Indian Journal of Plant Sciences ISSN: 2319–3824
An Open Access, Online International Journal Available at http://www.cibtech.org/jps.htm
2021 Vol.10, pp.69-85/Basuchaudhuri
Review Article

AUXINS IN ROOTING OF CUTTINGS

***P. Basuchaudhuri**
Formerly Senior Scientist, Indian Council of Agricultural Research,
New Delhi 110012, India
**Author for Correspondence: pranab.basuchaudhuri@yahoo.com*

ABSTRACT
Details of auxins action, function and its regulations are still unclear. Rooting of cuttings is usually achieved by exogenous auxins commercially available but details of the processes, molecular and genetic changes are not understood properly and clearly because of the complex biological nature of the processes and environmental effects. Here, it was tried to elaborate the topic with special emphasis on rooting of cuttings.

Keywords: *Auxins, Stem Cuttings, Rootings, Molecular Concept, Genetic Concept*

INTRODUCTION

In asexual mode of propagation, the vegetative parts such as stem cuttings are used, the progeny always resembles the mother plant in all respects and maintaining the progeny of elite plants with all qualities (Hendrique *et al*.,2006). Vegetative propagation is an integral part of tree improvement, as it is needed for establishing clonal seed orchards. Also, mass vegetative multiplication of selected genotypes is possible. In most tree species exogenous application of natural and synthetic auxins facilitates adventitious root production from branch cuttings(Hendrique *et al*.,2006) . Kester *et al*.(1990) reported that the most reliable rooting hormone is indolebutyric acid (IBA) although others such as napthaleneacetic acid (NAA) can also be used. Although there are reports that it may also be toxic to young/succulent cuttings of certain species, IBA is still probably the best hormone because of being non-toxic to plants over a wide range of concentration(Figure1).

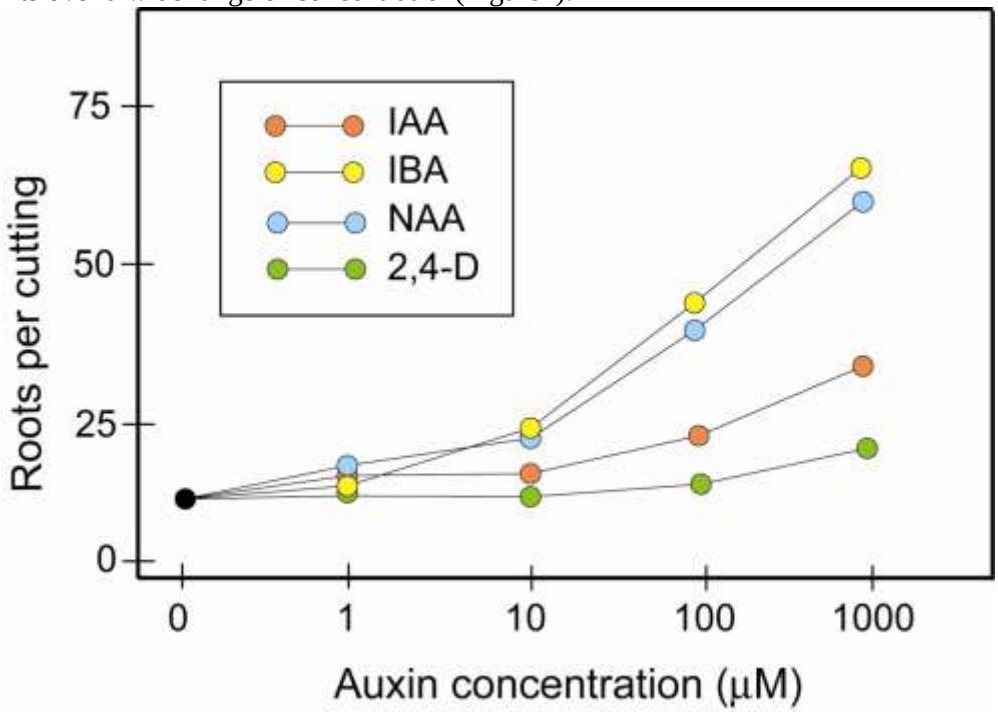

Figure1: Rooting with different auxins

Rooting of cuttings is not always successful and the reasons for rooting failure are not clearly understood. Factors such as cultivar and age of source tree, the collection date, length, diameter, and degree of hardening of the cuttings, injury and heat treatments of the cuttings and the treatment concentrations of auxin-like compounds can affect rooting (Tsipouridis *et al*., 2006). The indole butyric acid (IBA), a synthetic auxin induces rooting in peach cuttings, but its effect can vary with the type of cutting used(Couvillon,1985). Tsipouridis *et al*. (2003) found that IBA (2000 ppm) stimulated rooting of hardwood and semi-hardwood cuttings but rooting success varied with peach cultivar. In contrast, softwood cuttings treated for 24h with 25ppm solution of IBA rooted (Gur *et al*.,1986). In several species, rooting success had been related to endogenous auxin concentrations (Guerrero *et al*.1999) .

