

## Auxin Control in the Formation of Adventitious Roots

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### Abstract

Adventitious rooting is a complex process and a key step in the vegetative propagation of economically important woody, horticultural and agricultural species, playing an important role in the successful production of elite clones. The formation of adventitious roots is a quantitative genetic trait regulated by both environmental and endogenous factors. Among phytohormones, auxin plays an essential role in regulating roots development and it has been shown to be intimately involved in the process of adventitious rooting. Great progress has been made in elucidating the auxin-induced genes and auxin signaling pathway, especially in auxin response Aux/IAA and Auxin Response Factor gene families. Although some important aspects of adventitious and lateral rooting signaling have been revealed, the intricate signaling network remains poorly understood. This review summarizes some of the current knowledge on the physiological aspects of adventitious root formation and highlights the recent progress made in the identification of putative molecular players involved in the control of adventitious rooting. Despite much has been discovered regarding the effects and regulation of auxins on plant growth since the Darwin experiments, there is much that remains unknown.

**Keywords:** Aux/IAA, Auxin Response Factor, shoot-born roots, vegetative propagation

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### Introduction to adventitious roots

Vegetative propagation is extensively used in agriculture, horticulture and forestry for multiplying elite plants selected from natural populations or obtained in breeding programs (Hartmann *et al.*, 1990). The formation of adventitious roots is an essential step in vegetative propagation and therefore if cuttings do not form roots, losses occur (de Klerk *et al.*, 1999). Researchers developed new rooting treatments, examining the effects of plant growth regulators (Haissig and Davis, 1994) by a short exposure to a solution with a high auxin concentration or by dipping in rooting powder (auxin with talc). Noticeable progress has been made recently in the research on rooting, which is not a single process but a progressive process consisting of different steps, each with its own requirements (Kevers *et al.*, 1997).

Plant development is modulated by genetic and environmental factors, which have effects on auxin biosynthesis, metabolism, transport, and signaling pathway (Han *et al.*, 2009). Recent advances in studying mutations of *Arabidopsis* and rice increased the understanding of the role of auxin in the regulation of rooting mechanisms and molecular studies are essential to reveal the basic mechanisms operating in adventitious root formation (de Klerk *et al.*, 1999). Many dicots like *Arabidopsis thaliana* have a primary root that branches to generate several orders of lateral roots, while the root systems of crops like rice and

maize are predominantly composed of adventitious roots (Hochholdinger *et al.*, 2004; Osmont *et al.*, 2007).

**Definition.** Adventitious roots can arise naturally from stem tissue under stressful environmental conditions; they may also be induced by mechanical damage or following tissue culture regeneration of shoots (Li *et al.*, 2009). They are postembryonic roots which arise from the stem and leaves and from nonpericycle tissues in old roots (Geiss *et al.*, 2009). There are at least two pathways by which adventitious roots form: by direct organogenesis from established cell types such as the cambium or from callus tissue following mechanical damage (e.g. cuttings).

**Induction phases.** Rooting phases, which can be distinguished in various ways, have different hormone requirements (de Klerk *et al.*, 1999). De Klerk *et al.* (1997) showed that apple microcuttings are not very sensitive to auxin and cytokinin during the first 24 h. Dedifferentiation occurs during this lag period and cells can respond to auxin. The root primordia originates from the cells between the vascular bundles which accumulate starch during the initial 24 h. Between 72-96 h, activated cells become committed to the formation of root primordia by the rhizogenic action of auxin in the induction phase, when auxin pulses induce the highest number of roots. On the histological level the starch grains present at 24 h are degraded during the next 24 h, the first cell divisions occurring on the first 48 h and by 96 h meristemoids are present. Auxin is not required after 96 h and the concentrations favorable

for the meristemoids formation become inhibitory during this phase. The meristemoids develop into root primordia and further into roots during the differentiation phase (de Klerk *et al.*, 1999).

### The plant hormone auxin

Auxins, cytokinins, gibberellins, abscisic acid and ethylene are the five classical groups of plant hormones (Kende and Zeevaert, 1997). Ideas about hormone function have evolved from numerous experiments in which the application of the hormones have shown to affect cell division in the vascular cambium, cell expansion and control of differentiation into different types of cambial derivatives (Melrowicz *et al.*, 2001).

