikelet axis. Each floret is enclosed in a pair of other leaf-like structure (called the palea and the lemma). The floret is very peculiar because it contains almost only reproductive organs (stamens and ovary), the petals being reduced to tiny specific structures, the lodicules (fig.14).

In maize, the monitoring of floral meristem development spotted the presence of a small bump on its axil, associated to a cryptic bracts *l.s.* (fig.15C). As the flower grows, the cryptic bract *l.s.* disappears fast. In addition, *TASSELSHEATH4* (*TSH4*), an homolog of *SPL15* and *SPL9*, is expressed in the cryptic bract domain, in a mutually exclusive manner from the meristematic markers *RAMOSA2* (*RA2*) (Chuck *et al.* 2010). Hence, in both *A. thaliana* and Maize, bract inhibition leaves remnants structure transiently visible on microscopic observations. This domain is also characterized in both species by a lack of meristematic features, and the presence of specific markers.

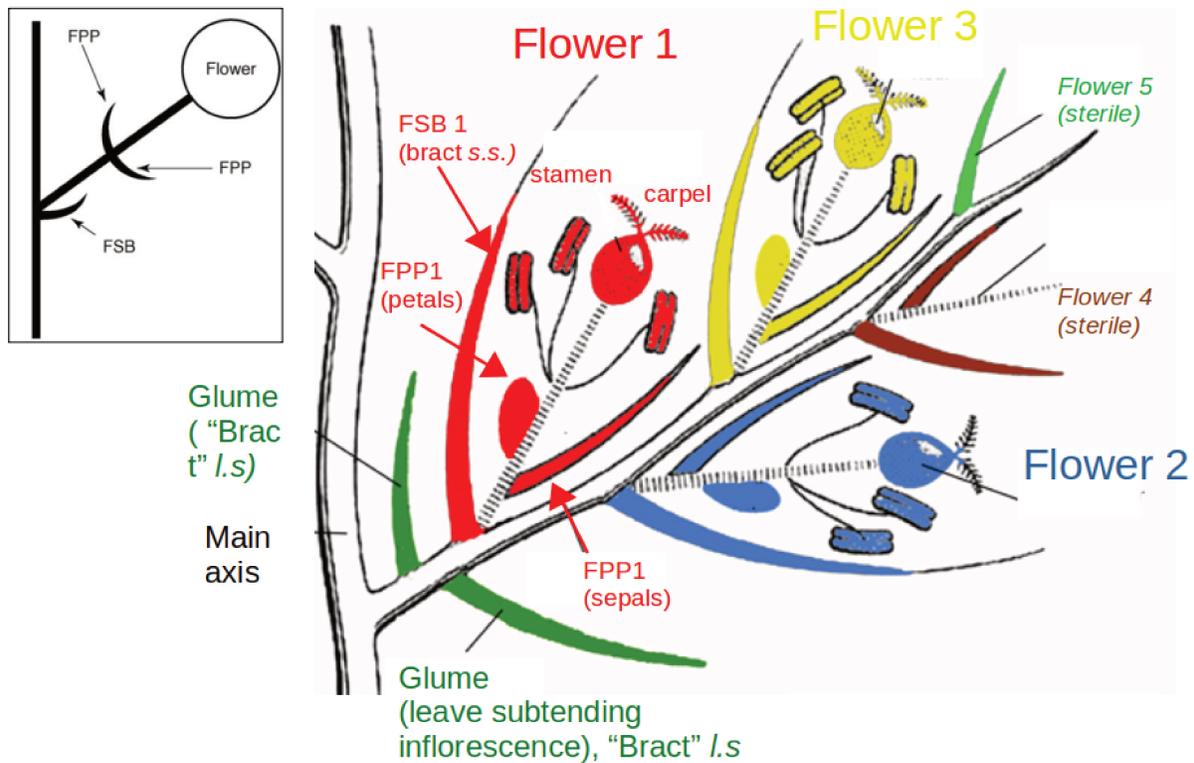


Figure 14: **Schematic representation of a typical Poaceae's inflorescence.** The inset shows a schematic representation of different types of leaves that can subtend, or precede flowers. Leaves that precede flowers (or Flower Preceding Prophyll, FPP) can be modified sepals or petals. The leaf presents at intersection between the axil of the flower, and the stem, are called Flower Subtending Bract, or bract *stricto-sensu*, and constitutes the main focus of my study. From Prenner *et al.* 2009. The right picture show a typical Poaceae inflorescence. Each flower (called "floret"), are represented in a specific color. In some species, last florets can be extremely reduced and sterile. The two glumes are bracts *lato sensu*, because they are leaves subtending an inflorescence. Within the inflorescence, each flower is subtended by a bract *stricto-sensu*, called the lemma. A FPP, consisting of a modified sepals, precede the "floret". Another FPP, the lodicules, consist of a modified petals. The floret comprises only the reproductive organs: stamens and carpels. In some species, like maize, male and females flowers are separated in the general plant architecture, with the tassel (male) on the top, and the ear (female) on the bottom part of the plant. Adapted From <https://botarela.fr/Poaceae/Description-detail/Epillet.html>, and Prenner *et al.* 2009 for the inset.

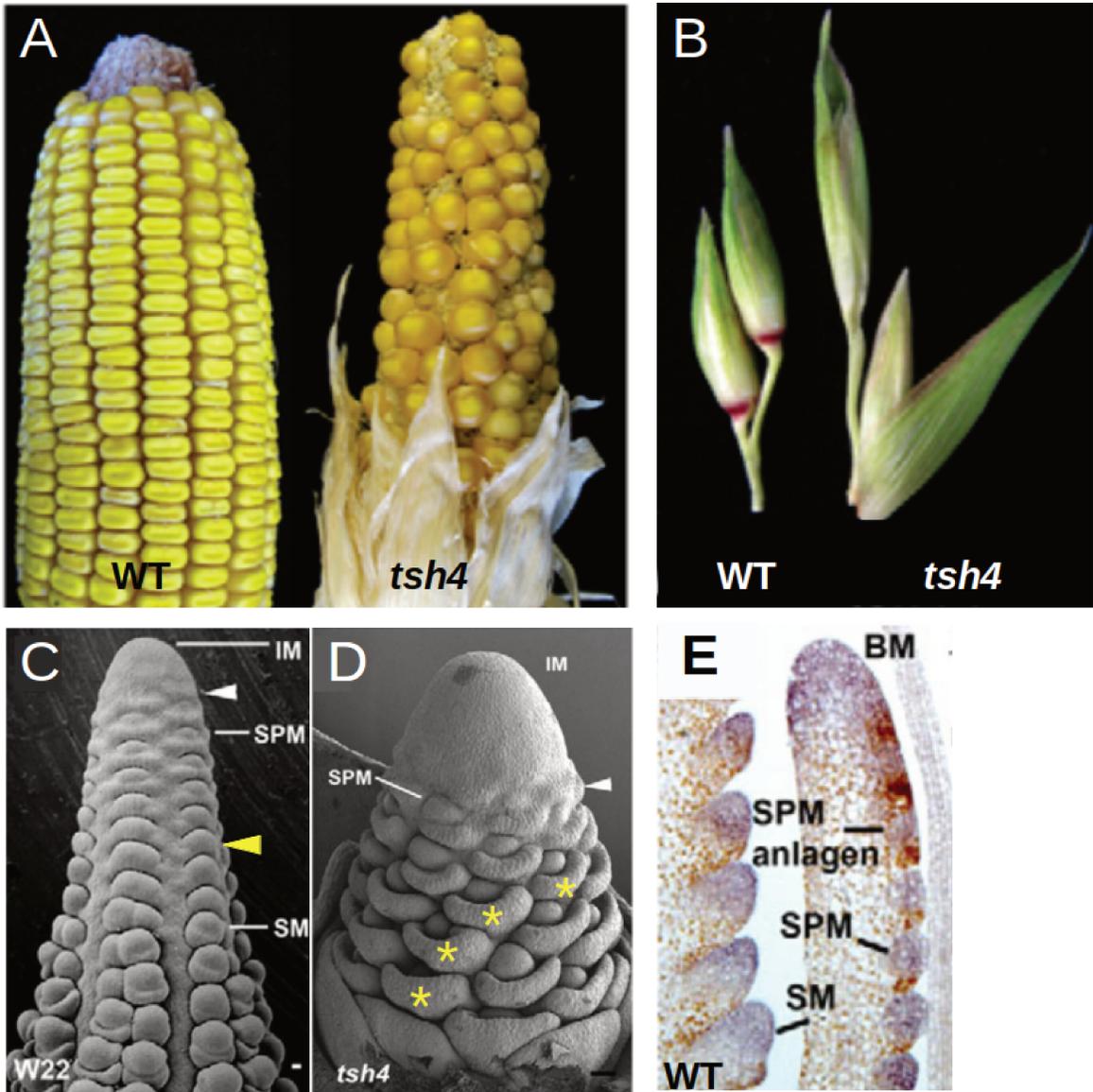


Figure 15: **Cryptic bract *l.s.* and bract *l.s.* mutants in maize.** (Bracts *lato sensu*, see section 2 for bract *stricto-sensu* definition). A: Comparison of mature WT ears (left), and of *tsh4* mutants (right) ears. *tsh4* mutants show a disturbed phyllotaxy of the kernel rows, with a reduced number of grains. B: Mature spikelet pair (i.e. palea and lemmas enclosing the florets) of WT (left) and *tsh4* mutant (right), showing an ectopic bract *l.s.* in the mutant. C-D: Scanning electron micrograph of WT (right) and *tsh4* mutant (left). C: cryptic bract *l.s.* are transiently visible in the WT top ear (white arrowhead), but disappear fast and are no more visible after Spikelet pair meristem growth (yellow arrowhead). D: bracts *l.s.* are not repressed in the *tsh4* mutants, and are bigger than in the WT (some bracts are marked with a yellow asterisk). E: Double labeling of MIR156 (blue) and *TSH4* (gold) in wild-type tassel with branch. Their expression patterns appear complementary. Adapted from Chuck *et al.* 2010.

2.3 Basal bract emergence in bractless species: experimental and natural conditions

2.3.1 Experimental restoration of bracts involves defects in flower development

In both bractless Poaceae and Brassicaceae, bract development has been investigated using mutants that fail to inhibit bracts, revealing interesting genes whose alteration induces bract development.

In maize, bract *l.s.* loss seems to be triggered by *TSH4* gene (Chuck *et al.* 2010). *TSH4* is a protein involved in lateral organ inhibition, and marks the boundary between leaf and lateral meristem. In *tsh4* mutant, bracts *l.s.* are de-repressed, while the size and number of axillary meristems and internodes are reduced, and the shape and size of the grains and the phyllotactic pattern is perturbed (fig.15A-B). Altogether, these phenotypes suggest a role in partitioning cells fate within the phytomer. Moreover, *TSH4* is a target of *MIR156*, a micro-RNA involved in floral transition (Xu, Hu, J. Zhao, *et al.* 2016) and expressed in the floral meristem, antagonistic to *TSH4* expression pattern (fig.15). Sequestration of *TSH4* in this boundary region by *MIR156* during Spikelet Pair Meristem emergence could hence be responsible for bract inhibition (Chuck *et al.* 2010).

Bract *l.s.* loss has been studied in other Poaceae species, including barley, wheat, or rice. Different pathways have been involved in bract loss (fig.16). Several of these genes are also involved in floral transition pathways, such as *SPL* and *NECK LEAF 1 (NL1)* in rice (L. Wang, Ming, *et al.* 2021; L. Wang, Yin, *et al.* 2009), *TSH4* in maize (Chuck *et al.* 2010), *THIRD OUTER GLUME 1 (TRD1)* in barley (Houston *et al.* 2012), or *VERNALIZATION PROTEIN 1 (VRN1)* in wheat (C. Li *et al.* 2019).

Other pathways can be involved in bract suppression in Poaceae, such as leaf initiation (through *PLASTOCHRON* genes in rice (Kawakatsu, Taramino, *et al.* 2009; Kawakatsu, Itoh, *et al.* 2006)), or meristem maintenance (through *RIL1* in rice (Ikeda *et al.* 2019)).

In *A. thaliana*, the homologous of *TSH4*, *SPL9* and *SPL15*, are still involved in floral transition with *MIR156*, but do not seem to inhibit bract, as double mutant does not show any bract phenotype (Schwarz *et al.* 2008).

In *A. thaliana*, several mutants bearing "bracts" (*s.s.*, *l.s* or *fpp*) have been described in

the literature. The pathways involved here mostly refer to the acquisition of flower identity or the specification of floral organs. Hence, the mutants also show defects of the flower identity itself. Indeed, 35S:LFY (Weigel & Nilsson 1995), UNUSUAL FLOWER (UFO) KO mutants (*ufo*) (Hepworth, Klenz, *et al.* 2006), BLADE-ON-PETIOLE (BOP) KO mutants (*bop1*, *bop2*) (Hepworth 2005), PUCHI KO mutants (*puchi1*) (Karim *et al.* 2009), JAGGED dominant (*jag-5D*) (Dinnyeny 2004; Ohno 2004), *agl6* OE (Koo *et al.* 2010) are described as having "bracts", even though the nature of the leaf is difficult to address, as flower identity is itself strongly impaired (fig.17, and fig.16). However, mutants in floral transition pathways can also lead to bract emergence, as it is the case for *agl24*, *svp*, *soc1* (Liu *et al.* 2009), *ft* (Müller-Xing *et al.* 2014), but these plants also show severe sign of flower impairment (fig.16).

Thus, it appears that in all these mutants, bract inhibition and floral identity seems strongly related. The link between flower identity and bract suppression has also been investigated by Nilsson *et al.* After specific destruction of floral cells, using a dipterin toxin A under the control of the *LEAFY* promoter, bract-like structures replaced flowers, suggesting that absence of flower removes bract inhibition (Nilsson *et al.* 1998). Still, disturbing floral transition pathways, in *A. thaliana*, can trigger bract emergence. In fact, *fd* mutants plants with pFD::MIR156 construct produced more branches, and present flowers with "bracts", but with inflorescence defects (J.-W. Wang *et al.* 2009)

It is noteworthy that in several studies, bract has been taken in a *lato sensu* or *fpp* sense, rather than *stricto sensu*. In fact, the diversity of flower shapes in Angiosperms shows that leaves can precede flowers (i.e. be located in the same axis), and that leaf-like sepal exists which are sometimes called "bracts" (here, they will be referred as bract *fpp*). For instance, in strong *ap1* mutants, the 1st flower whorls can be transformed into bract-like structure (Xu, Hu, McKim, *et al.* 2010). Mutants converting flower whorls into leaf-like shape have been already described in the literature (Bowman, Alvarez, *et al.* 1993; Bowman, Sakai, *et al.* 1992), which highlights the leaf homology of the floral organs. Still, such leaves do not correspond to bracts *s.s.*, as they are produced *by* the floral meristem and not *subtending it* within the phytomer module. Besides, some genes of the ABC family participate to the establishment of bracts *fpp* (S. Kim *et al.* 2005)). Taken together, these data suggest that bracts described in the literature can have several origins: those resulting from a modification of floral whorls (*fpp*),

Species	Genotype	Type of bract	Description of the phenotype	Reference
Rice	<i>spl7, spl7/14, spl7/17, spl7/14/17</i>	bract l.s.	Growth defects, Flower patterning defects, Branching defects	Wang et al. 2021
Rice	<i>nl1 (neck leaf 1)</i>	bract l.s.	Growth defects, Flowering time defects	Wang et al. 2009B
Rice	<i>ril1</i>	bract l.s.	Defects in meristem maintenance, Flower patterning defects	Ikeda et al., 2019
Rice	<i>Pla1, Pla2, Pla3 (plastochron)</i>	bract l.s.	Defect in flower determination, Defects in leaf formation	Kawakatsu et al., 2006, Kawakatsu et al., 2009
Barley	<i>trd1 (third outer glume1)</i>	bract l.s.	Ectopic leaves at the collar, Ectopic floral features at the peduncle node	Houston et al., 2012
Wheat	<i>vrn1/ful2, vrn1/ful3/ful3</i>	bract l.s.	Defect in flower determination	Li et al., 2019
Maize	<i>tsh4</i>	bract l.s.	Defect in flower determination, Defects in flower patterning (see fig. 15)	Chuck et al., 2010
<i>A. thaliana</i>	<i>bop1/bop2, puchi1/bop1/bop2,</i>	bract s.s.	Defects in flower determination (branch-like flowers), defects in floral patterning (open flowers, sepals defects). (see fig. 17)	Norberg et al. 2005, Hepworth et al. 2005 Karim et al. 2009
<i>A. thaliana</i>	<i>jag5-D</i>	bract s.s.	Defects in flower determination (leafy shoots) (see fig. 17)	Dinnerly et al., 2004
<i>A. thaliana</i>	<i>Lfy-12</i>	bract l.s.	Defect in flower determination (branch-like flowers) (see fig. 17)	Xu et al., 2010
<i>A. thaliana</i>	<i>ap1</i>	bract fpp	Defect in flower determination (flowers at the axil of sepals) (see fig. 17))	Xu et al. 2010
<i>A. thaliana</i>	<i>gAGL6::VP16</i>	bract s.s.	Defects in flower patterning (multiple floral organs)	Koo et al., 2010
<i>A. thaliana</i>	<i>soc1/agl24/svp</i>	bract s.s.	Defect in flower patterning (multiple floral organs)	Liu et al. 2009
<i>A. thaliana</i>	<i>ft</i>	bract l.s.	Defect in flower determination (floral reversion)	Müller-Xing et al. 2014

Figure 16: **Bract mutants in Poaceae, and in *A. thaliana*** Mutants of Poaceae and *A. thaliana* harbors pleiotropic phenotypes, among which the main ones affect the flower. "Defects in flower determination" regroups phenotypes showing branching features (production of flowers within flowers, iteration, leafy shoots). "Defects in flower patterning" regroups phenotypes showing a modification in floral organs number or position (multiple flower, disturbed phyllotaxis, open flowers). The reference associated to each mutant is specified in the right column.

those that subtend branch-like flowers (*l.s.*) and bracts that are the leaf subtending flowers (*s.s.*). Comparison between non homologous organs may lead to some misconceptions.

If the study of mutants did not allow us to decouple bract loss from proper flower identity, this can be reached in wild-type plants, under specific condition, but on the first flowers only.

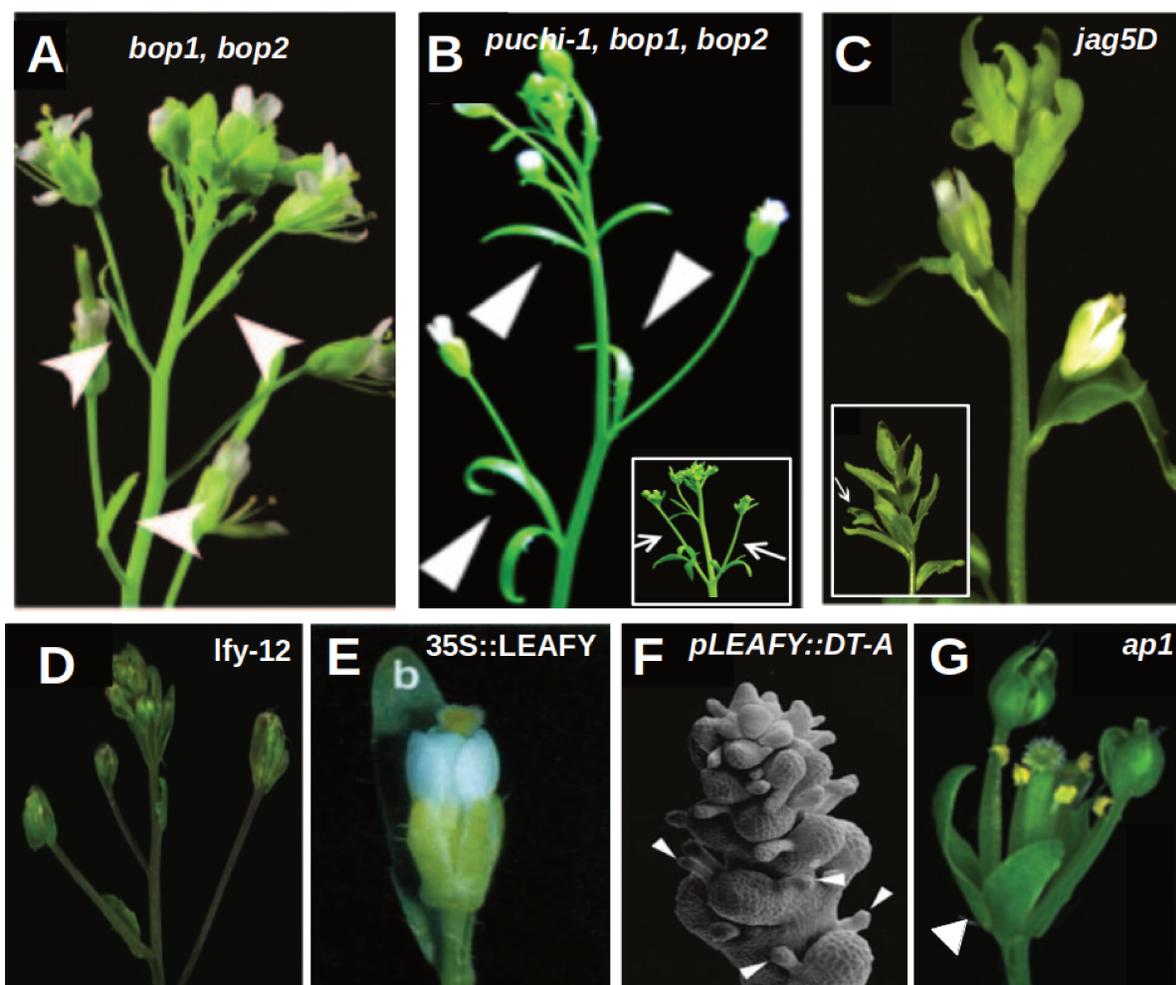


Figure 17: **An overview of some bract mutants in the literature.** A) *bop-1, bop-2* KO mutants showing bracts *s.s.* (arrowhead) subtending flowers with open structure and sepals defects. Adapted from Norberg 2005. B) *puchi1, bop1, bop2* showing bracts *s.s.* under flowers (arrowheads). The inflorescence of this triple mutants often fails to produce flowers, and instead produces secondary inflorescence (arrow in the inset). This questions whether bract-flowers are not actually a conversion of such inflorescence into proper flowers. Adapted from Karim *et al.* 2009. C) *Jag-5D* homozygous inflorescence, with flowers subtended by bracts *s.s.*. Flowers are reduced, and leafy shoots appears often in this mutants (inset). Adapted from Dinneny 2004. D) *lfy-12* mutants showing "bract" (*l.s.?*) subtending flowers transformed into inflorescence. From Xu, Hu, McKim, *et al.* 2010. E) 35S::LFY flower subtended by a bract (*s.s.?*). From Weigel & Nilsson 1995. F) Scanning electron micrograph of pLFY::DT-A. No flower meristem are visible. Instead, leaf-like structures with stipules (arrowhead). From Nilsson *et al.* 1998. G) *ap-1* mutant showing bracts *fpp* (arrowhead). These bracts *fpp* result from flower growth in the axil of a sepal. Adapted from Xu, Hu, McKim, *et al.* 2010.

From 1995, Hempel and Feldman observed that WT plants can bear bract on their first flowers and they call the corresponding phytomers as chimeric ones. The frequency of chimeric phytomers has been shown to increase when plants were exposed to a strong continuous light condition (Hempel, Zambryski, *et al.* 1998). Time lapse dissection of vegetative meristem shows that the first flower is produced immediately after the transfer to a flower-inductive condition, and does not bear bract (Hempel & Feldman 1994; Hempel & Feldman 1995). So they concluded that bract-flowers are the result of a conversion into flower of aM initiated before the floral induction. The phytomer would keep a "developmental bias" with a vegetative identity, hence keeping a leaf as branches do, but the fate of the aM could still be reoriented to a floral identity (Hempel, Zambryski, *et al.* 1998). To support their hypothesis of a branch-to-flower conversion, they report the frequent occurrence of floral indetermination, with chimeric shoot-flower phenotypes (14% of bracts-flowers have chimeric shoot-flower phenotypes) (Hempel & Feldman 1995; Hempel, Zambryski, *et al.* 1998). The frequency of shoot-flower phenotypes increased when plants were exposed to a strong light condition (up to 50% of plants with chimeric shoot-flower phenotype). Their results extend the relationship between bract inhibition and flower identity to wild type population. Nevertheless, observing bracts on the first flowers of WT plants is frequent. In *A. thaliana*, a number of natural accessions display basal bracts on the first flowers of the raceme, without being exposed to a particular intense light condition (personal observation), questioning how widespread such chimeric phytomers are.