ROOTING OF CUTTINGS

Rooting of cuttings is a natural phenomenon there are plants in which rooting of cuttings is easy. But in many plants stem cuttings do not root easily, even rooting is not possible except undergone some treatments. These are known as difficult to root. Many economically and ecologically important hardwood tree species have a low genetic and physiological capacity for adventitious root formation and are considered recalcitrant to routine, commercial scale vegetative propagation via rooted cuttings (Pijut *et al*.,2011) . Propagation of tree planting stock by rooted cuttings can overcome the problems with seed viability, germination, and storage and dormancy associated with seed; shorten the time to flowering or encourage consistent flowering; maintain superior genotypes; and contribute to the genetic uniformity(Macdonald,1986) . This method can allow for the production of clones of elite, pest or disease resistant or genetically improved plants for planting and breeding programs. Disadvantages associated with clonal reproduction and adventitious root formation may be less branched roots, more horizontal roots, poor root distribution around the stem or too few roots.

Adventitious root formation is different from lateral root formation, as the development of roots on excised aerial plant parts or from an unusual point of origin on the plant. De Klerk *et al*.(1999) summarized the successive phases in rooting of apple micro-cuttings as dedifferentiation, was the activation of cells by wounding related compounds and auxin. The induction phase was the initiation of cell division where auxin stimulates the formation of root meristemoids. During out growth in the stem phase, meristemoids develop into typical dome shape root primordial. Root primordial elongate and develop during the differentiation phase and finally grow out of stem.

Wound induced roots are the major type of root in stem cuttings. Once the stem (or shoot) is removed from the plant (wounding) a series of wound responses occur and de novo adventitious root regeneration proceeds(Hartmann *et al*.,2002). At the wound sealing off (protection from desiccation and pathogens) occurs by the production of suberized, protective cells. Cells begin to divide, and a layer of parenchyma cells (callus) then forms at the wound site. The use of auxin during adventitious rooting enhances the formation of callus in addition to inducing the formation of roots. Cells in the vicinity of the vascular cambium and phloem begin to divide and initiate adventitious roots (Hartmann *et al*., 2002).

FACTORS IN ROOTING OF STEM CUTTINGS

Stem cuttings need rooting regulators called auxins, to make roots. The plant itself produces these natural auxins, in limited quantities within the leaves and meristems. When cutting are taken from the mother plant, the auxins are stored at the basal ends. The auxins move from cell to cell by polar transport. Polar transport takes place in the xylem, phellogen, and other transport vessels in the shoots and stems. The plant makes use of natural transport proteins. These proteins allow the auxins to be taken into the top of the plant cell, move through the cell, and be released at the bottom of the cell. Cell by cell, auxins are transported downward. Polar transport of the auxins occurs at a speed of approximately one centimeter per hour. The auxins accumulate in the basal ends of the cuttings. When the amount of auxins at the basal ends of the cuttings exceeds a boundary level then cells reprogram to become root cells. Usually natural auxin production in the cutting is not sufficient. So, auxins, usually synthetic

auxins are supplied at the basal end of the cuttings to achieve consistent and uniform roots. Rapid and steady rooting occurs around the base of the cuttings. Important synthetic auxins are indole-3-butyric acid, 1-napthalneacetic acid etc. More than one auxin can also be applied, sometimes with other chemicals for better rooting suggesting synergistic effects(Tables 1&2). However, it is noted that environmental and endogenous factors also influence rooting e.g. hormones, light quality, light quantity, oxygen, carbon dioxide, nitric oxide, free radicals, relative humidity, pH of the growth media, physical structure of the growth media, antioxidants, wounding, polyamines and concentration and types of nutrients in the media etc. For most of these factors, the biological role is uncertain. Some may enhance rooting simply by keeping shoots healthy while rooting process takes place. Polyamines may act more directly by affecting the production or distribution of endogenous factors such as auxin (Naija *et al*.2009) . NO may be acting as an intermediary in auxin signaling (Pagnussat *et al*., 2003).