The formation of adventitious roots is a process induced and regulated by environmental and endogenous factors, such as temperature, light, hormones (especially auxin), sugars, mineral salts and other molecules. Phytohormones have direct (involved in cell division or cell growth) or indirect (interacting with other hormones or molecules) effects on plants. Over the last years, a multitude of models have been proposed to show how plant hormones interact to control plant development (Jaillais and Chory, 2010; Nemhauser *et al.*, 2006; Santner and Estelle, 2009).

Auxins are a group of tryptophan-derived signals, which are involved in most aspects of plant development (Woodward and Bartel, 2005). Auxin plays a major role in controlling growth and development of plants, early stages of embryogenesis, organization of apical meristem (phyllotaxy) and branching of the plant aerial parts (apical dominance), formation of main root, lateral and adventitious root initiation (Went and Thimann, 1937). Auxin is also involved in gravitropism and phototropism (Kepinski and Leyser, 2005). These multiple effects across the plant result from its control of cell division, cell elongation and certain stages of differentiation (Davies, 2004). Auxin is synthesized mainly in young leaves and is actively transported to other tissues to coordinate growth and facilitate responses to environmental variations.

**Discovery and history.** The first observations suggesting the presence of a chemical substance modifying plant growth in response to unilateral illumination date from 1880, when Darwin published "The power of movement in plants". Tips of plants exposed to light curved toward the light, which led Darwin to believe that a chemical messenger transports a signal from the coleoptile to the rest of the plant (Darwin, 1880). In 1913, Peter Boysen-Jensen removed the coleoptiles and plants growth stopped and then he replaced the coleoptile with a piece of agar and placed the coleoptile on top of the agar block. He proved that a signal must be transported from the coleoptile to the rest of the plant, as Darwin had originally deduced (Moore *et al.*, 1995). In 1918, Arpad Paal accumulated the first evidence showing that growth signal is chemical in nature (Moore *et al.*, 1995). In 1926, Went showed that

the active growth substance from oat coleoptile tips can diffuse into a block of agar and maintain its stimulative effect of the growth of coleoptiles. He developed a bioassay called Avena test to identify and quantify the biological activity of this growth substances he called AUXIN (from Greek *auxein*, to increase, to grow) (Went, 1935). In the 30s, Kögl *et al.* (1933) isolated from human urine various substances including IAA (indole-3-acetic acid). After few mistakes, the IAA was finally identified as the molecule that stimulates coleoptile growth (Jacobs, 1979) and some years later was isolated from plants. Auxin has been described by Van der Lek (1941) and Haissig and Davis (1994). Practical application of auxin for rooting became possible when it was discovered that it also acts when added on the cut surface of cuttings (Hitchcock and Zimmerman, 1936). After the discovery of IAA, IBA (indolic-3-butyric acid) and NAA (1-naphthalene acetic acid) were synthesized chemically, their capability to induce roots was discovered by Zimmerman and Wilcoxon, 1935, and talc powder was introduced as a carrier for auxin (Grace, 1937).

IBA is used for rooting in commercial operations, followed by IAA and NAA and chemical analogues synthesized and examined for auxin-like activity. Auxin enters cuttings mostly via the cut surface (Kenney *et al.*, 1969), even in microcuttings that are known to have a poorly functioning epidermis (Guan and De Klerk, 2000) and is rapidly taken up in cells by pH trapping (Rubery and Sheldrake, 1973) and by influx carriers (Delbarre *et al.*, 1996). Auxin metabolism studies on adventitious rooting have been done on cuttings exposed for a prolonged period to auxin, but in other studies cuttings have been exposed to auxin for short periods (Diaz-Sala *et al.*, 1996; Liu and Reid, 1992) An optimal auxin concentration for one of the three phases may be supraoptimal or suboptimal for the next. It was observed that apple microcuttings cultured continuously on medium with auxin (IAA, IBA or NAA) show the best rooting performance (a large number of roots is formed over a broad range of auxin concentrations) when cultured with IAA (de Klerk *et al.*, 1997). Although roots may be induced by auxin, wounding is usually required to achieve rooting and it was suggested that WRCs (wounding-related compounds) play a main role in the dedifferentiation phase (de Klerk *et al.*, 1999).

