Part III

Characterization and mapping of a new locus involved in the regulation of branching in Arabidopsis

Abstract

The final plant architecture depends on the number and the position of lateral inflorescences, and its development is the result of several meristems activity. The main inflorescence controls the activity of axillary meristems through a mechanism called apical dominance. This process is regulated by different environmental and hormonal factors, including strigolactones (SL), a class of hormones that inhibit the activity of the axillary meristems. *scap1-3* mutant plants show obvious phenotypic alterations at the level of the inflorescence architecture. In particular, these plants are characterized by a reduced apical dominance and the presence of a greater number of secondary inflorescences compared to wild type plants. Phenotypic analysis might suggest a role for *SCAP1* in branching regulation. Strigolactones (SL) form a new class of plant hormones that inhibit growth of lateral buds. Mutants impaired in SL synthesis or signalling (*more axillary meristems*, *max*) show a bushy phenotype, reminiscent of *scap1-3*. Expression profile analyses show down-regulation of genes involved in SL biosynthesis or signalling, in *scap1-3* plants, a result that correlates with the *scap1-3* observed phenotype. *SCAP1* knock-down alleles obtained by generation of artificial microRNAs specifically targeting SCAP1 did not recapitulate the *scap1-3* phenotype. Furthermore, the cross between *scap1-3* (No-0 background) and wild type Col produced a F2 progeny with altered branching that were genotypically wild type for *SCAP1*. These data demonstrate that *scap1* is not responsible for the branching phenotype (renamed *more axillary meristem 7*, *max7*). Mapping experiments were made with the purpose to determine the genomic location of *max7* mutation, taking advance of several polymorphisms between Col-0 and No-0 ecotype. A future prospective will be to identify the *MAX7* gene. To this end we are characterising independent T-DNA insertion alleles for each of the *MAX7* candidates (located in the relevant genomic region previously mapped) and testing if any of them recapitulates the *max7* phenotype.

Introduction

The overall architecture of a plant derives from the activity of the primary shoot apical meristem (SAM) together with the activities of axillary (secondary) meristems, which are formed post-embryonically. The complexity of the pattern of branching depends on the number and distribution of the secondary inflorescences. The secondary inflorescences derive from the activity of axillary meristems, which are derived from totipotent cells at the base of both rosette and cauline leaves (Grbić and Bleecker, 2000). Axillary meristems can immediately form an axillary bud or remain dormant. Axillary buds may, in turn, develop few leaves and then arrest their development or remain dormant (Sussex et al., 2001). An important and well-known phenomenon that controls the activity of axillary meristems is apical dominance. Apical dominance is the control imposed by the primary SAM on the development of secondary buds (Thimann and Skoog, 1934). The dormant axillary buds can outgrow and replace main stem in case this is damaged or removed (Ward and Leyser, 2004). The formation and activity of axillary meristems is influenced by environmental stimuli, such as the length of the photoperiod, light quality, temperature and nutrients availability (Halle *et al.,* 1999; Ungerer *et al*., 2002; Müller and Leyser, 2011).

Regulation of apical dominance is mediated by a complex network of hormonal signals that interact each other and move systemically throughout the plant (Müller and Leyser, 2011) (Figure 1). Several studies have revealed the antagonistic interaction between the hormones auxin and cytokinins (CK) in the regulation of the development of axillary buds (Thimann and Skoog, 1934; Wickson and Thimann, 1958; Li and Bangerth, 2003; Miyawaki et al., 2004). Auxin produced in the apical meristem is actively transported downwards and inhibits the development of the buds, while CK moves upwards in the plant, promoting bud outgrowth (Figure 1). Therefore bud activity depends on the ratio between these two hormones (Shimizu-Sato et al., 2009; Tantikanjana et al., 2001). Recently a third class of hormones involved in bud outgrowth have been detailed, the strigolactones (SL), which move upwards entering the bud and inhibiting its development (Umehara et al., 2008; Gomez-Roldan et al., 2008; Smith et al., 2012; Smith, 2013) (Figure 1).

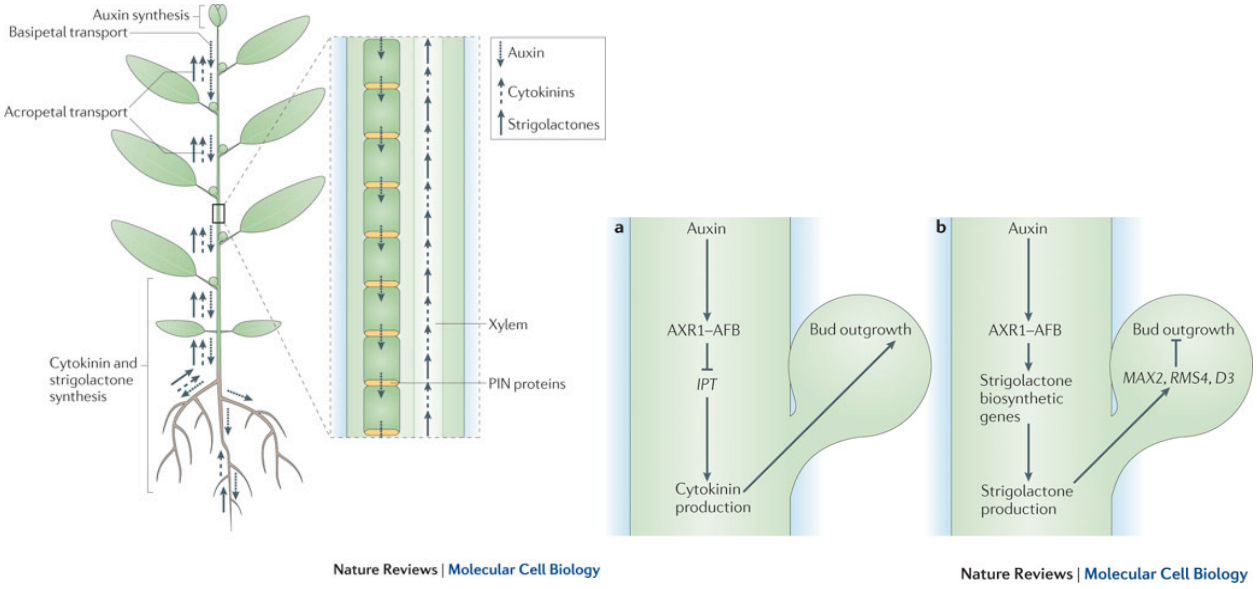

Figure 1 - *Schematic representation of hormonal regulation of branching.*
Auxins, CK and SL are three classes of hormones involved in the control of axillary meristem activity. These hormones are transported in the plant, forming a network that allows the integration of information from different plant organs. Auxin is synthesized mainly in SAM and it is actively transported in a basipetal. SL and CK are mainly produced in the roots, but also locally in the shoots, and are transported in a manner acropetal into the xylem. (a) Auxin regulates the biosynthesis of CK, down-regulating family members IPT. (b) The auxin up-regulates SL, acting on genes for their biosynthesis (image derived from Müller and Leyser, 2011).

Auxin is a key regulator of apical dominance. Decapitation experiment showed that the removal of the SAM (the main site of auxin biosynthesis) releases dormancy of axillary buds. If auxin is exogenously applied to the cut stem growth inhibition of axillary buds is restored (Thimann and Skoog, 1933). The importance of auxin in the apical dominance is supported by studies with mutant plants. Increasing the level of endogenous auxin causes a reduction in branching, while decreasing promotes branching (Klee and Lanahan, 1995). Auxin is synthesized mainly in the shoot apex and is then actively transported via basipetal movements (apex towards the roots) (Ljung et al., 2001). The mechanism by which auxin inhibits growth of axillary meristems remains poorly unclear although auxin acts indirectly because it does not enter the buds (Sachs and Thimann, 1967; Booker et al., 2003). Physiological and genetic analyses led to the formulation of two hypotheses. The first is that the transport of auxin in the main stem can inhibit bud outgrowth by affecting the ability of axillary buds to export auxin in the stem (Li et al., 1999; Morris, 1977; Sachs, 1981; Bennett et al., 2006; Mouchel and Leyser, 2007; Ongaro and Leyser, 2008). The second is the hypothesis of the second messenger, according to which auxin acts by regulating a mobile signal that moves acropetally, entering the bud and directly regulating its activity (Snow et al., 1929; Sachs et al., 1967; Bangerth, 1994). In both cases, auxin and CK and SLs influence each other to regulate the production of secondary inflorescences (Figure 1).