Table 1: Vegetative propagation of some tree species

Tree species	**Treatment of cuttings (mM)**	**Rooting (%)**	**References**
Acer rubrum	4.9IBA in talc	59	Henry and Preece, 1997
Acer saccharum	12.3 or 24.6 IBA; 13.4 or 26.9 NAA; IBA + NAA	<30	Alsup *et al*., 2004
Betula spp.	4.9 IBA	24-100	Barnes, 2002
Castanea dentala	20.7K-IBA	3	Preece *et al*., 2001
Fagus grandifolia	9.8IBA + 5.4 NAA	25	Barnes, 2003
Juglans cinerea	0-74 IBA : 0-62K-1BA	6.3-88	Pijut and Moore, 2002; Pijut, 2004.
Prunus serotina	0-74 IBA; 0-62K-IBA	50-54	Pijut and Espinosa, 2004
Quercus alba	49.2 IBA	0-30	Zaczek *et al* ., 1997
Quercus bicolor	29.5 IBA	88-91	Amissah and Bassuk, 2007
Quercus nigra	49.21 BA	20-27	Zaczek *et al* ., 2000
Robinia pseudoacacia	1.3-4.0 NAA; 1.2-3.7 IBA	35-83	Swamy *et al*., 2002 a,b

Table 2: Vegetative propagation of some fruit tree species

Tree species	Treatment	Rooting (%)	Reference
Artocarpus heterophyllus Lam	5000ppm IBA+ etiolation	75	Chatterjee and Mukherjee, 1980
Prunus ulmifolia (Plum)	2500ppm IBA	88	Ivanika and Pastyrick, 1978
Peach	250ppm IBA	56	Tworkishi and Takeda, 2007
Malus (Azayesh)	2500ppm IBA in Cocopeat+perlite (1:1)	31.48	Dvin *et al.*, 2011
MM106 apple	Agrobacterium+ Sorbitol+4000ppmIBA	30	Karakurt *et al.*, 2009
Psidium guajava L	4000ppm IBA+shade	40.11	Kareem *et al.*, 2016
Annona muricala	2000mg per kg	70	Santos *et al.*, 2011

MOLECULAR ASPECTS

AUXIN BIOSYNTHESIS AND METABOLISM

IAA is believed to be synthesized mainly from precursors generated via the shikimate pathway. The IAA precursor L-Trptophan is synthesized from chorismate, the final product of the shikimate pathway. Although, L-Trp dependent biosynthesis of IAA is believed to be the main route of IAA biosynthesis in plants, evidence for a tryptophan – independent pathway of IAA synthesis branching from indole-3-glycerol phosphate (IGP) also exists (Ouyang *et al.*,2000) . The genes and enzymes involved in tryptophan- independent IAA are still largely unknown, and the existence of this alternative pathway is based mainly on feeding studies using stable labeled IAA precursors and different tryptophan biosynthesis mutants(Wright *et al*., 1991; Normanly *et al*., 1983) . In addition, the four-carbon side chain indole-3-butyric acid (IBA) has also been suggested to function as an endogenous IAA precursor, being converted to IAA in peroxisomes by β-oxidation (Starder and Bartel, 2011) . Tryptamine is found in very low levels compared with IAA and L-Trp in plants, and is believed to be the product of tryptophan decarboxylases. It is possible that TRA could function both as a precursor for IAA and in indole alkaloid and serotonin biosynthesis in different plant species (Mano and Nemoto, 2012) . Indole-3-acetamide is present in many plant species, and IAM hydrolases convert IAM to IAA (Nemoto *et al*., 2009). L-trp also acts as a precursor of camalexin (CAM) and indole glucosinolates (IGS) (Normanly, 2010) and perturbations in these pathways have been shown to affect IAA biosynthesis, thus highlighting the link between auxin biosynthesis and the biosynthesis of IGS (Novak *et al*., 2012) . The cytochrome P450 mono-oxygenases CYP79B2 and CYP79B3 catalyze the conversion of L-Trp to IAO_x (Sugawara *et al*., 2009) (Figure 2) .

