Chapter 3
Axillary Shoot Branching in Plants

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3.1 Introduction

Multiple shoot branching refers to the ability of a plant to produce an extra number of axillary shoots. This phenotype usually reflects healthy and yield-promising plants because increases in shoot branching can be translated to greater vegetative biomass, fruit and seed production. Historically, multiple shoot branches was a desirable trait in some crop plants, such as rice, in which multiple shoot branches (tillers) are associated with increased yield. In contrast, maize cultivars have been selected for a low number of axillary branches to improve the quality of the ears and kernels by concentrating plant resources.

High-yield production can be achieved by genetically altering the number of shoots per plant and/or by modifying other processes related to plant growth and development, as axillary branch formation is controlled by a complex interaction between genetically regulated developmental processes and the environment. Multiple shoot branching can also be achieved, to some extent, by augmenting the amount of fertilizers used in the field. However, increasing the fertilizer usage does not proportionally augment the yield because wild-type plants have a limited biochemical capacity to metabolize these artificially supplied inorganic nutrients. In fact, the application of high amounts of fertilizer to increase the number of shoot branches produced per plant would not only enhance input costs to farmers, but also lead to an accumulation of unused fertilizers in the soil which would ultimately pollute the groundwater. Therefore, the optimum situation is to use reasonable amounts of supplied nutrition and to genetically alter the number of shoot branches

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to obtain the desired plant architecture to maximize yield in crop plants. This requires an understanding of the mechanisms controlling plant architecture.

Significant progress has been made towards gaining a better understanding of the mechanisms responsible for axillary meristem initiation and development due, in part, to the availability of modern reverse genetics and genetic mapping technologies. Reverse genetic approaches, which are based on determining the phenotypic effect of losing a functional gene, have facilitated the identification of genes involved in multiple branching phenotypes. Genes identified so far using these technologies have been shown to display different degrees of regulatory relationships with known branching mechanisms, and to encode products involved in hormonal mobilization, gene transcription, protein ubiquitation or degradation networks. Discoveries achieved in the area of shoot branching physiology were previously thoroughly discussed in several excellent reviews which cover topics such as the physiology of secondary bud initiation (Prusinkiewicz 2004; McSteen and Leyser 2005; Dun et al. 2006), the role of hormones in shoot branching (McSteen and Leyser 2005; Doust 2007; De Smet and Jurgens 2007; Ongaro and Leyser 2008) and the genes involved in shoot branching (Wang and Li 2006; Doust 2007). In this chapter, we discuss the progress achieved so far in understanding shoot branching mechanisms in plants and the metabolic pathways controlling this process.

3.2 Axillary Shoot Development

The initiation of axillary branching and development is a complicated process and was found to be controlled by a range of genes. The identification of genes controlling this process is crucial since they would provide key targets which can be manipulated to improve plant architecture in order to increase crop yield. Most of the genes involved in the initiation of axillary branching were discovered by studying the phenotypic effect of a mutation within a given gene. Therefore, in some cases there is an indirect relationship between these genes and the actual axillary branching mechanism. Nevertheless, the role which these genes play in controlling this process and the other factors which these genes influence have been thoroughly studied. For example, the genes discovered so far were found to control branching through alteration of different transcriptional or hormonal pathways. Also, different types of transcription factor genes were characterized to be keys in regulating these pathways by controlling the expression of gene networks involved in axillary branching. This indicates that the coordinated expression of an array of genes may be necessary in order to achieve multiple branching phenotypes. In addition, other identified proteins implicated in axillary branching are involved mainly in hormonal regulation pathways, suggesting that hormones are vital for the regulation of axillary branching. This chapter discusses the recently identified genes which show significant effects on axillary branching. It is possible that, at a certain stage of bud initiation and development, both wild-type and multiple axillary mutants share a common molecular pathway. After this point, other factors are responsible for leading to the observed differentiation in axillary

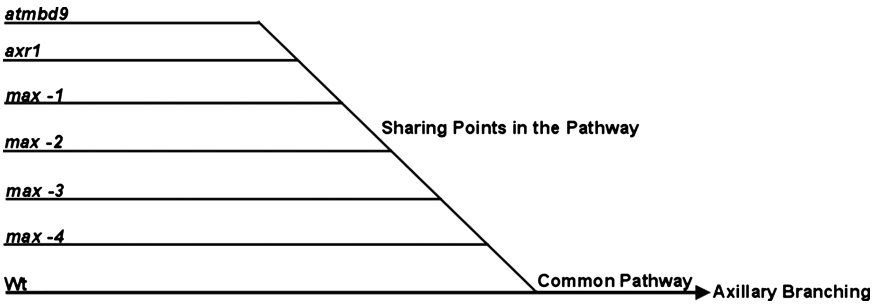

Fig. 3.1 Genetic modifications lead to multiple axillary branching in Arabidopsis. Schematic representation of the proposed axillary branching pathways involved. The different genotypes share a common pathway of axillary bud initiation and outgrowth at some point

branch numbers giving rise to the multiple branching phenotype in the mutants thus far studied (Fig. 3.1). The known information on these genes is discussed, as well as the possible pathways in which they are involved.