CK are positive regulator of branching and act inside the bud. Auxin exerts its inhibitory effect on secondary inflorescence development via negative regulation of CK synthesis (Nordström et al., 2004; Müller and Leyser, 2011; Tanaka et al., 2006). It has been proposed that after decapitation CK are synthesized directly in the bud and not transported from the roots (Tanaka et al., 2006). In support of this claim, *IPT* (adenosine phosphate isopentiltrasferasi) gene expression, encoding the enzyme involved in the first step of CK biosynthesis, is activated in axillary buds soon after decapitation (Kakimoto, 2001; Takei et al., 2001; Nordström et al., 2004; Tanaka et al., 2006). Auxin that derives from the apex also controls CK degradation through the transcriptional regulation of *CKX* (cytokinin oxidase) in the stem, an enzyme able to inactivate irreversibly the CK (Eklöf et al., 1997; Jones and Schreiber, 1997; Nordström et al., 2004; Shimizu-Sato et al., 2009) (Figure 1). Unlike CK, SLs inhibit branching by regulating the transport of auxin. It has been demonstrated that SL reduce the transport of auxin in the stem through the control of PIN expression. Thus, plants impaired in SL biosynthesis or signalling have an increased auxin transport (Bennett et al., 2006; Prusinkiewicz et al., 2009; Crawford et al., 2010). However, auxin promotes SL production (Figure 1). Decapitation results in a significant decrease in the transcript levels of SL biosynthetic *MAX* (*more axillary growth*) genes, which results in a decreased production of SL (Nordström et al., 2004; Brewer et al., 2009; Hayward et al., 2009). SLs are synthesized from carotenoids, mainly in the roots and transported acropetally inside axillary buds (Figure 1) (Beveridge et al., 1996; Napoli et al., 1996; Alder et al., 2012; Sorefan et al., 2003; Booker et al., 2004; Booker et al., 2005; Umehara et al., 2008). The SLs biosynthesis pathway involves the action of a series of enzymes, *DWARF27*, *MAX3*, *MAX4* and finally *MAX1* (Lin et al., 2009; Janssen and Snowden, 2012; Brewer et al., 2013; de Saint et al., 2013) (Figure 2).

Several components of SLs signalling are known including the F-box protein *MAX2* (Beveridge et al., 1996; Booker et al., 2005; Simons et al., 2007; Stirnberg et al., 2002; Stirnberg et al., 2007) and the SL receptor, *AtD14* (Arite et al., 2009; Water et al., 2012), which physically interacts with MAX2 to promote SL degradation (Hamiaux et al., 2012). Since the function of the F-box proteins is to drive the degradation of other protein, the interaction between D14 and MAX2 suggests that they may form a complex that recruits other proteins for degradation (Lechner et al., 2006; Umehara et al., 2008; Gomez-Roldan et al., 2008, Hamiaux et al., 2012; Jiang et al., 2013; Zhou et al., 2013). MAX2 interacts with BES1 (bri1-EMS-suppressor 1) and regulates SL-specific target genes expression. BES1 is a positive regulator of axillary bud growth as mutant plants *bes1* suppress the *max2* phenotype. In the presence of active SLs, AtD14 interacts with MAX2 and degrade BES1 to inhibit axillary bud outgrowth (Wang et al., 2013) (Figure 3).

In addition, SLs act directly in the bud by regulating transcription of a TCP transcription factor, BRC1 that has a negative role in the branching process. In *max* mutant plants there is a reduction of *BRC1* transcript (Aguilar-Martinez et al., 2007; Finlayson et al., 2010) whilst *brc* mutant plants show increased branching (Figure 2) (Hubbard et al., 2002; Minakuchi et al., 2010).

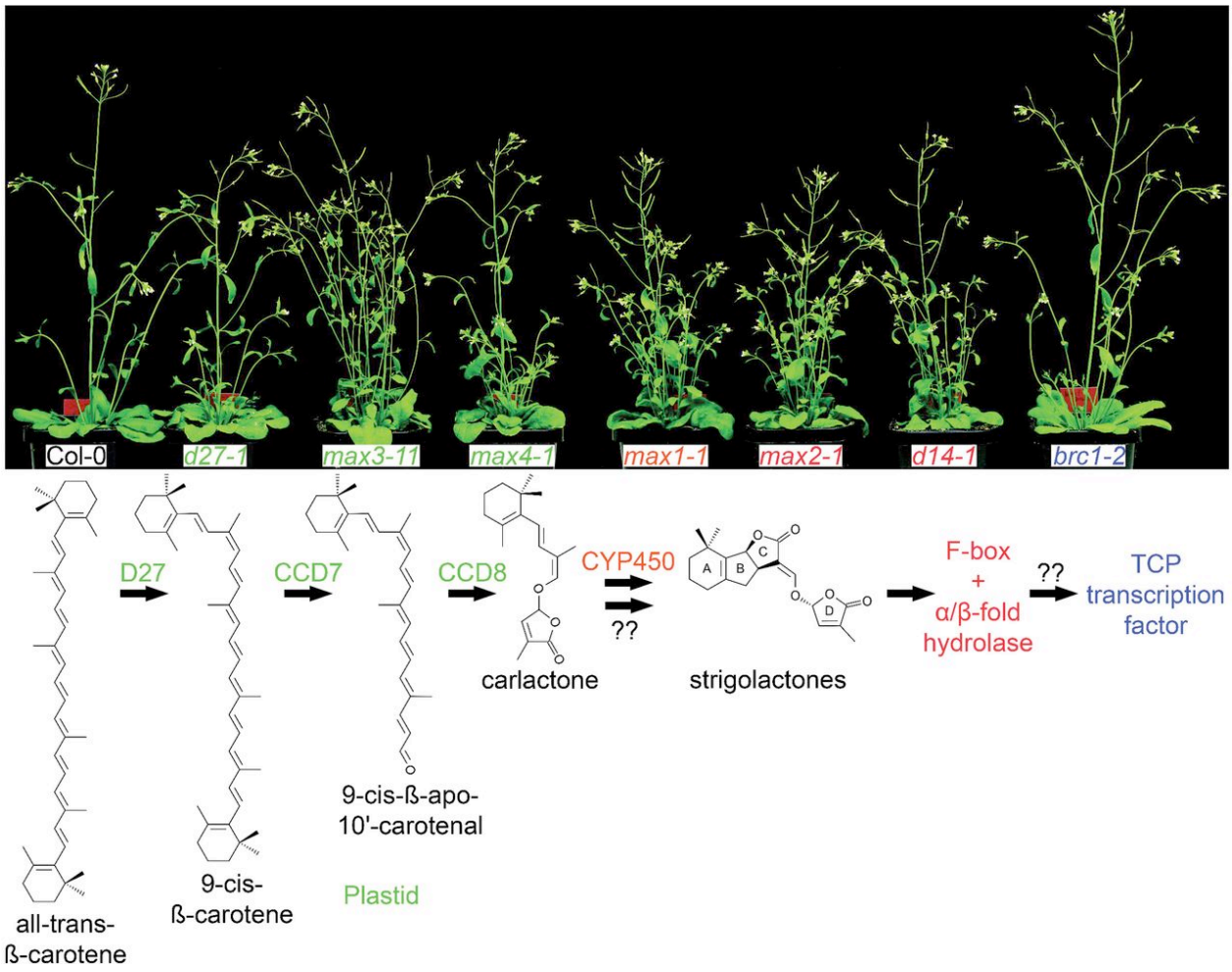

Figure 2 - *Schematic representation of genes involved in SL biosynthesise or signalling pathway.*
The plants are shown in the ecotype Col-0. Below each mutant are shown the steps of the SL pathway (image from Brewer et al., 2013).

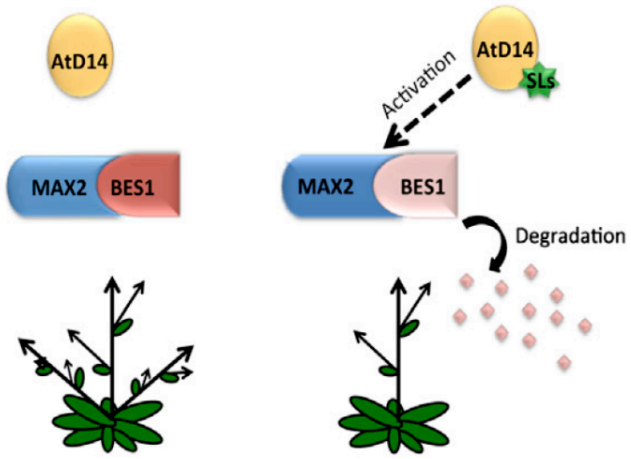

Figure 3 - *Scheme of SL signalling pathway in Arabidopsis.*
The F-box protein MAX2 interacts with BES1. The binding of SL to AtD14 allows the degradation of BES1 by MAX2, in order to inhibit the branching (image from Wang et al., 2013).