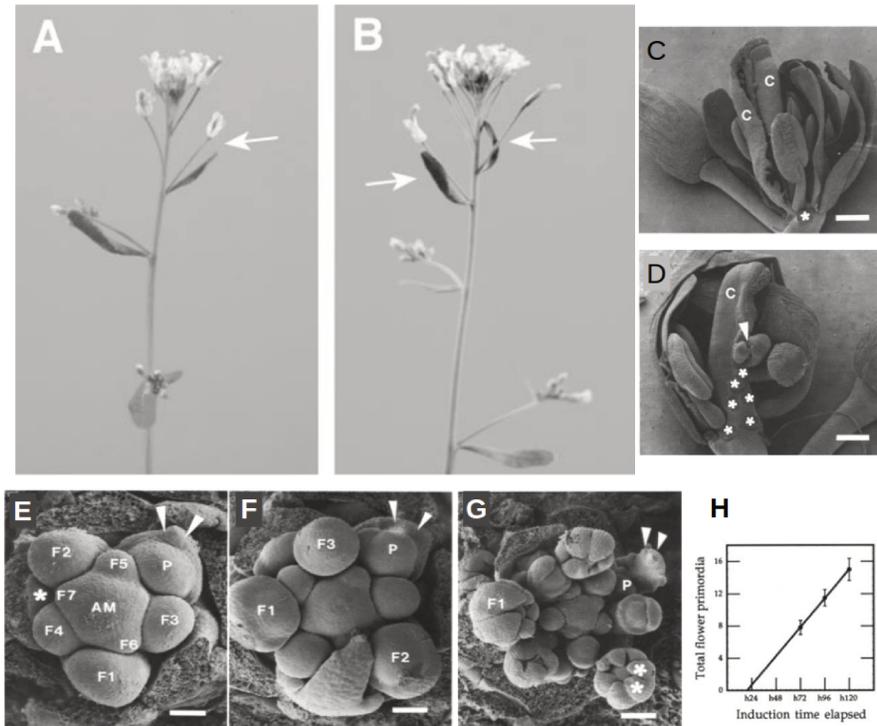


Figure 18: **Observation of chimeric phytomers in Wild-type plants under continuous light condition.** A-B) Bract-flowers, called “chimeric phytomers” (arrow) in Wild Type Landsberg *erecta* (*Ler*) plants, cultured under continuous light condition after 30 days in short days condition. Only the flower(s) at the base of the inflorescence present such chimeric phytomers. From Hempel, Zambryski, *et al.* 1998. C-D) Chimeric shoots observed at the base of Wild type Nössen plants. C) Half-flower/half-branch, with partially fused carpels (c), and secondary flowers (which one was removed – asterisks). D) Half-flower/half-branch shoot. Flower development are illustrated by the presence of a carpel (c), while an axillary meristem (arrowhead), is located between the remaining young flowers. Asterisks denote flowers that were removed. Such types of flowers were observed in 51% of plants exposed to strong light induction, and 12% of plants with normal flower induction. From Hempel & Feldman 1995. E-H): Time-lapse dissection of meristem at floral transition show that chimeric phytomers are initiated immediately prior to the switch from photoinductive condition. E: SAM at Day 3 of continuous-light induction. 7 flowers primordias have been initiated, and axillary meristem (P) develop at the axil of the youngest leaf (two arrowheads). Another axillary meristem develops on the axil of an older leaf (asterisks). F: SAM at Day 4 after continuous-light induction. An axillary meristem (P) develops at the axil of the youngest leaf (two arrowhead). A primordia initiate from this new growth axis. G: SAM at day 5 after continuous light induction. Arrowheads denote the youngest leaf primordium. Split sepals are marked with asterisk, and are common on the first flowers of Nössen plants. H: Rate of flower primordia initiation following photoperiodic induction. The graph represents the mean number of flower primordium at each time point (72h, 96h and 120h) after continuous-light induction (at h=0), after a 30 days growth in short day condition. The linear curve-fit through the 72, 96 and 120h mean values extrapolates a conservative estimate of the average time the first flower primordium was initiated. N=15 SAM/day. Adapted from Hempel & Feldman 1995

2.3.2 Natural bract development without floral defects

Absence of bract is a specificity of Brassicaceae. However, upon closer inspection, it appears that some genera of Brassicaceae present bracts, sometimes in the entire inflorescence (in *Onuris* clade, Salariato *et al.* 2015) but mostly only in the lowermost flowers of the inflorescence (see Results, Chapter 1). The latter is very common and can easily be observed in Europe. In addition, basal flowers with bracts lack visible floral defects (personal observations, see Results - Chapter 1).

In this introduction, I have reviewed how the concept of phytomer can enlighten the diversity of angiosperm architecture, both between species and along the different plant developmental stages. In other terms, the study of phytomer can be useful to unravel the species-specific determinants of plant architecture. It also addresses the control of plant developmental plasticity through the phytomer remodeling that accompanies developmental transitions. Understanding the mechanisms controlling this essential module are thus of tremendous importance in plant biology. However, our knowledge is very unbalanced: the literature is abundant on flower development, but much less is known about the remodelling affecting internodes or leaves. The leaf however undergoes exceptional remodelling at floral transition in *A. thaliana*, as it does not grow. Attempts to study bract inhibition have relied on mutants or natural accessions bearing bracts. Such bract-flowers have been observed in various environmental and genetic contexts. Several pathways may converge to this phenotype, triggered by one or a combination of different factors such as a specific environment, a disrupted developmental switch, or a specific genetic background. Mechanisms leading to bract inhibition are not always related to flower identity, as it can be deduced from the observation of wild type plants. This genetic context can thus help to isolate the mechanisms of bract loss, in order to better understand the developmental and evolutionary pathway(s) leading to bract loss.

3 Objectives of this thesis

Unlike flowers, bract development has received very little attention. Yet, convergent bract loss with the maintenance of a cryptic form in several angiosperms constitute a relevant biological context to address trait loss during evolution. How do different species converge to loose bracts? And why do distantly related species maintain a cryptic bract (cb)? To date, studies on bract inhibition relied on mutants producing ectopic bracts in bractless species. However, such mutants also display malformed flowers with indeterminate shoot-like phenotype. Do the corresponding genes really participate to bract inhibition, or do the presence of bract on these mutants indirectly result from the indeterminate branch-like flower?

Hempel and Feldman have addressed the question of bract loss by studying wild-type plants under specific conditions. Still, the bract-flowers observed here resulted from a photo-induced conversion of branches into flowers with frequent branch-like flower phenotypes. Thus, it was commonly accepted that bracts and flowers development are not compatible in *A. thaliana*. Nevertheless, botanists have for long reported bract-flowers in basal flowers of Brassicaceae inflorescences, including *A. thaliana*. In fact, upon close inspection, it appears that several populations of *A. thaliana* present bract-flowers at the base of the inflorescence, that are not affected by indeterminate branch-like phenotype, like *Tsu-0*. What are the mechanisms of bract inhibition at floral transition? And how can bract re-emerge specifically at floral transition?

Thanks to the increasing affordability of next generation sequencing technologies, it is now possible to newly question the mechanisms of bract loss in *A. thaliana*. I used the natural accession *Tsu-0* as a genetic tool to revisit the genetic and developmental mechanisms underlying bract inhibition at floral transition in *A. thaliana*. I first explored the bract-flowers phenotype in *Tsu-0* as well as in other accessions and different Brassicaceae species (Results - Chapter 1). I also compared bract-flower in *Tsu-0* from those described in the literature. Because bract-flowers affected the first flowers produced at floral transition, I tried to capture the differences in the GRN of floral transition between *Tsu-0* and *Col-0*, and how these differences could explain the bract-flower phenotype of *Tsu-0* plants (Results - Chapter 2). I finally mapped the genetic basis of bract-flower phenotype. By coupling genomic and transcriptomic approaches, I tried to reduce the list of relevant genetic candidates in the mapped interval, that would be

suitable for further investigations (Results - Chapter 3).

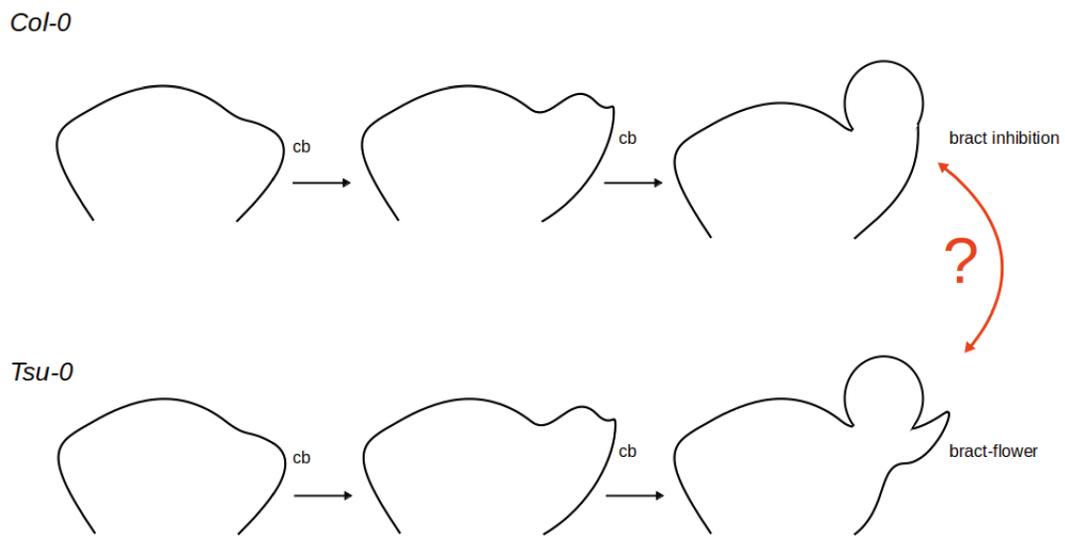


Figure 19: **What are the determinants that explain the presence of bracts in *Tsu-0*'s first flowers?** At floral transition, *A. thaliana* reference plant *Col-0* produces flowers with a cryptic bract (cb). This cryptic bract do not develop into a full bract-flower. In another natural, *Tsu-0*, bracts fully develop on the axil of the first flowers, which remain normal. This work explores bract inhibition at floral transition using *Tsu-0* natural accession.

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1

2 Revisiting bract inhibition with 3 natural population of *Arabidopsis* 4 *thaliana*

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8 Abstract

9 In *Arabidopsis thaliana*, experimental restoration of bracts has been almost always accompanied
10 with defects of floral determinacy and/or identity. However, a certain number of *Brassicaceae* bear
11 basal bracts on the first few flowers of the racemes, with no visible defects. Here, we show that
12 basal bracts are common in natural accessions of *A. thaliana*, and could be linked to flowering
13 time. In addition, we observed the same sequence of flower development between a bracteate
14 wild-type *Cleomaceae* species and a bract bearing *A. thaliana*'s mutant. In fact, the bract grows
15 before the floral meristem becomes visible. It can thus be hypothesized that the same process
16 happen in bract-bearing natural accessions but only restricted to the floral transition. We
17 propose natural accessions of *A. thaliana* as a new tool to investigate bract inhibition
18 independently from flower determination. Such studies on natural accessions of *A. thaliana* can
19 help to better understand the mechanisms of bract inhibition as well as its evolution.

21 Introduction

22 A plethora of studies investigated the mechanisms of flower development. However, in the model
23 plant *A. thaliana*, flower development is also accompanied with the inhibition of its subtending
24 leaf: the bract. Unlike flower, little is known about the mechanisms of bract development. Bract
25 suppression happened independently in different clades, but to what extent they share common
26 features remains elusive. Some mutants bearing bracts have been generated in *A. thaliana*, as well
27 as in maize or rice, but did not allow to really understand bract loss. In *Arabidopsis thaliana*, mu-
28 tants bearing bracts display pleiotropic phenotypes that also affect flower development. Flower
29 impairment in these mutants made it difficult to uncouple the mechanisms of bract loss from
30 flower emergence. Bract emergence has also been observed in wild type plants, when exposed to
31 a continuous and strong light condition ((Hempel *et al.*, 1998)). In this context, some bract-flowers
32 displayed signs of indeterminate shoot-flower phenotypes: the authors suggested it resulted from
33 a conversion of branches into flowers. So far, no study has focused on phenotypes where bract
34 inhibition was uncoupled from a proper flower development. However, botanists are for long fa-
35 miliar with the presence of bracts in some *Brassicaceae* species, under normally developing flowers
36 often located at the basis of inflorescence. Hence, in some *Brassicaceae*, flowers can develop nor-
37 mally despite the presence of bract. In this chapter, we describe the existence of bract-flowers in
38 some natural populations of the model plant *A. thaliana*. We propose this developmental context
39 as a new standard to understand the mechanism of bract loss and its evolutionary origin.

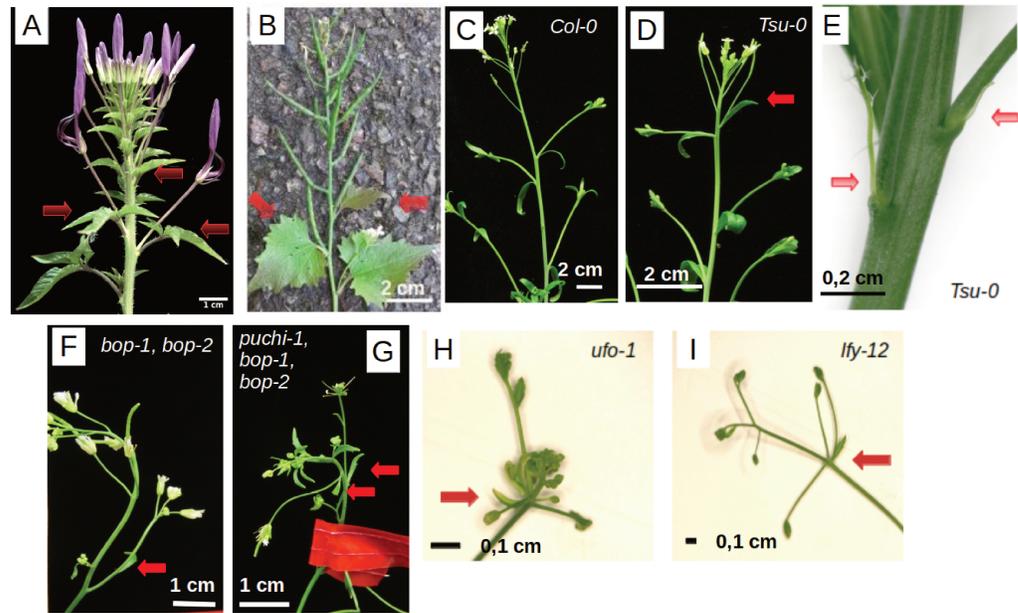


Figure 1. Natural basal bracts in *A. thaliana* accessions resemble wild-occurring bracts but not mutant bracts. Red arrows point to bracts. A-E) Bracts in wild type plants. Pictures show the main stem of the inflorescence raceme, at the transition between branches and flowers. A) In *Tarenaya hassleriana*, a *Cleomaceae* species, each flower is subtended by a bract, like branches by a leaf (only the three first bracts-flowers are pointed by red arrows). B) In *Allaria petiolata* (*Brassicaceae*) the few first siliques of the raceme are subtended by bracts (basal bracts) while younger flowers are bractless. C) In *textitA. thaliana* (*Brassicaceae*, *Col-0* reference accession): branches with cauline leaves are directly followed by bractless flowers. D) *A. thaliana* *Tsu-0* accession: a single basal bract subtend the first flower (red arrow); the other flowers are bractless. E) in *Tsu-0*, basal bracts are sometimes rudimentary outgrowth (coined as "needle" bracts). F-J) Inflorescence details of several known "bract-making" mutants in *textitA. thaliana*, showing severe flower phenotypes. F) a *bop-1, bop-2* double mutant (the flower has turned to shoot, note the shifted position of the bract on the peduncle). G) a *puchi-1, bop-1, bop-2* triple mutant (shoot-like flowers) H) *ufo-1* (tiny and sterile flowers-like); I) *lfy-12* (tiny and sterile flowers-like).

40 Results

41 Bracts can be present in some *Brassicaceae* species and are different from mutants

42 Bracts are usually present in *Cleomaceae* species (fig.1A) but not in the closely related model *Bras-*
 43 *sicaceae* plant *A. thaliana* (fig.1C). Nevertheless, bracts have been observed on the first flowers of
 44 some natural isogenic accessions of *A. thaliana*, like *Tsushima-0* (*Tsu-0*) (fig.1D) as well as in other
 45 *Brassicaceae* plants (fig.2, fig.1B). They will be hereafter called "basal bracts" because of their exclu-
 46 sive bottom position in the inflorescence. In *Tsu-0*, basal bracts displayed a range of continuous
 47 phenotypes from a large and fully developed leaf (fig.1B) to a very reduced extrusion (fig.1E). The
 48 flowers, on the other hand, did not present any phenotypic alterations, contrary to what can be ob-
 49 served for bract-bearing mutant flowers (fig.1F-I). In fact, the triple mutant *puchi1, bop1, bop2* and
 50 double mutant *bop1, bop2*, as well as *lfy-12* mutant present flower indetermination phenotypes,
 51 with multiple flowers, or branch-flower phenotypes. Because of their lack of flower defects, bracts
 52 in wild type plants, including *Tsu-0*, seem to arise from a different developmental mechanism.

53 *Cleomaceae* and *Brassicaceae* differences within the shoot apical meristem

54 As all aerial organs, bracts are produced by the shoot apical meristem (SAM) of both *Brassicaceae*
 55 and *Cleomaceae* plants. We thus dissected the SAM of *Col-0*, *Tsu-0*, the bract mutant *puchi1, bop1,*
 56 *bop2* and plants from a wild-type stock of *Tarenaya hassleriana* to look for morphological differ-
 57 ences. In *A. thaliana* wild types, flower primordia are visible very early with a typical round shape.

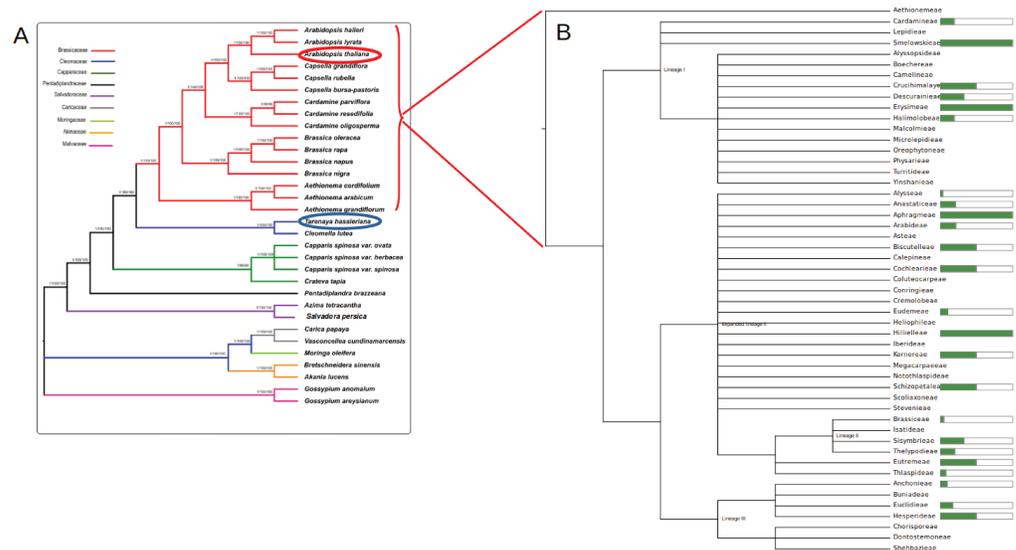


Figure 2. Phylogenetic relationships between the bracteate *Cleomaceae* family and the ebracteate *Brassicaceae* family and distribution of basal bracts in *Brassicaceae*. A) Phylogeny of 31 *Brassicales* species, established by (Khan *et al.*, 2021). 60 shared genes dataset was analyzed using Bayesian inference, maximum parsimony (MP) and maximum likelihood (ML). Numbers above the branches represent bootstrap values in the MP and ML. Each clade have been represented with a specific color (see on the top left). *Arabidopsis thaliana* and *Tarenaya hassleriana* have been circled, as they will be described later (adapted from (Khan *et al.*, 2021)). B) Detailed cladogram of the *Brassicaceae* family by tribes. When available, the rate of species in a tribe bearing bracts at least in the lower half of the raceme is represented with a proportional green fill in the grey bar following the tribe's name. *A. thaliana* belongs to the Camelinae tribe. Data and cladogram generated from Brassibase (<https://brassibase.cos.uni-heidelberg.de>)

58 At the precise time of floral transition, it is possible to see both the first flowers and phytomers
 59 of the future lateral branches underneath. While axillary meristems are not really visible before
 60 the floral transition (see Results - chapter 2 fig.4, and (*Hempel and Feldman, 1994*)), they rapidly
 61 bulge out at the leaf axil in a basipetal wave once the first flower has been produced. (fig.3A). On
 62 the contrary, in *T. hassleriana*, the emergence of the round flower meristem starts later, on the axil
 63 of an already developed leaf: the bract (fig.3B). Interestingly, in the bract-making mutant *puchi1*,
 64 *bop1*, *bop2*, floral meristem also initiate at the axil of a sharp leaf (fig.3C), even though, as dis-
 65 cussed previously, this meristem may have a mixed shoot-flower identity. In *Tsu-0* plants, we can
 66 observe the presence of bracts at the axils of the first flowers, but never after the 4th (fig.3D). We
 67 did not manage to observe in live the full developmental sequence of a "bract-flower", which is ex-
 68 tremely challenging. Hence, we cannot be sure that the bract develops *before* the flower. Still, our
 69 images show that the bract does not develop later, protruding from the floral meristem for exam-
 70 ple. Taken together, a bract develops before the flower in bracteate *Cleomaceae*. In the ebracteate
 71 *A. thaliana*, restoration of a bract in mutants or wild-type *Tsu-0* accession involves a similar devel-
 72 opmental event, in coherence with the evidence supporting the presence of a cryptic bract (see
 73 introduction). Hence, the basal bracts observed in *Tsu-0* can be considered as bracts *stricto sensu*,
 74 homologous to bracts in bracteate species. Interestingly, these first flowers are no longer able to
 75 prevent bract growth.

76 **Basal bracts emerges at floral transition in the main and the lateral shoots**

77 In *Tsu-0*, bracts were always present on the first few flowers, never exceeding the 4th (data not
 78 shown). *Tsu-0* produced on average one bract per cauline branch, regardless of the branch position
 79 along the main stem (fig.4C). On the contrary, in *Col-0*, bract production seems correlated with
 80 cauline branch position, with the higher branches producing slightly more bracts than the bottom
 81 branches (fig.4C). Besides, the number of cauline branches in *Col-0* is reduced compared to *Tsu-0*
 82 (fig. 4B). *Col-0* shows on average 8 cauline branches when cultured 20 days in SD before switch
 83 to LD, while they are on average 11 in *Tsu-0*. Hence, in *Col-0*, cauline branches exceeding the 10th
 84 position shows 0,25 bract on average, but their frequency is rare, so that high branches bearing
 85 bracts are still rare. We next defined the bract mean frequency as the average number of bracts
 86 per cauline branches per plant for a given genotype (see methods).

87 **Basal bracts are frequent in natural populations of *A. thaliana*, and may be linked 88 to flowering time**

89 In order to evaluate to what extent basal bracts are widespread in *A. thaliana*, we screened the
 90 presence of bracts in different natural accessions (fig.5A). Bracts have been observed on the first
 91 flowers of several accessions such as *Sha*, *Star-8*, or *Bor-4* (fig.5A1). Several natural accessions
 92 present a similar bract mean frequency as *Tsu-0*, such as *Mc-1*, *Bik-1*, or *Berg-1* (fig.5A2), even though
 93 statistical analysis cannot be meaningful with such small number of plants. Still, this first rapid
 94 screen indicated for the first time that unexpectedly (see Brassibase), basal bracts are not rare in
 95 *A. thaliana* natural populations grown in laboratory conditions.

96 The basal position of bracts indicates that their formation is restricted to the floral transition. To
 97 test whether the flowering time is linked with basal bract production, we determined bract mean
 98 frequency with respect to flowering time in five different natural accessions (fig.5B). Flowering time,
 99 assessed as the day when the first flower opens, ranged from day 22 to day 44. Isogenic *Tsu-0* plants
 100 show a negative correlation between bract mean frequency and flowering time (5). Surprisingly, the
 101 trend was different in the other natural accessions: in *Kn-0*, *Ler-0* and *Col-0*, bract emergence did
 102 not depend on flowering time; and in *Wu-0*, the later a plant flowers, the more it bears bracts.
 103 Thus, the link between bract formation and flowering time is complex and distinct mechanisms
 104 could explain the formation of basal bracts in different accessions.

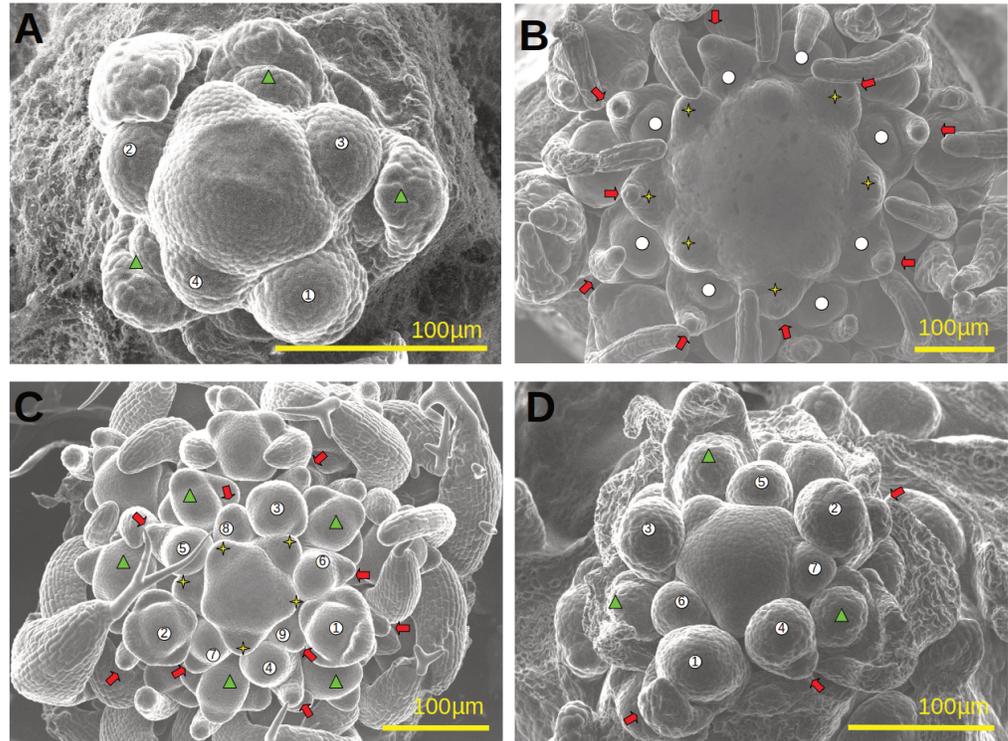


Figure 3. Observation of bract formation by scanning electronic microscopy (SEM). Imaging by SEM of inflorescence shoot apical meristems (SAM). Green triangle: vegetative axillary meristems. Star: bract without a visible meristem on its axil. White circle: floral meristem, numbered from the oldest to the youngest, when possible. Red arrow: bract or bract-like leaf subtending a meristem. *A/* SAM *A. thaliana*, *Col-0* accession, at the floral transition. The very first flower of the raceme (n° 1) is bractless, while underneath, axillary meristems bulge out at the axil of leaves (green triangles). *B/* Inflorescence SAM of *T. hassleriana*. Bracts are initiated first and can be recognized early (stars). Visible floral meristems arise at the axil of existing bracts (white circles). *C/* In the *puchi1, bop1, bop2* triple mutant of *A. thaliana* (*Col-0* background), bracts are clearly visible (stars) before the floral meristem emerges (white circle n°9). All branch-like flowers from the first flower of the raceme bear a bract. *D/* Inflorescence SAM *A. thaliana*, *Tsu-0* accession, at the floral transition. The first flower of the raceme (n°1) is still young (at the onset of sepal outgrowth), but already bears a large bract. At least two subsequent flowers (n°2 and 4) display small adaxial outgrowth suggesting a bract rudimentary development. The next flower initiations do not show clear solitary bract stages, as in *Col-0*. Scale bars are represented on the bottom right of each picture.