3.2.1 Bud Initiation

Based on available information, plant species share some similarities in their control of shoot initiation. In dicotyledons, plant growth stops once a genetically predetermined structure has completely formed (determinately), or can continue to develop throughout the life of a plant (indeterminately). The meristem produces phytomers, which are shoot units consisting of a leaf, axillary bud and a stem segment. Arabidopsis mutant plants of unfunctional Terminal Flower1 (TFL1) transcription factor (Shannon and Meeks-Wagner 1991) have a determinant meristem. TFL1 controls the growth of the phytomer by delaying the expression of floral-related genes, such as *LEAFY* (*LFY*) and *APETALA1* (*AP1*), and hence gives more time for the vegetative axillary buds to initiate and grow (Ratcliffe et al. 1999). Determination of the mechanism by which an axillary bud initiates and develops is important to genetically design plants with a desired branching habit. In fact, there are mainly two different hypotheses describing the process of shoot apical meristem initiation in dicotyledons (McSteen and Leyser 2005). The first is that the new apical meristem starts to form and grow at the leaf axils (Snow and Snow 1942). The second is that the apical meristem results from the growth of active clusters of meristem cells which were originally present in the apical meristem at the time of leaf initiation (Garrison 1955; Sussex 1955). A new hypothesis on axillary bud initiation, which merges the first and the second hypotheses, has been developed based on the molecular functional characterization of a gene which encodes for the transcription factor LATERAL SUPRESSOR (LS) in tomato (Schumacher et al. 1999) and its ortholog of Arabidopsis (LAS; Greb et al. 2003). It is postulated that the LS/LAS prevents the complete differentiation of the leaf axil and thereby maintains its meristematic potential.

In monocotyledons, vegetative branches are called tillers, and usually arise from the basal node of the first formed phytomer. Beside tillers, grasses produce secondary or axillary branches which hold the ears, as in maize. Loss of function of the *MONOCULM1* (*MOC1*) gene (Li et al. 2003), an ortholog of *LS*/*LAS*, resulted in absence of tillers in rice, providing strong evidence of a common shoot branching mechanism in monocotyledons and dicotyledons. Overexpression of the *MOC1* gene increases the number of tillers, implying that this gene can promote apical meristem outgrowth and initiation. A similar defect was noted in the *uniclum2* (*clu2*) barley mutant, in which mutant plants lacked tillers (Babb and Muehlbauer 2003). However, in this case, the defect does not affect apical meristem initiation but cannot guarantee their meristematic activity and development. In contrast, *TEOSINTE BRANCHED 1* (*TB1*) genes in maize (Doebley et al. 1997; Wang et al. 1999; Hubbard et al. 2002) and its ortholog in rice (*OsTB1*; Takeda et al. 2003) suppress tiller and branch growth. Mutations leading to loss of function in these genes result in multiple axillary branch development in maize and a greater tiller number in rice. Despite these valuable findings on genes which control apical meristem initiation in monocotyledons, other genes need to be identified and characterized, because important information is required to clarify some of developmental processes during apical meristem initiation and development. The involvement of other key genes is not unexpected, given the occurrence of significant QTLs other than the *TB1* loci in a genome of some species, such as foxtail millet (Doust et al. 2004).

3.2.2 Genes Control Axillary Shoot Branching

Tremendous progress has been achieved recently towards the identification of new genes which influence axillary shoot branching. These genes have been identified mainly in Arabidopsis or rice plants. However, because axillary branching pathways are common amongst plants, this allows isolation of new genes from different species. Several transcription factors have been found to control axillary branches (Table 3.1). Amongst these transcription factors are the tomato *Blind* (*BL*) gene which encodes for a myb transcription factors (Schmitz et al. 2002), the Arabidopsis *AtMBD9* which encodes for a methyl-CpG binding domain (Peng et al. 2006; Yaish et al. 2009), and the rice *OsNAC2* which codes for a NAC (NAM, ATAF1, 2, CUC2) transcription factor family protein (Mao et al. 2007). These transcription factors have the opposite effect on enhancing axillary shoot formation in plants. While a functional BL protein is required for normal lateral meristems in tomato, and the overexpression of the *OsNAC2* in rice plant increases the number of tillers, mutations within *AtMBD9* also lead to an increase in the axillary branches in Arabidopsis. This indicates the indirect functional control of these transcription factors. Therefore, this fact implies the need to identify the downstream gene products which are controlled by these transcription factors.