Results

Molecular characterization of a new allele of *SCAP1*

In order to understand the role of *SCAP1* we analysed an independent *scap1* allele derived from the RIKEN collection (*scap1_54-3788-1*). RIKEN lines were generated in the Arabidopsis thaliana ecotype Nossen (No-0) using a transposable element Ds as a mutagen (Kuromori et al., 2004). In the *scap1_54-3788*-1 line (hereafter dubbed *scap1-3*) a Ds transposon insertion is located 354 nucleotides downstream of the ATG codon (Figure 4). Homozygous *scap1-3* plants were selected for subsequent phenotypic and molecular analysis. A full-length *SCAP1* transcript was not detected in *scap1-3* plants but a shorter version of *SCAP1* corresponding to the 5' region (upstream of the transposon insertion) could be detected. This could suggest that *scap1-3* insertion allele may not be null (Figure 4).

The *scap1-3* mutants show altered plant architecture

scap1-3 mutant plants present evident phenotypic alterations. Homozygous plants have higher numbers of secondary inflorescences that develop from axillary meristems located at the base of rosette leaves (Figures 5). However this was not correlated with an increased production of vegetative leaves. Further phenotypic analyses revealed that *scap1-3* plants had a smaller rosette and a shorter main inflorescence than wild type (Figure 6). Also the activity of the axillary meristems located at the base of cauline leaves was altered. The main inflorescence of *scap1-3* plants is more branched and produces coflorescenze up to the third level, generally not observed in wild type plants (Figure 7). The phenotypic analysis suggest that this gene has an important role in the control of branching, inhibiting growth of new axillary buds positioned at the axils of vegetative and cauline leaves.

The bushy phenotype depends on transcript changes in strigolactones pathway genes

Several phytohormones are involved in the regulation of branching (Gomez-Roldan et al., 2008; Umehara et al., 2008; Thimann and Skoog, 1934; Wickson and Thimann, 1958). We decided to focus our attention specifically on SLs. Mutants impaired in SL synthesis or signalling (*more axillary meristems*, *max*) show a bushy phenotype, reminiscent of *scap1-3* plants. We analysed the SLs related genes *MAX1*, *MAX3* and *MAX4*, and *MAX2*. Our expression analysis of these genes revealed a general down-regulation in *scap1-3* mutant compared to wild type (Figure 8). In

particular, down-regulation was more pronounced in the aerial part of plants compared to the root system, and was stronger for the genes operating at later stages of SL biosynthesis (*MAX4* and *MAX1*).

The bushy phenotype is SCAP1 independent

We already knew that *SCAP1* is involved in guard cell maturation (Negi et al., 2013) and stomata cell lineage specification. The *scap1-2* and *amiRNA-SCAP1* mutant plants (in Ler and Col background, respectively) did not show any branching alterations. This could suggest that either the bushy phenotype observed in *scap1-3* plants was caused by an independent mutation in the genome or was ecotype (No-0) specific (i.e. derives from the interaction between *scap1* and a No-0–specific modifier).

We first wanted to evaluate the heritability of the *scap1-3* – derived phenotype in the No-0 and Col-0 background. The *scap1-3* allele is recessive because heterozygous plants are indistinguishable from wild type (Figure 5). However, we did detect phenotypic variations in *scap1-3* homozygous plants, suggesting an incomplete penetrance of the bushy phenotype in No-0 (Figure 5). We next scored an F2 population derived from a cross between *scap1-3* (No-0) and Col-0 wild type plants for plants displaying the characteristic bushy phenotype of *scap1-3* (No-0). The frequency of plants with bushy phenotype was close to a mendelian segregation of 1:3 (98 bushy and 376 wild type). This finding suggests that a single mutation is responsible for the bushy phenotype. If *scap1* was responsible for the branching alterations, it is expected that all the plants with bushy phenotype were homozygous for *scap1-3* mutation. For this purpose 290 plants were genotyped including all plants with a bushy phenotype (n = 98) and 192 plants with a wild-type phenotype. Out of 98, one plant with a bushy phenotype was also heterozygous for *scap1-3* and 12 wild –type looking plants were genotyped as *scap1-3* homozygous. These results clearly indicate that the bushy phenotype derives from an independent mutation other than *SCAP1*. However, since almost all plants with increased branching were also homozygous for *scap1-3* is plausible that the causal mutation (renamed *max7*) is physically linked to *SCAP1*.

The Mapping of *max7* mutation

There is a strong genomic variability across the different Arabidopsis ecotypes, such as Col and No-0. This variability appears as polymorphisms in nucleotide sequence, and allows us to discriminate whether a particular chromosomal region comes from Col-0 or No-0. We exploited these

polymorphisms, located at different distances from *SCAP1* to genotype the F2 population generated by crossing *scap1-3* (No-0) and Col-0 wild type. This would allow us to accurately map the position of the *MAX7* locus since *MAX7* will be located with higher probability in a region with a lower recombination rate between No-0 and Col-0 (higher linkage). It must be noted that the RIKEN informs that the genotype of some transposon lines (including ours) might not be completely Nossen (which could interfere with our analysis). By analysing in detail the polymorphic markers in the original line *scap1-3* we found that indeed some chromosomal positions in the *scap1-3* line carry polymorphisms characteristic of the Col-0 background (Figure 9). We circumvented this problem by also generating another mapping population consisting of a cross between *scap1-3* (No-0) and No-0 wild type to account for those chromosome regions that were originally Col.

Recombination rate analysis

As we knew that *max7* was closely associated with *scap1-3*, we initially generated 8 polymorphic markers for No-0 and Col-0 located at different positions with respect to the *scap1-3* transposon insertion (-5 Mb, Mb -1.5, -500 kb, -250 kb + 250 kb, kb +500, +700 and +720 kb). By analysing the genotype of F2 bushy plants in our No-0 X Col mapping population we observed a higher number of recombinant plants upstream of *SCAP1* as inferred by the enrichment of Col-0-derived polymorphisms (Fig?). Therefore, we decided to refine our search in a genomic region downstream of *SCAP1* by generating additional polymorphic markers. The recombination rate decreased in the region between *SCAP1* and the +250 kb marker and between the +740 kb marker and the telomere. At +760 kb no recombinant plant could be found. We hypothesized that *max7* was located in the regions between *SCAP1* and the +250 kb marker or between +740 kb marker and the telomere of chromosome 5. The two candidate regions as locus for *max7* were further validated. We evaluated the recombination rate in F2 plants with wild type phenotype but resulted *scap1-3* homozygous from the previous genotyping. These plants analysed for the marker downstream to *SCAP1*, as +740 kb marker, always recombined suggesting that the gene controlling branching could effectively be there. As the telomere region was poor in annotated ORFs we focused on the region between +740 kb and the telomere, which corresponds to about 20.6 kb region, precisely from position 26,954,659 on chromosome 5 to the beginning of the telomere region (starting at about 26,975,315 bp). Within this region 7 candidate genes are predicted including *AT5G67580*, *AT5G67590*, *AT5G67600*, *AT5G67610*, *AT5G67620*, *AT5G67630* and *AT5G67640* (Figure 10). Expression analysis of these genes in the *scap1-3* plants did not however reveal any significant difference compared to wild type (Figure 11). For each of these genes we also analysed independent homozygous insertional mutants

alleles (Figure 10). Our initial data suggest that none of the homozygous plants present any developmental aberrations similar to *max7*.