### Auxin effect on adventitious rooting

Auxin and ethylene are often described as activators, while cytokinins and gibberellins are seen as inhibitors of adventitious root formation, even when some positive effects have been observed. The widely used sources of growth hormones for cuttings rooting are the IBA, NAA, IAA and commercialization root promoters (root-growing powders). The successful formation of adventitious roots is an obligatory phase of vegetative propagation in many woody plants; this being related to the presence of auxin

(Kim *et al.*, 1998; McClelland *et al.*, 1990). IAA was the first used to stimulate rooting of cuttings (Cooper, 1935) and soon after another auxin which also promoted rooting, IBA, was discovered and was considered even more effective (Zimmerman and Wilcoxon, 1935). Nowadays IBA is used commercially to root microcuttings and is more efficient than IAA (Epstein and Ludwig-Müller, 1993).

Auxin is one of the major endogenous hormones known to be intimately involved in the process of adventitious rooting (Wiesman *et al.*, 1988) and the physiological stages of rooting are correlated with changes in endogenous auxin concentrations (Heloir *et al.*, 1996). High endogenous auxin concentration is normally associated with a high rooting rate at the beginning of the rooting process (Blažková *et al.*, 1997; Caboni *et al.*, 1997). Auxins have been shown to be effective inducers of adventitious roots in many woody species (de Klerk *et al.*, 1999; Diaz-Sala *et al.*, 1996; Goldfarb *et al.*, 1998; Selby *et al.*, 1992) and are usually synthesized in the stem tip and tender leaves of aerial parts of plants and then transported to the action site (Ljung *et al.*, 2001). When applying exogenous auxin on cuttings, the endogenous auxin concentration reaches a peak after wounding (Gaspar *et al.*, 1996; Gatineau *et al.*, 1997) coinciding with the initiation of the rooting process. The importance of the auxin during the expression phase was demonstrated in *Populus sp.* (Bellamine *et al.*, 1998). Rice mutants affected in the expression of *PIN-FORMED1* (*OsPIN1*) gene, potentially involved in auxin polar transport, are affected in adventitious root emergence and tillering confirming that the auxin concentration and distribution in the different tissues is important (Xu *et al.*, 2005).

The pattern of auxin action, despite its crucial role in adventitious root development, is still poorly understood. In *Arabidopsis*, the *superroot* (*sur1* and *sur2*) mutants accumulate IAA and develop numerous adventitious roots on the hypocotyl and cuttings of different organs in the case of *sur1* (Boerjan *et al.*, 1995; Delarue *et al.*, 1998). Recently, differential roles for IAA and IBA have been found in the regulation of adventitious root formation from stem segments of *Arabidopsis* (Ludwig-Müller *et al.*, 2005).

Several gain-of-function *iaa* mutations affect production of lateral or adventitious roots (Fukaki *et al.*, 2002; Rogg *et al.*, 2001; Tatematsu *et al.*, 2004). TIBA, an auxin polar transport inhibitor, applied to the top of the hypocotyls lowered the rate of root formation (Fabijan *et al.*, 1981). It was suggested that endogenous IAA and exogenous IBA might interact to promote adventitious rooting in *Arabidopsis* stem segments. The performance of IBA versus IAA can be explained by several possibilities: higher stability, differences in metabolism, differences in transport and IBA as a slow release source of IAA. The conversion of IBA to IAA occurs in many plant species (Ludwig-Müller *et al.*, 2005). However, in microcuttings of *Malus sp.*, IBA induced more roots than IAA although it was converted to IAA only at very low levels, suggesting that either IBA

itself was active or that it modulated the activity of IAA (Van Der Krieken *et al.*, 1992). Although many researchers thought growth hormone treatment would promote rooting of cuttings, improve rooting number and lower the rate of partial rooting, the rooting ratio decreased while the hormone concentration increased. High hormone concentrations have side effects on the root development (Edson *et al.*, 1991; Mason, 1989). Auxin can increase the rate of ethylene biosynthesis (Riov and Yang, 1989) and stimulate the production of ethylene correlating with the fact that the *ACC synthase4* gene has been found to be an early auxin-induced gene (Abel *et al.*, 1995). Auxin and ethylene relationship in root development has been shown by a number of isolated mutants that have resistance to both hormones. The potential auxin efflux component, *AtPIN2*, allelic to the *ethylene-insensitive root1* (*EIR1*) is an example (Muller *et al.*, 1998). Analogously, *axr2* is a dominant mutant that gives resistance to both ethylene and auxin (Wilson *et al.*, 1990). IAA induced ethylene production may be a factor involved in the stimulation of adventitious rooting (Pan *et al.*, 2002).