Table 3.1 Genes involved in shoot branching

Gene name	Plant species	Class	Function	Reference
AUXIN-RESISTANCE (*AXR1*)	Arabidopsis	Ubiquitin-activating enzyme e1	Controls auxin response	Leyser et al. (1993)
BLIND (*BL*)	Tomato	Transcription factor	Regulation of apical meristem initiation	Schmitz et al. (2002)
BRANCHED1 (*RRC1*)	Arabidopsis	Transcription factor	Prevents axillary bud formation	Aguilar-Martínez et al. (2007)
BUSHY AND DWARF1 (*BUD1*)	Arabidopsis	Kinase	Controls auxin polar transportation	Dai et al. (2006)
DECREASED APICAL DOMINANCE1 (*DAD1*)	Petunia	Dioxygenase	Controls branching	Snowden et al. (2005)
LATERAL SUPPRESSOR (*LAS*)	Arabidopsis	Transcription factor	Controls axillary meristem formation	Greb et al. (2003)
LATERAL SUPPRESSOR (*LS*)	Tomato	Transcription factor	Controls initiation of axillary meristems initiation	Schumacher et al. (1999)
METHYL-CPG BINDING9 (*AtMBD9*)	Arabidopsis	Transcription factor	Controls axillary branching	Peng et al. (2006), Yaish et al. (2009)
MONOCULM1 (*MOC1*)	Rice	Transcription factor	Controls tiller initiation and outgrowth	Li et al. (2003)
MORE AXILLARY GROWTH1 (*MAX1*)	Arabidopsis	Cytochrome P450	Repressor of vegetative bud outgrowth	Stirnberg et al. (2002), Greb et al. (2003), Booker et al. (2005)
MORE AXILLARY GROWTH2 (*MAX2*)	Arabidopsis	F-box LRR	Involvedx in max. signalling pathway	Stirnberg et al. (2002), Greb et al. (2003)
MORE AXILLARY GROWTH3 (*MAX3*)	Arabidopsis	Dioxygenase	Catalyzes the biosynthesis of carotenoid-derived regulators of axillary bud outgrowth inhibitors	Booker et al. (2004)
MORE AXILLARY GROWTH4 (*MAX4*)	Arabidopsis	Dioxygenase	Involved in the biosynthesis of carotenoid-derived axillary bud inhibitors	Sorefan et al. (2003)
NAC (*NAM*, *ATAF1*, *2*, *CUC2*) (*OsNAC2*)	Rice	Transcription factor	Controls tillering	Mao et al. (2007)

(*continued*)

Table 3.1 (continued)

Gene name	Plant species	Class	Function	Reference
RAMOSUS (*RMS*)	Pea	Dioxygenase	Regulates shoot branching	Sorefan et al. (2003)
REVALUTA (*REV*)	Arabidopsis	Transcription factor	Regulation of apical meristem initiation	Otsuga et al. (2001)
SUPPERSHOOT (*SPS*)	Arabidopsis	Cytochrome P450	Involved in axillary bud initiation and growth	Tantikanjana et al. (2001)
TEOSINTE BRANCHED1 (*OsTB1*)	Rice	Transcription factor	Negatively regulates lateral branching	Takeda et al. (2003)
TEOSINTE BRANCHED1 (*TB1*)	Maize	Transcription factor	Controls lateral bud outgrowth	Doebley et al. (1997)

In addition to transcription factors, hormone-related proteins have been shown to have a direct effect on axillary branches. Classically, auxin was known to control axillary branches through the apical dominance phenomena. Therefore, loss of the apical meristem usually leads to increases in the number of axillary branches. Mutation within proteins involved in auxin polar transportation leads to a dwarf and bushy phenotype in the *bud1* mutant (Dai et al. 2006). Likewise, loss of function of the Arabidopsis *AUXIN-RESISTANCE* (*AXR1*) gene reduces the response of Arabidopsis to auxins and increases the axillary branches in Arabidopsis (Leyser et al. 1993).

More recently, novel hormone-like molecules controlled by a group of genes know as *MAXIMUM AXILLARY GROWTH* (*MAX1-4*) were identified and found to be involved in the synthesis and transportation of a non-classical growth regulator, carotenoid-derived signalling molecules (Stirnberg et al. 2002). The *MAX* gene family has homologs in pea *RAMOSUS* (*RMS*; Sorefan et al. 2003) and in petunia *DECREASED APICAL DOMINANCE1* (*DAD1*; Snowden et al. 2005).

3.3 Hormones Involved in Axillary Bud Formation

Shoot branching is determined by the outgrowth of axillary buds, which is regulated by a wide range of endogenous and environmental factors. The most important endogenous factors are the plant hormones. So far, three hormones are known to be involved in axillary bud outgrowth and, consequently, shoot branching. These hormones include auxin and cytokinin, as well as new, chemically unidentified metabolite-like hormones. The following section highlights the different proposed models for the hormonal network-regulated shoot branching.

3.3.1 Auxin, Cytokinin and Novel Hormone

The physiological role of auxin and cytokinin in shoot branching has been studied extensively. Auxin is the first plant hormone shown to be involved in shoot branching, and it has been established that it controls the shoot tip apical dominance and, consequently, inhibits axillary bud outgrowth. Additionally, the replacement of the shoot apex with exogenous auxin maintains the inhibition of axillary buds (Cline 1996). Cytokinins show the opposite physiological role to auxin, since they act directly to promote axillary bud outgrowth. Studies have demonstrated that either exogenous cytokinin application or overexpression of genes encoding enzymes involved in cytokinin biosynthesis often induce bud outgrowth (King and Van Staden 1988; Medford et al. 1989; Miguel et al. 1998). In addition, some of the mutants with a greater level of cytokinin show more shoot branching (Dun et al. 2006).