Conclusions and future perspectives

Our work identified a new locus, *MAX7*, involved in the regulation of branching. This gene could represent a new locus in the branching signalling network as no genes involved in branching control have not been previously described in this particular genomic location. Our data indicates that *MAX7* is able to inhibit development of axillary meristems of both vegetative and cauline leaves. The branching alterations observed in *max7* plants resemble those of *MAX* gene mutants involved in SL biosynthesis or signalling. This might suggest that *MAX7* is involved in the pathway of SLs. Expression analysis of *MAX* genes in *max7* plants revealed a down-regulation of their transcript compared to wild type. SLs have a role in inhibiting axillary bud outgrowth. Hence a reduction of SLs level in *max7* plants is consistent with the observed bushy phenotype. MAX7 may thus act as an upstream regulator of SLs biosynthesis. To identify the *MAX7* gene we performed an RNA-seq experiment aimed at understanding transcriptomic differences between *max7* and wild type plants. We revealed quantitative and qualitative differences of transcripts in regions downstream of *SCAP1*. In the region between the +740 kb markers and the telomere of chromosome 5, we found single nucleotide substitution that resulted in conservative mutations, or in 5' and 3' untranslated regions (UTR). Our mapping approach also revealed another candidate region between *SCAP1* and the +250 kb marker. Analysis of the RNA-seq data on this region showed significant polymorphism compared to wild type. Further studies on the genes in this region will be necessary to determine if one of them encodes *MAX*7. A role of *MAX7* in the SL pathway might be further substantiated treating *max7* mutant plants with the GR24 (a SL synthetic analogue). This could inform whether *MAX7* is involved in the SL biosynthesis or signalling. Finally, it would be useful to analyse the relationship between auxin, cytokinin and *MAX7*, by testing the expression pattern of genes involved in auxin or CK biosynthesis or signalling in *max7* plants.

In higher plants the level and pattern of branching is the major determinants of the plant architecture. Although, significant advances in the characterization of branching mutants have been made, the control of the branching process is still a poorly understood phenomenon. Consequently, it will be of high significance to map and characterize the *MAX7* gene to provide a new the molecular mechanism underlying the control of branching.

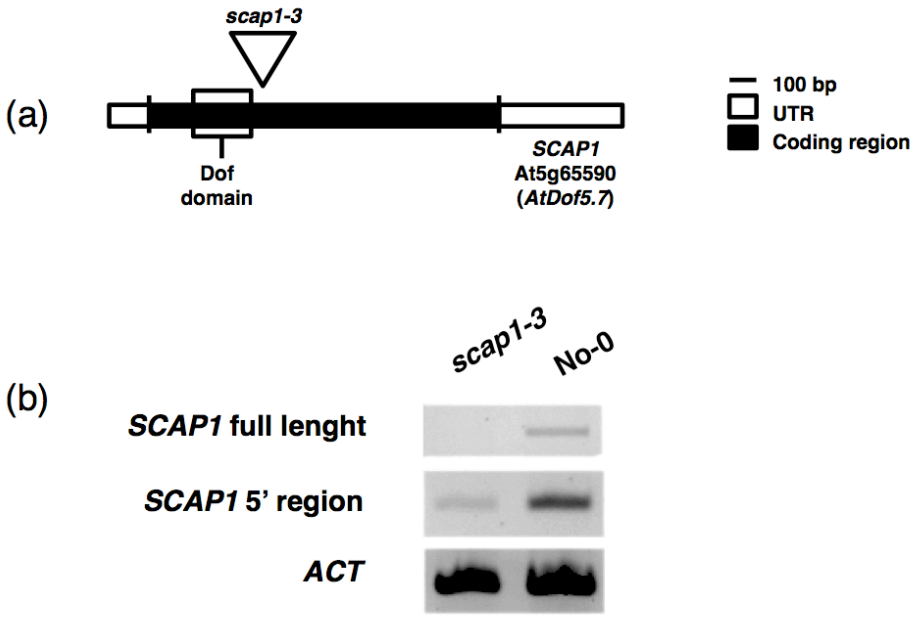

Figure 4 - *Schematic representation of SCAP1.*
(a) Schematic representation of the transposon RIKEN 54-3788-1 within the *AT5G65590* gene. The black box indicates the exon, the withe represent the 5 'and 3' UTR. The transposable element is represented as a triangle. (b) Analysis of *SCAP1* transcript by semi-quantitative RT-PCR. No-0 is used as a control wild type and actin is used as an internal control.

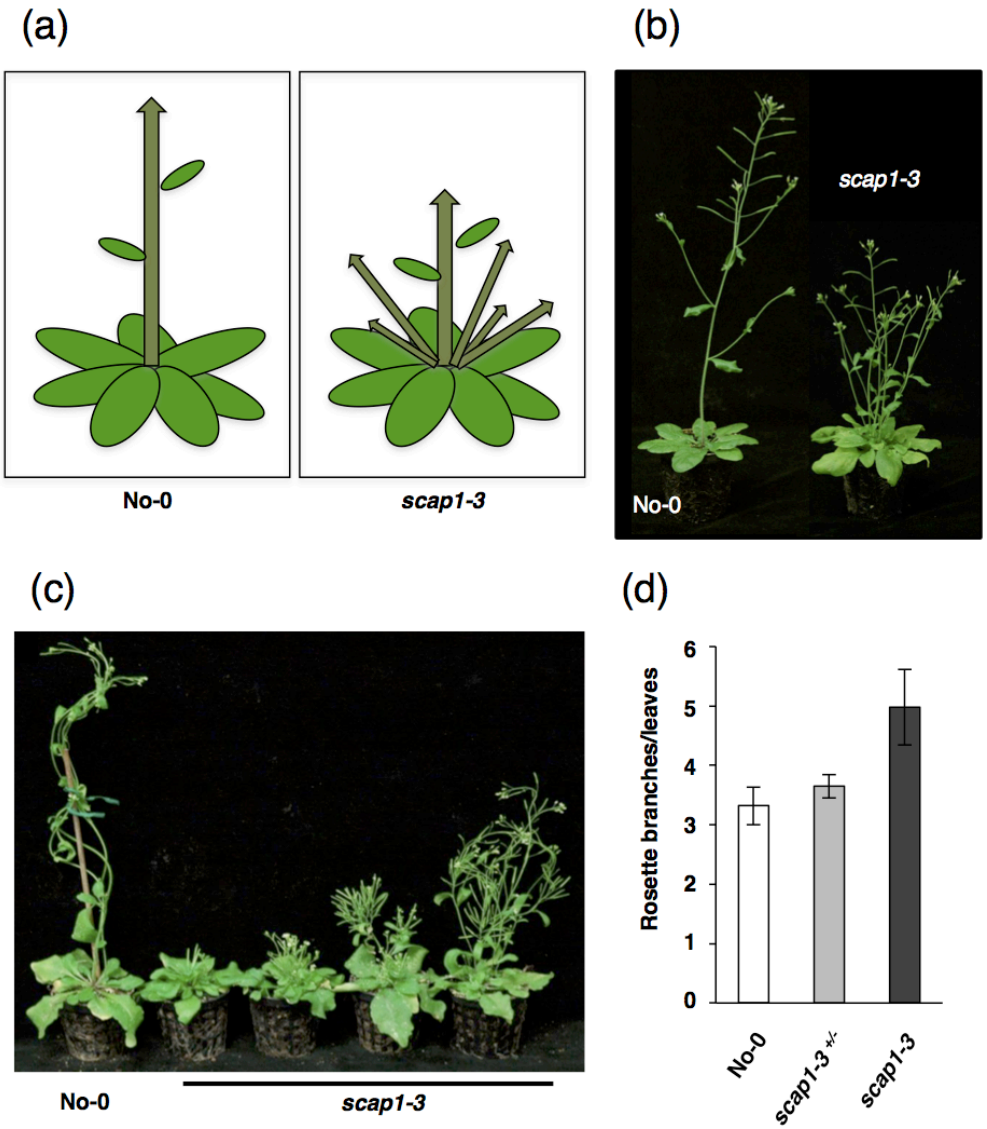

Figure 5 - *Phenotypic analysis of scap1-3 mutant No-0.*
(a) Schematic representation of rosette leaves branching observed (b) in *scap1-3* mutant and wild type. (c) Different intensity of bushy phenotype in *scap1-3* plants compared to wild type No-0. (d) Number of secondary inflorescences originated from rosette leaves in homozygous and heterozygous *scap1-3* plants.