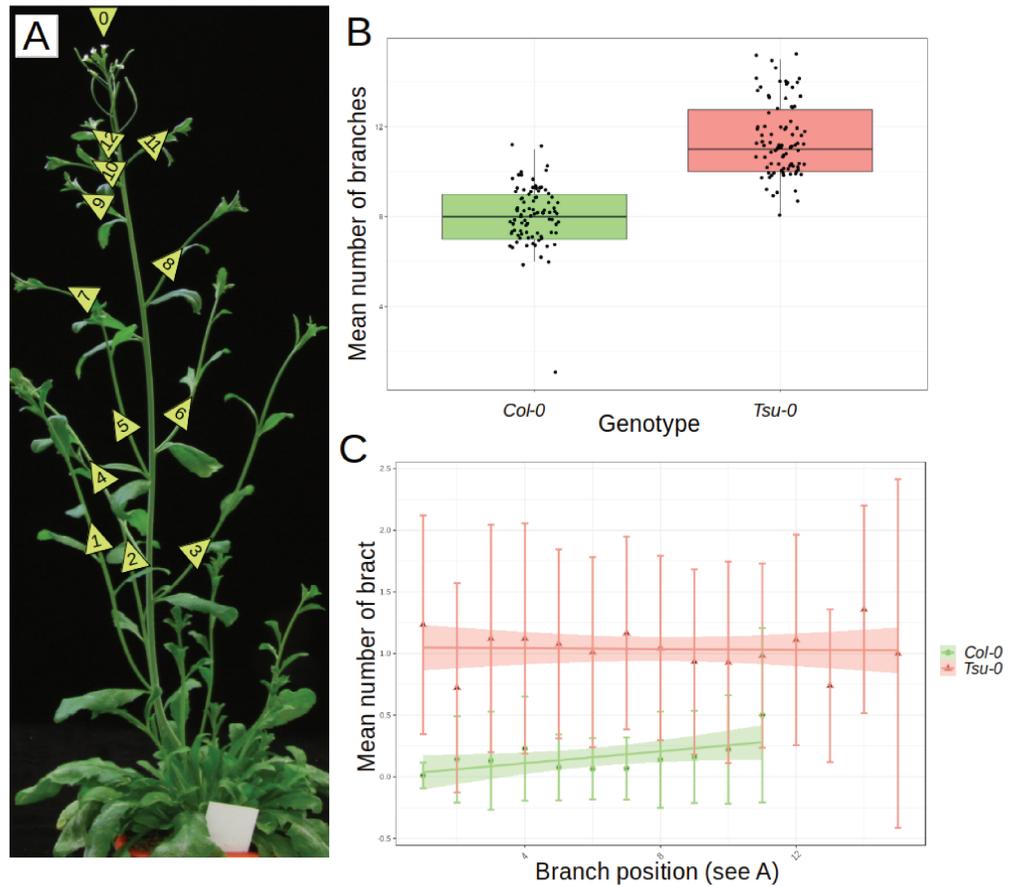


Figure 4. Basal bracts can appear on each cauline branches. A) To measure the trait quantitatively, bracts were scored along the main stem (0), and on every secondary cauline branches (numbered from 1 onward) to generate a bract mean frequency. B) Number of branches in *Tsu-0* (red) and *Col-0* (green). C) Number of bract depending on the position of the cauline branch along the main stem in *Col-0* (green) and *Tsu-0* (red). In *Tsu-0*, basal bracts are as frequent in every axis (main stem and branches) while a slight correlation may exist in *Col-0* between cauline branch position and bract mean frequency: bract mean frequency increase on higher branches ($R=0.62$, $p\text{-value}= 0,04$). $N > 80$ plants per genotype.

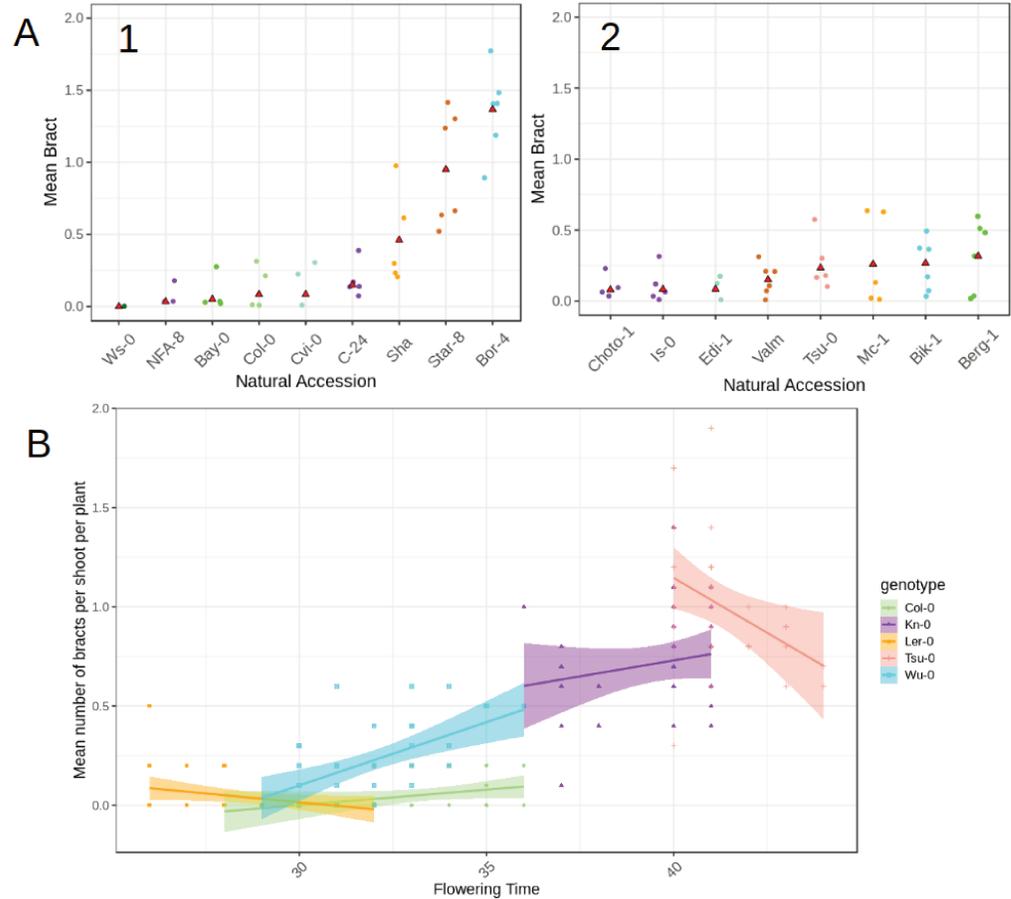


Figure 5. Bracts in different accessions of *A. thaliana* (A) and their relation with flowering time (B). A) bract mean frequency in different natural accessions in two independent experiments. N = 6 plants/genotype. The red triangles represent the average value for each genotype. B) bract mean frequency in some natural accessions relating to their flowering time. Flowering time have been reported as the day the first flower bloom. N > 30 plants/genotype. Pearson test show a significant correlation (p-value < 0,05), for *Tsu-0* (R=-0,4, p-value = 0,01) and for *Wu-0* (R=0,5, p-value = 0,0001)). This might represent a complex relationship between bracts and flowering time depending on genotypic background.

105 Discussion

106 Leaves are a basic component of the phytomers, but they are not present at the reproductive phy-
 107 tomers of several clades such as *Brassicaceae*, or *Poaceae* (see introduction). The mechanisms of
 108 bract inhibition have not been fully resolved. So far, studies investigating bracts inhibition relies
 109 on mutants of *A. thaliana*, maize or rice, that also presented flower impairment, making bract inhi-
 110 bition mechanism difficult to isolate. We describe a new system to address the question of bract
 111 inhibition using natural populations of *A. thaliana* that make bracts with normal flowers. Bracts in
 112 wild-type plants were produced only at the base of inflorescence, during floral transition.

113 Floral transition offers a specific developmental context in which bract can emerge in some
 114 genotypes. The timing of floral transition is linked with bract production. However, the relation-
 115 ship between flowering time and bract emergence seems dependent on the genetic background.
 116 Various environmental cues could explain the variability of the phenotype observed even within
 117 the same genetic background. Assessing some of them could be interesting: in particular, we will
 118 test in the next chapter 2 the role of the photoperiod in bract production.

119 The spatial and temporal restriction of bract formation suggest a particular developmental sig-
 120 nature associated with floral transition, where the regulation of gene expression could be less
 121 canalized. It has been described that the first flower presents higher morphological variations
 122 ((*Monniaux et al., 2016*)). Moreover, the phenotypes of bract-mutants flowers were often gradual
 123 from the base to the top, with the most severe phenotypes at the base. This illustrates that remod-
 124 eling of the GRN controlling floral transition may not be sharp, but rather presents a high variability
 125 at this precise developmental transition. Examples of the particular status of the floral transition
 126 step have been also studied at an inter-species level. Transcriptomes of several nightshades de-
 127 scribed higher divergence at the floral transition too, which could participate to architecture evo-
 128 lution ((*Lemmon et al., 2016*)). A transcriptomic analysis over floral transition in *Tsu-0* and *Col-0*
 129 is described in the next chapter 2.

130 Affordability of DNA sequencing can now facilitate the investigation of the genetic determinants
 131 of bract production in natural populations. Beside, as bract is produced only at floral transition,
 132 transcriptome related to its formation can be circumscribed. In chapter 3, we will describe the
 133 complex genetic determinants of basal bract in *Tsu-0*. However, as bracts are present in a lot of
 134 different genetic backgrounds, we can address whether the genetic determinants of bract produc-
 135 tion is identical in each accession. In fact, bracts have been suppressed independently in several
 136 species, and this can be the result of convergent or divergent mechanisms. On the other hand,
 137 bracts are observed in different mutants, showing that several genes participate to its suppres-
 138 sion in *A. thaliana*. It will not be surprising that (re)emergence of bracts at the base of inflorescence
 139 could result from different genetic variants, that would have yet in common to decanalize the con-
 140 trol of bract inhibition at floral transition. The investigation of a broad panel of natural accessions
 141 could thus teach us about the convergence/divergence of bract inhibition within *A. thaliana*.

142 Methods and Materials

143 Please refer to the "Methods and Material" section.

144 Authors contributions

145 S.D. carried out all the experiments and the analysis. S.D. and F.B. designed the experiments and
 146 interpreted the results. S.D. wrote the first draft of the manuscript, and F.B. helped edit it.

147 Acknowledgments

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 149 from Wageningen University (WUR, Holland) for kindly providing the *Tarenaya hassleriana* seeds.
 150 We are very grateful to our culture team for taking great care of the plants. The research leading
 151 to these results received funding from the PhD program of ENS Paris-Saclay.

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1

2 Bract formation in *Arabidopsis* 3 *thaliana* reveals divergent 4 developmental trajectories at floral 5 transition between two natural 6 accessions

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9

10 **Abstract** Bracts are leaves that subtend most flowers in Angiosperms, but have been lost in
11 the model plant *Arabidopsis thaliana*, as in most *Brassicaceae*. While the Gene Regulatory Network
12 (GRN) of flower development is very well established, the regulation of bract development is yet
13 to be explored. How bract development is inhibited during flower formation in *A. thaliana* is still
14 an open question. However, we have reported the presence of basal bracts -i.e. bracts formed at
15 floral transition- in some natural accessions of *A. thaliana*, including *Tsu-0* (see Results - Chapter
16 1). Deciphering how floral transition can provide a bypass to bract inhibition could enlighten the
17 mechanisms of bract suppression. To this aim, we compared floral transition in two accessions:
18 *Tsu-0*, making basal bracts, and *Col-0*, that does not. We investigated some parameters that could
19 explain their divergent developmental trajectories. In *Tsu-0*, bract formation does correlate
20 neither with the photoperiod nor the plastochron. We show some subtle transcriptomic changes
21 during floral transition between the two natural accessions, that could explain the phenotypic
22 difference of the first flowers. This study provides a temporal framework for the GRN linked to
23 floral transition in two different accessions. It can contribute to a better understanding of the
24 mechanism of bract loss in *A. thaliana*.

25 Introduction

26 At the floral transition, determinate floral meristems are produced, which stop the growth axis,
27 impacting angiosperm's architecture ((Prenner *et al.*, 2009)). In addition, in some groups like *Bras-*
28 *sicaceae*, including the model plant *A. thaliana*, floral transition leads to bract inhibition. The study of
29 mutants bearing "bracts" allowed to identify several genes involved in bract inhibition ((Dinneny,
30 2004; Ohno, 2004; Norberg, 2005; Penin, 2008): *lfy* ((Weigel and Nilsson, 1995)), *ufo* ((Hepworth
31 *et al.*, 2006)), *puchi* ((Karim *et al.*, 2009)), *bop* ((Hepworth, 2005)), *jagged* ((Dinneny, 2004; Ohno,
32 2004)). However, those mutants displayed pleiotropic phenotypes, especially with defects in flower
33 development. Observing that proper bract and flower developments are somehow incompatible
34 in *A. thaliana*, it has been suggested that the mechanism of bract inhibition is tightly coupled to the
35 flower developmental program.

36 However, several studies have now reported the coexistence of proper bracts with wild-type
37 flowers in *A. thaliana*. These bract-flowers have only been observed on the first flowers at the ba-
38 sis of the inflorescence, that is on flowers produced at floral transition. Floral transition thus offers
39

40 a temporal window where the mechanisms of bract suppression can be decoupled from flower
 41 development. In chapter 1, we described that several *A. thaliana* natural accessions make "basal"
 42 bract. Such basal bracts are also observed in many other *Brassicaceae* species, sometimes with a
 43 much higher frequency ((*Salariano et al., 2015*)). In a series of seminal studies, Hempel and Feld-
 44 man investigated the production of basal bracts appearing in particular accessions cultured under
 45 strong photo-inductive conditions ((*Hempel and Feldman, 1995; Hempel et al., 1998*)). Using pre-
 46 cise time course observations following photo-induction, they concluded that bract-flowers belong
 47 to phytomers produced *before* floral transition. They were likely indeterminate axillary meristem
 48 converted to floral fate after floral transition, thus keeping their subtending leaf. The authors pro-
 49 posed a temporal mechanism to explain the bypass of bract suppression by floral meristems. Be-
 50 fore floral transition, leaves are normally initiated with a vegetative axillary meristem. After floral
 51 transition, the initiated leaf is not suppressed. However, its associated axillary meristem can still
 52 be converted into floral fate depending on the timing and the strength of floral induction. Their
 53 hypothesis is supported by the observations of a gradation of indeterminate shoot-flower pheno-
 54 types, interpreted as partial conversions of vegetative axillary meristems into flowers. Since these
 55 works, though, no further studies have elucidated the precise developmental pathways involved,
 56 such as those controlling the conversion of axillary meristem fates. It is also unclear whether the
 57 observations described in this particular context are applicable to other *A. thaliana* accessions or to
 58 other culture conditions. In this chapter, we present our data comparing floral transitions between
 59 two accessions, one making basal bracts, *Tsu-0*, versus the bractless laboratory reference accession
 60 *Col-0*. We quantified various parameters that could explain bract development. We investigated
 61 shoot apical meristems undergoing floral transition at different scales and profiled their associ-
 62 ated transcriptomes. We showed that bracts in *Tsu-0* did not result neither from a strong photo-
 63 induction, nor from a conversion of branches into flowers. We rather spotted subtle changes in
 64 the transcriptome of *Tsu-0* during floral transition, and especially during the emergence of the first
 65 flower. Altogether, this study helps to better understand the GRN and the developmental context
 66 associated with basal bract production in *A. thaliana*.

67 Results

68 Basal bracts in *Tsu-0* does not recapitulate previous studies

69 In *Tsu-0*, bract emergence is not impacted by the photoperiodic condition
 70 The chimeric phytomers described by Hempel and Feldman at the floral transition were obtained
 71 with plants of either *Ler-0* and *Nössen* accessions, submitted to a strong photo-induction: they
 72 were germinated in Short Day (SD) and switched to intense Continuous Light (CL) conditions after
 73 30 days ((*Hempel and Feldman, 1995*)). To test whether bract production in *Tsu-0* results from a
 74 strong photo-induction, we cultured plants in different photoperiodic conditions. We quantified
 75 bract mean frequency in Continuous Light (CL), in Long Day (LD), and in biphasic condition where
 76 plants were first exposed to Short Day (SD) for 20, 28, or 35 days before switch to LD or CL. We also
 77 tested the impact of restoring short days environment after an inductive LD treatment of 20 days.
 78 Increasing photoperiod did not induce an increase in bract mean frequency neither in *Tsu-0* nor
 79 in *Col-0*. Besides, none of the different photoperiodic condition was linked to a significant change
 80 in bract mean frequency in neither *Col-0* nor *Tsu-0* plants (fig.1A). In all conditions, *Tsu-0* plants
 81 presented more bracts than *Col-0* plants, highlighting a strong genetic determinant invariant to a
 82 wide range of photoperiodic conditions. Hence, contrary to previous studies, basal bracts in *Tsu-0*
 83 are independent from photo-induction, even though the light intensity we used was different from
 84 these studies.

85 Because none of the condition we tested increased the level of bracts, we choose the 20SD →
 86 LD condition for the rest of this chapter as it allows to synchronize plants and to get plants suitable
 87 for dissection.

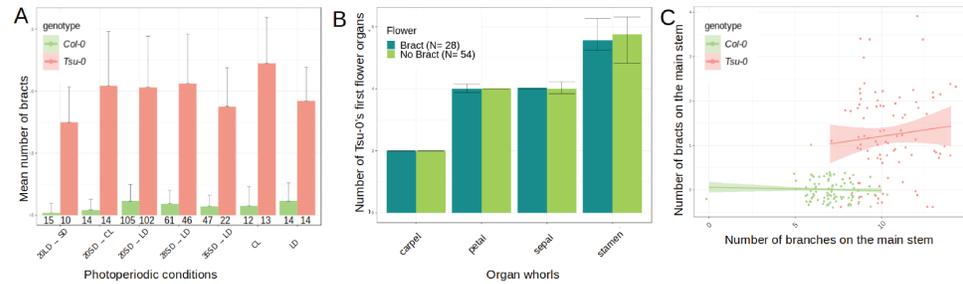


Figure 1. Basal bracts in *Tsu-0* are insensitive to photoperiodic conditions and are unlikely to be associated to branch-to-flower conversions A) Bract mean frequency upon on different photoperiodic conditions for *Col-0* and *Tsu-0* genotypes (numbers of plants scored in each condition indicated below each bar). SD: Short Day, LD: Long Day, CL: Continuous Light, 20SD → LD: 20 days in Short Day condition, before switching to 'Long Day' condition. Differences between the two genotypes are significant at each condition (Anova, p-value <0.05). B) Quantification of carpels, petals, sepals and stamens in *Tsu-0* flowers with (blue, N=28) or without (green, N=54) bract. No difference exist between organs number in flower with or without bract (p-value » 0.05). C) Bract number is not correlated to branch number in the main stem in *Col-0* (green) nor in *Tsu-0* (red) (person test, p-value > 0.05). N= 80 plants/genotypes

88 Basal bracts are not associated with a conversion of branches into flowers.
 89 To test further Hempel and Feldman's hypothesis of a 'branch-to-flower' conversion, we also mon-
 90 itored first flower emergence after transfer to inductive light conditions. We regularly dissected
 91 and imaged the SAM of both natural accessions. In *Tsu-0*, the first flower emerged between day
 92 11 and 16 after transfer to LD condition: in our culture conditions, the floral transition was not
 93 triggered immediately by transfer to long days. Hence, basal bracts were not produced during SD
 94 condition. Moreover, despite numerous observations of first flowers from *Tsu-0* plants (N>7310),
 95 bearing bracts or not, we never observed chimeric "shoot-flower" phenotypes (or other sign of inde-
 96 terminacy). Precise counting of floral organs indicates that all first flowers on racemes, bracteate or
 97 ebracteate, were wild-type (fig.1B). These observations did not support the hypothesis of a strong
 98 flowering signal able to convert axillary meristem identity. To challenge this hypothesis, we reason-
 99 ed that if basal bracts resulted from a branch-to-flower conversion, the more basal bracts,
 100 the less branches should remain. However, such a negative correlation between the number of
 101 branches and the number basal bracts was not observed in our dataset (fig.1C). Conversely, de-
 102 spite the absence of phenotypic changes on the flower, the bract itself presented a range of phe-
 103 notypes, from little "needles" to fully developed bracts (see Results - Chapter 1). This gradation of
 104 phenotypes shows that developmental modifications in *Tsu-0* mostly concern the bract, and not
 105 its associated meristem.

106 Basal bracts are not associated with a faster plastochron

107 If basal bracts do not result from a branch-to-flower conversion, flower meristem and its subtend-
 108 ing bract may have been initiated together. We next hypothesized that basal bracts could be pro-
 109 duced in a short time window at floral transition, when floral identity would not be well fully estab-
 110 lished yet. In this period, a mix between vegetative and reproductive transcriptomes would result
 111 in the production of bract-flowers. A prediction is then that a faster rate of phytomer production
 112 (the plastochron) should result in more basal bracts on a stem. To test this, we measured the
 113 plastochron in both *Col-0* and *Tsu-0* plants throughout floral transition, by counting the number of
 114 leaves surrounding the meristem until the formation of the first flowers in batches of synchronized
 115 plants. The average difference of plastochron is not significantly different, and the variability be-
 116 tween replicate is higher than between accessions (fig.2). This indicates that plastochron variation
 117 is not sufficient to explain the presence of bracts in *Tsu-0*.

118 Taken together, our data show that in *Tsu-0* plants grown in our laboratory conditions, basal
 119 bract formation resulted from different mechanisms that the ones previously described in the

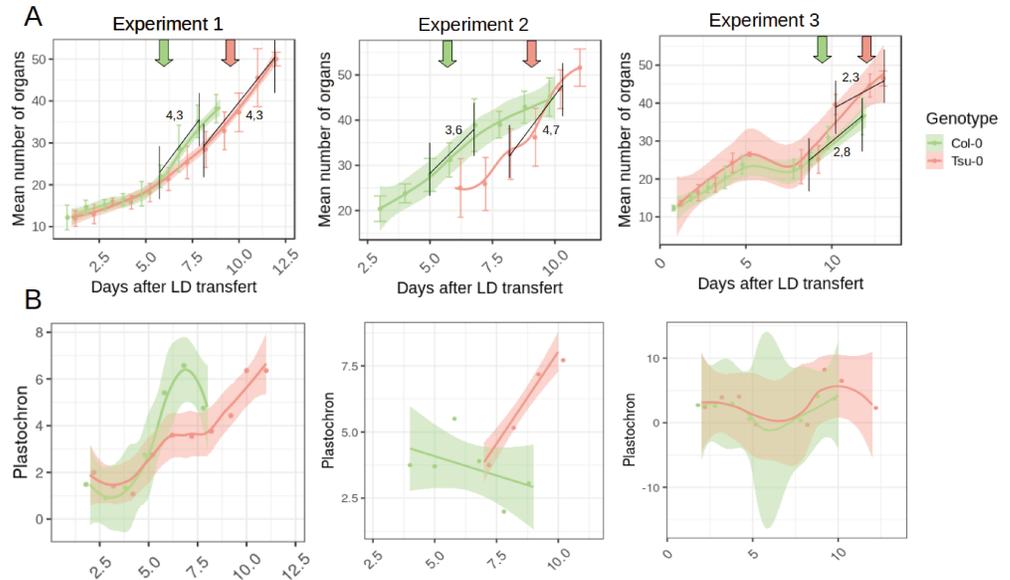


Figure 2. Variations in plastochron dynamics at floral transition does not explain basal bract formation. A) Cumulative counts of organs produced over time before, during and after floral transition for *Col-0* (green) and *Tsu-0* (red) plants in 3 independent experiments. At each time point, organ number is counted by SAM dissections in a subset of plants ($5 < N < 10$) from a batch of numerous synchronized plants (error bars are standard deviation). A global fit is computed using a local regression loess function (confidence intervals in corresponding color shades). Vertical green and red arrows indicate floral transition in *Col-0* and *Tsu-0*, respectively, observed from dissection. Instantaneous plastochron at floral transition (black numbers, in organs/day) is estimated from the local slope of the curve between indicate time points (black line), corresponding to the day at which at least one plant flowered, and the day all dissected plants flowered. The variable plastochron over the three experiments is not significantly different between the two genotypes. B) Plastochron, *i.e.*, the speed of organ production (in organ/day) at each time point calculated between the day before and the day after each point. Remark that as expected, plastochron of day 1 and of the last day are missing. Plastochron is very variable depending on the experiment, and do not show significant difference between the two genotypes (t-test, p-value > 0.05).