Studies have emphasized that polyamines play a role in adventitious rooting (Biondi *et al.*, 1990; Hausman *et al.*, 1994; Heloir *et al.*, 1996) and a possible interrelationship between polyamines and auxin controlling rooting induction was suggested (Hausman *et al.*, 1995).

#### Genes associated with the adventitious root formation

Some mutants affected in hormone homeostasis or signaling are also affected in adventitious root formation. The ABA-deficient tomato mutants *flacca* and *notabilis* produce an excess of adventitious roots on the stems (Tal, 1966). Recently, it has been shown using *notabilis* mutant that adventitious root phenotype can be restored to wild type by expressing the *LeNCED1* gene involved in ABA biosynthesis, suggesting that ABA can be a negative regulator of adventitious roots (Thompson *et al.*, 2004). Mutants overproducing auxin in *Arabidopsis*, like *sur1* and *sur2* (Boerjan *et al.*, 1995; Delarue *et al.*, 1998) or *yucca* (Zhao *et al.*, 2001) produce adventitious roots on hypocotyls of light grown seedlings. *SUR1* and *SUR2* genes encode a C-S-lyase protein and the cytochrome P450 Cyp83B1, both involved in the indole glucosinolate pathway (Bak *et al.*, 2001; Barlier *et al.*, 2000; Mikkelsen *et al.*, 2004). *YUCCA1* gene encodes a flavin monooxygenase suitable for converting tryptamine in N-hydroxyl tryptamine *in vitro* (Zhao *et al.*, 2001, 2002) and belongs to a family of YUC flavin mono-oxygenases from which 4 have a role in auxin biosynthesis (Cheng *et al.*, 2006).

Temperature-sensitive *Arabidopsis* mutants (*rrd1*, *rrd2* and *rrd4*) affected in *root redifferentiation* were identified (Sugiyama, 2003) and it was suggested that *RRD1* and *RRD2* genes have a role in fundamental processes for active cell proliferation. *RRD4* is involved in the acquisition step of cell proliferation during callus initiation in hypocotyl

explants. Other temperature-sensitive mutants defective in various stages of adventitious root formation were isolated (Konishi and Sugiyama, 2003). The *root growth defective* mutants *rgd1*, *rgd2* and *rgd3* became defective after the establishment of the root apical meristem. The *ROOT INITIATION DEFECTIVE 5 (RIDS)* gene was identified as the *MOR1 / GEM* gene encoding a microtubule-associated protein (Konishi and Sugiyama, 2006). The *rid 2-1* mutant is recessive temperature-sensitive mutant of *Arabidopsis* that was isolated by screening using adventitious root formation as an index phenotype. *RID2* gene encodes an evolutionarily conserved methyltransferase-like protein, which was localized in the nucleus, contributing to the nucleolar activity for pre-rRNA processing (Ohbayashi et al., 2011).

The auxin insensitive rice mutant *crl1/ar1* (*crown rootless1/adventitious rootless1*) defective in adventitious roots formation was identified (Inukai et al., 2005; Liu et al., 2005). *CRL1/ARL1*, an auxin-responsive gene, encodes a nuclear protein containing ASYMMETRIC LEAVES2 (AS2)/LATERAL ORGAN BOUNDARIES (LOB) domain (Inukai et al., 2005; Liu et al., 2005). *CRL1/ARL1* can be considered as a positive regulator for crown root formation in rice. Phylogenetic reconstructions revealed that the allelic rice genes *CRL1* and *ARL1* (Liu et al., 2005), the maize gene *RTCS* (Taramino et al., 2007) and the *Arabidopsis* genes *LBD16* and *LBD29* (Shuai et al., 2002) are closely related. The genes have been involved in different aspects of root formation, *CRL1/ARL1* and *RTCS* genes are localized in syntenic regions of the genomes and their loss of function results in similar phenotypes indicating orthologous functions during shoot-borne root formation (Hochholdinger and Zimmermann, 2008; Inukai et al., 2005). In *Arabidopsis* *LBD16* and *LBD29* genes are involved in lateral root formation (Okushima et al., 2007) and are activated by *ARF7* and *ARF19* which indicates that these LOB domain genes are early auxin responsive genes. Therefore, these closely related monocot and dicot LOB domain proteins all probably act early in auxin signaling in the root, yet in different developmental contexts: The rice gene is involved in shoot-borne and lateral root formation, the maize gene in shoot-borne root formation and the *Arabidopsis* genes in lateral root initiation (Hochholdinger and Zimmermann, 2008).