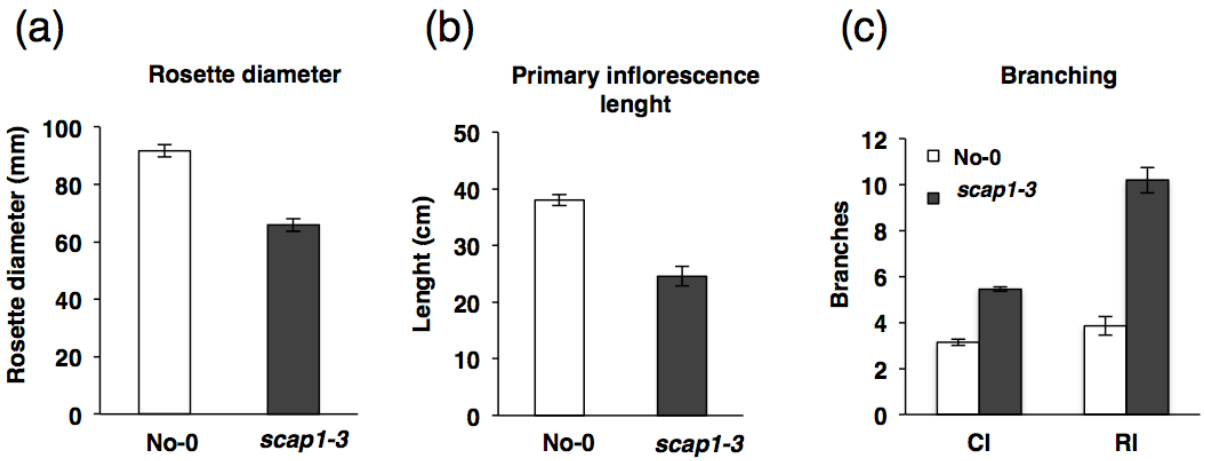

Figure 6 - *Phenotypic analysis of scap1-3 mutants.*
(a) Rosette diameter of one month-old *scap1-3* plants and No-0 wild type plants. Error bars represent the standard error (n = 24). (b) Primary inflorescence length. Error bars represent the standard error (n = 18). (c) Number of branches of the first order of the rosette (RI) and coflorescenze (CI). Error bars represent the standard error (n = 18).

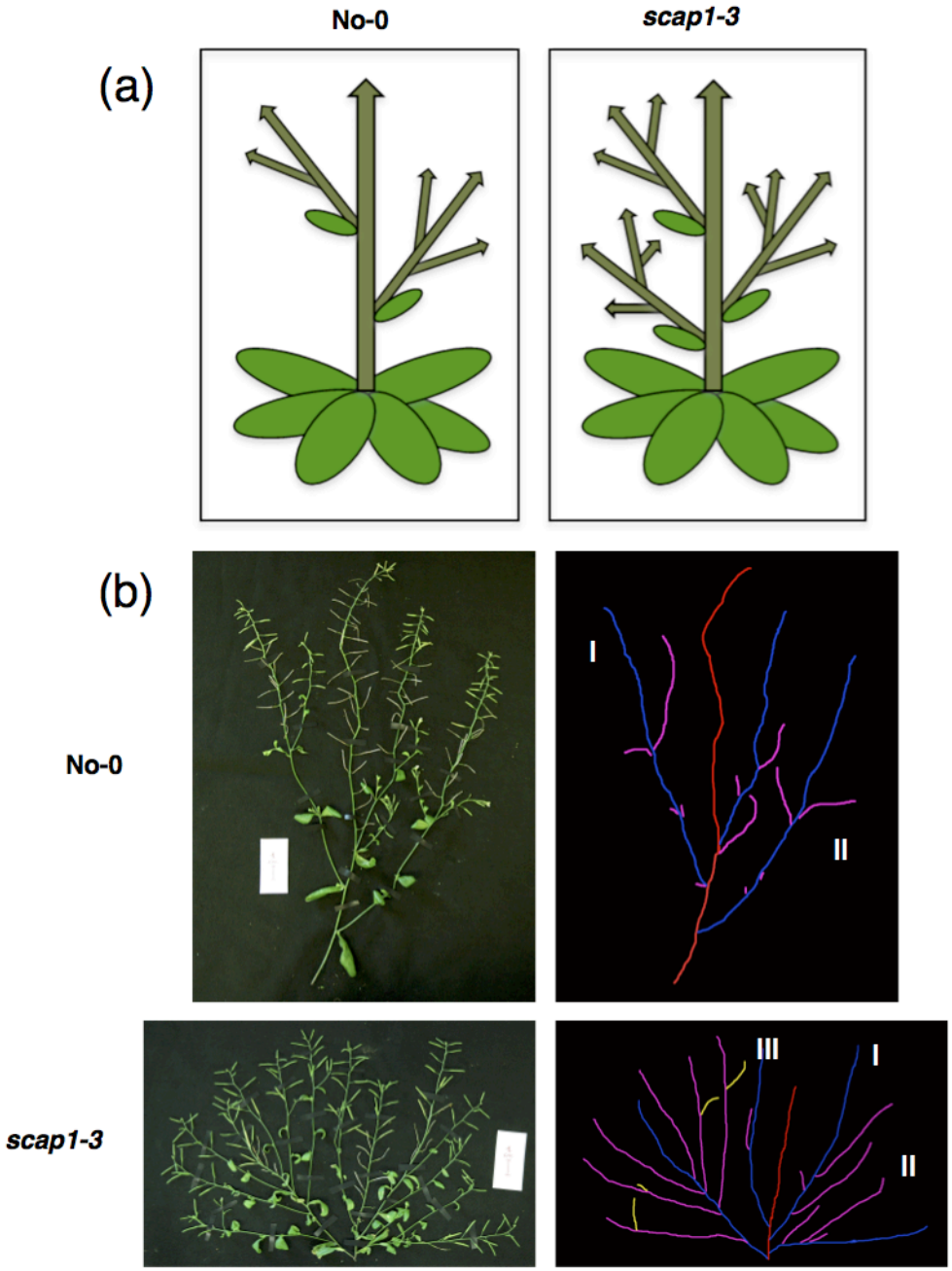

Figure 7 - *Analysis of branching in scap1-3 and No-0 plants.*
(a) Schematic representation of increased branching at the level of cauline leaves. (b) On the left, images of the main inflorescence with its secondary coflorescenze. On the right, schematic representation of the main inflorescence (red) and coflorescenze of first (blue), second (pink) and third level (yellow, present only in *scap1-3* mutant).