Another carotenoid-like plant hormone with as yet unknown chemical structure was proposed to be involved in regulating bud outgrowth, by the analysis of the

branching mutants in Arabidopsis, pea and petunia. It was shown that the loss of function of the *MAX1*, *MAX2*, *MAX3*, *MAX4*, *MAX5* in Arabidopsis, *RMS1*, *RMS2*, *RMS3*, *RMS4*, *RMS5* and *RMS6* in pea, or *DAD1*, *DAD2*, *DAD3* in petunia resulted in increasing the shoot branching compared to the wild types (Rameau et al. 2002; Stirnberg et al. 2002; Sorefan et al. 2003; Bennett et al. 2006). Most of the *MAX*, *RMS* and *DAD* genes have been cloned and appeared to be orthologous (reviewed in Ongaro and Leyser 2008).

3.3.2 Axillary Bud Outgrowth Hypotheses

Three hypotheses were proposed for the role of the plant hormones auxin and cytokinin in shoot branching (Dun et al. 2006). These are the classical hypothesis, the auxin transport hypothesis and the bud transition hypothesis. The classical hypothesis proposed that auxin regulates shoot branching by influencing the level of other signals required for bud outgrowth inhibition (Dun et al. 2006). These signals are referred to as second messengers for auxin action (McSteen and Leyser 2005). Evidence for the role of second messengers was obtained from various studies which found a link between the cytokinin biosynthetic pathway and bud outgrowth. For example, decapitation in legumes resulted in a concomitant increase in the endogenous cytokinin concentrations in axillary buds, possibly mediated by an increase in the expression of the cytokinin biosynthesis genes (isopentenyl transferase IPT1 and IPT2) in the stem. This increase is partially removed by auxin application (Tanaka et al. 2006) and, consequently, reduces the cytokinin supply to the bud (McSteen and Leyser 2005). It was suggested that novel hormone, in addition to cytokinin, might serve as second messenger for auxin action (McSteen and Leyser 2005).

The second hypothesis is based on the auxin transport stream. Auxin is synthesized in the apical meristems and transported to the basal plant organs thorough the polar auxin transport stream (Ljung et al. 2001). It was suggested that this stream is saturated with auxin and thus prevents the flow of the auxin from the axillary buds in the plants where the axillary bud outgrowth is inhibited (Li and Bangerth 1999; Leyser 2005). Several proteins are involved in active auxin transport. In *Arabidopsis*, the main shoot PIN1 (Auxin efflux carrier) appears to be particularly important for polar auxin transport (Okada et al. 1991). Supporting the transport hypothesis, Bennett et al. (2006) reported a higher level of labelled auxin and PIN1 expression in the stem of the *max4* mutant. Similar results were observed in the *rms* pea mutant (Beveridge et al. 2000). Enhancing auxin movement in the branching mutants may be an indicator for the auxin flow enhancement in these plants, which is responsible for the increased shoot branching. However, the shoot endogenous auxin content may not have a direct correlation with shoot branching. For example, grafting *rms2* scion on the wild-type rootstock inhibits bud outgrowth without reducing a high auxin concentration in the *rms2* internodes, which might be due to the feedback regulation which allows the signals from the wild-type region to inhibit bud outgrowth (Foo et al. 2005; Morris et al. 2005; Beveridge 2006).

The third hypothesis for auxin in shoot branching is the bud transition hypothesis. Based on this hypothesis, bud development can be classified in three stages: dormancy, transition and sustained growth (reviewed in Dun et al. 2006). It seems that bud location on the stem influences its outgrowth potential and its response to cytokinin or to decapitation. For example, cytokinin application is effective in inducing the outgrowth of the axillary buds at pea node 2. However, this treatment does not promote the growth of the axillary buds at node 3 or node 4 (King and Van Staden 1988). It was proposed that bud growth is determined by the bud stage, and the auxin can act to inhibit the bud outgrowth only in the transition stage. This hypothesis is supported by the findings of Morris et al. (2005), who reported the occurrence of a rapid signal which led to the dormant bud entering into the transition stage after decapitation. This includes the initial but not the sustained bud growth. Thus, the current understanding can be integrated with the classical hypothesis, which proposes that auxin may inhibit the growth of the bud in the transition stage by affecting the cytokinin response.

3.3.3 Abscisic Acid and Branching

It is well known that "cross talk" exists in the hormonal networks which are involved during different developmental stages throughout the plant's life cycle. Therefore, in addition to the well-known role of auxin and cytokinin, we cannot exclude the possibility of the participation of other hormones such as abscisic acid (ABA), also a carotenoid derivative, in controlling axillary bud outgrowth and, consequently, shoot branching. Several studies have been carried out to elucidate the role of the plant hormone ABA in shoot branching and its interaction with auxin. To date, the precise role of this hormone in the branching network is not clear. ABA was also implicated as a secondary messenger which modulates auxin-induced repression of axillary bud growth. However, evidence to support this is lacking (Chatfield et al. 2000). The possible role of ABA in controlling axillary bud outgrowth is supported by the fact that ABA is a "dormancy hormone", and the exogenous ABA application inhibits the growth of active buds. Decapitation is also accompanied by a reduction of the lateral bud ABA content (Geuns et al. 2001). For example, the increase of endogenous indole-3-acetic acid (IAA) at the terminal buds and internodes of soybeans, when exposed to shaded light of a low red:far-red ratio, induced an increased synthesis of ABA in the axillary buds (Begonia and Aldrich 1990). Also, the ABA-insensitive *AB13* mutant inhibited vegetative growth and was expressed abundantly in dormant axillary buds (Rohde et al. 1999 http://aob.oxfordjournals.org/cgi/content/full/98/4/ - B28). Work on the ABA-insensitive *Arabidopsis* mutants, *abi1-1* and *abi2-1*, demonstrated that auxin inhibition of axillary bud outgrowth is ABA-independent and excludes the involvement of ABA in apical dominance (Chatfield et al. 2000). Furthermore, compared to wild type, the leaves of the pea *rms2* mutant are similar in ABA content and responses to ABA on stomatal conductance (Dodd et al. 2008). Interestingly, recent work using