Researchers have identified several genes that regulate indeterminate root growth in *Arabidopsis*. *SHORT-ROOT (SHR)* is a regulator of radial patterning and indeterminacy of *Arabidopsis thaliana* primary root. *SHR* mutant fails to initiate cell division following germination. *SHR* is also required for the initiation and patterning of lateral root primordia, to maintain the indeterminate growth of lateral and anchor roots, regulating root-related developmental processes (Lucas et al., 2011).

Another related protein, SCARECROW (*SCR*), has a similar role alongside *SHR* (Benfey et al., 1993; DiLaurenzio et al., 1996). *SHR* and *SCR* genes encode closely

related transcription factors belonging to the *GRAS* gene family (DiLaurenzio et al., 1996; Helariutta et al., 2000). *SHR* has been demonstrated to directly regulate the expression of genes including *SCR* (Levesque et al., 2006) and a number of cell cycle components including the D-type cyclin, *CYCD6; 1* (Sozzani et al., 2010).

Genes related to the induction of adventitious rooting in forest species have been described (Goldfarb et al., 2003; Lindroth et al., 2001a, 2001b; Sanchez et al., 2007). Recently, two genes in pine were characterized, a *P. radiata SCARECROW-LIKE1* gene (*PrSCL1*) (Sanchez et al., 2007) and *P. radiata SHORT-ROOT (PrSHR)* (Sole et al., 2008), both genes may play a role during the earliest stages of adventitious root formation. The expression of *PrSHR* gene during adventitious rooting is also affected by the presence of MDPU (methylendioxyphenyl urea) which could interact, directly or indirectly, with the auxin-signalling pathways in rooting-competent cuttings during adventitious rooting (Ricci et al., 2008). *SCARECROW (SCR)* is a putative transcription factor, expressed in cortical and endodermal initials, and it is required for the asymmetric cell division that gives rise to cortex and endodermis and to other tissues in aerial organs of *Arabidopsis thaliana* (DiLaurenzio et al., 1996; Heidstra et al., 2004; Wysocka-Diller et al., 2000). *Arabidopsis SCR (AtSCR)* is also involved in the establishment of quiescent center identity and in the maintenance of the stem cell status of the surrounding initial cells during embryonic pattern formation and postembryonal development (Sabatini et al., 2003), and its expression is associated with auxin distribution in the root apical meristem (DiLaurenzio et al., 1996; Sabatini et al., 1999).

*VvPRP1* and *VvPRP2*, induced in stem cuttings of *Vitis vinifera* L. during rooting, encode proline-rich proteins. Induction of these genes is not enhanced by IAA treatment and the expression of the *VvPRP1* is wound-inducible. The results suggest that the genes have an important role in the initiation of new roots by altering the cell wall mechanical properties to enable root emergence increasing the plasticity of the cell wall (Thomas et al., 2003).

The gene expression pattern during adventitious root development of *Pinus contorta* was investigated. During the root initiation phase, genes involved in cell replication and cell wall weakening and a transcript encoding a PINHEAD/ZWILLE-like protein were upregulated, while genes related to auxin transport, photosynthesis and cell wall synthesis were downregulated. During the root elongation phase downregulation of transcripts encoding proteins involved in cell replication and stress occurred (Brinker et al., 2004). Transgenic lines expressing an active form of the *Populus* type-B cytokinin response regulator PtRR13 ( $\Delta$ DDKPtRR13) have a delayed rooting phenotype and cause misregulation of *CONTINUOUS VASCULAR RING1*, a negative regulator of vascularization. Inappropriate cytokinin action via  $\Delta$ DDKPtRR13 expression appeared to disrupt adventitious root develop-

ment 24 h after shoot excision, when root founder cells are hypothesized to be sensitive to the negative effects of cytokinin (Ramirez-Carvajal *et al.*, 2009).