120 literature. We next described molecular and transcriptomic characteristics of floral transition in
 121 *Tsu-0* that could be linked with basal bract production.

122 **Acquisition of floral identity in *Tsu-0* and *Col-0* during floral transition revealed by** 123 **live confocal imaging attempt**

124 To address the molecular characteristics of *Tsu-0*'s first flower, we next attempted to explore the
 125 expression pattern of *LFY*, a marker of floral identity, during the emergence of the first flowers
 126 in the two natural accessions. We dissected transitioning meristems of pLFY::2mCherry-N7 lines
 127 in *Col-0* and *Tsu-0* backgrounds. However, dissection of meristems before bolting, in the rosette
 128 stage, leads to important tissue damage that hamper the acquisition of good quality images. Be-
 129 sides, PI staining is hardly suitable for damaged tissues, and 2mCherry fluorescent dye and PI
 130 cannot be separated. Hence, despite three replicates with > 30 dissected plants/ replicates, very
 131 few images were exploitable. Moreover, *LFY* signal was weaker at transition stage than at bolting
 132 stage when reporter lines plant were initially screened (fig.3). Taken together, the following image
 133 analysis needs to be taken with a lot of reserve. Our images suggest that pLFY::2mCherry-N7 is
 134 expressed in bract-flower in *Tsu-0*, but not in axillary meristems, confirming the floral identity of
 135 bract-flowers (fig.3). The lack of good quality images did not allow any quantification. Nevertheless,
 136 no visible difference has been observed so far in the expression pattern of *LFY* between the two
 137 accessions. Similarly, visual comparison between the first and the second flower did not show any
 138 change in the expression of *LFY*. Hence, the expression pattern, as well as the expression level of
 139 *LFY* in the first flower of *Tsu-0* did not show obvious visible discrepancies. Moreover, the sequence
 140 of the pLFY promoter in *Tsu-0* and *Col-0* did not show any variation. It can thus be suggested that
 141 production of basal bract in *Tsu-0* seems to be *LFY*-independent. Despite the absence of quantifi-
 142 cation, low pLFY::2mCherry signal suggests that *LFY* expression continues to increase even after
 143 floral transition, making the first flowers a "LFY-reduced" flower. Moreover, the expression of *LFY*
 144 seemed highly variable in the first flowers, with number of apices that present no visible signal
 145 (data not shown). Taken together, these results insinuate that *LFY* expression level and pattern
 146 did not seem to distinguish *Tsu-0* from *Col-0* flowers, at least not visually. It also could hint that
 147 flowers dissected at T stage express a lower level of *LFY* than flowers dissected at bolting, that
 148 might indicate an incomplete floral fate commitment. Nevertheless, confocal analysis only shows
 149 expression and spatial data for targeted cloned genes. We then carried a global transcriptomic
 150 analysis of apices throughout floral transition in the two accessions to catch molecular hints that
 151 might lead to basal bract formation.

152 **Transcriptional divergence culminates at floral transition in *Tsu-0* where meris-** 153 **tems show contrasting hallmarks of immaturity and maturity**

154 Matching developmental stages in *Tsu-0* and *Col-0* with morphological clues indicate a
 155 similar but delayed profile

156 In our culture conditions, floral transition of *Tsu-0* plants is delayed by 2-3 days compared to *Col-0*
 157 (fig. 4)). To see how this affects meristem development at a microscopic scale, we monitored its
 158 morphological evolution across floral transition. We dissected and imaged meristems from both
 159 accessions before, during and just after floral transition. Despite their different flowering time,
 160 four identical morphological stages can be easily identified in both *Col-0* and *Tsu-0*. The Vegetative
 161 stage (V stage) corresponds to a small and flat meristem, only surrounded by leaf primordia, that
 162 plants harbor before they are transferred to long day conditions (day 1). The Late Vegetative stage
 163 (L stage) corresponds to a bigger and domed vegetative stage (5 days after switching to inductive
 164 conditions). At the Transitioning stage (T stage), the first flower primordia is produced, and the axil-
 165 lary meristem starts to expand at the axil of grown leaves. The Floral Stage (F stage) corresponds to
 166 big and domed meristems surrounding by flower primordia, clearly recognizable with their round
 167 shape. T and F stages occurred later in *Tsu-0* plants than in *Col-0*: in *Tsu-0*, the T stage happened

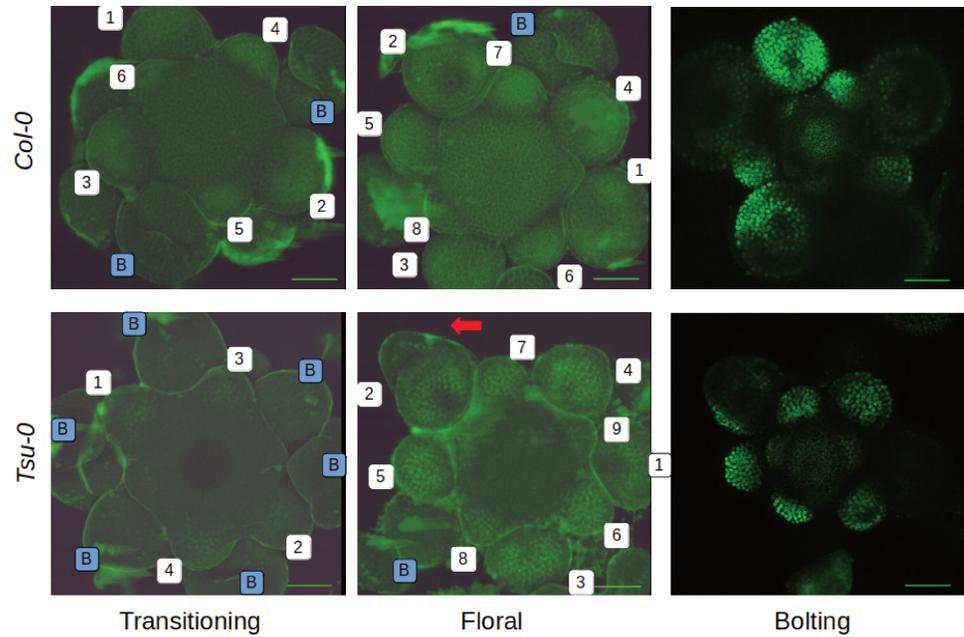


Figure 3. Confocal images of pLFY::2mCherry-N7 in *Col-0* (up) and *Tsu-0* (bottom) reporter lines. Shoot apices of pLFY reporter lines in *Col-0* and *Tsu-0* background at T and F stage, and later (bolting). Apices of T and F stages have been stained with PI. LFY reporter expression is visible on *Tsu-0* bract-flower (flower 2 in *Tsu-0* F stage, the red arrow shows the bract). LFY reporter expression is visible on the first flower of each accession (flower 1 in T stage), while branches (marked with blue tag) does not show any expression. LFY expression is very low at T and F stage, and increases later (bolting).

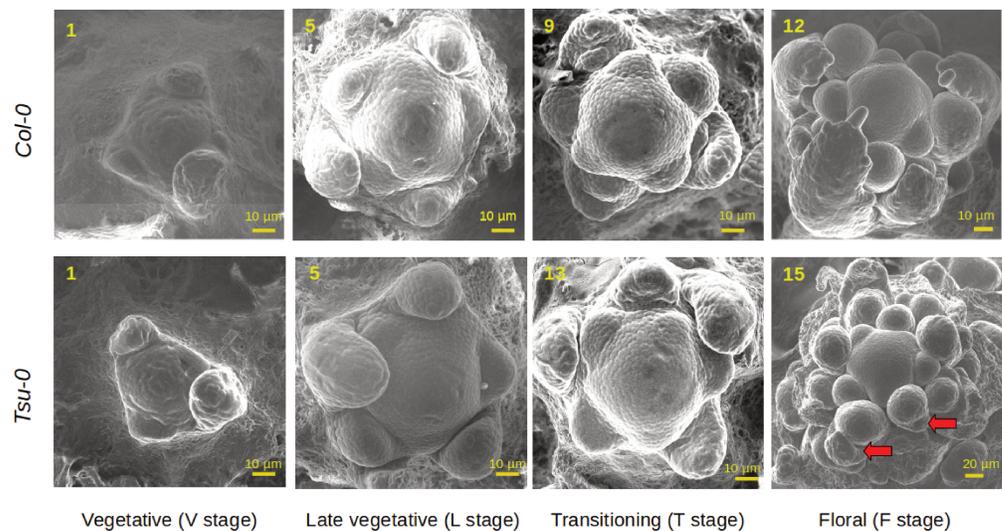


Figure 4. SAM of *Col-0* and *Tsu-0* during floral transition and identification of four morphological stages. SEM pictures of dissected SAM of *Col-0* (up) and *Tsu-0* (bottom) plants cultivated in SD condition for 20 days, and then transferred in LD condition at day 1. Day after transfer are noted on the upper left of each picture. Scale bar represents 10 or 20 μm . Vegetative (V) stage correspond to meristem at day 1: the meristem is small and flat. Late vegetative (L) stage are meristem in 5 days: the vegetative meristem is domed. Transitioning (T) stage corresponds to the emergence of the first flower, and axillary meristems starts to grow. Floral (F) stage corresponds to a complete switch of the apical meristem to inflorescence stage: it is large, domed and surrounded by flowers.

168 around day 13, while in *Col-0*, T stage was observed around day 9. These stages are similar to the
 169 stages described previously ((*Klepikova et al., 2015*), (*Kinoshita et al., 2020*))

170 Matching similar morphological stages in the the two accessions allow us to compare function-
 171 ally equivalent developmental stages although plants differ in absolute age. Notably, important
 172 molecular parameters related to the control or the consequences of these developmental trans-
 173 formations should be synchronized and display similar levels between matched stages. Conversely,
 174 variations of some molecular parameters between matched stages could point to the cause of why
 175 similar developmental stages end up in different outcomes (here the production or inhibition of a
 176 bract), as nicely shown in nightshades meristems within and across species (*Lemmon et al., 2016*)

177 A de-correlation between morphological and transcriptomic signature of floral transition
 178 in *Tsu-0* and *Col-0*

179 We performed RNA-sequencing on the shoot apices of *Col-0* and *Tsu-0* for the four developmental
 180 stages described above. A Principal Component Analysis (PCA) shows that developmental stages
 181 constitutes the main driver of transcriptomic dynamic differences between the two accessions
 182 (fig.5A). Developmental stages well recapitulate floral transition as shown by the expression of key
 183 regulators of floral transition (fig.1). Besides, all replicates are grouped together despite sampling
 184 time difference (fig.5A), showing that the morphological staging is robust to at least 4 days of sam-
 185 pling disparity (see Appendix). Genotypes constituted the second main driver of transcriptomic
 186 divergence. Notably, the developmental stages of the two accessions displayed transcriptomic
 187 shifts despite their similar morphological features. In particular, the T stage, that corresponds to
 188 the expected moment for basal bract emergence, displays the highest transcriptomic shift: T stage
 189 in *Tsu-0* appears older than the T stage in *Col-0* (fig.5A). However, if a basal bract made of cells
 190 expressing a leaf-like transcriptome emerges at the stage T *Tsu-0*, one could expected to detect
 191 a more juvenile transcriptome in *Tsu-0* compared to *Col-0*. In order to better characterize the dy-
 192 namic of gene expression in both accessions, we selected a subset of genes that are differentially
 193 expressed between at least two stages in at least one of the two accessions (fig.5B). This subset
 194 represents 7498 genes that we coined hereafter "dynamical genes" (fig.5C). In a quite expected
 195 manner, a Gene Ontology (GO) term enrichment analysis indicates that this pool is enriched for
 196 genes involved at the molecular scale in the regulation of transcription and whose function is as-
 197 sociated to responses to stimuli and hormones and post-embryonic development. A PCA on these
 198 dynamical genes shows similar results as with the whole dataset: the main driver of variation is the
 199 developmental stages, while the second driver is the genotype (fig.5D). Because sampling dates of
 200 T and F stages were different between *Col-0* and *Tsu-0* (fig.4 and Supplemental data), we established
 201 the relation between the sampling date and the "transcriptomic age", defined as the projection of
 202 the different samples in the first component found by the PCA. Fig.5E shows that the older tran-
 203 scriptome of T stage in *Tsu-0* is not only due to an older sampling date. In fact, *Tsu-0* is delayed
 204 on both axis: the sampling date and the transcriptomic age. In *Tsu-0*, the transition from L to T
 205 stage is as divergent as the transition from L to F stage in *Col-0*. Reaching T stage involves the
 206 highest change in gene expression, and it is more pronounced in *Tsu-0* (fig.5E and fig.2). On the
 207 other hand, few transcriptomic divergences exist between the T and F stage in *Tsu-0*, contrary to
 208 *Col-0*. Hence, comparing development between *Col-0* and *Tsu-0* reveals a complex heterochrony
 209 at stage T: while meristems look alike (doming), *Tsu-0* plants both have juvenile traits (bracts) and
 210 older transcriptome.

211 To better understand the gap between morphological and transcriptomic characteristics in *Tsu-*
 212 *0*, we next investigated the dynamics of gene expression in both accessions.

213 **A reduced down-regulation of genes in *Tsu-0*'s floral transition**

214 To better assess which genes participate to this heterochrony, we carried a clustering analysis
 215 based on the dynamics of genes across floral transition in both accessions. To group genes based
 216 on the variations of expression levels rather than their absolute levels, the expression levels of each

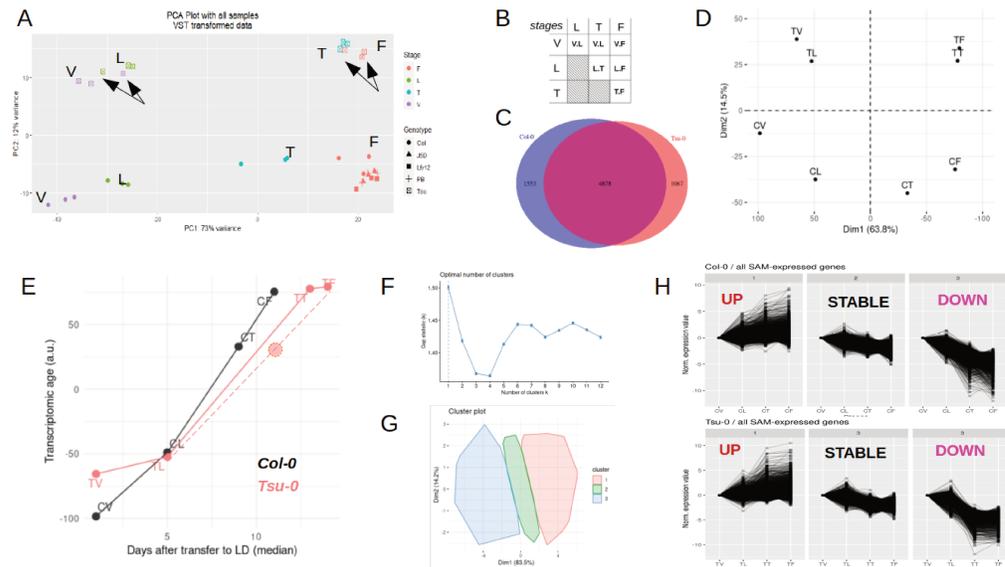


Figure 5. Different transcriptomic trajectories across floral transition in *Col-0* and *Tsu-0* revealed by time course RNA-sequencing. A) A global PCA analysis of all the triplicates samples corresponding to fig. 4 in *Tsu-0* and *Col-0*. *jag-5D*, *puchi-1*, *bop-1*, *bop-2*, and *lfy-12* mutants have been taken at F stage only (see fig.6). The developmental stages account for the majority of sample dispersion. V, L, T, and F stage are well separated in *Col-0* but less in *Tsu-0* where V and L, T and F are respectively closer together. B) Analysis of the dynamical genes: table showing how we extracted dynamical genes by comparing between stage differential expressed genes. C) Venn diagram of the number of dynamical genes in *Col-0* (blue) and *Tsu-0* (red). D) PCA analysis of dynamical genes, showing the same global profile than the global PCA, with *Tsu-0* T and F stage closer together. E) Transcriptomic age and sampling age of each sample. The transcriptomic age corresponds to a projection of each point of the PCA in D to the developmental age axis (horizontal axis). Sampling dates for stage V and L were identical between the two genotypes, but different for stage T and F. Still, transcriptomic age of *Tsu-0* T stage appears older than *Col-0*. The predictive transcriptomic age if *Col-0* and *Tsu-0* had it similar is represented in a red dotted line circle. F-H) k-means clustering of the dynamical genes into several groups. F) K statistics depending on the number of groups for the clustering. G) Distribution of the different groups in *Col-0*, showing no overlapping regions. H) Normalized expression profile across floral transition for the 3 gene clusters in *Col-0* (up) and *Tsu-0* (bottom). The "Up" group corresponds to genes whose expression level increases across floral transition. The "Stable" group corresponds to genes whose expression level slightly decreases across floral transition. And the "Down" group corresponds to genes whose expression level decreases across floral transition. *Col-0* and *Tsu-0* globally presents the same dynamical groups profile.

Figure 5-Figure supplement 1. Expression level of key regulators of floral transition during the time course RNA sequencing

Figure 5-Figure supplement 2. Differentially expressed genes during floral transition

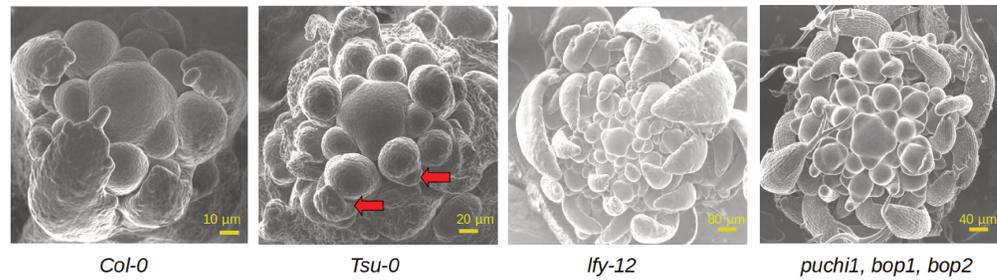


Figure 6. Shoot apical meristem of *Col-0*, *Tsu-0*, *lfy-12* and *puchi1, bop1, bop2* mutants at F stage. At F stage, *lfy-12* and *puchi1, bop1, bop2* mutants produced indeterminate branch-like meristems, subtended by leaves. After transitioning, the SAM of these mutants presented an important number of leaves with developing meristems, and have been used as a "leaf with meristem" transcriptomic signature.

217 gene were normalized to their level at initial V stage. Optimal number of clusters was comprised
 218 between 3 and 4 for the two accessions (fig.5F-G), but 3 clusters better fitted the data. Normalized
 219 gene expression dynamics of the three major clusters are represented in fig.5H. In both accessions,
 220 the clustering aggregates genes based on three global trends: "Up", "Stable" and "Down" grouping
 221 genes whose expression level globally increases, slightly decreases or drops, respectively. How-
 222 ever, the genes belonging to each cluster in *Tsu-0* and *Col-0* differed, and especially for genes be-
 223 longing to the cluster "Down". In fact, 46% of "down" genes in *Col-0* are found in another dynamical
 224 group in *Tsu-0*, while only 3% of "Up" genes in *Col-0* are lost in *Tsu-0*. *Tsu-0* transcriptional program
 225 is thus characterized by less down-regulation and more "Stable" or "Up" gene expression profiles
 226 during floral transition. The genes promoting basal bract formation could thus correspond to a set
 227 of genes that are not (or less) down-regulated in *Tsu-0*, while they are in *Col-0*. We could then pre-
 228 sume that such gene would be necessary for leaf emergence. To test whether the differences of
 229 gene cluster composition between the two genotypes could be associated to particular biological
 230 functions, we then performed a comparative Gene Ontology (GO) term enrichment analysis across
 231 the two accessions for each cluster. However, we failed to identify any relevant enriched biological
 232 functions that were lost or gained in any of the clusters. Surprisingly, the pool of dynamical genes
 233 missing from the "Down" cluster (almost 500 genes) in *Tsu-0* compared to *Col-0* does not induce an
 234 obvious change in GO term enrichment. In particular, we could not identify a modification related
 235 to leaf development.

236 We then took advantage of mutants to better identify leaf transcriptome within *Tsu-0* dataset.

237 **Capturing bract signature with mutant transcriptomes**

238 Several mutants produce flower-like meristems subtended by leaves (fig. 6) after floral transition,
 239 such as *lfy-12* ((*Xu et al., 2010*)), *jag5-D* ((*Dinneny, 2004*)) or *puchi1, bop1, bop2* ((*Karim et al., 2009*)).
 240 We then cultured these mutants in the same conditions as *Col-0* and *Tsu-0* plants and sampled their
 241 inflorescence meristems at the time the SAM produce visible meristems for the RNAseq analysis
 242 (roughly stage F, but the meristems are not flowers, but instead branch-like, see methods). Compar-
 243 ing their transcriptome with *Col-0* at F stage, we defined a list of 90 genes whose expression
 244 can be associated with leaf-making inflorescence meristems. Unfortunately, this set is too small
 245 to identify any GO term enrichment. Moreover, intersecting it with the genes that are also differ-
 246 entially expressed between *Tsu-0* and *Col-0* at T stage did not reveal particular relevant functions,
 247 especially nothing obviously related to leaf development.

248 In conclusion, we were unable to identify relevant biological functions associated to the tran-
 249 scriptomic heterochronic changes at the floral transition in *Tsu-0*. However, at the time of writing
 250 this manuscript, this dataset is still under investigation. In addition, in the next chapter, we will
 251 re-use our RNAseq data as a clue to better precise the genetic determinants of bracts formation.

252 Discussion

253 Bract loss has happened several times independently in *angiosperms*, like *Brassicaceae* or *Poaceae*.
 254 Attempts have been made to investigate bract loss mechanisms using mutants. However, the
 255 isolation of bract inhibition mechanisms have been challenged by the pleiotropic effect of the mu-
 256 tants, questioning whether mutant bracts could be qualified as bracts *stricto sensu* (s.s.). Never-
 257 theless, the presence of bracts s.s. associated with wild-type flowers in some natural accessions of
 258 *A. thaliana* (like *Tsu-0*) offers a new opportunity to study bract formation and decipher the mech-
 259 anism of its inhibition. Their exclusively basal position on the inflorescence suggests a strong link
 260 with floral transition. In this chapter, we then used bract-making *Tsu-0* plants in comparison with
 261 non-bract making *Col-0* plants and focused precisely on floral transition to understand the devel-
 262 opmental features that characterize basal bract formation.

263 In *Tsu-0*, the absence of floral indeterminacy and the range of bract phenotypes in bract-flowers
 264 suggest a perturbation of bract inhibition only, independently of floral determinism. This is further
 265 confirmed by the very similar transcriptomic profiles of floral determinism genes, both globally
 266 and for canonical flowering genes such as AP1 or LFY in *Tsu-0* and *Col-0*. Hence, acquisition of
 267 floral identity is highly conserved between the two natural accessions. Besides, bract-flowers are
 268 impacted neither by varying photoperiodic conditions, contrary to previous observations ((*Hempel*
 269 *et al., 1998*)), nor by the plastochron. The *Tsu-0* accession offers a robust genetic context in which
 270 bract inhibition is compromised, at least during the short time window of floral transition. Like
 271 *Tsu-0*, a number of other natural accessions bear bracts on their first flowers (see Results -Chapter
 272 1). To what extent bract-flowers in other natural accessions is governed by the same mechanisms
 273 as in *Tsu-0* is still an open question. Of course, the question is even more relevant at the levels of
 274 *Brassicaceae*, or of Angiosperms.

275 The main specificity of bracts in wild type *A. thaliana* plants is their position, exclusively restricted
 276 to the first flowers. Bract-flowers have not been observed after the fourth flower in *Tsu-0*. This in-
 277 dicates the existence of a particular context during floral transition that perturb bract inhibition in
 278 *Tsu-0*. To study floral transition, we identified four developmental stages that have identical meris-
 279 tem morphology. Transcriptome profiling over floral transition in *Tsu-0* and *Col-0* reveals insightful
 280 characteristics of *Tsu-0*'s floral transition. First and foremost, the four developmental stages very
 281 well depicted the floral transition process in both accessions. Despite their different sampling time,
 282 they display a similar global profile. This is expected from two natural genetic backgrounds within
 283 the same species: the gene regulatory network that triggers floral transition is conserved. Notably,
 284 as described previously in different species ((*Lemmon et al., 2016*)), the T stage, that corresponds
 285 to the emergence of the first flowers, is characterized by a climax of gene expression change when
 286 compared to other stages. Most of the differential gene expression between *Col-0* and *Tsu-0* hap-
 287 pened during T stage. This higher transcriptomic change at T stage in *Tsu-0* could maybe favor a
 288 faster adaptation to flowering signals: the transcriptome associated to flowering is reached before
 289 the visible production of flower. How far these characteristics can be correlated with basal bract
 290 production needs to be addressed by adding new natural accessions transcriptomes at floral tran-
 291 sition. Interestingly, our result at the level of two *A. thaliana* natural accessions recapitulates previ-
 292 ous study of floral transition in nightshades ((*Lemmon et al., 2016*)). *Lemmon et al.* showed that the
 293 divergence of transcriptomes peaks at floral transition between different species, despite an iden-
 294 tical meristem morphology. Besides, they show how heterochronic shifts, *i.e.* desynchronization of
 295 gene expression dynamics with respect to the matched similar phenotype, can be responsible for
 296 the evolution of plant branching system. Here, we provide the first example of such model at an
 297 intra-specific level. In this case, our results suggest that transcriptomic divergence during T stage
 298 could also lead to phenotypic evolution, here the presence or absence of basal bracts. Besides, like
 299 in nightshades, we also depicted the distortion between morphological and transcriptomic status
 300 of the meristem: *Tsu-0*'s T stage transcriptome was closer to *Col-0*'s F stage, but morphologically
 301 identical to *Col-0* T stage.