decapitated shoots of *Ipomoea nil* (Japanese morning glory) and *Solanum lycopersicum* (Better Boy tomato) revealed that, unlike auxin, apically applied ABA did not restore apical dominance, but ABA was able to repress lateral bud outgrowth when applied basally (Cline and Oh 2006). These findings imply a possible interaction between ABA, auxin and the unidentified carotenoid-derived hormone, whereby ABA is able to restore apical dominance via acropetal transport up the shoot (Cline and Oh 2006). The finding opens up new avenues of investigation on the role of ABA in apical dominance. Thus, despite the evidence for the involvement of ABA in the inhibition of the axillary bud outgrowth, details about its role and its interaction with auxin and cytokinin still need further clarification.

3.4 Regulatory Pathways Involved in Shoot Branching

Shoot system architecture is regulated by the establishment of axillary meristems and the outgrowth of axillary buds. While auxin is the primary effector of shoot branching, auxin does not enter the lateral buds to inhibit bud growth. Instead, other secondary messengers are involved in the repression of bud outgrowth, and their actions are mediated by auxin. This section describes the diverse set of molecules which interact with auxin to control the shoot system architecture.

3.4.1 Carotenoid-Derived Signalling Molecules

Carotenoids are a class of isoprenoid-derived compounds which are produced in the plastids. Carotenoids can absorb light energy and dissipate excess energy, and are precursors for hormone biosynthesis. A novel carotenoid-derived compound with unknown chemical structure has been shown recently to be required for the inhibition of axillary bud growth. This was demonstrated through the analysis of the *DAD1*, *MAX4* and *RMS1* mutants in petunia, *Arabidopsis* and pea respectively, which displayed an increase in lateral branching (Sorefan et al. 2003; Snowden et al. 2005; Bennett et al. 2006). The *DAD1*, *MAX4* and *RMS1* mutants result from lesions in the gene which encodes a carotenoid-cleavage dioxygenase (CCD). Therefore, the increase in branching in these mutants is due to the inability to synthesize a carotenoid-derived signalling molecule capable of inhibiting axillary meristem development (Schwartz et al. 2004; Bennett et al. 2006).

The carotenoid-derived signalling molecule is synthesized via the *MAX* (*More axillary branching*) pathway in *Arabidopsis*. There are four genes (*MAX1* to *MAX4*) in this pathway, and the synthesis of the acropetally mobile molecule depends on the actions of MAX1, AtCCD7 (MAX3) and AtCCD8 (MAX 4). Analysis of the recombinant proteins showed that AtCCD7 catalyzes a 9–10 cleavage of β-carotene to produce the 10′-apo-β-carotenal and β-ionone, while AtCCD9 catalyzes a 13–14 cleavage of the 10′-apo-β-carotenal to produce 13′-apo-β-carotenone (Schwartz

et al. 2004). *MAX1* encodes a cytochrome P_{450} and acts downstream of MAX3 and MAX4. *MAX2* encodes an F-box LRR family protein and is responsible for perceiving the signal. It has been proposed that the MAX-dependent pathway branching signal interacts with auxin and cytokinin hormone networks (Wang and Li 2008).

In addition to the MAX-dependent pathway branching signal, another carotenoid-derived signalling molecule has been identified based on work done on the *bypass1* (*bps1*) *Arabidopsis* mutant. The *bps1* mutant displayed loss of shoot apical meristem activity as a result of a constitutively produced graft-transmissable signal capable of arresting shoot growth (Van Norman and Sieburth 2007). The synthesis of this signal requires β-carotene but not the activity of CCDs and, therefore, does not require AtCCD7 or AtCCD8.

Taken together, it is clear that the carotenoid pathway is important for the synthesis of mobile signals which regulate shoot development. The next goal is to determine the chemical structure of these novel signalling molecules in order to examine the mechanism involved in regulating shoot branching. Since these carotenoid-derived signalling molecules also move acropetally from the roots to the shoots, and modulate auxin-mediated repression of bud outgrowth, it will also be important to determine whether these novel carotenoid-derived signals interact with ABA to modulate auxin-mediated repression of bud growth.