An Arabidopsis transgenic line overexpressing *ARF17* developed fewer adventitious roots than wild-type plants, confirming the potential role of *ARF* genes in the regulation of adventitious root development by auxin (Sorin *et al.*, 2005). It was shown that *ARF17*, a target of *miR160*, is a negative regulator, and *ARF6* and *ARF8*, targets of *miR167*, are positive regulators of adventitious rooting. These results provide evidence of microRNA control of phenotypic variability and are an important step in understanding the molecular mechanisms regulating adventitious rooting (Gutierrez *et al.*, 2009). The proteomic analysis of *ago1-3*, *sur1-3*, *sur2-1* and the *sur2-1 ago1-3* double mutant led to the identification of 11 proteins, including three auxin-inducible GH3-like proteins, whose expression was altered by mutations, particularly in adventitious rooting formation. The results strongly suggest that those proteins will be valuable markers for quantitative genetic analysis of adventitious root development (Sorin *et al.*, 2006).

*MsAPK1*, member of the plant kinases family containing the Ankyrin-Protein Kinases (*APKs*), is induced by osmotic stress in roots of *Medicago sativa* and is related to two *APK* genes in *Arabidopsis thaliana*, *AtAPK1* and *AtAPK2*. Promoter-GUS fusions assays revealed that Arabidopsis *APK* genes show distinct expression patterns in roots and hypocotyls. The DN mutant lines showed increased capacity to develop adventitious roots when compared with control or *MsAPK1*-expressing plants (Delphine *et al.*, 2008).

In a study made on tomato mutants, the *epi* (*epinastic*) mutation increased adventitious root formation and the *Nr* (*Never ripe*) mutation reduced the number of adventitious roots (Negi *et al.*, 2010). This indicates a negative role for ethylene in lateral root formation of tomatoes and a positive role in adventitious root formation with modulation of auxin transport as a central point of ethylene-auxin crosstalk (Negi *et al.*, 2010). The treatment of tomatoes with AVG (aminoethoxyvinylglycine) and NPA (1-N-Naphthylphthalamic acid) resulted in a reduction of adventitious roots in waterlogged plants. Ethylene, perceived by the *Nr* receptor, stimulated auxin transport. Auxin accumulation in the base of the plant induces growth of adventitious roots, forming a new root system capable of replacing the one damaged by submergence (Vidoz *et al.*, 2010).

Auxin efflux carrier, *PINI*, is a highly conserved gene family, which may play a key role in polar auxin transport (Friml and Palme, 2002). The finding that *OsPINI* is involved in auxin-dependent adventitious root emergence and tillering provides a new insight into the function of the *PINI* family in rice (Xu *et al.*, 2005). The rice gene *OsCAND1*, the homolog of Arabidopsis *CAND1*, is involved in auxin signaling to maintain the G2/M cell cycle

transition in crown root meristem and the emergence of crown root, providing new information about the molecular regulation of the emergence of crown root (Wang *et al.*, 2011).

#### ARF and AUX/IAA involved in adventitious rooting

Great progress has been made in recent years in understanding the auxin response genes and auxin signaling (Parry and Estelle, 2006; Quint *et al.*, 2009). The response to auxin includes a rapid initial cell growth response that may involve auxin-induced changes in pH, calcium and gene expression. Auxin response is regulated by *AUX/IAA* proteins and the *ARF* (*Auxin Response Factor*) proteins (Overvoorde *et al.*, 2005). The *AUX/IAA* genes are induced in response to auxin, encoding small nuclear proteins that share four domains (I, II, III and IV) and function as transcription factors that regulate downstream auxin responses (Guilfoyle and Hagen, 2007; Reed, 2001). *AUX/IAA* genes were identified in screens for mRNA transcripts induced rapidly by auxin. *AUX/IAA* proteins likely function as homodimers and/or heterodimers and have been found to interact in homotypic and heterotypic associations in yeast two-hybrid experiments, and these interactions were dependent on the presence of domains III and IV (Kim *et al.*, 1997; Rouse *et al.*, 1998). Arabidopsis has 29 *AUX/IAA* proteins which have four conserved domains called I–IV. Domain I is a transcriptional repression domain and can repress auxin gene induction responses (Kim *et al.*, 1997; Tiwari *et al.*, 2003). Domain II is recognized by SCF<sup>TIR1</sup> and probably other closely related E3 ubiquitin ligases (Kepinski and Leyser, 2005). Domains III and IV constitute a dimerization domain and can interact with similar motifs in ARF proteins (Ulmasov *et al.*, 1999). Gain-of-function mutations in motif II of several *IAA* genes stabilize the corresponding protein and affect developmental responses to auxin. In several cases these mutations decrease auxin-induced gene expression (Tatematsu *et al.*, 2004). *AUX/IAA* proteins have short half-lives, suggesting a primary role for protein degradation in the regulation of their activity. Guilfoyle *et al.* (1998) assumed that domain II was responsible for the rapid turnover of these proteins, because mutations in this domain (like *axr3* mutants) led to a semidominant gain-of-function phenotype (Guilfoyle *et al.*, 1998). Worley *et al.* (2000) suggested that rapid degradation of *AUX/IAA* proteins is essential for a normal auxin response. It was also found that overexpression of the *IAA17* protein in Arabidopsis resulted in plants with an *axr3*-like phenotype (Worley *et al.*, 2000). *AUX/IAA* proteins do not appear to bind DNA themselves but can affect the transcription of ARF-regulated genes by dimerising with ARFs (Guilfoyle and Hagen, 2007; Tiwari *et al.*, 2003).