HOMEOSTASIS AND CATABOLISM

Homeostatic mechanism operating in plant cells to maintain auxin level by conjugation (mainly to amino acids and sugars) and by degradation (Normanly, 2010; Rosquete *et al.*, 2012). IAA conjugates are either reversible or irreversible storage compounds, although the function of IAA conjugates, and the genes that

regulate their formation is still under investigation (Figure 3). The metabolites 2-oxoindole-3-acetic acid (oxIAA) and oxIAA-glucose (oxIAA-Glc) are the major degradation products of IAA (Novak *et al*.,2012) , but the genes involved in IAA catabolism have so far not been identified (Figure 4).

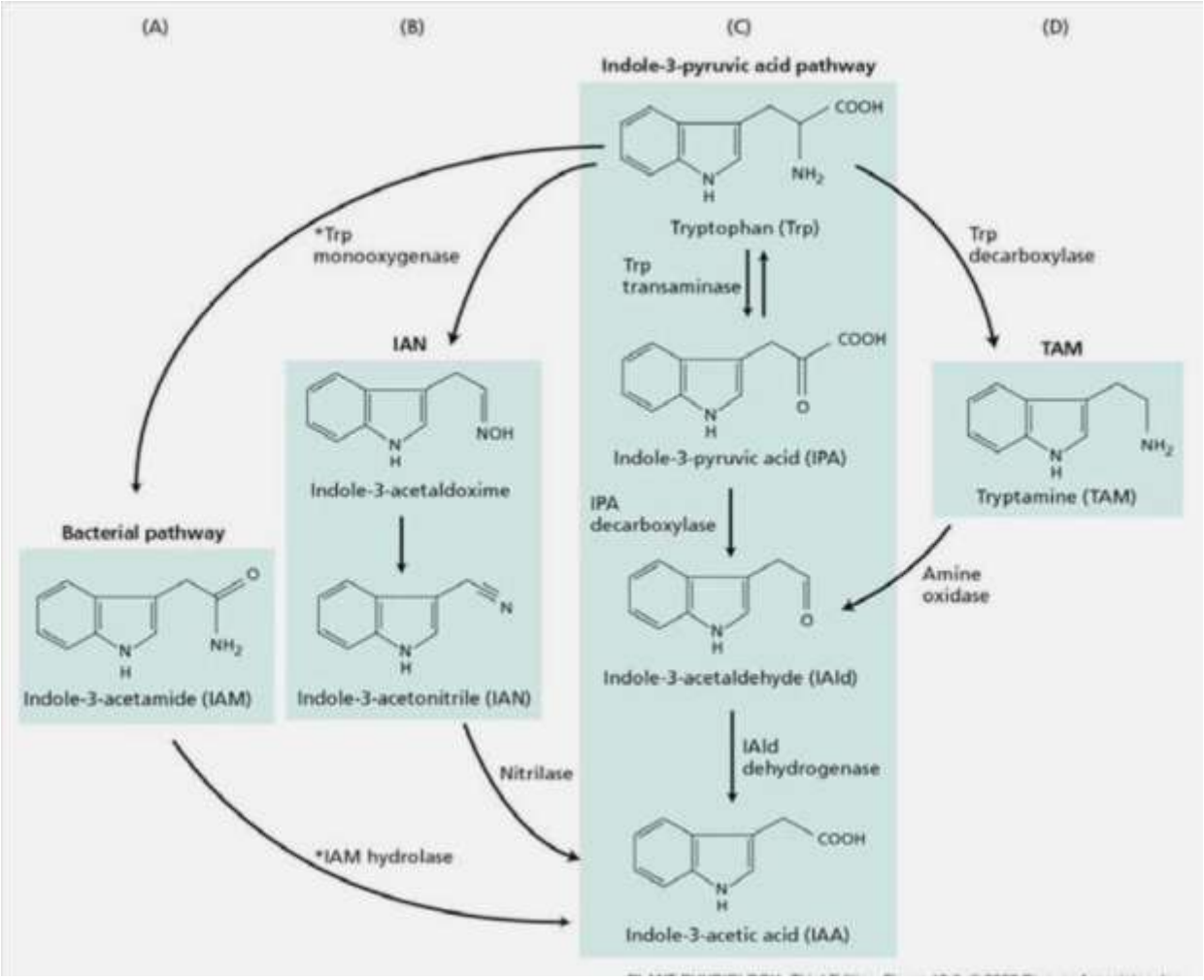

Figure 2: Auxin biosynthesis

LOCALIZATION OF AUXIN METABOLISM

The localization of enzymes involved in IAA biosynthesis, conjugation and deconjugation suggests that different sub-cellular compartments are involved in IAA metabolism as well as in the storage of these compounds. Both the shikimate and L-Trp biosynthesis pathways are believed to be localized to plastids, based on protein localization studies and the presence of specific plastid transit peptides in the enzymes involved in these pathways (Mano and Nemoto, 2012) . By contrast, the pathways downstream of L-Trp are believed to be localized to the cytosol. Some suggested the enzyme can be localized both to the cytosol and to the cytosolic face of the ER membrane. Several IAA-amino acid conjugate hydrolases have been shown to be located at the ER(Woodward and Bartel, 2005) . Vacuoles and the apoplast could be important for metabolism, storage and transport of IAA and its different IAA metabolites (Figures 5&6).