3.4.2 Polyamines

Polyamines are aliphatic nitrogen compounds implicated in playing important roles in plant growth and development. The involvement of polyamines in apical dominance was demonstrated using *isopentyl transferase* (*ipt*)-transformed tobacco. It was observed that the defoliation of upper nodes of *ipt*-transformed tobacco plants led to an enhanced concentration of cytokinins in the axillary buds. This resulted in the release of the axillary buds from dormancy, and a concomitant change in polyamine composition occurred, whereby putrescine and spermidine levels decreased and spermine levels increased in the axillary buds (Geuns et al. 2001). It has been proposed that polyamines may play an important role in the subsequent growth and development of axillary buds into shoots after their release from dormancy (Geuns et al. 2001). Similar patterns have been observed in other plants. For example, the *Arabidopsis* bushy and dwarf mutant, *bud2*, shows severe alterations in apical dominance. The *bud2* mutant results from the complete deletion of the gene which encodes an *S*-adenosylmethionine decarboxylase (SAMDC; Ge et al. 2006). This SAMDC is required for the synthesis of the polyamines spermidine and spermine from putrescine. Consequently, the *bud2* mutant had higher levels of putrescine and lower levels of both spermidine and spermine, and this alteration in polyamine homeostasis led to the termination of dormancy of axillary buds. However, the response of *bud2* to auxin and cytokinin remains to be determined. Further work on this mutant may provide insights into the precise role polyamines play in shoot branching (Ge et al. 2006).

Recent work by Falasca et al. (2008) showed that spermidine, putrescine and α-1,4-linked oligogalacturonides (OGs) enhanced the formation of cytokinin-induced adventitious vegetative shoots in tobacco leaf explants. The effect of putrescine was less pronounced than that of spermidine. However, unlike spermidine, the effect of OG on the enhancement of adventitious vegetative shoot formation was calcium-independent, and the stimulatory effect of spermidine was enhanced in the presence of auxin (Falasca et al. 2008). Moreover, exogenous application of calcium and auxin to tobacco leaf explants led to an enhancement in the expression of genes encoding enzymes involved in polyamine biosynthesis, whereas exogenous OG repressed their expression. This implies that while polyamines affect cytokinin-induced vegetative shoot regeneration, calcium and auxin may modulate their effects during shoot growth (Falasca et al. 2008). Therefore, future work should take into account the interplay between auxin, cytokinin, OGs and calcium in mutants defective in polyamine biosynthesis to determine their importance and the mechanism controlling plant architecture.

3.4.3 Inositol Phosphates

Inositol phosphates (IPs) are a group of phosphorylated C6-cyclitols, and are important secondary messengers in eukaryotic cells. For example, inositol 1,4,5-triphosphate (IP_3) and inositol 1,3,4,5-tetrakis-phosphate (IP_4) are secondary messengers which regulate cytosolic calcium concentration in animal cells (Berridge 1993). In *Arabidopsis*, the inositol polyphosphate 6-/3-kinase genes (*AtIpk2a* and *AtIpk2β*) encode enzymes capable of converting IP_3 to inositol 1,4,5,6-tetrakis-phosphate, a precursor for phytate synthesis (Stevenson-Paulik et al. 2002). Recently, *AtIpk2β* has been shown to play a role in axillary shoot branching by controlling auxin signalling (Zhang et al. 2007). *Arabidopsis* plants with the overexpressed *AtIpk2β* gene possessed more axillary shoot branches, and had greater bud outgrowth rates compared to wild type (Zhang et al. 2007). Moreover, *Arabidopsis* plants with the overexpressed *AtIpk2β* gene had repressed levels of the *MAX4* transcript. Interestingly, *AtIpk2β* was induced by exogenous auxin, and *AtIpk2β* overexpression lines displayed altered auxin responses, as well as deviations in auxin distribution and accumulation. Therefore, these findings strongly imply that *AtIpk2β* regulates axillary shoot branching in *Arabidopsis* by interacting with the auxin-signalling pathway and the *MAX*-dependent pathway branching signal.

The role that *AtIpk2β* plays in axillary branching via auxin signalling is intriguing. The recent determination of the crystal structure of the auxin receptor TIR1 supports a further role for phytate, the end product derived from IP_3 via actions of AtIpk2β. The auxin-binding pocket of TIR1 is stabilized by phytate (Kepinski 2007), and this finding provides insights into the mechanism controlling auxin-mediated signalling via the actions of *AtIpk2β*.

In conclusion, recent work has demonstrated that secondary messengers are crucial for auxin-mediated repression of bud outgrowth which shapes plant architecture. Future work should focus on the interplay between secondary messengers and the hormone networks which modulate their activity to unravel the mechanism controlling shoot branching.

3.5 Future Perspectives

Multiple axillary branching should be considered for increasing crop biomass formation and yield. Engineering plants with maximum axillary shoot number is not a simple task because several mechanisms involved are still unclear. Also, the exact function of auxin and cytokine receptors, as well as the nature of the *MAX* gene products and their role in inducing axillary buds need further investigation. The information released from high-throughput microarray and metabolic pathway data, along with additional genetic and physiological studies, may better clarify the axillary shoot branching processes.