302 Observing this older transcriptome in *Tsu-0*, was surprising as bracts could have been seen
 303 as the remnants of a vegetative phase. So far, we could not however identify particular gene ex-
 304 pression characteristics, such as leaf GO term enrichment, associated to such vegetativeness at T
 305 stage. But identifying a GRN responsible for basal bracts formation in our transcriptomic dataset
 306 is of course very challenging. Bract development or inhibition occurs in a very narrow domain. As
 307 the transcriptomes have been sampled from the whole inflorescence meristems, it is uncertain to
 308 isolate differential gene expressions related to bract formation from the numerous unrelated vari-
 309 ations expected in two different natural genetic backgrounds. A single cell RNA sequencing could
 310 allow to isolate, based on the expression of abaxial "cryptic bract" genes (see Introduction), the
 311 specific transcriptome of bract domain between the two accessions. As this technology is getting
 312 more affordable, we will be able to consider using scRNAseq to precise bract-related transcrip-
 313 tome. Finally, this transcriptomic approach is based on the assumption that bract formation in
 314 *Tsu-0* results from a difference in the transcription of a set of genes, but this can be wrong, and
 315 other scenarii could be investigated, that will be discussed in Chapter 3.

316 One of the possible mechanisms of bract formation is a mis-localization of some genes specify-
 317 ing bract versus floral domain. Even if *LFY* expression level was identical between *Col-0* and *Tsu-0* all
 318 throughout the four stages, it was interesting to address its spatial localization in the first flowers
 319 of *Tsu-0* and *Col-0*. However, observing the emergence of the first flowers requires to dissect and
 320 image vegetative meristems, which is a challenging task. Besides, *LFY* signal in transgenic reporter
 321 lines were selected on bolted meristems, but it appears that the *LFY* signal of vegetative meristem
 322 was way below the one observed after bolting. For these reasons, as well as time constraints,
 323 we had not enough good quality pictures to clearly describe the expression pattern of *LFY* during
 324 the emergence of the first flower in *Tsu-0* and *Col-0*. Apart from *LFY* imaging, it would also be of
 325 great interest to image identity markers of the cryptic bract domain such as *FIL*, or *SPL 10*. In fact, as
 326 shown in maize, a mis-patterning of cryptic bracts versus flower domain can lead to bract formation
 327 (*Chuck et al., 2010*). To this end, generating appropriate transgenic bract domain reporter lines is
 328 crucial. Identifying genes with more bract/leaf specificity and less pleiotropic expressions (such as
 329 in flowers for instance) will be helpful. To this end, pinpointing the causal gene(s) responsible for
 330 basal bract formation in *Tsu-0* can thus not only inform on the mechanisms of bracts suppression,
 331 but might provide good candidate reporters to monitor and/or perturb bract development.

332 Conclusions and perspectives

333 Previous studies of bract development in *A. thaliana* relied on phenotypes showing signs of floral
 334 indeterminism. Here, we explored the formation of basal bracts in *Tsu-0*, a natural accession that
 335 does not show signs of floral indetermination. It appears that the mechanism of bract production
 336 in this context is different from the one previously described, as bracts are not a conversion of
 337 branches into flower after strong photo-induction. Because bracts are restricted to the first flow-
 338 ers, we captured the transcriptome related to floral transition in *Tsu-0* that depicted sign of hete-
 339 rochrony at T stage, with an older transcriptome. Taken together, this chapter exposes the first
 340 study of transcriptomic variations over floral transition within the same species. Interestingly, it
 341 shows that hundreds of genes display a significant change in their expression dynamics, and most
 342 of these differences are moderate (changes in expression levels or heterochronic shifts) rather
 343 than opposite trends. Moreover, despite a macroscopic phenotypic output (basal bract forma-
 344 tion), most of the known major regulator genes previously involved in bract formation and floral
 345 determinism show an astonishing similar expression profile. Although this needs to be confirmed,
 346 this intermediate result suggests that other unexpected genes (and possibly pathways) would ex-
 347 plain basal bract polymorphism in natural *A. thaliana* populations. In line with this hypothesis, the
 348 importance of overlooked genes in population-scale micro-evolution in nature has been showed
 349 for flowering time in *A. thaliana* (*Brachi et al., 2010*), although our experimental set-up does not
 350 mimic natural growth conditions. As we will detail in the next chapter, transcriptomic dataset can
 351 also help for the identification of the causal gene of basal bract production in *Tsu-0*. These results

352 will bring new insights on bract inhibition in *A. thaliana*.

353 **Methods and Materials**

354 Please refer to the general section "Methods and Materials".

355 **Authors contributions**

356 S.D. carried out all the experiments. S.D. and F.B. designed the experiments, proposed the com-
357 puter analysis, analyzed and interpreted the results. B.J. and F.B. carried out the RNA-sequencing
358 analysis. S.D. wrote the first draft of the manuscript, and F.B. helped edit it.

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367 using the eLife free template.

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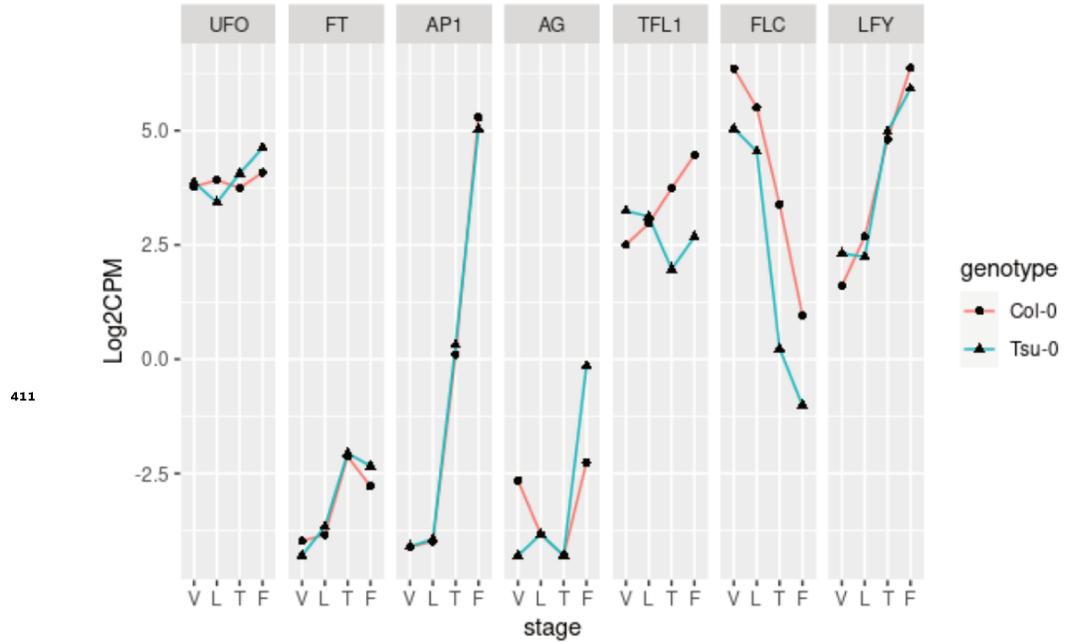


Figure 5-Figure supplement 1. Expression level (log2CPM) of *UFO*, *FT*, *AP1*, *AG*, *TFL1*, *FLC* and *LFY* in *Col-0* (red) and *Tsu-0* (blue) during the time course RNA-seq experiment. *FT*, *AP1*, *AG*, *FLC* and *LFY* increase throughout floral transition and do not present significant difference between *Tsu-0* and *Col-0*. *TFL1* show contrasting expression between *Tsu-0* and *Col-0*, with global increase in *Col-0* and decrease in *Tsu-0*.

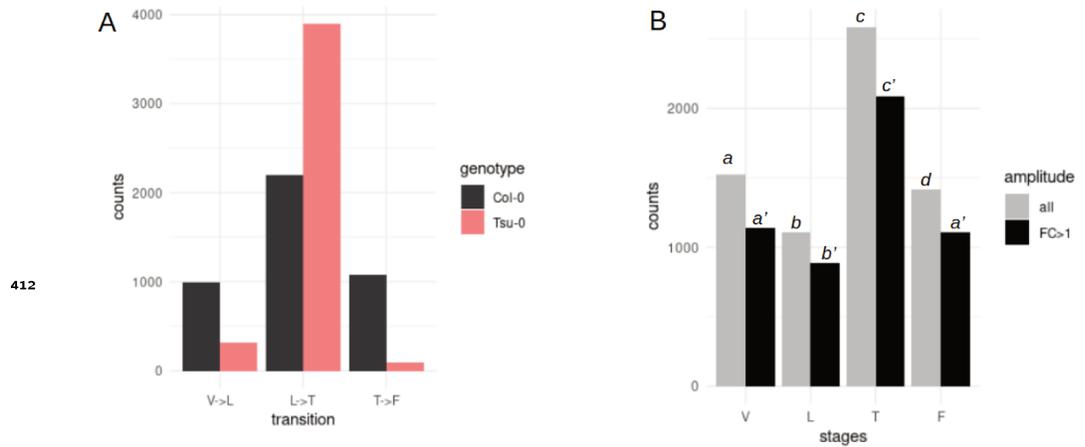


Figure 5-Figure supplement 2. A) Differentially expressed genes (FC>1) between two consecutive stages in *Col-0* (black) and *Tsu-0* (red). The switch from L to T stage correspond to the highest number of DE genes, mostly in *Tsu-0* ecotype. B) Differentially expressed genes at each stage between *Col-0* and *Tsu-0*, without (grey) or with (black) filter genes which Fold Change (FC) > 1.

1

Genetic determinants of bract transient formation in a natural accession of *Arabidopsis thaliana*

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Abstract

While the model plant *Arabidopsis thaliana* has lost bracts under its flower, we identified a natural accession, *Tsu-0*, that frequently bears bracts but only on its first flowers produced at the basis of flowering branches. We therefore used this accession, in association with the bractless reference *Col-0* accession, to map the genetic determinants controlling the formation of "basal" bracts that appear at floral transition. By using both bulked F2 segregant populations and Recombinants Inbred Lines, we established that the genetic determinism of this trait is complex and located in two major loci in chromosome 1. We then crossed evidence from different datasets to select the best candidates within the mapping region. These includes the effects of natural polymorphisms, the dynamics of gene expression over floral transition and expression data from bract mutants. We propose interesting candidate genes that could give possible insight on the mechanisms and evolution of bract loss in *A. thaliana*.

Introduction

Phenotypic evolution proceeds through a variety of changes, including transformations, gains, losses or reversions. The nature of the heritable genomic changes that produce such phenotypic evolution is a central question in biology. Given the high gene conservation among even distantly related organisms, trait gains or losses are mostly not caused by simple gene gains or losses. As observed in many mutagenesis experiments, a loss of gene function do not simply results in a loss of trait, but rather in pleiotropic effects. Therefore, we need more examples of how precise phenotypic changes occur in nature to characterize their causal genomic variation and their underlying developmental regulatory network. One surprising phenotypic change that participate to the evolution of forms are the complete or partial loss of a morphological trait. However, existence of atavism, i.e the resurgence of an ancestral trait, suggests the maintenance of the genetic information required to build the trait. It appears that small changes in gene activity could channel the development of a structure into its complete, partial or lost form. Sometimes, cryptic structures are maintained. Bract development in the model plant *Arabidopsis thaliana* offers a case study to investigate the nature of such small heritable genetic changes. Bracts, the leaves subtending flowers, have been lost in *Brassicaceae* clade. Nevertheless, at least in *A. thaliana*, a bract starts to form just before each flower grows out, but its development stops early and the bract remains cryptic (Penin, 2008; Kwiatkowska, 2006). However, many *Brassicaceae* species keep making a few bracts at the basis of inflorescences (see Chapter 1). Although the mechanism of floral induction is very well established, the inhibition of bract is still poorly understood. Moreover, the mechanisms of its resurgence at the basis of some inflorescences are completely unexplored. Like in previous studies, (Hempel and Feldman, 1994; Hempel et al., 1998; Hempel and Feldman, 1995), we have reported

43 the presence of fully developed bracts in some natural populations of *A. thaliana*. Bracts were only
 44 subtending the first flowers at the basis of the racemes, as it was observed in some other *Bras-*
 45 *sicaceae* species, and were called "basal bracts" (see chapter 1). In *A. thaliana*, such bracts can be
 46 triggered by specific photo-induction (*Hempel and Feldman, 1994, 1995*), or observed in standard
 47 laboratory culture conditions for many natural accessions (see chapter 1). Hence, in *A. thaliana* as
 48 in many *Brassicaceae* species, we propose that basal bracts are an atavic structure. That is to say,
 49 the developmental program of bracts production beyond the cryptic stage are retained. However,
 50 except for such rare bracts, this developmental program is usually not triggered, and/or inhibited.
 51 Comparing bracteate and ebracteate accessions of *A. thaliana* offer a way to identify natural genetic
 52 variations that trigger this developmental program, at least during floral transition. The nature of
 53 such genetic variation is so far totally unknown.

54 In this chapter, we present how using the natural accessions *Tsu-0* and *Col-0* enabled us to map
 55 the genetic variations associated with transient bract formation at the floral transition. Our ge-
 56 nomic mapping identified several QTL controlling bract formation, revealing its complex genetic
 57 determinism. We re-analyzed genomic data to comprehensively annotate the putative functional
 58 effects of natural polymorphisms in *Tsu-0*, especially in the genetically mapped intervals. We also
 59 used transcriptome profiles during floral transition to capture differential gene expression corre-
 60 lating with the production of bract in *Tsu-0* and some bract-mutants. By crossing all these data, we
 61 propose a list of candidate genes that could be at the origin of atavic bracts at floral transition in
 62 the *A. thaliana* natural accession *Tsu-0*.

63 Results

64 Bulk Segregant Analysis (BSA) identifies a complex genetic determinism governed 65 by several candidate loci in Chrom 1 and Chrom 5

66 To map the gene(s) responsible for atavic bract formation at floral transition using bulk segregant
 67 analysis (BSA), we first generated a mapping population (*Col-0* x *Tsu-0*). We measured the bract
 68 mean frequency (defined as the mean number of bracts per branch for each individuals) of the
 69 parents (N= 90/genotype), the F1 (N= 176) and the F2 (N= 684). For practical reasons, we split this
 70 quantification into four replicates including all genotypes (fig.1). As mentioned earlier (see Chapter
 71 1), bract formation is variable even in a fixed isogenic background: mean bract per branch in a plant
 72 ranges from 0 up to 0.5 in *Col-0* and from 0 to 1.9 in *Tsu-0*. This stochastic variability diminishes the
 73 statistical power of associative mapping. Except for the first replicate, the distribution of bracts per
 74 branch was normal in *Tsu-0*, so we calculated its mean value for each genotype. The phenotype of
 75 the F1 population was intermediate between *Col-0* and *Tsu-0*, as F1 bore on average 0.44 (+/- 0.28)
 76 bracts/branch, *Col-0* 0.11 (+/- 0.13) and *Tsu-0* 1.0 (+/- 0.40) (fig.1A). This indicates that at least one
 77 co-dominant allele is involved in bract formation. No transgressive phenotype was observed in the
 78 F2 as bract distribution ranges within the parental *extrema*. F2 bract mean frequency did not mark
 79 any peaks around parental and F1 means, incompatible with a mendelian segregation of a single
 80 locus. Instead, F2 distribution was close to the F1: F2 bore on average 0.42 bract/branch (+/- 0.31)
 81 (fig.1A). Thus, several genes may be involved in the formation of basal bracts. We then performed
 82 a BSA by selecting F2 plants presenting high and low bract frequency (named hereafter *Tsu-like*
 83 and *Col-like* plants, respectively), measured as the mean bract number per branch in a plant. We
 84 defined *Tsu-like* plants as plants showing higher bract frequency than the highest F1 plant of the
 85 same replicate, resulting in a selection of only 17 individuals over the 684 scored F2 plants. We
 86 defined *Col-like* as plants showing no bracts in replicates 1, 2 and 4, representing 56 individuals
 87 (fig.1A). After bulk sequencing of the DNA extracted from *Col-like* and *Tsu-like* pools, we scanned
 88 the variations in *Col-0* versus *Tsu-0* allele frequency along the genome using the deltaSNPindex
 89 method (*Takagi et al., 2013*). To generate an accurate list of natural genomic variations between
 90 the parental strains of our two bulks, we re-sequenced the genome of the *Col-0* and *Tsu-0* parental
 91 strains used in our experiments and performed a genomic variant analysis. The loci linked with

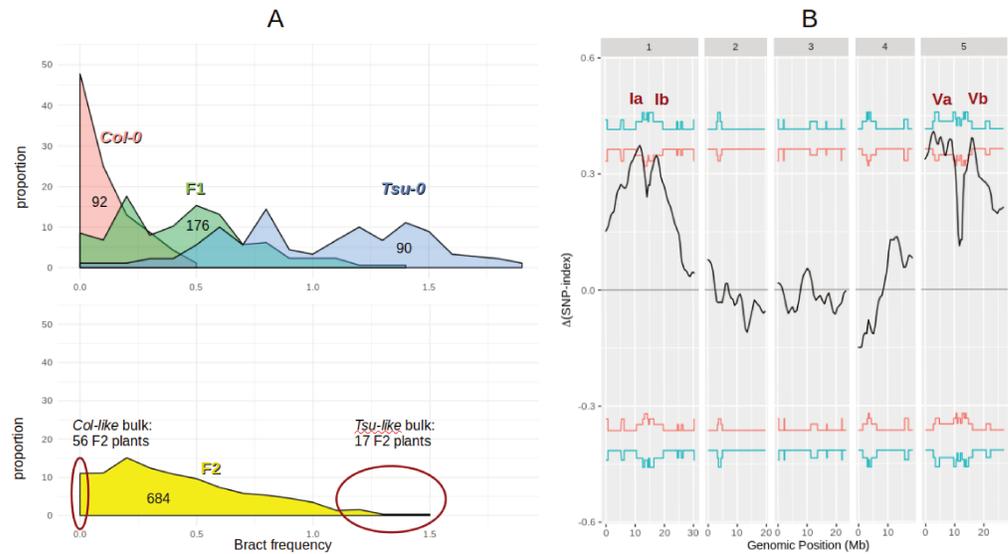


Figure 1. Genetic mapping of basal bract formation using a BSA approach A) Genetic transmission of the basal bract phenotype in an experimental *Col-0* × *Tsu-0* cross. Distribution of *Col-0* (red), *Tsu-0* (blue), F1 (green) and F2 (yellow, below) depending on bract mean frequency (the mean number of bract per branch per plant). Number of individuals for each genotypes are reported. *Col-like* and *Tsu-like* bulks are circled in red. B) Genetic mapping deduced from allele frequencies in the two selected bulks (QTLseqR package (Takagi et al., 2013)). The Δ SNP index (Mansfeld and Grumet, 2018) along each chromosome (1-5) and the 99% (blue) and 95% (red) confidence interval associated for each position (in Mb). Two peaks are present in chromosome 1 (Ia, Ib, the latest being just below the 95% confidence interval). Two other peaks are present in chromosome 5 (Va, Vb)

Figure 1-Figure supplement 1. Detail of the four replicates used for the BSA

92 bract formation are expected to have a significantly higher *Tsu-0* allele frequency in the *Tsu-like*
 93 than in the *Col-like* bulk. The results presented in Figure 1B point two chromosomes bearing each
 94 two peaks. The first peak was located in chromosome 1 between 11Mb and 13Mb and spanned
 95 2Mb. A second peak was noticed in chromosome 1, between 17 and 18Mb, even though it did
 96 not reach the 95% confidence interval (fig.1B). Two other peaks appeared within chromosome 5:
 97 one peak between 2Mb and 10Mb, the other between 16 Mb and 17Mb. Taken together, these
 98 different regions identified by BSA adds up to a mapping region of 12Mb.

99 **QTL mapping using RILs confirms the presence of two QTLs in Chrom 1 associated** 100 **with basal bracts**

101 The intervals provided by the BSA were excessively large, especially with the Va and Vb peaks,
 102 probably due to the low number of individuals selected for each bulks (notably in the *Tsu-like* bulk).
 103 To confirm and restrict the mapping, we then used a publicly available set of Recombinants Inbred
 104 lines (RILs) between *Tsu-0* × *Col-0* strains. All these RILs have been genotyped for 79 markers spread
 105 on the five chromosomes, defining mapping intervals of 1.48 +/- 0.6 Mbp on average (5.3 +/- 2.9 cM)
 106 (Simon et al., 2008). We then directly selected a limited number of RILs with appropriate genotypes
 107 guided by our BSA results. In total, we analyzed 56 RILs with or without recombination event within
 108 chromosome 1 and/or chromosome 5 (Supplemental table). Correlations between genotypes and
 109 phenotypes were analyzed using both R/qtl (Broman et al., 2019) and gCIM (Zhang et al., 2020)
 110 software (see methods). As shown in 2, two additive QTLs in the chromosome 1 are significantly
 111 associated with the presence of basal bracts, but none in the chromosome 5. The I α and I β peaks
 112 were found at respectively 5,6 Mb (15.8cM) and 22,2 Mb (64.8 cM) in Chr1, with both softwares (fig.
 113 1). However, the low genotyping marker density limits the final resolution that can be obtained.
 114 In addition, we notice a significant shift between the position of QTLs detected by RIL and BSA

115 mapping in chromosome 1 (I α was located at 12 Mb while I α at 5,6 Mb, and I β was located at 17 Mb
 116 while I β was at 22 Mb). These results clearly point to the existence of two principal additive QTLs in
 117 chromosome I controlling transient bract formation in *Tsu-0*. We decided to focus on chromosome
 118 1 as it resulted from both BSA and RILs analysis. Within the chromosome 1, we will keep the interval
 119 coordinates from both techniques to provide a transparent and well-informed analysis.

120 **On the main stem, the presence of bracts is genetically linked to cauline branch** 121 **number.**

122 We next investigated the association of QTLs I α and I β with different pleiotropic phenotypes that
 123 could provide interesting insights on the underlying allelic variations responsible for basal bracts.
 124 In the RILs dataset, we noticed a positive correlation between the number of bracts and the num-
 125 ber of cauline branches on the main stem (3A). Yet, the latest generally increases with the flow-
 126 ering time (supplemental data), which is, as we showed in Chapter 1, also associated with bract
 127 frequency. To investigate whether these different traits can be linked with bract frequency, we
 128 extracted from our phenotyping data the percentage of plants having bracts specifically on the
 129 main stem as well as the mean number of cauline branches in RIL assays. We treated these two
 130 new phenotypes as co-variables in the R/qtl analysis. Interestingly, the number of cauline branches
 131 and the percentage of plants having bracts on the main shoot are both linked with peak I β (fig. 3B)
 132 but not QTL I α . This suggests that, in *Tsu-0*, the two QTLs in chromosome I contribute to transient
 133 bract formation through distinct mechanisms, and that one of these mechanisms would be linked
 134 to the control of cauline branch number.

135 **Transgressive traits in some RILs evoke bract mutants phenotypes**

136 Despite the RIL analysis indicates an additive contribution of QTL I α and I β for bract frequency, we
 137 noticed other interesting transgressive phenotypes that could be linked with bracts. However, they
 138 were not systematically scored in all RILs and cannot be analyzed as co-variables and mapped in
 139 a quantitative manner. The first transgressive phenotype is flower indeterminacy. Two indeter-
 140 minacy phenotypes were observed, including branched flowers (4A) and prolonged organogene-
 141 sis inside carpels, and neither of them were never observed in the control parental strains *Col-0*
 142 and *Tsu-0* (N>7310). As mentioned earlier, most of the known bract-making mutants also display
 143 defects in floral indeterminacy. Such transgression in RILs could indicate that in *Tsu-0*, a similar
 144 pleiotropic allele exists. Nevertheless, *Tsu-0* may not present floral indeterminacy because of a
 145 particular allelic combination that favors basal bract formation while compensating floral indeter-
 146 minacy. Genomic recombination in RILs would break this epistatic inhibition. However, the incom-
 147 plete scoring of these traits preclude their genetic mapping: we could not determine whether they
 148 are associated with one or several QTLs and whether they overlap with I α and I β controlling bract
 149 frequency.

150 The second transgressive phenotype is a shift of bract position on the flower pedicel, that was
 151 noticed in at least 13 RILs in our dataset. As pictured in 4B-C, shifted bracts are not attached to the
 152 stem at the node, but rather upstream on the flower pedicel. Interestingly, similar shifted bracts
 153 have been observed in bract-bearing mutants like *bop-1*, *bop-2* or *jag-5D* (Dinneny, 2004; Ohno,
 154 2004; Norberg, 2005) (see also Chapter 1). In some RILs (470, 165, 008) almost all bracts are shifted,
 155 while in other RILs this occurs only sporadically. Here again, precise mapping and relations of
 156 this trait with QTL I α and I β could not be investigated. However, no obvious correlation with bract
 157 frequency was detected, as RIL 008 had 0.2 bract/branch, while RIL 165 1.52 bracts/branch.

158 Altogether, the observation of the transgressive phenotypes can be used as additional clues to
 159 identify and prioritize the best candidate genes.