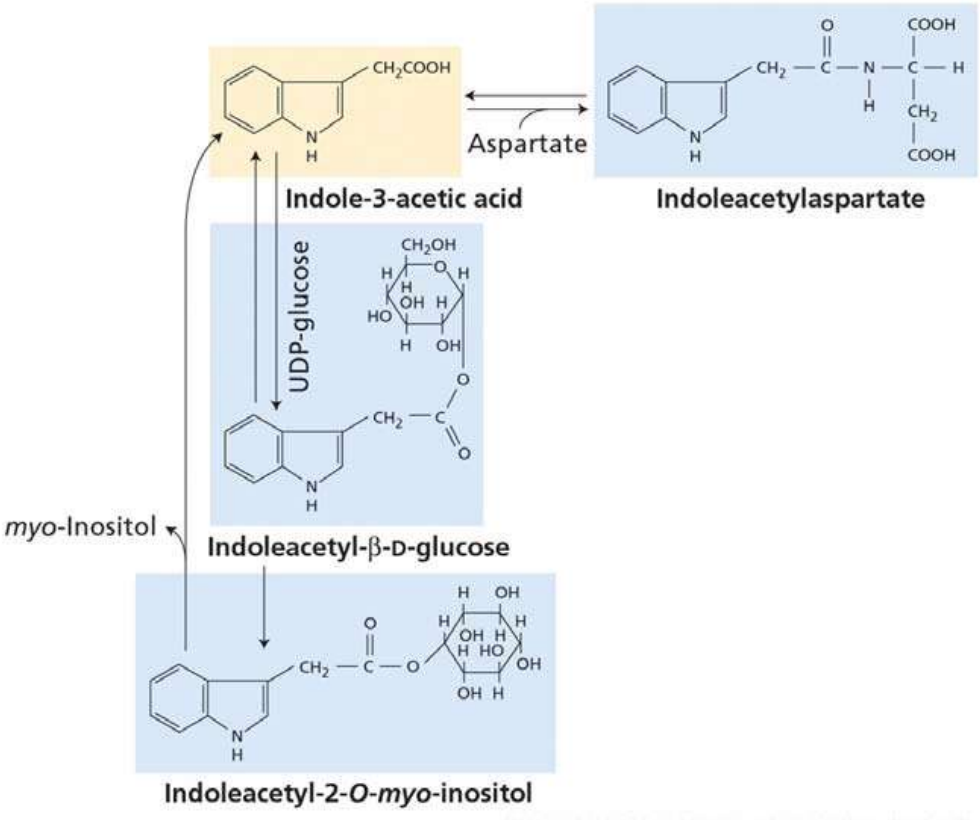

Figure 3: Auxin conjugation

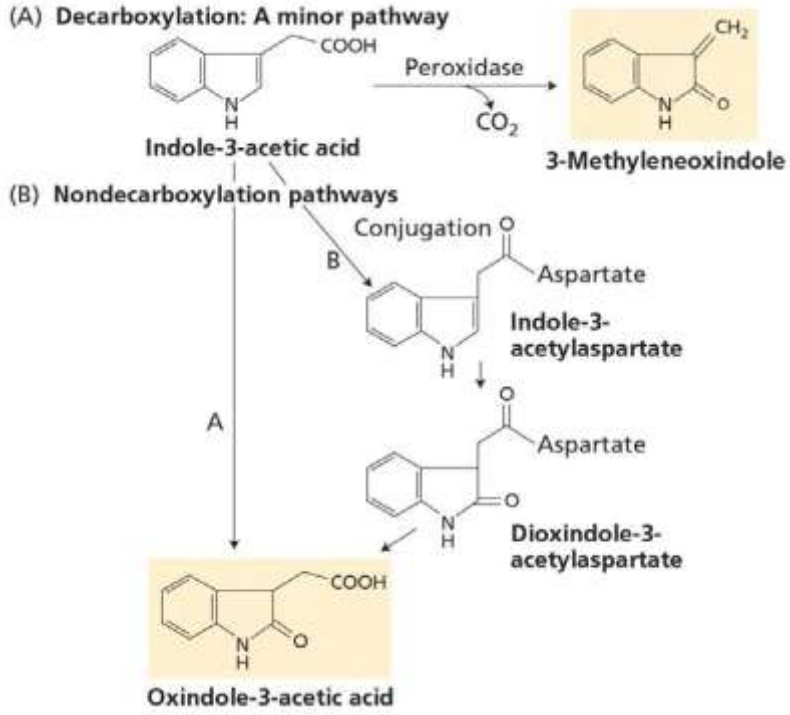

Figure 4: Auxin degradation

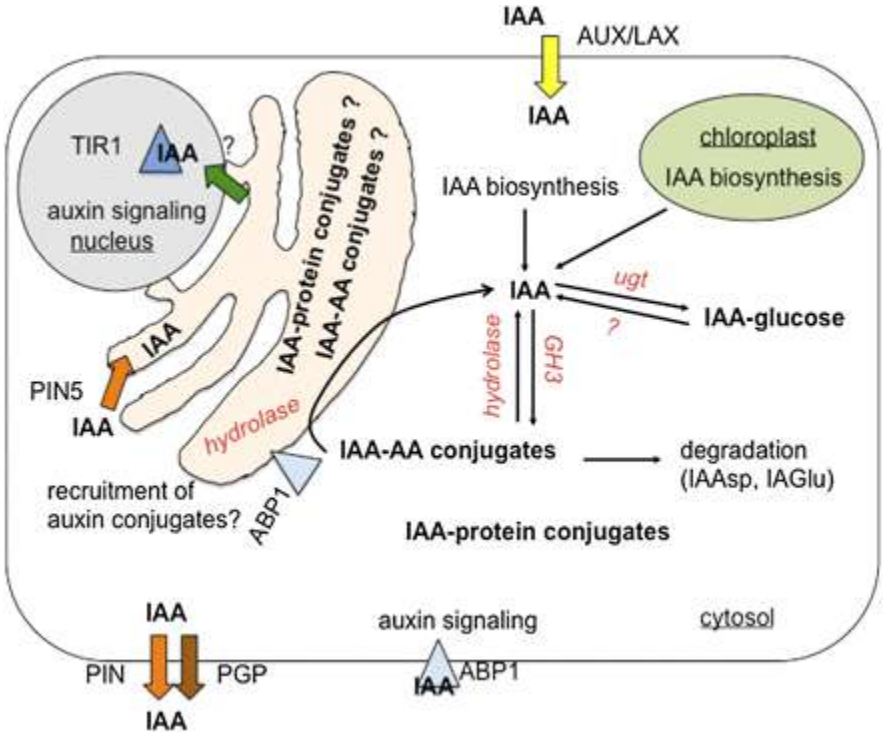

Figure 5: Localization of auxin metabolism (Jutta Ludwig-Müller, 2011).

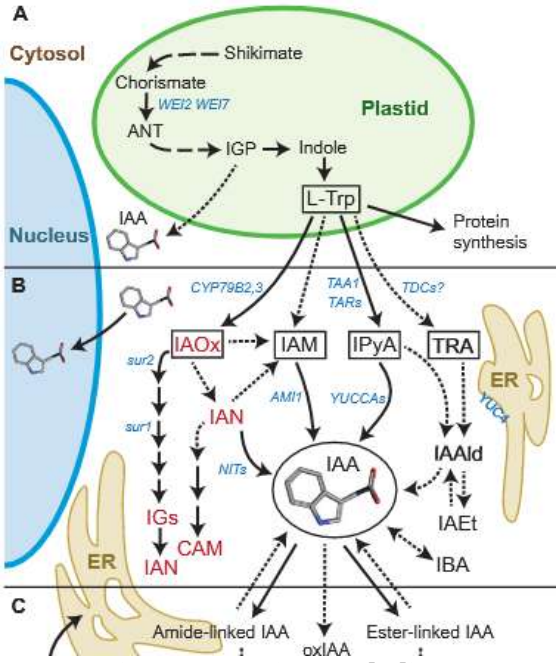

Figure 6: Auxin metabolism

AUXIN TRANSPORT

Auxins are weak organic acids, the carboxyl group is protonated at low pH, making the molecule less polar ($IAA^- + H^+$ = IAA-H). In this form it can diffuse across cell membranes, whereas the molecule in its unprotonated negatively charged form (IAA^-) is too polar to diffuse. The pH in different cellular compartments varies, being 5.0-5.5 in the apoplstic fluid of the cell wall and in vacuoles and 7.0 in the