References

Aguílar-Martinez JA, Poza-Carrion C, Cubas P (2007) *Arabidopsis BRANCHED1* acts as an integrator of branching signals within axillary buds. Plant Cell 19:458–472

Babb S, Muehlbauer GJ (2003) Genetic and morphological characterization of the barley uniculm2 (cul2) mutant. Theor Appl Genet 106:846–857

Begonia GB, Aldrich RJ (1990) Changes in endogenous growth regulator levels and branching responses of soybean to light quality altered by velvetleaf (*Abutilon theophrasti* Medik.). Biotronics 19:7–18

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

Berridge MJ (1993) Inositol trisphosphate and calcium signalling. Nature 361:315–325

Beveridge CA (2006) Axillary bud outgrowth: sending a message. Curr Opin Plant Biol 9:35–40

Beveridge CA, Symons GM, Turnbull CG (2000) Auxin inhibition of decapitation-induced branching is dependent on graft-transmissible signals regulated by genes *Rms1* and *Rms2*. Plant Physiol 123:689–698

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

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

Chatfield SP, Stirnberg P, Forde BG, Leyser O (2000) The hormonal regulation of axillary bud growth in Arabidopsis. Plant J 24:159–169

Cline MG (1996) Exogenous auxin effects on lateral bud outgrowth in decapitated shoots. Ann Bot 78:255–266

Cline MG, Oh C (2006) A reappraisal of the role of abscisic acid and its interaction with auxin in apical dominance. Ann Bot 98:891–897

Dai Y, Wang H, Li B, Huang J, Liu X, Zhou Y, Mou Z, Li J (2006) Increased expression of MAP KINASE KINASE7 causes deficiency in polar auxin transport and leads to plant architectural abnormality in *Arabidopsis*. Plant Cell 18:308–320

De Smet I, Jurgens G (2007) Patterning the axis in plants - auxin in control. Curr Opin Genet Dev 17:337–343

Dodd IC, Ferguson BJ, Beveridge CA (2008) Apical wilting and petiole xylem vessel diameter of the *rms2* branching mutant of pea are shoot controlled and independent of a long-distance signal regulating branching. Plant Cell Physiol 49:791–800

Doebley J, Stec A, Hubbard L (1997) The evolution of apical dominance in maize. Nature 386:485–488

Doust AN (2007) Grass architecture: genetic and environmental control of branching. Curr Opin Plant Biol 10:21–25

Doust AN, Devos KM, Gadberry MD, Gale MD, Kellogg EA (2004) Genetic control of branching in foxtail millet. Proc Natl Acad Sci USA 101:9045–9050

Dun EA, Ferguson BJ, Beveridge CA (2006) Apical dominance and shoot branching. Divergent opinions or divergent mechanisms? Plant Physiol 142:812–819

Falasca G, Capitani F, Della Rovere F, Zaghi D, Franchin C, Biondi S, Altamura MM (2008) Oligogalacturonides enhance cytokinin-induced vegetative shoot formation in tobacco explants, inhibit polyamine biosynthetic gene expression, and promote long-term remobilisation of cell calcium. Planta 227:835–852

Foo E, Bullier E, Goussot M, Foucher F, Rameau C, Beveridge CA (2005) The branching gene *RAMOSUS1* mediates interactions among two novel signals and auxin in pea. Plant Cell 17:464–474

Garrison R (1955) Studies in the development of axillary buds. Am J Bot 42:257–266

Ge C, Cui X, Wang Y, Hu Y, Fu Z, Zhang D, Cheng Z, Li J (2006) BUD2, encoding an *S*-adenosylmethionine decarboxylase, is required for *Arabidopsis* growth and development. Cell Res 16:446–456

Geuns JM, Smets R, Struyf T, Prinsen E, Valcke R, Van Onckelen H (2001) Apical dominance in *Pssu-ipt*-transformed tobacco. Phytochemistry 58:911–921

Greb T, Clarenz O, Schafer E, Muller D, Herrero R, Schmitz G, Theres K (2003) Molecular analysis of the *LATERAL SUPPRESSOR* gene in *Arabidopsis* reveals a conserved control mechanism for axillary meristem formation. Genes Dev 17:1175–1187

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. Genetics 162:1927–1935

Kepinski S (2007) The anatomy of auxin perception. Bioessays 29:953–956

King RA, Van Staden J (1988) Differential responses of buds along the shoot of Pisum sativum to isopentyladenine and zeatin application. Plant Physiol Biochem 26:253–259

Leyser O (2005) The fall and rise of apical dominance. Curr Opin Genet Dev 15:468–471

Leyser HM, Lincoln CA, Timpte C, Lammer D, Turner J, Estelle M (1993) *Arabidopsis* auxin-resistance gene *AXR1* encodes a protein related to ubiquitin-activating enzyme E1. Nature 364:161–164

Li C-J, Bangerth F (1999) Autoinhibition of indoleacetic acid transport in the shoot of two - branched pea (*Pisum sativum*) plants and its relationship to correlative dominance. Physiol Plant 106:15–20

Li X, Qian Q, Fu Z, Wang Y, Xiong G, Zeng D, Wang X, Liu X, Teng S, Hiroshi F, Yuan M, Luo D, Han B, Li J (2003) Control of tillering in rice. Nature 422:618–621

Ljung K, Bhalerao RP, Sandberg G (2001) Sites and homeostatic control of auxin biosynthesis in Arabidopsis during vegetative growth. Plant J 28:465–474

Mao C, Ding W, Wu Y, Yu J, He X, Shou H, Wu P (2007) Overexpression of a NAC-domain protein promotes shoot branching in rice. New Phytol 176:288–298

McSteen P, Leyser O (2005) Shoot branching. Annu Rev Plant Biol 56:353–374

Medford JI, Horgan R, El-Sawi Z, Klee HJ (1989) Alterations of endogenous cytokinins in transgenic plants using a chimeric isopentenyl transferase gene. Plant Cell 1:403–413