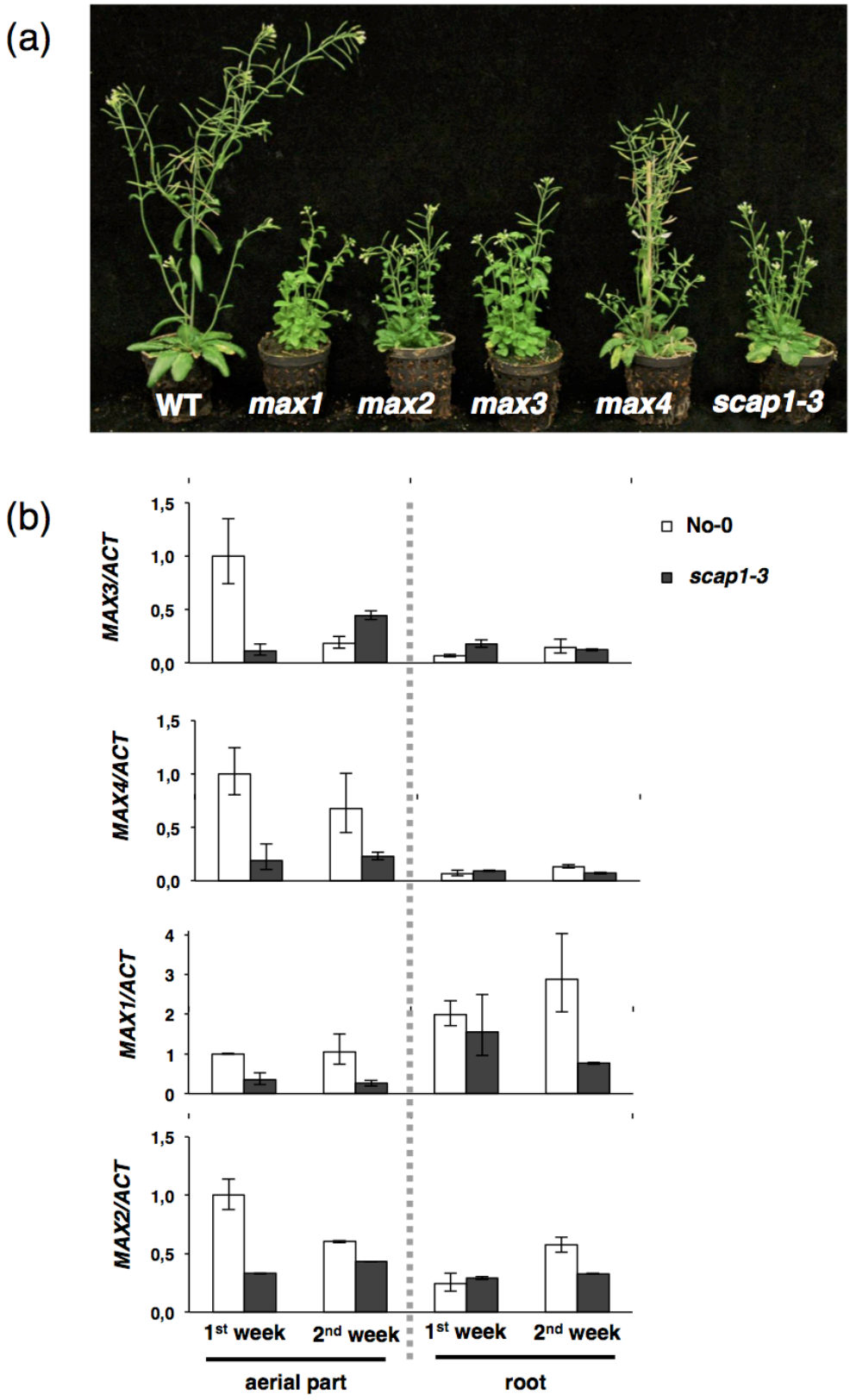

Figure 8 – *Characterization of scap1-3 plants.*
(a) The *scap1-3* plants phenoocopy SL biosynthetic mutant plants. (b) Expression level of *MAX1*, *MAX2*, *MAX3* and *MAX4* genes in *scap1-3* (grey) and No-0 plants (white). Quantitative RT-PCR performed on total RNA of 7 (week 1) or 14 (week 2) day-old seedling. Error bars indicate standard deviation. The results are the average of three biological replicates.

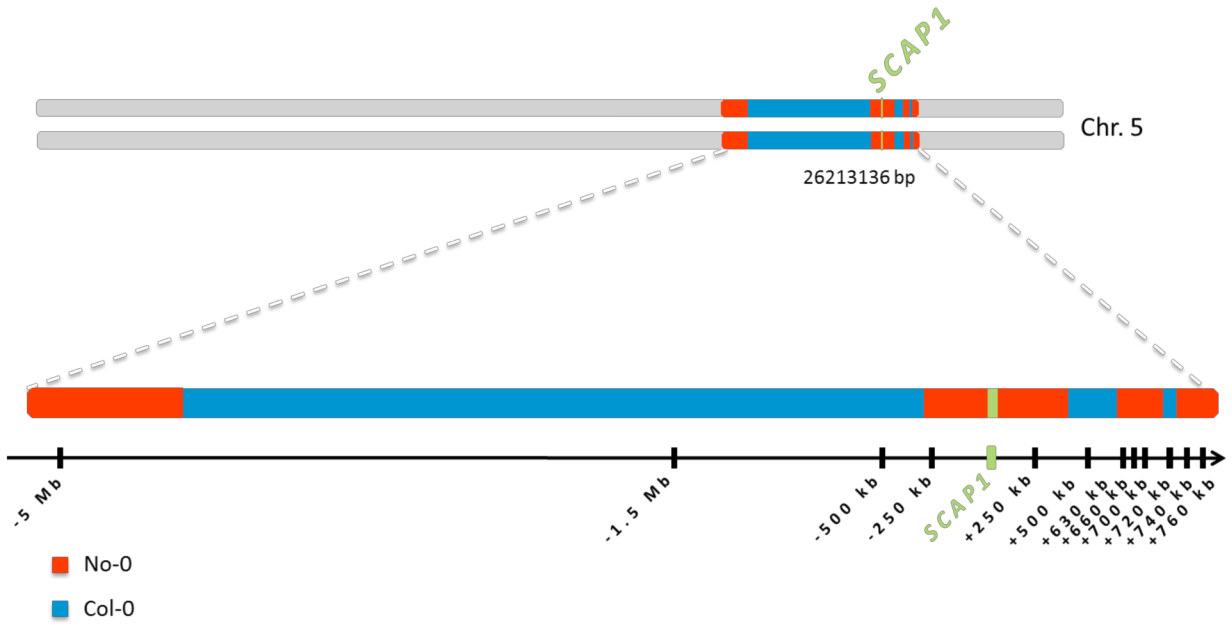

Figure 9 - *Schematic representation of chromosome 5 of scap1-3 line.*
At the bottom is shown an enlargement of the region analysed for the mapping. The location of the *SCAP1* gene and markers is shown. Regions from No-0 and Col ecotype are coloured in red and blue, respectively.

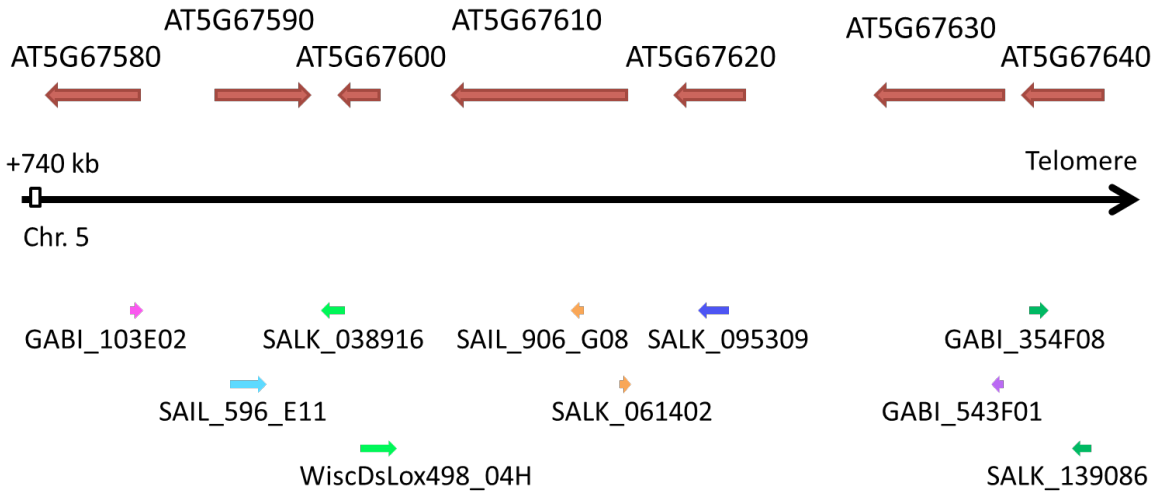

Figure 10 - *Schematic representation of genomic location of the T-DNA lines.*
The black line represents the chromosome 5 from position +740 kb until the beginning of the telomere. The red arrows on the top indicate the positions of the 7 genes that are located in this region. The arrows at the bottom indicate T-DNA insertion lines.

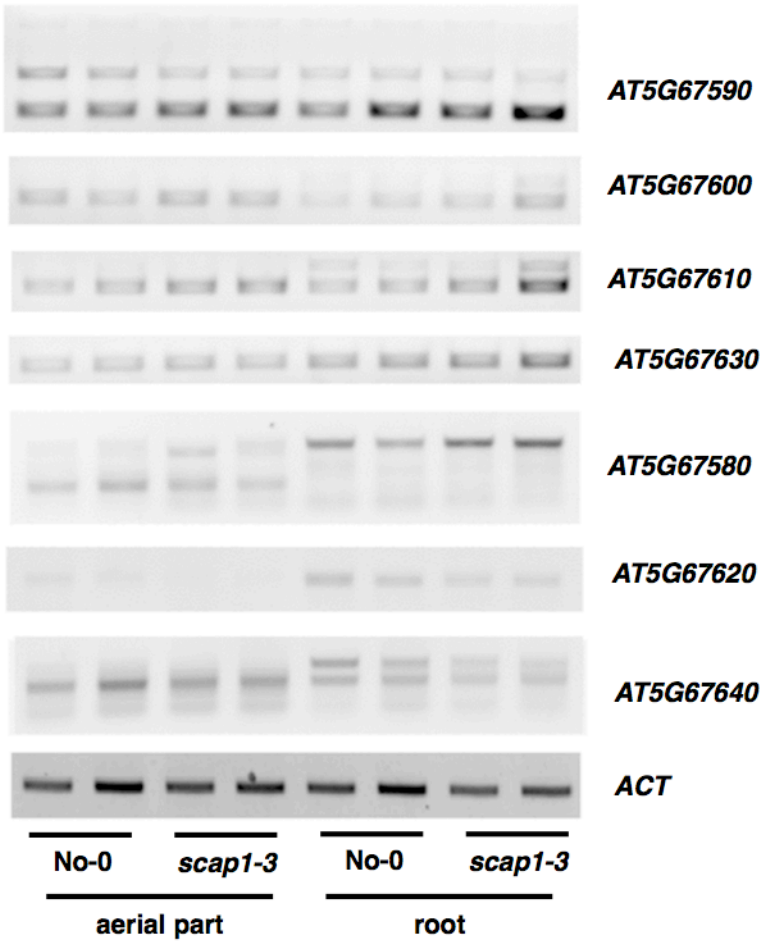

Figure 11 - *Expression analysis in scap1-3 and No-0 plants.*
Level of *AT5G67580*, *AT5G67590*, *AT5G67600*, *AT5G67610*, *AT5G67620*, *AT5G67630* and *AT5G67640* transcripts resulted from semi-quantitative RT-PCR. For both genotypes was analysed in the aerial part and in the roots. Actin was used as internal control.