cytosol. IAA-H in the apoplast and in vacuoles can thus diffuse over the surrounding membrances, whereas IAA^- is trapped within the cell and cannot escape from the cytosol without the aid of specific transporters(Rosquete *et al*.,2012) . Two families of IAA efflux carrier proteins have been identified (PIN and ABCB). There are also specific IAA influx carrier proteins such as the AUX1/LAX family, which are important for increasing IAA transport into specific cell types(Swarup and Peret,2012). A new group of transport proteins - the PINLIKES or PILS proteins, were identified and are postulated to have a function in IAA transport between the cytosol and the ER(Barbez *et al*.,2012) . The localization of influx and efflux carriers at the plasma membrane directs the transport of IAA in and out of the cells. Auxins stimulates cell elongation by stimulating wall loosening factors, such as elastins, to loosen cell walls(Figure 7).

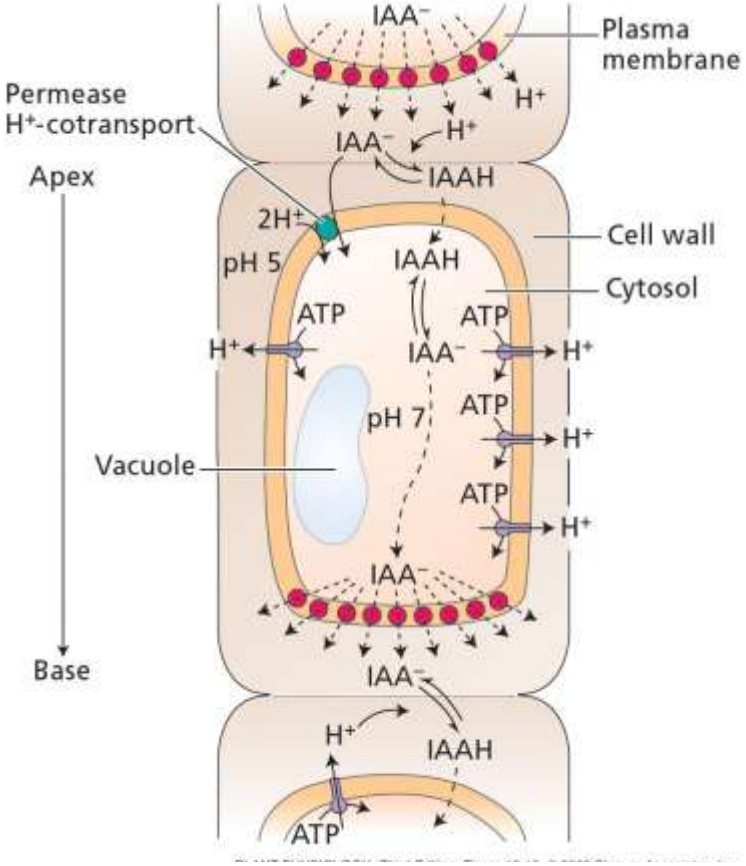

Figure 7: Auxin polar transport in cells

The effect is stronger if gibberellins are also present. Auxins also stimulate cell division if cytokinins are present. When auxin and cytokinin are applied to callus, rooting can be generated if the auxin concentration is higher than cytokinin. Xylem tissues can be generated when auxin concentration is equal to cytokinin. Auxins also induces sugar and mineral accumulation at the site of application. It had been noted that there is importance of interactions of auxins with non-hormones in cell differentiation, such as auxin and sugar (vascular tissue), auxin and low sugar (xylem), auxin and high sugar (Phloem), auxin and moderate level of sugar (xylem and phloem). The coordination of cell repair, DNA replication, cell division, and cell elongation processes necessary for rooting of cuttings requires the investment of considerable energy and structural carbohydrates (Husen, 2008) , and the production of proteins(Hutchison *et al*.,1999) . These are more of a supportive role of root growth rather than initiation.

Sucrose was transported for use in cell repair and cell division, establishing the wounded tissue as a sink, which require transporters.