Auxin response factors (*ARF*) are transcription factors that regulate the expression of auxin response genes (Guilfoyle and Hagen, 2007; Tiwari *et al.*, 2003). Arabi-

dopsis has 22 ARF proteins encoded by 22 *ARF* genes, each containing a conserved DNA binding domain near their N terminus and a dimerization domain near their C terminus that can interact with a corresponding domain in *AUX/IAA* proteins (Ulmasov *et al.*, 1999). The role of *ARFs* in plant development and growth has been revealed by studies on *arf* mutants. Mutations were identified: *arf7/nonphototropic hypocotyl 4 (nph4)*, which present defects in hypocotyls tropisms and resistance to auxin and ethylene (Harper *et al.*, 2000; Waller *et al.*, 2002) and *arf19*, which show insensitivity to auxin and ethylene (Li *et al.*, 2006). *arf* double mutants (Remington *et al.*, 2004) have stronger phenotypes than the single mutants, suggesting that related ARFs might have redundant roles in Arabidopsis. Single mutants of *arf7* and *arf19* have reduced lateral and adventitious root numbers, but *arf7 arf19* double mutants have extremely reduced numbers of adventitious and lateral roots (Okushima *et al.*, 2007; Wilmoth *et al.*, 2005). While no phenotypic defects were reported for single mutants *arf10* or *arf16*, *arf10 arf16* double mutants have root cap defects and abnormal root gravitropism (Wang *et al.*, 2005).

Genetic studies have implicated different *ARFs* in diverse growth processes. Highly specific and/or dynamic patterns of gene expression have been observed for *ARF7* in seedlings, roots and embryos development (Hardtke *et al.*, 2004; Okushima *et al.*, 2005; Wilmoth *et al.*, 2005), *ARF10* in the root caps development, vascular tissue of roots and leaves (Wang *et al.*, 2005), and *ARF19* in seedlings and roots development (Li *et al.*, 2006; Okushima *et al.*, 2005; Wilmoth *et al.*, 2005). Mutations in *ARF19* in combination with mutations in *NPH4/ARF7*, encoding the most closely related adventitious root, cause phenotypes like a drastic decrease in lateral and adventitious roots.

## Conclusions

The process of adventitious root formation is very complex and plays a key role in the vegetative propagation of difficult-to-root genotypes. Although the importance of auxin in root development is well known, this plant hormone is not always efficient and the molecular mechanisms involved in the formation of adventitious roots are still partly unknown, despite the efforts done by researchers all over the world. For improving rooting conditions of economically important genotypes, scientists need to elucidate these molecular mechanisms through which auxin regulate adventitious rooting. Characterizing adventitious rooting mutants and identifying the genes responsible for the mutations leads to a better understanding of the regulation of the adventitious rooting mechanisms. New information on *ARFs* and *AUX/IAA* roles in plant growth and development has been revealed in the past years by forward and reverse genetic studies. Candidate genes regulated by *ARFs*, which may function in these mechanisms, have been identified experimentally or predicted

from data mining. Unfortunately, the adventitious root response genes are still not entirely identified, therefore this process represents an area open for research.

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