160 **Fine mapping the QTLs of chromosome I using Near Isogenic Lines (NIL)**

161 BSA and RIL genetic mapping were not precise enough to isolate the genes responsible for basal
 162 bract in the *Tsu-0* natural accession, so we generated Near Isogenic Lines to further reduce our

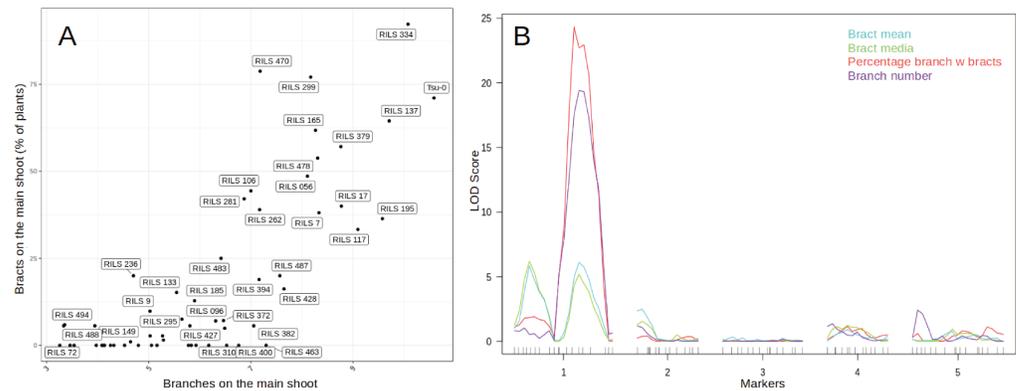


Figure 3. The production of basal bracts could be genetically linked to the production of cauline branches A) Scatter plot showing a positive trend in the RILs between the presence of basal bract(s) on the main stem (expressed as the percentage of plant in the RIL) and the number of cauline branches on the same main stem. B) LOD score corresponding to the correlation between genotypes and several traits: the per RIL mean (blue) and per RIL median (green) of the number of bract/branch/plant, the percentage of branches with bract (red) and the per RIL mean number of cauline branches on the main stem (purple). Analysis were carried using R/QTL software (*Broman et al., 2019*), with the MQM package (). Each markers are presented in the X axis, grouped by chromosomes. 56 different RILs genotypes were used, with >20 plants per genotype. The line mean number of bract is associated with two peaks in chromosome 1, while the percentage of branches bearing at least one bract is only linked with the second peak on chromosome 1.

Figure 3-Figure supplement 1. The number of branches increase with flowering time in *Tsu-0*

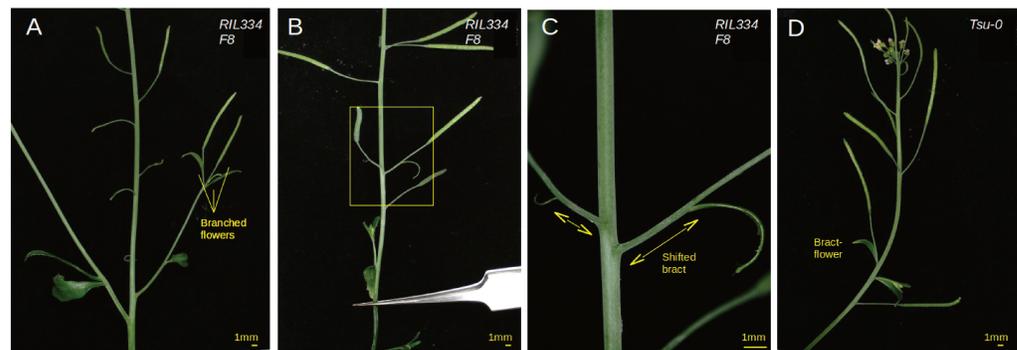


Figure 4. Transgressive phenotypes in some RIL F8. A) Branched flowers observed in RIL 334. Three (bract) flowers at the same node: 2 with a normal carpel development, the third with an atrophied carpel. B-C) Shifted bract and needle in RIL 334. C picture is a magnified of B on the shifted bract and needle. The bract is not located at the node, but rather upstream on the flower pedicel. D) *Tsu-0* classical bract-flower. The bract is directly attached on the main stem, and not on the flower pedicel.

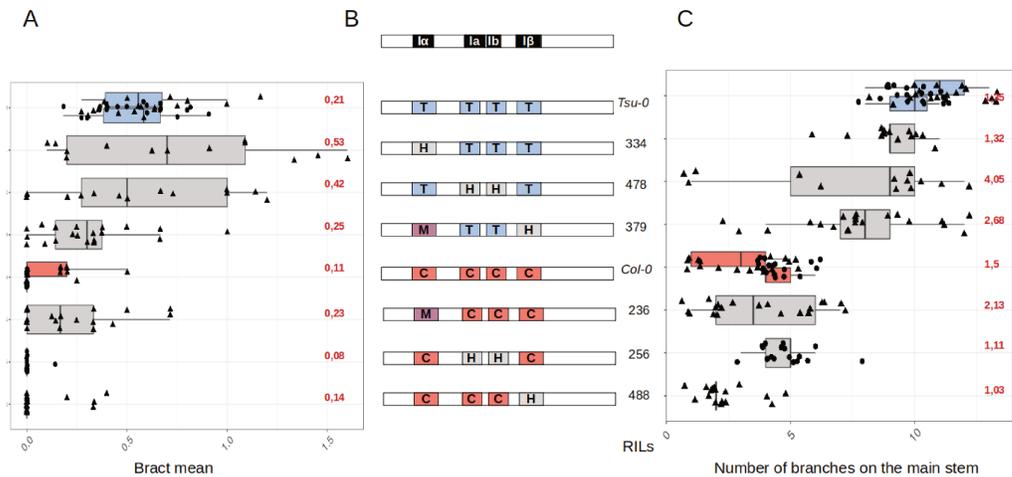


Figure 5. Using segregating RILs to confirm and select QTLs with major effects on basal bracts and cauline branch number. A) Bract mean frequency for each line. The standard deviation is indicated at the end of each line. B) A schematic view of the chromosome 1 with the localization of the $l\alpha$, l_a , l_b and $l\beta$ QTL. The expected genotype of each F8 - RIL at these loci are colored in red (when *Col-0* background), blue (when *Tsu-0* background), grey (when heterozygous background), or purple (when mixed background). C) Mean number of cauline branches on the main stem for each line. The standard deviation is indicated at the end of each line. The precise genotypes of each RIL used for this study are available in Supplemental data. $N > 13$ for each lines.

163 candidate QTLs $l\alpha$ and $l\beta$. At the time of the manuscript writing, this work is still ongoing. We
 164 selected several RILs heterozygous at markers in large regions covering $l\alpha$, l_a , l_b and $l\beta$
 165 (fig.6). We scored the corresponding segregating progeny in the F8 generation in order to detect
 166 possible segregation of bract phenotype. We choose RILs that are heterozygous in $l\alpha$, l_a , l_b or $l\beta$,
 167 and *Tsu-0* for the other target QTLs. Hence, if the heterozygous region contains a major bract gene,
 168 its segregation could be visible in the bract mean frequency of the offspring with "tsu-like" and
 169 "col-like" individuals. All RILs 334, 478, and 379 show higher standard deviation, with individuals
 170 presenting a lower bract frequency than *Tsu-0*. Hence, the segregation of each of these lines might
 171 reflect the segregation of a "bract gene". Surprisingly, RILs 478 and 334 displayed new phenotypes,
 172 with shifted bracts (478) and indetermination (334). We kept these lines for further studies. We next
 173 investigated the $l\alpha$, l_a , l_b and $l\beta$ intervals with RILs that are heterozygous in one of the QTLs and
 174 *Col-0* for the others. Presence of segregation was difficult to assess as *Col-0* showed a high bract
 175 mean distribution reaching 0.5 bract/branch for one individual. Besides, as show in fig.6, the two
 176 replicates of *Col-0* were not equivalents.

177 Because only 13 to 20 individuals were used for each genotype, the number of individuals that
 178 have fixed *Tsu-0* or *Col-0* haplotypes on one entire QTLs region is low (below 1/32 individual). Plus,
 179 even in a *Tsu-0* or *Col-0* homogeneous background, the distribution of bract frequency in *Col-0* and
 180 *Tsu-0* overlap. So, in order to address the contribution of each QTL, and eventually reduce it, it is
 181 necessary to significantly increase the number of plants. To date, this is ongoing.

182 RILs population allowed us to explore loci that correlates with the phenotype, but the complex-
 183 ity of the trait made it challenging to reduce it. In fact, each RILs plant have different genotype in
 184 other regions, that may be epistatic. We started to build NILs in order to get rid of any epistatic
 185 interaction outside the QTL we wanted to test, and to study the implication of each target QTLs
 186 on the basal bract phenotype. To this end, we started with RIL 478 and 334 because of their high
 187 standard deviation that could indicate a visible bract-allele segregation. Our aim is to get NILs that
 188 are, for each markers within the target region, either *Col-0*, heterozygous or *Tsu-0*. We will then
 189 be able to measure the effect of each of these regions to bract production, as well as the epistatic
 190 relation between every locus.

191 Altogether, BSA and RIL strategies point to a major role of the chromosome 1 with several QTLs.
192 BSA identified two QTLs: one QTL of 2MB between the position of 11Mb and 13Mb, and one QTL
193 of 1Mb between the position of 17Mb and 18Mb. RILs analysis identified 2 peaks associated with
194 the markers at 5.6 and 22.2 Mb. We thus allocate the surrounding markers at 4.2 and 7.2 Mb and
195 at 20.4 and 23.4, leading to two target regions of 3Mb. The construction of NILs that segregates at
196 each of these loci will allow to better precise the relevance of each QTL identified. In addition, BSA
197 identified also a large QTL in chromosome 5, that was not confirmed with the RILs analysis, so we
198 decided to exclude the later from further analysis. In fact, the Va and Vb QTL probably result from
199 a lack of power due to a low amount of individuals per bulk (see Discussion).

200 Taken together, and considering all the chromosome 1 QTLs, our strategy retrieve intervals that
201 correspond to 3743 potential candidate annotated genes, suitable for further researches. While
202 waiting for the establishment of the NILs population to precise the QTL, we studied our potential
203 candidate genes through additional dataset to isolate some interesting genes that could be tested
204 rapidly.

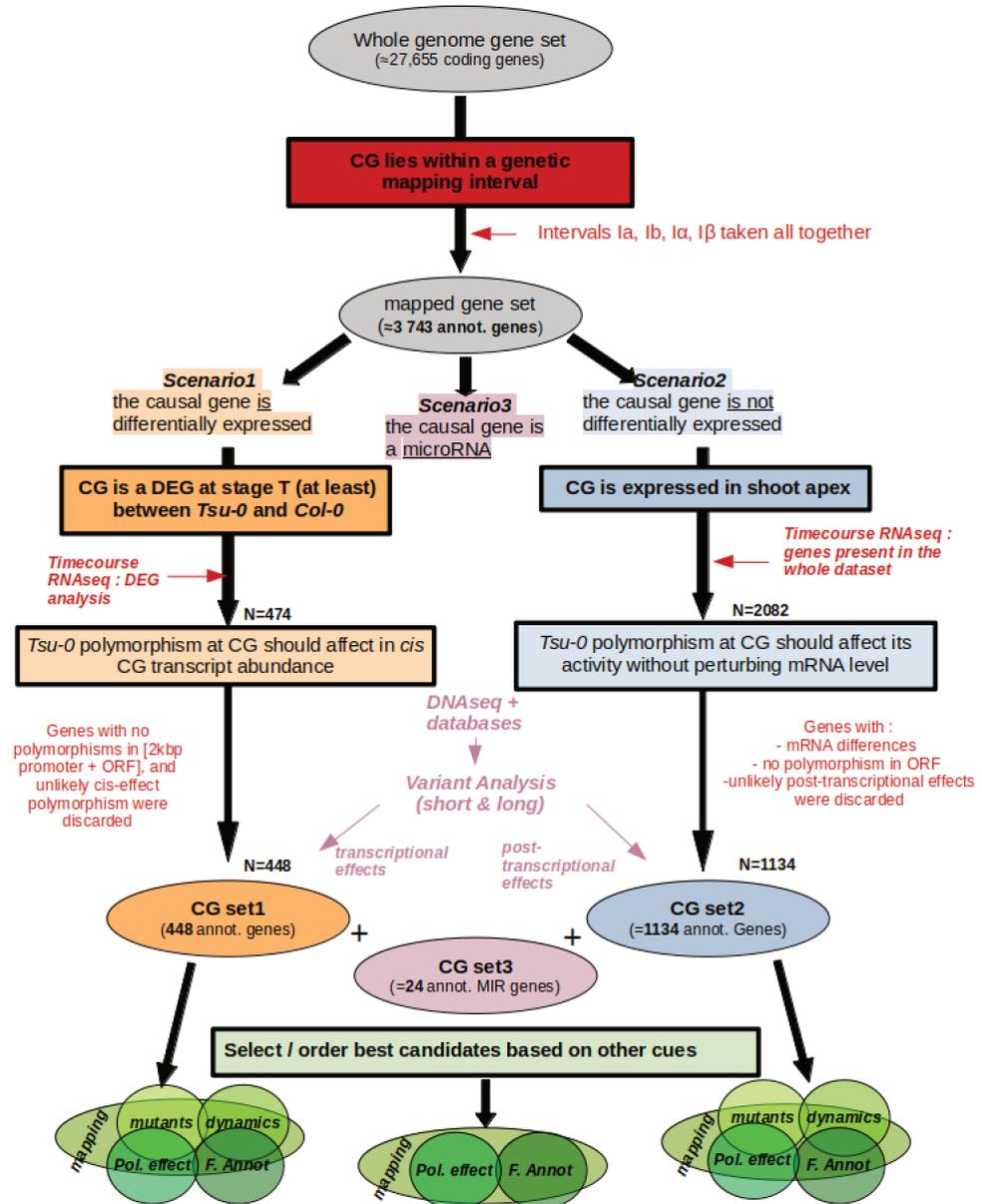
Looking for causal gene(s) (CG) of basal bracts in *Tsu-0* accession (*A. thaliana*)

Figure 6. Data mining strategy to investigate the candidates genes. All scenarii are based on the mapping regions, that comprise the α , Ia, Ib and Ib QTL. The first scenario (orange) explore the genes that are differentially expressed at floral transition between *Tsu-0* and *Col-0*, base on our previous RNA-sequencing results (see Chapter 1). Among the 474 genes that are differentially expressed, we discarded the one showing no polymorphism in their ORF and the 2kbp upstream. We also removed the 2 genes with polymorphism that are unlikely related to any cis-effect. The CG set 1 comprise then 448 genes that can be investigated based on other clues such as: the functional annotation, the dynamic across floral transition (see Chapter 1), the differential expression on the mutants, as well as the predicted effect of the mutation. The second scenario (blue) explore the genes that are not differentially expressed between the two different accessions according to our RNA-sequencing data. The polymorphism should then affect the ORF of the gene but not its transcript level. We also discarded the genes showing polymorphism with unlikely post-transcriptional effects. The CGset 2 leads then to a pool of 1134 genes, suitable for further investigations. The Third scenarii (majenta) explore the micro RNA located in the QTL region based on DNA sequencing data. Their transcriptional effect or post-transcriptional effect could affect one (several) of the CG set1 or CG set2 respectively. We identified 24 micro RNA in this region.

205 **Data-mining of candidate genes involved in bract formation by transcriptomics**

206 Coupling BSA and RILs phenotyping was not sufficient to reduce the interval to an individually
 207 testable number of candidate genes. We then aggregated to the genetic evidence other data that
 208 are the following: -genetic intervals from BSA (QTLSeqR software) and from RILs (*r/qtl2* software)
 209 -prediction of the functional impacts of natural genomic *Tsu-0* polymorphisms on every mapped
 210 genes
 211 -functional prediction of the mapped gene based on GO annotations
 212 -expression profiles of the mapped genes in *Tsu-0* and *Col-0* meristems before, during and after
 213 floral transition (see chapter 2)
 214 -expression profiles of the mapped genes in meristem of "bract-making" mutants
 215 -expression profiles of the mapped genes in published public dataset ((*Klepikova et al., 2016*)).

216
 217 We propose three different scenarii concerning the genes responsible for basal bracts in *Tsu-*
 218 *0* (hereafter called "bract-gene"). Depending on the scenario, the diverse data require to meet
 219 different expectations. We first assumed that bract-gene are located in chromosome 1, within one
 220 of the 4 QTLs described earlier.

221 **Scenario 1: bract-genes may be differentially expressed during the emergence of the first**
 222 **flower in *Tsu-0* compared to *Col-0***

223 We first hypothesized that a confident bract-gene candidate may be differentially expressed dur-
 224 ing the production of the first flower in *Tsu-0* compared to *Col-0*. We have previously carried a
 225 RNA-sequencing of *Tsu-0* and *Col-0* shoot apex across floral transition, and in particular during the
 226 formation of the first flower. We thus identified the genes located in the mapping region and dif-
 227 ferentially expressed during T stage, that corresponded to the emergence of the first flower. 474
 228 genes were retained. Among them, 448 genes bear polymorphism(s) that could explain their differ-
 229 ential expression level in *Tsu-0* genetic background and constitute Candidate Gene Set 1 (CGS1). We
 230 investigated the dynamics of those gene expression across floral transition as described in chapter
 231 2. 54 genes are dynamical during floral transition (their expression level varies across the 4 devel-
 232 opmental stages) that belong to a different dynamic group in *Col-0* compared to *Tsu-0*, including
 233 *NAC019* or *ASL9*. Interestingly, *ASL9* is also downregulated in *lfy-12* and *jag5-D* mutants, that are
 234 "bract-bearing" mutants. Expression pattern of *ASL9* is mainly located in plant hypocotyl, vascular
 235 tissues and mark boundary regions ((*Klepikova et al., 2016*)). Its homologous *ASL2* is known for its
 236 role in leaf development and polarity (*Machida et al., 2015*). Altogether, *ASL9* gather interesting
 237 characteristics and could constitute a good candidate bract-gene.

238 **Scenario 2: Genes responsible for bracts may have protein coding differences in *Tsu-0***

239 We also explored the hypothesis that bract gene(s) code for a protein which present amino acid
 240 differences in *Tsu-0*, inducing bract formation at its first flower. According to our transcriptomic
 241 dataset, 2082 genes are expressed in the SAM and located in the mapping region, but do not show
 242 differential expression with *Col-0* at T stage (contrary to GeneSet1). From this first set, 1134 genes
 243 bear genomic variation within the open reading frame (ORF) that could cause plausible effects on
 244 the gene (and/or protein) function (see Methods): we group these genes into the Candidate Gene
 245 Set 2 (CGS2). We focused on the genes presenting high impact coding variation (see Methods), that
 246 represented 110 genes. One of them is *ARF1*, a repressive transcription factor mediating the auxin
 247 transcriptional responses (*Tiwari et al. (2003)*), that present a splice donor variant at c.1163G>C,
 248 and thus could become an interesting candidate gene to test.

249 **Scenario 3: miRNA misregulation in *Tsu-0* during floral transition**

250 Because miRNA play a major role in floral transition, we next considered all the miRNA present
 251 in the mapping intervals. On the 24 miRNAs present in the mapping region, none of them have
 252 known function in floral transition or flower determinism. Still, they could highlight unexpected

253 roles in bract inhibition.

254 Discussion

255 In bractless species belonging to the *Brassicaceae* or *Poaceae* families, the formation of transient
 256 bracts offers the opportunity to study the genomic basis underlying the development of such atavic
 257 traits. Bracts have been described in a certain number of mutants in *A. thaliana* or maize but the
 258 mechanisms of bract inhibition and/or atavic reformation remain elusive. In these examples, the
 259 pleiotropic effects of mutated genes, which also affect flower development, preclude the study of
 260 how a wild type flower inhibit bract development in a normal context. Natural variation captures
 261 phenotypes that are often not uncovered by mutant screens. In *Tsu-0*, a bract develops on the
 262 first flower without affecting flower development. In that sense, this genetic background provides
 263 an example of a developmental variation that breaks pleiotropy and avoid deleterious additional
 264 effects. Such natural variation represents a plausible evolutionary path for a trait reversion and,
 265 by symmetry, for its loss.

266 In this chapter, we presented all the work performed in order to identify the genetic determi-
 267 nants that allow plants with a *Tsu-0* genotype to develop transient atavic bracts at the floral transi-
 268 tion. We showed that coupling BSA and RILs analysis allow to circumscribe the mapping region into
 269 several loci in chromosome 1 representing a total of 9Mb, but not to precisely identify bract-gene
 270 variations.

271 Contrary to mutant strains originated from laboratory mutagenesis, two random natural acces-
 272 sions bear a huge amount of genomic polymorphisms which hurdle the identification of the causal
 273 variations responsible for the phenotypic difference. This challenge is particularly important when
 274 it comes to subtle and quantitative phenotypes, like bract mean frequency. Here, we combined
 275 two genomic approaches, BSA and RILs, to map bract gene(s). The BSA allows to first determine
 276 the genetic architecture of the trait and estimate the mapping interval(s). Because of their length,
 277 we used a set of RILs to fine map the previous intervals and reduce the amount of candidate genes.
 278 By coupling both approaches, we mapped bract genes into at most a 9Mb intervals spread into
 279 several smaller QTLs within chromosome 1, but failed to shorten them. We noticed a major dis-
 280 crepancy between BSA and RIL approaches. While both methods pinpoint 2 loci in chromosome
 281 1 (although with different coordinates), the BSA outputs a 17-Mb signal in chromosome 5, which
 282 is absent from RIL analysis. Several hypotheses could explain this. First, some experimental de-
 283 tails may have biased our BSA results. Indeed, we took less than 20 F2 for the *Tsu-like* pool, when
 284 generally BSA mapping strategies relies on at least 40 F2 individuals per pools (*James et al., 2013*).
 285 Pooling a low number of individuals increases the risk of selecting confounding false positive un-
 286 related phenotypes. While a stringent pool selection minimizes the chance to have heterozygotes
 287 in *Tsu-like* bulks, it might reduce the precision of the mapping. On the contrary, the *Col-like* bulk
 288 contains individuals with no bracts at all: it may includes heterozygous as many F1 individuals had
 289 no bracts. Finally, because of technical issues at the DNA extraction step, we could not ensure pre-
 290 cisely an equal amount of DNA for each individuals (see methods), that may add some inaccuracies.
 291 On the other hand, the RIL approach may also have one caveat: the RIL set we used segregates a
 292 mutation in the beginning of chromosome 5, derived from the parental *Col-0* strain used to gener-
 293 ate the RILs. The mutated gene, *SG1* (*SHOOTGROWTH1*) (*Coustham et al., 2014*), resulted in drastic
 294 shoot growth phenotypes, not suitable for bract counting. We thus needed to discard all the RILs
 295 with *Col-0* genotype between markers c5_02900 and c5_04011 to avoid the mutated *sg1* allele. This
 296 restricted the choice of possible RILs that recombined into the Va QTL identified with the BSA. How-
 297 ever, we still were able to investigate RILs bearing *Tsu-0* haplotype between markers c5_02900 and
 298 c5_04011, that were not linked to an increase in bract levels. Hence, bract phenotype was mostly
 299 explained by the genotype in chromosome 1, rather than in chromosome 5. However, we cannot
 300 exclude that Chromosome 5 contains epistatic variations with minor effect that increase the bract
 301 mean frequency in *Tsu-0*. In fact, our *Tsu-like* bulk displayed a high bract mean frequency. It is
 302 possible that F2 plants in this "high" bulk were selected on both chromosomes: the major effect of

303 the variation in chromosome 1, and minor effect a the variation in chromosome 5. We also have
304 shown that bract mean frequency and floral transition were linked, and the QTL in chromosome 5
305 could contain flowering time genes that could indirectly promote the presence of bract. The exis-
306 tence of (one or several) QTLs for transient bract formation on chromosome 5 clearly needs more
307 experiments to be settled down. However, our genetic data demonstrates the polygenic determin-
308 ism of the trait and the major role played by chromosome 1 that most likely bears two QTLs. While
309 both BSA and RILs approaches captured two QTLs within chromosome 1, their coordinates were
310 not overlapping: the two BSA QTL (coined Ia and Ib) are located in-between the two RIL's QTLs ($I\alpha$
311 and $I\beta$).

312 To better map the gene, we are now trying to generate NILs. We look for recombination events
313 to shorten the intervals while limiting any epistatic relation elsewhere. Such NILs can give precision
314 on which of the QTLs identified are relevant. We planned to establish lines that are *Col-0*, *Tsu-0*
315 or heterozygotes at the loci $I\alpha$, Ia, Ib, and $I\beta$. Other genomic approaches can be used to better
316 map the bract genes. As bracts are common in natural accession of *A. thaliana*, it is suitable for
317 GWAS studies. GWAS approaches can help to identify common variations linked to bract formation
318 in a wild range of natural accessions. It can also identify other variations that participate in the
319 establishment of basal bracts, and how much each of these genomic variants contribute to the
320 frequency of the trait at the species level. Notably, it can be interesting to investigate to what extend
321 bracts can be linked with other traits (cauline branch number, flowering time, flower indeterminacy
322 or shifted bracts) as is was observed in some genotypes.