References

Aguilar-Martinez J.A., Poza-Carrion C., Cubas P. (20079. Arabidopsis BRANCHED1 acts as an integrator of branching signals within axillary buds. *Plant Cell,* 19, 458–472.

Alder A., Jamil M., Marzorati M., Bruno M., Vermathen M., Bigler P., Ghisla S., Bouwmeester H., Beyer P., Al-Babili S. (2012). The path from β-carotene to carlactone, a strigolactone-like plant hormone. *Science,* 335, 1348–1351.

Arite T., Umehara M., Ishikawa S., Hanada A., Maekawa M., Yamaguchi S., Kyozuka J. (2009). d14, a strigolactone-insensitive mutant of rice, shows an accelerated outgrowth of tillers. *Plant Cell Physiol,* 50, 1416–1424.

Bangerth F. (1994). Response of cytokinin concentration in the xylem exudates of bean (Phaseolus vulgaris L.) plants to decapitation and auxin treatment, and relationship to apical dominance. *Planta,* 194, 439–442.

Bennett T., Sieberer T., Willett B., Booker J., Lusching C., Leyser O. (2006). The Arabidopsis MAX pathway controls shoot branching by regulating auxin transport. *Curr Biol,* 16, 553–563.

Beveridge C.A., Ross J.J., Murfet I.C. (1996). Branching in pea (Action of Genes *Rms3* and *Rms4*). *Plant Physiol*, 110, 859–865.

Booker J., Chatfield S., Leyser O. (2003). Auxin acts in xylem-associated or medullary cells to mediate apical dominance. *Plant The Cel,l* 15, 495–507.

Booker J., Sieberer T., Wright W., Williamson L., Willett B., Stirnberg P., Turnbull C., Srinivasan M., Goddard P., Leyser O. (2005). MAX1 encodes a cytochrome P450 family member that acts downstream of MAX3/4 to produce a carotenoid-derived branch-inhibiting hormone. *Dev. Cell,* 8, 443–449.

Booker J., Auldridge M., Wills S., McCarty D., Klee H., Leyser O. (2004). MAX3/CCD7 is a carotenoid cleavage dioxygenase required for the synthesis of a novel plant signaling molecule. *Curr. Biol,* 14, 1232–1238.

Brewer P.B., Koltai H., Beveridge C.A. (2013). Diverse roles of strigolactones in plant development. *Mol Plant,* 6, 18–28.

Brewer P.B., Dun E.A., Ferguson B.J., Rameau C., Beveridge C.A.. (2009). Strigolactone acts downstream of auxin to regulate bud outgrowth in pea and Arabidopsis. *Plant Physiol,* 150, 482–493.

Crawford S., Shinohara N., Sieberer T., Williamson L., George G., Hepworth J., Müller D., Domagalska M.A., Leyser O. (2010). Strigolactones enhance competition between shoot branches by dampening auxin transport. *Development,* 137, 2905–2913.

De Saint G., Bonhomme S., Boyer F.D., Rameau C. (2013). Novel insights into strigolactone distribution and signalling. *Curr Opin Plant Biol*, 16, 583–589.

Eklöf S., Astot C., Blackwell J., Moritz T., Olsson O., Sandberg G. (1997). Auxin–cytokinin interactions in wild-type and transgenic tobacco. *Plant Cell Physiol,* 38, 225–235.

Finlayson S.A., Krishnareddy S.R., Kebrom T.H., Casal J.J. (2010). Phytochrome Regulation of Branching in Arabidopsis. *Plant Physiology,* 152 (4), 1914-1927.

Gomez-Roldan V., Fermas S., Brewer P.B., Puech-Pages V., Dun E.A., Pillot J.P., Letisse F., Matusova R., Danoun S., Portais J.C., Bouwmeester H., Becard G., Beveridge C.A., Rameau C., Rochange S.F. (2008). Strigolactone inhibition of shoot branching. *Nature,* 455, 189-194.

Grbić V., Bleecker A.B. (2000). Axillary meristem development in Arabidopsis thaliana. *The Plant Journal,* 21 (2), 215-223.

Halle F., Kurmann M.H., Hemsley A.R. (1999). Ecology of reiteration in tropical trees. *The evolution of plant architecture,* 93–107. Royal Botanic Gardens.

Hamiaux C., Drummond R.S., Janssen B.J., Ledger S.E., Cooney J.M., Newcomb R.D., Snowden K.C. (2012). DAD2 is an α/β hydrolase likely to be involved in the perception of the plant branching hormone, strigolactone. *Current Biolog,y* 22 (21), 2032-6.

Hayward A., Stirnberg P., Beveridge C.A., Leyser O. (2009). Interactions between auxin and strigolactone in shoot branching control. *Plant Physiol,* 151, 400–412.

Hubbard L., McSteen P., Doebley J., Hake S. (2002). Expression patterns and mutant phenotype of teosinte branched1 correlate with growth suppression in maize and teosinte. *Genetic,s* 162, 1927–1935.

Janssen B.J., Snowden K.C. (2012). Strigolactone and karrikin signal perception: receptors, enzymes, or both? *Front Plant Sci,* 3, 296.

Jiang L., Liu X., Xiong G., Liu H., Chen F., Wang L., Meng X., Liu G., Yu H., Yuan Y, Yi W., Zhao L., Ma H., He Y., Wu Z., Melcher K., Qian Q., Xu H.E., Wang Y., Li J. (2013). DWARF 53 acts as a repressor of strigolactone signalling in rice. *Nature,* 504 (7480), 401-5.

Jones R.J., Schreiber B.M.N. (1997). Role and function of cytokinin oxidase in plants. *Plant Growth Regul,* 23, 123–134.

Kakimoto T. (2001). Identification of plant cytokinin biosynthetic enzymes as dimethylallyl diphosphate: ATP/ADP isopentenyltransferases. *Plant Cell Physiol,* 42, 677–685.

Klee H.J., Lanahan M.B. (1995). *Transgenic plants in plant biology*. In Plant Hormones: Physiology, Biochemistry and Molecular Biology, edn 2. Edited by Davies PJ. Dordrecht, The Netherlands: Kluwer Academic Publishers. 340-353.

Kuromori T., Hirayama T., Kiyosue Y., Takabe H., Mizukado S., Sakurai T., Akiyama K., Kamiya A., Ito T., Shinozaki K. (2004). A collection of 11 800 single-copy Ds transposon insertion lines in Arabidopsis. *Plant J,* 37, 897-905.

Lechner E., Achard P., Vansiri A., Potuschak T., Genschik P. (2006). F-box proteins everywhere. *Curr. Opin. Plant Biol,* 9, 631–638.

Li C., Bangerth F. (2003). Stimulatory effect of cytokinins and interaction with IAA on the release of lateral buds of pea plants from apical dominance. *J. Plant Physiol,* 160, 1059–1063.

Li C., Bangerth F. (1999). Autoinihibition of indoleacetic acid transport in the shoot of two-branched pea (*Pisum sativum*) plants and its relationship to correlative dominance. *Physiol. Plant,* 106, 415–420.

Lin H., Wang R., Qian Q., Yan M., Meng X., Fu Z., Yan C., Jiang B., Su Z., Li J., Wang J. (2009). DWARF27, an iron-containing protein required for the biosynthesis of strigolactones, regulates rice tiller bud outgrowth. *Plant Cell,* 21, 1512–1525.