Miguel LC, Longnecker NE, Ma Q, Osborne L, Atkins CA (1998) Branch development in *Lupinus angustifolius* L. I. Not all branches have the same potential growth rate. J Exp Bot 49:547–553
Morris SE, Cox MC, Ross JJ, Krisantini S, Beveridge CA (2005) Auxin dynamics after decapitation are not correlated with the initial growth of axillary buds. Plant Physiol 138:1665–1672
Okada K, Ueda J, Komaki MK, Bell CJ, Shimura Y (1991) Requirement of the auxin polar transport system in early stages of Arabidopsis floral bud formation. Plant Cell 3:677–684
Ongaro V, Leyser O (2008) Hormonal control of shoot branching. J Exp Bot 59:67–74
Otsuga D, DeGuzman B, Prigge MJ, Drews GN, Clark SE (2001) *REVOLUTA* regulates meristem initiation at lateral positions. Plant J 25:223–236
Peng M, Cui Y, Bi YM, Rothstein SJ (2006) AtMBD9: a protein with a methyl-CpG-binding domain regulates flowering time and shoot branching in Arabidopsis. Plant J 46:282–296
Prusinkiewicz P (2004) Modeling plant growth and development. Curr Opin Plant Biol 7:79–83
Rameau C, Murfet IC, Laucou V, Floyd RS, Morris SE, Beveridge CA (2002) Pea *rms6* mutants exhibit increased basal branching. Physiol Plant 115:458–467
Ratcliffe OJ, Bradley DJ, Coen ES (1999) Separation of shoot and floral identity in *Arabidopsis*. Development 126:1109–1120
Rohde A, Van Montagn M, Boerjan W (1999) The *ABSCISIC ACIDINSENSITIVE* (*AB13*) gene is expressed during vegetative quiescence processes in *Arabidopsis*. Plant Cell Environ 22:261–270
Schmitz G, Tillmann E, Carriero F, Fiore C, Cellini F, Theres K (2002) The tomato *Blind* gene encodes a MYB transcription factor that controls the formation of lateral meristems. Proc Natl Acad Sci USA 99:1064–1069
Schumacher K, Schmitt T, Rossberg M, Schmitz G, Theres K (1999) The *Lateral suppressor* (*Ls*) gene of tomato encodes a new member of the VHIID protein family. Proc Natl Acad Sci USA 96:290–295
Schwartz SH, Qin X, Loewen MC (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
Shannon S, Meeks-Wagner DR (1991) A mutation in the Arabidopsis TFL1 gene affects inflorescence meristem development. Plant Cell 3:877–892
Snow M, Snow R (1942) The determination of axillary buds. New Phytol 41:13–22
Snowden KC, Simkin AJ, Janssen BJ, Templeton KR, Loucas HM, Simons JL, Karunairetnam S, Gleave AP, Clark DG, Klee HJ (2005) The *Decreased apical dominance1*/*Petunia hybrida CAROTENOID CLEAVAGE DIOXYGENASE8* gene affects branch production and plays a role in leaf senescence, root growth, and flower development. Plant Cell 17:746–759
Sorefan K, Booker J, Haurogne 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
Stevenson-Paulik J, Odom AR, York JD (2002) Molecular and biochemical characterization of two plant inositol polyphosphate 6-/3-/5-kinases. J Biol Chem 277:42711–42718
Stirnberg P, van De Sande K, Leyser HM (2002) *MAX1* and *MAX2* control shoot lateral branching in *Arabidopsis*. Development 129:1131–1141
Sussex IM (1955) Morphogenesis in *Solanum tuberosum* L.: Experimental investigation of leaf dorsiventrality and orientation in the juvenile shoot. Phytomorphology 5:286–300
Takeda T, Suwa Y, Suzuki M, Kitano H, Ueguchi-Tanaka M, Ashikari M, Matsuoka M, Ueguchi C (2003) The *OsTB1* gene negatively regulates lateral branching in rice. Plant J 33:513–520
Tanaka M, Takei K, Kojima M, Sakakibara H, Mori H (2006) Auxin controls local cytokinin biosynthesis in the nodal stem in apical dominance. Plant J 45:1028–1036
Tantikanjana T, Yong JW, Letham DS, 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 15:1577–1588
Van Norman JM, Sieburth LE (2007) Dissecting the biosynthetic pathway for the bypass1 root-derived signal. Plant J 49:619–628

Wang YH, Li JY (2006) Genes controlling plant architecture. Curr Opin Plant Biol 17:123–129
Wang Y, Li J (2008) Molecular basis of plant architecture. Annu Rev Plant Biol 59:253–279
Wang RL, Stec A, Hey J, Lukens L, Doebley J (1999) The limits of selection during maize domestication. Nature 398:236–239
Yaish MW, Peng M, Rothstein SJ (2009) AtMBD9 modulates Arabidopsis development through the dual epigenetic pathways of DNA methylation and histone acetylation. Plant J 59:123–135
Zhang ZB, Yang G, Arana F, Chen Z, Li Y, Xia HJ (2007) Arabidopsis inositol polyphosphate 6-/3-kinase (AtIpk2beta) is involved in axillary shoot branching via auxin signaling. Plant Physiol 144:942–951