323 In order to better investigate the candidate genes contained in the current interval, we used a
324 transcriptomic analysis of the SAM across floral transition in both natural accessions and used it to
325 explore the set of mapped genes. We also used a transcriptomic analysis of three bract-bearing mu-
326 tants, and online datasets such as expression profile or GO terms. We established three different
327 hypothesis to guide our data mining across the transcriptional dataset. The first was to hypothe-
328 size that bract-gene is differentially expressed during T stage in *Tsu-0* versus *Col-0*. This leads to 2
329 main interesting genes: *ASL9* and *NAC019*. The second hypothesis was to consider that the causal
330 polymorphism would affect only post-transcriptional steps in *Tsu-0*: *ARF1* is such a potential can-
331 didate gene. Finally, we investigated the miRNA lying in the QTL, but we did not retain interesting
332 candidate gene. However, none of the clues we used to better characterize the candidate genes
333 corresponding to each scenario are necessary nor sufficient to identify the exact gene responsi-
334 ble for bract emergence in *Tsu-0*. First, most of the variations have unclear functional effects: for
335 example, it is very hard to predict the real effect of SNP or short indels lying in the promoter of
336 regulating sequence of a coding gene. It must also be noted that we have not looked specifically for
337 large structural variations and that we might have missed such genomic variations in our mapped
338 intervals. Such analysis is now undergoing. A last caveat of variant prediction in natural accessions
339 is a frequent rate of false positive functional impacts, due to the fact that the reference genome and
340 annotation has been optimized on one particular accession ((*Gan et al., 2011*)). Second, available
341 expression pattern data (*Klepikova et al., 2016*) do not concern the precise localization in time and
342 space within the meristem, so it is difficult to target the domain of cryptic bract formation. Finally,
343 *Tsu-0* transcriptome present a number of genes with a different dynamical group than *Col-0*: very
344 likely, most of these genes have nothing to do with bract emergence. Hence, the clues are indica-
345 tors for further explorations, rather than definite proofs for the candidate genes. Moreover, this
346 data mining approach is certainly biased by our literature knowledge. In fact, among number of
347 genes responding to each scenarii, we picked the ones that seemed interesting candidate *for us*. As
348 developmental biologists, we considered genes involved in the developmental processes we know
349 the best. However, number of mutants developmental phenotypes can results from unexpected
350 genetic pathways. Finally, more than a third of the genes contains in our interval have unknown
351 functions. It is highly possible that the causal *Tsu-0* variation we look for affects such an unknown
352 gene. It is thus necessary to first reduce at maximum the number of potential candidate genes, so
353 the length of the QTLs.

354 The transcriptomic data allowed us to explore the genes within the mapping region with a read-
 355 ing criterion. However, it did not allow to unbiasedly detect the candidate gene responsible for
 356 bracts in *Tsu-0*, that would need further analysis. Taken together, the data mining approach we
 357 used allows only to highlight some interesting candidates that could deserve more attention. For
 358 example, a rapid confirmation of the importance of a candidate variation can be investigated by
 359 phenotyping natural accessions possessing the same variation. This work is ongoing.

360 We identified several QTLs that may be linked with basal bract formation in *Tsu-0*. The identifi-
 361 cation of the precise gene(s) controlling this process will need more investigations. In fact, several
 362 characteristics of this trait may explain this challenge: the phenotype is subtle (a few cells in a
 363 restricted time-window) harnessing its direct targeting (microscopy, RNAseq), it is also polygenic
 364 (potentially several genes with single limited effect) and a bit stochastic (high variability in an iso-
 365 genic population grown in the same culture condition). In addition, the drawback of working with
 366 natural variation is the high load of polymorphisms, increasing the number of potential candidate
 367 genomic variations even in small genetic intervals. Adding transcriptomic data did not bypass this
 368 challenge. Hopefully, we can make a better use of our RNAseq data using more elaborated analysis
 369 to detect heterochronic shifts and infer modification in gene networks linked to functional develop-
 370 mental modules (see chapter 2). But this chapter illustrates how key is the initial precision of the
 371 mapping, to concentrate the effort of cross-data mining on a smaller number of candidates. To
 372 this end, we are currently establishing NILs populations to reduce the length of the QTLs.

373 This study highlight the remaining challenge to identify natural variations responsible for subtle
 374 phenotypes. Nevertheless, tackling the challenge will be extremely useful to benefits all the oppor-
 375 tunities of natural variations in developmental and evolutionary biology.

376 Authors contributions

377 S.D. carried out all the experiments and the analysis. S.H. carried out the genotyping leading to the
 378 establishment of the NIL populations. S.D. and F.B. designed the experiments and the computer
 379 analysis and interpreted the results. S.D. wrote the first draft of the manuscript, and F.B. helped
 380 edit it.

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 387 in Overleaf, using the eLife free template.

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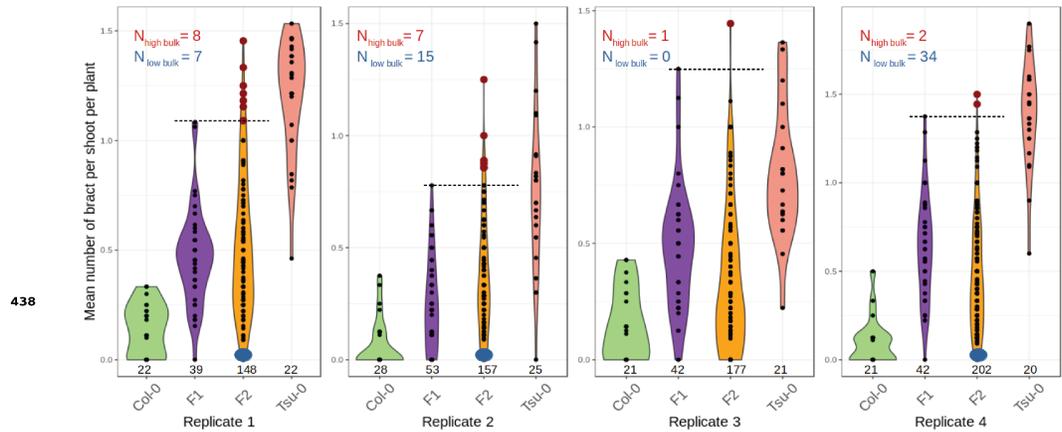


Figure 1-Figure supplement 1. Bract mean frequency on each branch are reported in parental, F1 and F2 lines for the four replicates separately. Individuals bulked in the *Tsu-like* group are highlighted with red dots (17 individuals). Individuals bulked in the *Col-like* group are plants with zero bracts for replicate 1, 2 and 4 (56 individuals). The detailed size of each bulks are specified in each graph.

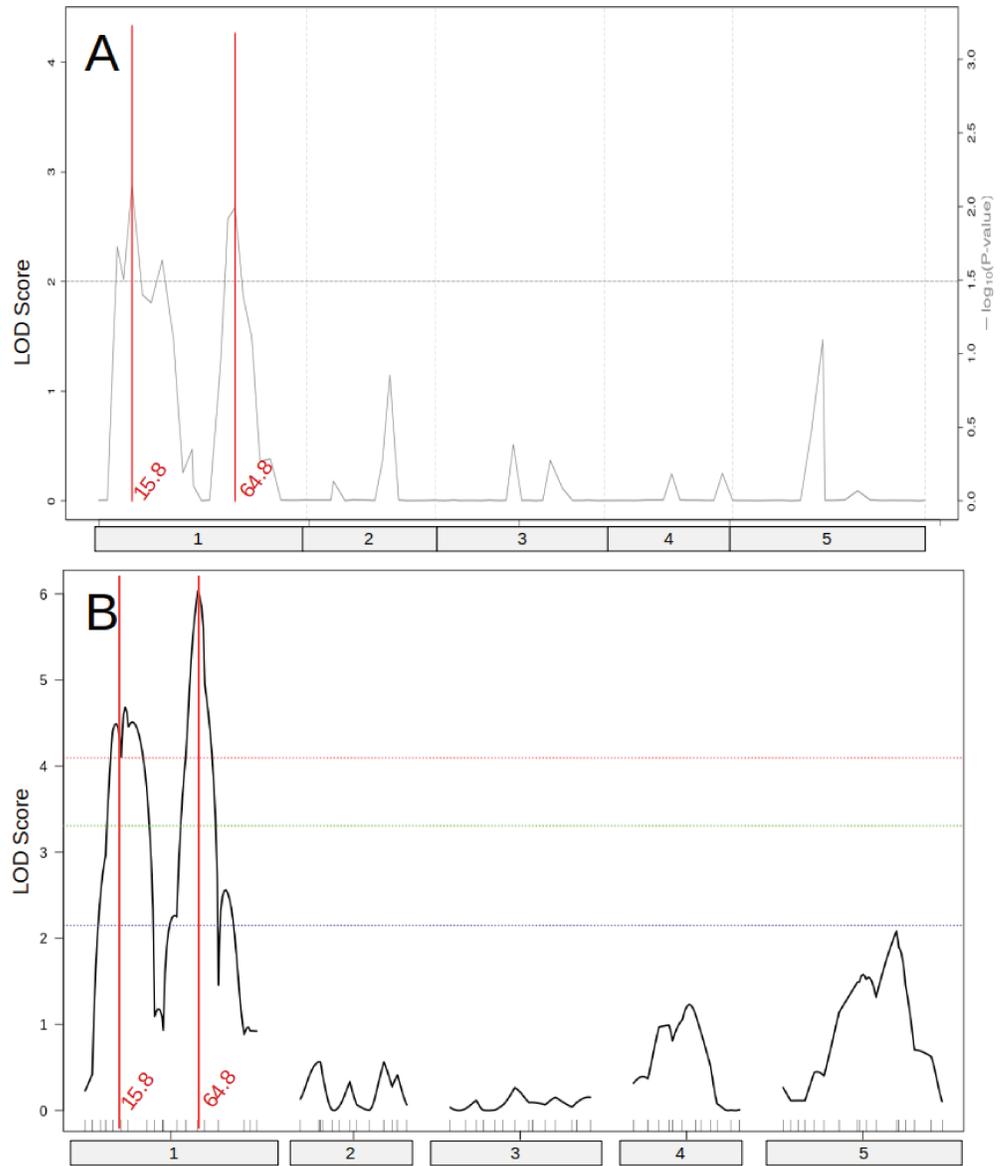


Figure 2-Figure supplement 1. A) QTL identification using gCIM(Zhang *et al.*, 2020). LOD score associated with each markers show two peaks I_{α} at 5,6 Mb, and I_{β} at 22,2 Mb. B) QTL identification using R/QTL (Broman *et al.*, 2019). The same two peaks are identified.

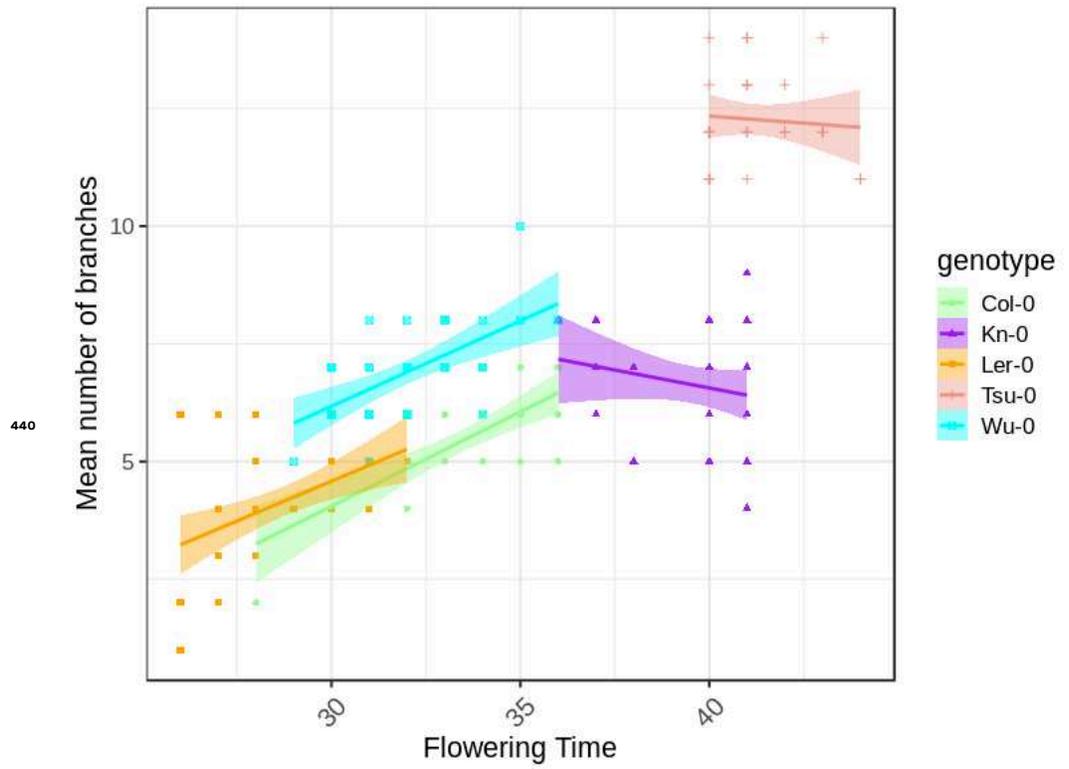


Figure 3-Figure supplement 1. Relation between the flowering time and the number of branches in a set of RILs, *Tsu-0* and *Col-0*. Globally, the number of branches increase with flowering time

METHODS AND MATERIALS

1 Plant material and culture condition

1.1 Culture condition

Seeds were sown on peaty-clay soil (clay: 60kg.m⁻³), stratified at 4°C for two days, and watered with fertiliser (18-10-18 N-P-K) under LED lighting (Valoya©, sunlight spectrum NS12, 150 µmol.m⁻².s⁻¹). Three different day/night regimes were used in the experiments: short-days (SD) with 8h light and 16h dark; long-days (LD) with 16h light and 8h dark and continuous light (CL) with 24h light. Temperature and humidity are controlled as follows: 22°C and 60% humidity during light for LD and CL conditions, and 18°C and 70% humidity constantly in SD and during nighttime in LD. Seedlings of 1 to 2 weeks were transplanted in individual pots. For the Bulk Segregant Analysis, plants have been cultured 20 days in SD before switching to LD. For the RILs, plants have been directly cultured in LD.

1.2 Plant materials

The natural accessions came from the Versailles *Arabidopsis* Stock Centre (*Col-0* : 186AV, *Tsu-0* : 91AV), as were the RILs (RIL set name: 3RV). F1 and F2 plants used for the BSA were generated by crossing the parental lines *Tsu-0* and *Col-0* on both sides. *ufo-1* seeds came from the Nottingham *Arabidopsis* Stock Centre (<http://arabidopsis.info>) (N16361, *Col-2* background), *bop-1*, *bop-2*, and *puchi-1*, *bop-1*, *bop-2* were published in Karim *et al.* 2009 (*Col-0* background), *lfy-12* was published in Maizel & Weigel 2004 (*Col-0* background). *Tarenaya hassleriana* seeds were kindly provided by Pr Eric Schranz and Frank Becker, from Wageningen University (WUR, Holland). *Allaria petiolata* were found in the wild in Massy.

1.3 pLFY::2mCherry-N7 construction and plant transformation

Reporter lines pLFY::2mCherry-N7 (Chapter 2) were generated by first producing a T-DNA transgene using the multisite Gateway cloning technology (Thermo Fisher Scientific). As no polymorphism has been detected in pLFY sequence between the two accessions, *LFY* promoter was amplified by PCR from *Col-0* DNA at -2277pb from the I ATG site using 5'GGGGACAACTTTGTATAGAAAAGTTGATCCATTTTTTCGCAAAGG and 5'GGGGACTGCTTTTTTTGTACAAACTTGAATCTATTTTTTCTCTCTCTCTATC primers. PCR fragments were purified and inserted into pDONR P4-P1R. This plasmid was then recombined in a three-fragment gateway reaction with with 2mCherry pDONR211 (containing double mCherry coding sequences), N7-tag pDONR P2R-P3 (containing the nuclear tag N7 and a stop codon) and the destination vector pK7m34GW (containing kanamycin resistance gene for *in planta* selection), resulting in the final pLFY::2mCherry-N7 construct in *E. Coli* DH5 α strain.

The construct was then transformed into *Agrobacterium tumefaciens* C58pMP90 strain by electroporation and then transformed into both *Col-0* and *Tsu-0* plants by floral dip method (Clough & Bent 1998).

2 Meristem dissection and imaging

2.1 Meristem dissection

Meristems were dissected from *A. thaliana* plants after 20 days of synchronization in SD conditions, at different times after transfer to LD depending on the experiment. Dissections have all been performed from 9:00 a.m and noon, by alternating between *Tsu-0* and *Col-0*. When dissected after bolting (for pictures in Chapter 1, Fig. 3B, and Chapter 2, Fig. 3), plant stems were cut at the base of the inflorescence and put in 2% agarose medium plates: flowers were removed with thin tweezers under a binocular loupe (Leica M125). When dissected from the rosette, before bolting, plants were completely removed from the soil, washed carefully with water, and big leaves were removed with thin tweezers. Then, remaining rosette axis and roots were carefully planted in a 5% agarose medium, and the smallest leaves were removed

with syringes (23G, 0.6x25mm), under the binocular loupe. In both cases, after dissection, plants were immediately put on an Apex Culture Medium (1/2 MS medium supplemented with 1% sucrose, 0.8% agarose, 1x vitamin solution (myo-Inositol 100mg/L, nicotinic acid 1mg/L, pyridoxine hydrochloride 1mg/L, thiamine hydrochloride 10mg/L, glycine 2mg/L) and 100nM N6-Benzyladenine (BAP)) waiting for imaging.

2.2 Microscopic meristem imaging

Meristems were imaged using a Scanning Electron Microscope (Hirox 3000 SEM), or with a confocal microscope Zeiss 700 LSM, according to the manufacturers instruction and without prior fixation. For plants expressing pLFY::2mCherry-N7, imaging, meristem cell walls have been stained with 100 µg/ml propidium iodide solution.

3 Plants phenotyping

3.1 Macroscopic imaging

Macroscopic pictures in Chapter 1, Fig.1 H, I were taken using the Keyence VHX-900F according to the manufacturer's instructions. Pictures in Chapter 1, Fig. A, C, D, F and G were taken by the Canon EOS 450D camera, according to the manufacturer instructions. The camera device of a Samsung Galaxy A10 was used to take the pictures in Chapter 1, Fig.1 B and E.

3.2 Bract quantification and flowering time measurement

Bract mean frequency was defined as the mean number of bracts per shoot per plant in the part derived from the main shoot (so we excluded secondary rosette branches). Practically, we counted the number of bracts visible to the naked eye on the main stem and the secondary cauline branches. To ensure that bracts were visible, phenotyping was performed after flowers of the last cauline branch were opened and internodes elongated.

We also used two other metrics to quantify the presence of bracts in genetic lines: bract median frequency was defined as the median number of bracts per shoot per plant, and the

percentage of branch with bract was defined as the average percentage of branch presenting at least one bract, per plant. Flowering time was measured in number of days after transferring plants in growth chambers when the first flower blooms (open petals).

4 DNA extraction and sequencing for Bulk Segregant Analysis (BSA)

The mapping population has been generated by crossing (*Col-0* x *Tsu-0*) at both sides. As no difference in bract frequency has been observed (data not shown), F1 plants coming from both side of the cross have been used. For practical reasons, the bulk segregant analysis have been split into four replicates. After stratification at 4°C for 2-3 days, plants have been grown 20 days in SD, then transferred in LD. A 1cm² leaf have been sampled for each F2 individuals at around 10 leaves rosette stage, and kept at -20°C for DNA extraction when the plant was selected in one of the bulk. To re-sequence the genome of the parental lines, DNA was extracted from a bulk of *Tsu-0* and *Col-0* seedlings respectively. All DNA was extracted and purified using a CTAB-based protocol (cetyl trimethylammonium bromide), following instructions as in Healey *et al.* 2014. To prepare each bulks, purified DNA from individual samples were then pooled in order to limit the variation of representation of each plants in the bulk sequencing. The final concentration was between 13 and 25 ng/μl. Pooled DNA bulks and parental DNA were then sent to BGI for library preparation and sequencing on BGISEQ-500WGS sequencing system to generate 5 Go data of 100bp paired-end reads per library (target coverage of 40X).

5 Time course RNA-sequencing over flowering

5.1 Tissue sampling and RNA extraction

Tsu-0 and *Col-0* meristems were dissected every day in LD condition after 20 days in SD, in order to capture the right developmental stages. The mutants *lfy-12*, *puchi1*, *bop1*, *bop2*, and *jag5-D* meristems have been dissected every days starting from 1 week after LD transfer, because only the F stage was targeted. Three different independent replicates have been used.

The number of meristems in each replicates can be find in the Appendix.

Morphological stages have been first established on a blank experiment, where *Col-0* and *Tsu-0* were dissected every days to monitor morphological clues happening before floral transition. The V stage has been defined as the starting stage, and dissected at the days plants were transferred to LD, so that plants have not experienced any LD condition at the time of the dissection. The L stage was sampled the same day for both *Col-0* and *Tsu-0* as they had the same meristem shape. The T stage has been defined as the stage in which the first flower emerges (the emergence of a round (rather that triangular) primordium can be guessed), and the emergence of axillary meristem starts to become visible at the axils of young leaf primordia. The F stage has been defined as the stage in which the flowers are clearly recognizable, with the differentiation of the first whorls on the first flower. For the mutants, the F stage was defined when several rounded primordia becomes visible at the SAM (that will become branch-like flowers, or only branch). Note that for the *leafy* mutant, homozygous *lfy-12* plants were easily distinguishable from a WT SAM (like in Chapter 1, fig.3C).

Meristem dissections were performed as described previously, with the difference that as soon as the meristems were dissected, they were put on micro tubes corresponding to the right stage into liquid nitrogen and stored at -80°C until RNA extraction. The speed of meristem dissection have been optimized to avoid the induction of stress-related gene expression: meristem dissection did not exceed 3min between the first organ removal and liquid nitrogen disposal. Meristem tissues were grounded with a RockyII tissue lyser according to the manufacturer's instructions. RNA extraction of each sample was performed with the PicoPure RNA Isolation Kit (Arcturus, Catalog KIT0202) according to the protocol. The final RNA concentration of each samples can be found in the Appendix. RNA concentration ranged from 3 to 64 ng/ μ l (average 23ng/ μ l), with a RIN value comprised between 5,6 and 7,6 (average 6,8).

5.2 RNA-sequencing

The RNA sequencing was performed by Helixio company. Library preparation was made using NEBNext® Ultra™ II Directional RNA Library Prep Kit for Illumina (New England Biolabs); NEBNext® Poly(A) mRNA Magnetic Isolation Module (New England Biolabs); and NEBNext® Multiplex Oligos for Illumina (Index Primers Sets 1, 2 et 3), from 40ng of ARN, in

order to obtain amplified cDNA. cDNA was sequenced using NextSeq500 from Illumina. Sequence quality was controlled using the Sequencing Analysis Viewer and sequences that did not pass the quality filter were removed. Remaining reads were on average 43 million sequences per sample.

6 Bio-informatic analysis

6.1 Computing environment

R (v3+ or v4+) (R Core Team 2013) was used for several bio-informatic analysis using specific R libraries and packages, as described below. Most plots were drawn using ggplot2 package (Wickham 2016).

6.2 DNA sequencing analysis and genomic variant analysis

Sequencing data consist in two parental plus two bulks of sequencing data. A genomic variant analysis was performed on each dataset following the workflow of short variant discovery previously described in Besnard *et al.* 2020, using the same software, version and parameters. This resulted in four gVCF files (one per sample) generated by the HaplotypeCaller tool of GATK (v3.8, McKenna et al., 2010). The reference genome of *Arabidopsis thaliana* used for read mapping is TAIR10 (*ENA Browser* 2021). Then, the two parental gVCF were first joint-genotyped using GATK's tool GenotypeGVCFs to emit a common vcf file for the two samples. This file was used to select a list of specific SNPs and small indels of *Tsu-0* (91AV stock) versus *Col-0* (186AV stock), filtering for positions with coverage metric $DP > 10$. This reference list of *Tsu-0* polymorphisms was then used as the `-dbsnp` option in a second pass of joint genotyping using all four gVCF as input (two parental samples plus the two bulks) to emit a common vcf file. Finally, relevant polymorphic positions from the reference list in the two bulks were selected after filtering for a depth ≥ 3 , and the resulting single vcf was converted to a table file (GATK's tool VariantsToTable), giving for each polymorphic site the global depth and the precise depth of each parental allele in the four samples. Subsequent analysis of read ratio for BSA was performed using QTLseqr (see below)

6.3 QTL identification from Bulk Segregant Analysis using QTLseqR

QTL identification were carried using the QTLseqr package (Mansfeld & Grumet 2018) according to the instructions. After filtering data with the following parameters (refAlleleFreq = 0, minTotalDepth= 10, maxTotalDepth = 90, minSampleDepth = 10, minGQ= 99, depthDifference = 15), the deltaSNP index (Takagi *et al.* 2013) was generated and loci that reached the 95% confidence interval were retained and formed the Ia, Ib, Va and Vb QTLs.

6.4 QTL identification using Recombinant Inbred Lines analysis

RILs *Col-0* x *Tsu-0*, F7 generation, were publicly available in the Versailles Stock Centre (<http://publiclines.versailles.inra.fr/rils/index>), as well as their genotyping data for 79 markers throughout the genome (including 19 within chromosome 1). The RILs analyzed were initially selected depending on their known genotypes within chromosome 1 and 5 using GGT 2.0 software (van Berloo 2008). The detailed genotypes of each line are available in supplemental data. We counted their bract frequency as described above. The correlation between genotype and phenotype was done using R/qtl software according to the instructions Broman *et al.* 2019, with the following modifications on parameters: number of permutations was set up at 1000; for all the tests, error prob was set at 1×10^{-5} . We also used gCIM software Zhang *et al.* 2020 to compare the two approaches. For the gCIM analysis, the following parameters were used: analysis was performed in the RIL mode, using random effects and a walk speed of 6 cM. Interaction between bract mean and percentage of plants with at least a bract on the main stem were assessed with R/qtl using MQM models according to the instructions.