Ljung K., Bhalerao R.P., Sandberg G. (2001). Sites and homeostatic control of auxin biosynthesis in Arabidopsis during vegetative growth. *The Plant Journal,* 28, 465–474.

Minakuchi K., Kameoka H., Yasuno N., Umehara M., Luo L., Kobayashi K., Hanada A., Ueno K., Asami T., Yamaguchi S., Kyozuka J. (2010). FINE CULM1 (FC1) works downstream of strigolactones to inhibit the outgrowth of axillary buds in rice. *Plant Cell Physiol,* 51 (7), 1127–1135.

Miyawaki K., Matsumoto-Kitano M., Kakimoto T. (2004). Expression of cytokinin biosynthetic isopentenyltransferase genes in Arabidopsis: tissue specificity and regulation by auxin, cytokinin, and nitrate. *Plant J,* 37, 128–138.

Morris D.A. (1977). Transport of exogenous auxin in two-branched dwarf pea seedlings (*Pisum sativum L.*). *Planta,* 136, 91–96.

Mouchel C.F., Leyser O. (2007). Novel phytohormones involved in long-range signaling. *Curr Opin Plant Biol,* 10, 473–476.

Müller D., Leyser O. (2011). Auxin, cytokinin and the control of shoot branching. *Annals of Botany,* 107, 1203–1212.

Napoli C. (1996). Highly branched phenotype of the petunia *dad1–1* mutant is reversed by grafting. *Plant Physiol,* 111, 27–37.

Negi J., Moriwaki K., Konishi M. Yokoyama R., Nakano T., Kusumi K., Hashimoto-Sugimoto M., Schroeder J.I., Nishitani K., Yanagisawa S., Iba K. (2013). A Dof Transcription Factor, SCAP1, is Essential for the Development of Functional Stomata in Arabidopsis. *Current Biology,* 23, 479–484.

Nordström A., Tarkowski P., Tarkowska D., Norbaek R., Astot C., Dolezal K., Sandberg G. (2004). Auxin regulation of cytokinin biosynthesis in Arabidopsis thaliana: a factor of potential importance for auxin–cytokinin-regulated development. *Proc Natl Acad Sci. USA,* 101, 8039–8044.

Ongaro V., Leyser O. (2008). Hormonal control of shoot branching. *J Exp Bot,* 59, 67–74.

Prusinkiewicz P., Crawford S., Smith R.S., Ljung K., Bennett T., Ongaro V., Leyser O. (2009). Control of bud activation by an auxin transport switch. *Proc. Natl Acad. Sci. USA,* 106, 17431–17436.

Sachs T., Thimann V. (1967). The role of auxins and cytokinins in the release of buds from dominance. *American Journal of Botany* 54, 136–144.

Sachs T. (1981). The control of patterned differentiation of vascular tissues. *Adv. Bot. Res,* 9, 151–162.

Schwartz S.H., Qin X., Loewen M.C. (2004). The biochemical characterization of two carotenoid cleavage enzymes from Arabidopsis indicates that a carotenoid-derived compound inhibits lateral branching. *J. Biol. Chem,* 279, 46940–46945.

Shimizu-Sato S., Tanaka M., Mori H. (2009). Auxin–cytokinin interactions in the control of shoot branching. *Plant Mol Biol* 69, 429–435.

Simons J.L., Napoli C.A., Janssen B.J., Plummer K.M., Snowden KC. (2007). Analysis of the *DECREASED APICAL DOMINANCE* genes of petunia in the control of axillary branching. *Plant Physiol* 143, 697–706.

Smith S.M. (2013). Plant biology: Witchcraft and destruction. *Nature,* 504, 7480.

Smith S.M, Waters M.T. (2012). Strigolactones: destruction-dependent perception? *Curr. Biol,* 22, R924–R927.

Snow R. (1929). The transmission of inhibition through dead stretches of stem. *Ann. Bot*., 43, 261–267.

Snow R. (1929). The young leaf as the inhibiting organ. *New Phytol,* 28 (5), 345–358.

Sorefan K., Booker J., Haurogné K., Goussot M., Bainbridge K., Foo E. Chatfield S., Ward S., Beveridge C., Rameau C., Leyser O. (2003). *MAX4* and *RMS1* are orthologous dioxygenase-like genes that regulate shoot branching in Arabidopsis and pea. *Genes Dev,* 17, 1469–1474.

Stirnberg P., van De Sande K., Leyser O. (2002). MAX1 and MAX2 control shoot lateral branching in Arabidopsis. *Development,* 129, 1131–1141.

Stirnberg P., Furner I.J., Leyser H.M.O. (2007). MAX2 participates in an SCF complex which acts locally at the node to suppress shoot branching. *The Plant Journal,* 50 (1), 80–94.

Sussex I.M., Kerk N.M. (2001). The evolution of plant architecture. *Current Opinion in Plant Biology,* 4, 3–37.

Takei K., Sakakibara H., Sugiyama T. (2001). Identification of genes encoding adenylate isopentenyltransferase, a cytokinin biosynthesis enzyme, in Arabidopsis thaliana. *J Biol Chem,* 276, 26405–26410.

Tamas I.A. (1995). Hormonal regulation of apical dominance. In Plant Hormones: Physiology, Biochemistry and Molecular Biology, edn 2. Edited by Davies PJ. Dordrecht, The Netherlands: Kluwer Academic Publishers. 572-597.

Tanaka M., Takei K., Kojima M., Sakakibara H., Mori H. (2006). Auxin controls local cytokinin biosynthesis in the nodal stem in apical dominance. *The Plant Journal,* 45 (6), 1028-1036.

Tantikanjana T., Yong J.W.H., Letham D.S., Griffith M., Hussain M., Ljung K., Sandberg G., Sundaresan V. (2001). Control of axillary bud initiation and shoot architecture in Arabidopsis through the *SUPERSHOOT* gene. *Genes Dev,* 15 (12), 1577–1588.

Thimann K.V., Skoog F. (1933). Studies on the growth hormone of plants. III. The inhibiting action of the growth substance on bud development. *Proc Natl Acad Sci. USA,* 19, 714-716.

Thimann K.V., Skoog F. (1934). Studies on the Growth Hormone of Plants. *Proc Natl Acad Sci,* 19 (7), 714–716.

Umehara M., Hanada A., Yoshida S., Akiyama K., Arite T., Takeda-Kamiya N., Magome H., Kamiya Y., Shirasu K., Yoneyama K., Kyozuka J., Yamaguchi S. (2008). Inhibition of shoot branching by new terpenoid plant hormones. *Nature,* 455, 195-200.

Ungerer M.C., Halldorsdottir S.S., Modliszewski J.L., Mackay T.F.C., Purugganan M.D. (2002). Quantitative Trait Loci for Inflorescence Development in *Arabidopsis thaliana*. *Genetics,* 160 (3), 1133-1151.

Wang Y., Sun S., Zhu W., Jia K., Yang H., Wang X. (2013). Strigolactone/MAX2-Induced Degradation of Brassinosteroid Transcriptional Effector BES1 Regulates Shoot Branching. *Developmental cell,* 27(6), 681-688.

Ward S.P., Leyser O. (2004). Shoot branching. *Current Opinion in Plant Biology,* 7, 73–78.

Waters M.T., Nelson D.C., Scaffidi A., Flematti G.R., Sun Y.K., Dixon K.W., Smith S.M. (2012). Specialisation within the DWARF14 protein family confers distinct responses to karrikins and strigolactones in Arabidopsis. *Development,* 139, 1285–1295.

Waters M.T., Brewer P.B., Bussell J.D., Smith S.M., Beveridge C.A. (2012). The Arabidopsis ortholog of rice DWARF27 acts upstream of MAX1 in the control of plant development by strigolactones. *Plant physiology,* 159 (3), 1073-1085.

Wickson M., Thimann K.V. (1958). The antagonism of auxin and kinetin in apical dominance. *Physiol Plant,* 49, 304–314.

Zhao L.H., Zhou X.E., Wu Z.S., Yi W., Xu Y., Li S., Xu T.H., Liu Y., Chen R.Z., Kovach A., Kang Y., Hou L., He Y., Xie C., Song W., Zhong D., Xu Y., Wang Y., Li J., Zhang C., Melcher K., Xu H.E. (2013). Crystal structures of two phytohormone signal-transducing alpha/beta hydrolases: karrikin-signaling kai2. *Cell Res,* 3, 436-9.