6.5 RNA-sequencing analysis

Sequenced reads were filtered based on their quality using Trimmomatic software (**Trimmomatic**). The percentage of reads that did not satisfy the filter criteria is less than 4% for the thirty-three samples. Filtered reads were then aligned to the reference genome (TAIR10) using STAR (Dobin *et al.* 2013) with the following parameters: non canonical splice junctions were removed, multi-mapping reads was limited to 10, and only reads mapped once were considered to determine splicing junctions. A post-alignment quality check was performed, assessing the

percentage of uniquely mapped reads: for the thirty three samples, 88 to 97% of the reads were mapped to a simple locus. Altogether, these quality checks showed that the sequencing and the alignment were of very good quality. Raw reads quantification was performed using the reference transcriptome (*ENA Browser* 2021) and the STAR software (Dobin *et al.* 2013). More than 80% of the reads were affected. Normalization of read counting was performed using the R Bioconductor packages DESeq (Love *et al.* 2014), with the following parameters: genes for which the total number of reads was below 10 were discarded, and data were transformed with Variant Stabilizing Transformation (VST) function (Anders & Huber 2010), to harmonize the variance. To check the consistency between biological replicates, a Principal Component Analysis on all samples with VST transformed data was generated using the same package. Biological triplicates of all conditions were very close to each other, allowing relevant comparisons per developmental stages and genotypes.

Differential analysis was made using the R Bioconductor package edgeR (McCarthy *et al.* 2012). Reads were first normalized using TMM (Trimmed mean of M-values) to reduce library-specific biases. Normalization factors were between 0.94 and 1.049 in our samples, showing that few genes were over represented in one sample compared to another. A generalized linear model was applied for the analysis of differentially expressed genes (DEG). 2 types of DEG were performed in this study: DEG at each stage between the different genotypes, and DEG for *Col-0* and *Tsu-0* between the different stages (see results - Chapter 2). Multiple DEG analysis were corrected using Benjamin-Hochberg correction, and genes with a p-value < 0.5 were retained.

6.6 Analyzing and comparing the global dynamics of the transcriptomes

From the matrices of DEG between developmental stages in both *Col-0* and *Tsu-0*, we first computed the set of "dynamical genes" (DynG), defined as the union of genes differentially expressed in at least one stage out of four in both accessions. PCA on the DynG was performed and visualized using FactoMineR (Lê *et al.* 2008) and factoextra (Kassambara & Mundt 2017) on R. PCA using the set of DynG produces similar sample separations as using all genes, in-

dicating that most the meaningful variations of the dataset is driven by these DynG. We then restricted our next analysis to this subset. To group genes with similar dynamics irrespective of their absolute read number in the data (because differences in CPM values between genes are not functionally relevant), we choose to use a k-mean clustering on vegetative-stage normalized CPM values. To normalize CPM values in each accession, we subtracted for each gene at each stage the expression value at stage V (thus, normalized expression at stage V is 0 for all genes). K-mean clustering was performed using `kmeans` function from the 'stats' R core package with the following parameters: `centers=3` and `nstart=100`. The number of clusters ('centers') was estimated from heuristic exploration of visual plots (drawn with 'fviz-cluster') and mathematical estimation using 'fviz-nbclust' and 'fviz-gap-stat' functions (last three functions from `factoextra` package). We used `agriGO` (v2.0, Tian *et al.* 2017) to detect Go term enrichments in each of these clusters with the Singular Enrichment Analysis (SEA) tool against the appropriate pre-calculated background (Arabidopsis genemodel TAIR9) and automatic default parameters (Fisher or Hypergeometric test, Yekutielli adjustment, significance level=0.05, minimum number of mapping entries=5).

6.7 Gene selection inside genetic mapping intervals

The R library 'GenomicFeatures' (Lawrence *et al.* 2013) was used to import gene information from the most recent annotation file of *A. thaliana* at the gff format (Cheng *et al.* 2017). Custom R scripts were used to intersect all gene loci falling within genetic mapping data. For each gene, custom scripts were used to import the results from the RNA-seq analysis (differential expression, dynamics, etc.) in a single table. In addition, we aggregated data on polymorphisms discovered from the variant analysis described above. We used `snpEff` (v5.0d, Cingolani *et al.* 2012) to predict the functional impacts of each of the genomic variant in the genetic mapping intervals. We next classified the different predicted impacts based on the variant ontology term attributed by `snpEff`, to define different categories (see classification of these ontology terms in the Appendix): all variations, variations with a putative functional effects that can be further split into HIGH/MODERATE/LOW/MODIFIER effects. Using custom scripts, we summed up the number of variations of different categories in the ORF and promoter (2 kbp upstream of the transcriptional start site) of each gene. For miRNA genes, we also consider

variations falling 500 bp downstream the ORF. Additional gene information (Aliases, names, curator summary and computational description) was retrieved from ThaleMine (Pasha *et al.* 2020) using AGI code. Finally, associated GO terms (including compartment, function and process) were also retrieved with AGI code using TAIR bulk data retrieval tool (*TAIR - Bulk Download - GO* 2021) and formatted in the table using custom script. The three different scenarii for causal gene identification (see chapter 3) resulted in three different files listing the relevant genes corresponding to each scenario in the genetic mapping intervals. The different information fields gathered per gene were used in a table file to further filter the most interesting candidates. These three files are provided in the appendix.

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DISCUSSION

"Les mots ne désignent pas, ils questionnent. A chacun de nous, chaque mot, chaque fois, redemande son sens. Et nous lui donnons chaque fois un sens nouveau"

Jean-René Huguenin

Bract designates a leaf that subtends a flower. It is also often used in a broader sense to define any leaf appearing in reproductive parts of the plant: flowers or inflorescence, as it is the case for Poaceae. In this manuscript, we are only using bracts in its *stricto sensu* sense, *i.e.*, as the leaf subtending the flower. Still, studies on bract loss in Poaceae are interesting regarding the mechanisms that control the suppression of leaf development (whatever organ this leaf is subtending). Because the basic composition of the phytomer is extremely well conserved among plant kingdom, addressing bract loss brings insights on both plant development and architecture evolution.

1 Natural population to study plant development

1.1 Advantages and drawbacks of natural variation studies

Many modifications of plant architecture roots at variations affecting the phytomer. Such variations can be observed in laboratory-generated mutants or in natural populations. Mutant screens are able to reveal severe phenotypes that could certainly not be maintained in natural conditions. The strong impact of the EMS mutagenesis often generates null alleles of genes, offering a direct way to investigate their function. Moreover, mutant approaches allow to focus on a single gene, avoiding uncontrolled epistasy except for carefully chosen other genes in controlled crosses.

On the other hand, natural populations result from several genetic variations, that together enrich the pool of phenotypic variations. So far, bract emergence with absence of floral defects have only been observed in natural population. In addition, natural variations are by definition viable mutations. Because they are present in naturally occurring *Arabidopsis thaliana* plants around the world, they can participate to species diversification. Hence, natural variations can facilitate the access to a plausible evolutionary path leading to architecture evolution, shedding light on its underlying genetic basis. It constitutes a good step before questioning bract loss at a larger evolutionary scale, between two distantly related species. Inhibition of bract constitutes an interesting step in the evolution of Brassicaceae's architecture, because they affect the composition of the well conserved phytomer. Still, presence of basal bract in natural populations have been observed from the early 90's, but affordable sequencing tools were missing to deeper explore the genetic basis of such phenotypes. Now, both the emergence of next generation sequencing and the availability of many seeds derived from natural population crosses, allow us to explore the GRN of basal bract emergence. Besides, natural variations can be used to precise the new functions of genes, as it has been the case for FRIGIDA (Zhang & Jiménez-Gómez 2020). Basal bract phenotype may be linked with genes that have not been studied yet, and can thus reveal, or precise, some genes functions. Natural populations make the perfect link between classical genetics and evolutionary studies. Their use constitute then a fantastic tool to question evolutionary developmental biology. To this end, we used the natural accession *Tsu-0*, that present frequent basal bract formation, to map their underlying genetic determinant(s).

However, catching the exact genetic divergence(s) that is (are) responsible for basal bract formation appeared quite challenging. First, because bract frequency is gradual and varies extensively even among a same batch of isogenic plants grown in the same conditions: *Tsu-0* plants can present no bract per branch as well as 2 bracts per branch. Second, because the trait is polygenic, which increases the quantitative spread of the trait value in crossing panels: for example, the F2 distribution spreads in between the two parental lines without any visible subclasses. Third, because the phenotype is subtle, and could be triggered by any genetic variation (affecting regulatory sequences, as well as protein coding sequences), contrary to strong impact mutation easily detectable in mutants. Because of the large QTL obtained

by BSA and RILs approaches, and because of the numerous genomic variations differentiating *Tsu-0* from *Col-0*, it was hardly possible to come up with a single confident gene name. To face this challenge, we are considering different strategies. We are currently establishing HIFs from the heterozygous RILs that will allow to isolate one of the targeted QTL, as the genotype of the outside region will be identical. We hope this could not only precise the implication of each QTLs, but also produce recombinants that reduce the length of the QTL. Complementary, and because basal bracts are common in natural populations, we consider studying a broad sample of natural accessions to carry a GWAS study. This approach is both complementary to confirm at a larger extend the implication of a QTL, but can also spots new QTLs involved in basal bracts in other populations. GWAS study can thus address the convergence or divergence of basal bract phenotype within the scope of natural populations of *A. thaliana*.

1.2 Using both mutants and natural variations approaches to question evolution and development

To complement natural population approaches, we used mutants bearing bracts to help understand basal bract emergence in *Tsu-0*. Several pathways have been involved in the lack of bract inhibition in both *A. thaliana* and Poaceae. Interestingly, two main genetic pathways can be retained: floral identity genes pathways represented by mutants like *lfy-12* (Weigel & Nilsson 1995), *ufo-1* (Hepworth *et al.* 2006), *puchi-1* (Karim *et al.* 2009), *bop1*, *bop2* (Hepworth 2005), *jagged5-D* (Dinneny 2004; Ohno 2004), *agl6* OE (Koo *et al.* 2010); and floral transition genetic pathways represented by mutants such as *agl24*, *svp*, *soc1* (Liu, Chen, *et al.* 2008), *ft* (Müller-Xing *et al.* 2014), *SNB* in rice (D.-Y. Lee & An 2012), and *tsh4* in maize (Chuck *et al.* 2010) (see Introduction).

In *Tsu-0*, the absence of any floral defects, as well as the climax of gene expression divergence at T stage, suggest a floral transition-related genetic pathway. Hence the SPL genes can be interesting to investigate. Notably, *SPL10* is located between the QTL I α and the QTL Ia, and present a deletion in its promoter, and two SNP in the 5'UTR and 3'UTR regions. *MIR157D* is also located between the QTL Ib and I β , and present SNP on its coding sequence (but not the conserved target binding region). Plus, *SPL10* is over expressed at T stage in *Tsu-0*, but

not at V and L stage, and the *MIR157D* targets *SPL3* and *SPL5* show an enhanced toggle in *Tsu-0* compared to *Col-0* (less expressed at L stage, and over expressed at T stage). A first glance at the implication of both genes have been assayed by phenotyping natural populations bearing the exact same variations as *Tsu-0*. However, no genotype-phenotype correlation have been observed for the 8 natural accessions tested, although the number of scored plants per accession was extremely reduced (n=6) in this first exploratory experiment.

We have no doubt about finding the candidate genes after a better QTL length reduction. The validation of a candidate gene will require to couple the use of natural population and mutants. Natural populations can be used to confirm the link between the candidate variants and basal bracts formation. In a second time, CRISPR technology can be used in *Col-0* at the target locus to validate the candidate genes. Such tool will then be crucial to investigate the role of the gene in uncoupling bract inhibition from flower emergence.

Natural accessions have been classically used to study flowering time (Brachi *et al.* 2010), or the environmental control of flowering (Lempe *et al.* 2005). However, the floral transition properly have not been investigated through the lens of natural variations. Because basal bract is temporally restricted to the first flowers, we investigated the dynamics of the transcriptome at the moment of floral transition between the two natural accessions.

2 Developmental switch at a population level

2.1 Heterochrony at a population level

The floral transition (FT) has been investigated in the two natural accessions by a morphological description of the SAM, by monitoring gene reporter lines for *LFY* expression, and by a transcriptomic analysis of the SAM over FT. Both the morphological features associated with FT, as well as the expression pattern of *LFY*, show no visible distinction. Hence, as the timing of FT was different, we were able to use the morphological feature to define 4 identical stages of FT, that were used for the transcriptomic analysis. The RNA-seq analysis revealed a morpho-temporal distortion between the two accessions at the time of FT. That is, a discrepancy between SAM morphology and transcriptomic signature. Such distortion was the highest at T stage, that corresponds to the emergence of the first flower. At T stage, *Tsu-0* transcriptomes

appeared "older" than *Col-0* ones, while they were closer before and after (at L and F stage). This result was surprising regarding the existence of bract in *Tsu-0*, because the presence of a leaf in a phytomer could be associated instead to a "more juvenile" (or "younger") stage. These data suggest that basal bract emergence does not result from a delay in the acquisition of floral identity, as confirmed also by the expression of *LFY* in flowers that do bear bracts. This reinforces the idea that flower identity and bract development can happen simultaneously in certain context.

2.2 Evolution of gene expression at floral transition is observed at a population level

Heterochronic shifts are major players in the evolution of developmental switch in both plants and animals (McKinney *et al.* 1988; Geuten & Coenen 2013; Mitchell & Diggle 2005). In insect, the timing of the ecdyzosteroid peak is associated with an important difference in insect life cycle (Truman & Riddiford 1999). In Solanaceæ, the timing of certain gene expression is associated with an important architecture remodelling (Lemmon *et al.* 2016). Our RNA-seq analysis enlightens for the first time the existence of such heterochronic shifts in the GRN of FT at a population scale. Whether this is responsible for bract emergence is a question that still needs to be investigated. Increasing the number of bract-bearing versus non bract-bearing natural accessions could help answer the question. It makes no doubt that the increasing affordability of RNA-sequencing and analysis will facilitate such investigation. Thus, the study of the transcriptome associated with FT in a large panel of natural accessions will help to better understand the types of transcriptomic remodelling related to architecture divergence at a population scale. In addition, we could shift the expression of some candidate genes in *Col-0* transgenic plants by putting them under the control of earlier/later expressed promoter, to see whether this might affect the presence of basal bract. To this end, we would need a better understanding on the genes that are shifted in *Tsu-0* compared to *Col-0*. Recently, Calderwood *et al.*, in the group of R. Morris, developed a curve registration method to better investigate time course transcriptomes (Calderwood *et al.* 2021). This method allows to study the complex multidimensional transformations between two global time courses and to identify

meaningful underlying differences. We are currently building a collaboration with R. Morris and R. Kristianingsih in order to make the most out of our RNAseq data.

2.3 Developmental switches: perfect times for the emergence of phenotypic variations?

Developmental switches enable plasticity in both animal and plant life cycle, through the intense phenotypic remodelling it triggers. The contrasted phenotypes before and after the switch often rely on a different set of genes. Developmental switches can be considered as a complete rewiring of the GRN operating in the tissues of interest. But this rewiring must also be tightly regulated to avoid the emergence of detrimental phenotypes. Investigating developmental switches at different scales is indispensable to understand how such a transition self-organizes. Interestingly, our work reveal that the "control" can be loose, because transient "chimeric" phenotype can appear in some situations. So why is FT favorable to the emergence of new transient phenotypes?

Transient phenotypes could arise from a combination of atypical gene expression levels. While transitioning from one state to the other, many genes are inevitably up or down-regulated. We could imagine that some genes with normally antagonistic effects would maintain intermediate expression levels for a short period of time, generating "chimeric" phenotypes. Such situation would not be stable in neither states of the transcriptome before and after the developmental switch, because of genetic feed back loops, and epigenetic marks. If the time to switch from one GRN to another is long relative to the time required to generate a phenotype, a third "transient" developmental state can be defined, which does produce macroscopic phenotypes. Reciprocal inhibition of antagonistic genetic modules made of several genes can involve a complex network of interactions between genes. We could then imagine that transient phenotypes are promoted by small modifications of genetic interactions in the network which delays the reciprocal inhibition, thereby extending the time window of the transient transcriptomic state.

Another possible explanation for transient phenotype could root at the dynamics of epigenetic marks during the GRN remodeling. In fact, Müller-Xing et al., showed the existence of a time window during which floral reversion can happen, in some Polycomb group mutants

(Müller-Xing *et al.* 2014). It appears that the rewiring of a new GRN at developmental switch involved two main phases. First, after signal transduction, new gene regulations are established involving down regulation of genes important for the earlier stage, and up-regulation of genes important for the next stage. In a second time, epigenetic marks "locks" on the new regulatory network, which can persist despite the absence of the initial trigger (flowering signals). This is supposed to be at the origin of the absence of vegetative reversion, even though flowering signals may become nonexistent. We did not investigate the epigenetic landscape of *Tsu-0* and *Col-0* during floral transition. Yet, it would be interesting to monitor the speed at which new epigenetic marks are deposited, such as repressive marks on "bract/leaf" genes. A delay in the deposition of such marks could be responsible for the absence of bract inhibition at the first flowers. The abrupt transition observed in *Tsu-0* transcriptome could then prevent from the rapid establishment of epigenetic marks.

This study presents a body of evidence that *Tsu-0*'s bracts are not the persistence of a vegetative signature, but instead result from a disturbed remodeling of GRN at floral transition. Bracts and flowers development are thus ontogenetically compatible, probably because they both belong to a much older developmental unit: the phytomer.

3 Bract loss: an evo-devo perspective

3.1 Is leaf a dispensable part of the phytomer?

The three elements of a phytomer (internode, leaf, meristem), are extremely well conserved in the plant kingdom. Despite a huge modification of their morphology, the fundamental composition of the phytomer has stayed identical through million years of plant diversification. This conserved modular unit may dictate strong developmental constraints. Leaves are the first organ to be formed in a phytomer. The establishment of leaf polarity and proper boundaries with the meristem are crucial to the formation of the meristem at its axil. This could explain the maintenance of a cryptic bract domain in species that do not present a complete bract development. Bracts, and any leaf, could be seen as an organizing center for the formation of the meristem. Several data can support this hypothesis: the presence of ectopic meristems at the leaf margin of tomato compound leaf (that includes both boundary and polarity molecular

features, as the axil of the leaf, Rossmann *et al.* 2015), the possible development of a second branch (accessory branch) at the axil of a cauline leaf in WT and some LATERAL ORGAN FUSION 1 OE (D.-K. Lee *et al.* 2009 and personal observations), as well as the presence of ectopic meristem at the axil of floral organs in some floral patterning mutants (Xu *et al.* 2010; Liu, Zhou, *et al.* 2007). Hence, all meristems develop at the axil of a leaf, and not (never?) in the middle of a leaf or an internode. Leaf axil combines several parameters, including genetic (adaxial and boundary markers,), hormonal (auxin minima), and mechanical characteristics (Wang *et al.* 2016). Whether the leaf dependence can be generalized to all meristems, including flowers, is an open question. But to test the role of the bract in the formation of the flower, we could control the development of the cryptic bract with both transgenic line as well as micro-laser dissections. Inspired by Nilsson *et al.* (Nilsson *et al.* 1998), we could destroy bract cells as the floral transition happens, and/or laser dissect bract domain in the early steps of flower development. To this end, I have built in both *Col-0* and *Tsu-0* genetic backgrounds reporter lines to mark the cryptic bract domain (with FIL promoter) and the flower (with LFY promoter). Yet, I could not carry out the ablation experiments because of time constraints.

Having considered the role of the leaf in meristem development, we will now question what evolutionary forces could have triggered its disappearance at the axil of flowers.

3.2 Bract loss: a way to optimize energy allocation to flower development?

Bract, and to a broader extent leaf loss appear independently in several clades, from which some are major plant models. Interestingly, both *Picea* and *Arabidopsis* have an annual life cycle, so the apparition of flowers also sign plant death. In other species, a lesser selection on photosynthetic performance could have instead allow the acquisition of new function, like protection, or attraction. In annual and pollinator-free plants, bract loss could have allow a better reallocation of energy into flower development, rather than leaf growth. This would not be surprising as the role of the source-sink status in plant architecture, notably in bud outgrowth, appear crucial (Barbier *et al.* 2015). Studying the role of strigolactone and sugar metabolism in the context of the flower versus bract development, as it was in the context of

axillary bud versus stem and leaf, would need to be considered.

To have a first look at the relative growth of bract and flower in both *Col-0* and *Tsu-0*, it would be necessary to live-image meristems at the moment of first flower growth. Then, we could compute the relative growth of the flower versus (cryptic) bract: in *Tsu-0*, we can expect (cryptic) bract to grow faster in the first flower, or floral meristem to grow slower. The delicate rosette dissection, as well as the short timing in which the first flower can be captured, made this experiment highly challenging. It appears then interesting to manipulate bract/flower growth rate in transgenic *Col-0* and *Tsu-0* lines, whether by using hormonal signaling, or cell cycle arrest. A reduction of bract frequency when increasing flower growth rate, and the other way around, would argue for an important role of the flower-bract resource competition. Such role can be rapidly investigated using non-model Brassicaceae species that bears bract on the lower half part of the inflorescence, because bract-flowers would then be easier to catch. A comparison of relative growth between (cryptic) bract and flower in such species, versus in species where bracts are totally absent would be interesting.

Taken together, our work re-explore the question of bract loss using genomic, transcriptomic, and classical developmental approaches. Exploring bract loss can be interesting to shed light into the mechanisms of trait loss at a both developmental and evolutionary scale. It can also bring new perspectives about the role of bracts in flower development.

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Appendices

Appendix related to Chapter 1, Chapter 2 and Chapter 3

Appendix 1: Phenotyping tables

This file contains the raw phenotyping dataset of the following experiments: Natural Accessions (see Chapter 1, fig.5A), Flowering Time and Natural Accessions (see Chapter 1, fig.5B), Photoperiod (see Chapter 2, fig.1), Flowers (see Chapter 2, 1B), Plastochron (see Chapter 2, fig.2), BSA (see Chapter 3, fig.1A), and RILs (see Chapter 3, fig.2A, 3A and 5). Each sheet corresponds to one of these experiments. The first sheet "README", details all the information needed.

Appendices related to Chapter 2

Appendix 2: RNA-seq Quality Check of the samples

This file contains the concentration and quality check performed by HELIXIO on our RNA samples.

Appendices related to Chapter 3

Appendix 3-5: List of the Candidate Gene set1, 2 and 3

These files contain the list of CGSet1, CGSet2 and CGSet3 as described in Chapter 2, fig.6. The first sheet "README" contains the details of each columns. See Appendix 6 for the list of SnpEff effects retains for the filters.

Appendix 6: SnpEff effects retains for the establishment of the Appendix 5

This file contains the list of the SnpEff effects and their definition. It specifies the one that were not considered for the establishment of the CGset2 (ie. the filter "unlikely post-transcriptional effect").

Appendix 7: List of genomic variants between *Col-0* and *Tsu-0*.

This vcf file contains the list of the variants that were identified between Col-0 and Tsu-0 after re-sequencing analysis.

Appendix 7: RILs genotypes.

This file contains the detail of each RILs genotypes used in this study. The first sheet "README" details how to read the table.

Inhibition de la bractée lors de la transition florale chez *Arabidopsis thaliana*

Résumé de la thèse

Le phytomère constitue l'élément de base du développement continu des plantes. Il se compose d'un noeud constitué d'une feuille et d'un méristème à la base de la feuille, ainsi que d'un entrenoeud, segment de tige séparant deux noeuds. Le passage du stade végétatif au stade reproductif se traduit par un remodelage du phytomère. Chez les Brassicaceæ, comprenant la plante modèle *Arabidopsis thaliana*, cela s'illustre notamment par la perte de la feuille, appelée bractée. Cependant, de précédentes études ont rapporté la présence de bractées à l'aisselle des premières fleurs de certaines accessions naturelles de *A. thaliana*, lorsqu'elles sont cultivées dans des conditions environnementales spécifiques. Ces fleurs dites "chimeriques" résulteraient d'une conversion de branche en fleur. Au cours de ma thèse, nous montrons que la présence de bractées au niveau des premières fleurs est fréquente chez certaines accessions naturelles d'*A. thaliana*, mais pas chez l'accession de référence *Col-0*. Chez *Tsu-0*, notamment, la présence de ces bractées ne dépend pas des conditions environnementales. De plus, elles ne résultent pas d'une conversion de branches en fleurs, contrairement à ce qui avait été proposé par les études précédentes. Afin d'établir les bases moléculaires à l'origine du développement de ces bractées, nous avons procédé à l'analyse transcriptomique de méristèmes apicaux caulinaires au cours de la transition florale chez les deux accessions naturelles. Ces transcriptomes révèlent des hétérochronies dans la dynamique d'expression des gènes. Leur lien avec la présence de bractées reste à établir. Notre étude décrit pour la première fois les différences de dynamique d'expression de gènes au cours de la transition florale à l'échelle de la population d'*A. thaliana*. Nous avons pu montrer que la différence d'expression des gènes entre ces deux accessions culmine au moment précis de la transition florale, faisant écho à ce qui avait été montré à l'échelle de l'espèce. De plus, nous avons cartographié les variants génétiques responsables de la présence de bractées chez *Tsu-0* en utilisant deux approches indépendantes, l'analyse génétique d'individus F2 ségrégants en masse et l'utilisation de lignées recombinantes ségrégantes. Nous avons ainsi pu identifier deux régions situées dans le chromosome 1 et associées au phénotype. Nous avons donc recoupé les éléments des analyses génétiques et ceux des analyses transcriptomiques afin de proposer de potentiels gènes candidats. Ainsi, cette thèse revisite l'étude des mécanismes qui contrôlent l'inhibition de la bractée au moment de la transition florale chez *A. thaliana*, en se basant sur une comparaison entre deux populations naturelles. Par conséquent, ces résultats peuvent également nous permettre de mieux comprendre l'origine évolutive de la perte des bractées chez les Brassicaceæ.