In addition, adventitious root formation is generally promoted by auxin, and auxin signaling and transport has been shown to control plant root length, number of adventitious roots, root hair and root growth direction . Nag *et al*.(2001) reported that auxin was an essential factor for induction rather than initiation of roots in plants, which verified the hypothesis that the adventitious root formation initially occurred in two phases: an auxin-sensitive phase and an auxin-insensitive phase . As a synthetic auxin, NAA is commonly used at relatively low dose to elicit auxin-type responses in cell growth, cell division, fruit setting, rooting, etc . The adventitious root production was increased rapidly at lower NAA concentration, while the number of roots was decreased at higher concentration.

The activities of enzyme in the rooting zone of cuttings provided an easy, fast and reliable means of assessing cellular differentiation into roots. Sato *et al.* (1993) reported that a particular peroxidase catalyzed the process of cell wall lignification during rooting in *Zinnia* cuttings. Polyphenol oxidase catalyzes the oxidation of polyphenols and the hydroxylation of monophenols and lignification of plant cells in trees . Furthermore, an auxin-induced change in peroxidase and IAAOxidase occurred during the rooting processes.

Coordinated DNA replication and cell division was necessary for the development of new meristems (Sedira *et al*.,2007). Brinker *et al*.(2004) identified cyclin-dependent kinases, CDC2, which was upregulated during rooting of cuttings of *Pinus contorta*. CDC2 may have a role in establishing cell division competence for organogenesis(Brinker *et al*.,2004) . Auxin induced organogenesis is also mediated by cytokinins by inhibiting proteinase inhibitor(PIN) auxin efflux carriers (Laplaze *et al*.,2007) . Auxin molecules present in cells may trigger responses directly through stimulation or inhibition of the expression of sets of genes or by means independent of gene expression. Auxin transcription ally activates four different facilities of early genes, so called because the components required for activation are pre-existing, leading to a rapid response. The families are glutathiones – transferases, auxin homeostasis proteins like GH3. SAUR genes are of currently unknown function, and the Aux/IAA repressors. The Aux/IAA repressors are leading to auxin induced changes of gene expression. This pathway involves the protein families TIR1 (transport inhibitor response 1), ARF (auxin response factor), Aux/IAA transcriptional repressors, and the ubiquitin ligase complex that is a part of the ubiquitin-proteasome protein degradation pathway. ARF proteins have DNA binding domains and can bind promoter regions of genes and activate or repress gene expression. Aux/IAA proteins can bind ARF proteins sitting on gene promoters and prevent their activities. TIR1 proteins are F-box proteins that have three different domains giving them the ability to bind to three different ligands : an SCF ubiquitin ligase complex (using the F-box domain), auxin (so TIR1 proteins are auxin receptors), and Aux/IAA proteins (via a degron domain). Upon binding of auxin, a TIR1 protein's degron domain has increased affinity for Aux/IAA repressor proteins, which when bound to TIR1 and its SCF complex undergo ubiquitination and subsequent degradation by a proteasome. The degradation of Aux/IAA proteins frees ARF proteins to activate or repress gene at whose promoters they are bound.

Within a plant system, elaboration of the Aux/IAA repressor pathway takes place via diversification of the TIR1, ARF and Aux/IAA protein families. Each family may continue many similar acting proteins, differing in qualities such as degree of affinity for partner proteins, amount of activation or repression of target gene transcription, or domains of expression.

Another protein, auxin-binding protein 1(ABP1), is a putative receptor for a different signaling pathway, but its role is as yet unclear. Electrophysiological experiments with protoplasts and anti ABP1 antibodies suggested –ABP1 may have a function at the plasma membrane, and cells can possibly use ABP1 proteins to respond to auxin through means faster and independent of gene expression.

In response to excision, a new developmental programme is initiated in particular responsive cells in the stem base near the wound, ultimately leading to the regeneration of a new root system.

Depending on the plant and type of explant, diverse cell types, here referred to as AR source cells, may generate ARs (Altamura, 1996). AR formation in stem tissues has repeatedly been observed to originate in the cambium or vascular tissues, where it involves sequential phases (da Costa *et al.*, 2013). The initial phase, generally referred to as the induction phase, is char-acterized as an anatomical lag phase devoid of cellular changes, during which the initial cell reprogramming occurs. If the AR source cells are root-competent already, they can be fate-con-verted directly to AR root founder cells by a root-inducing signal. However, often the cells from which AR starts first have to ac-quire root competence involving dedifferentiation before they can respond to a root-inducing signal (Altamura, 1996; Ikeuchi *et al.*, 2016). After determination of AR founder cells, the initiation of ARs starts with qualitative changes in cell structures, fol-lowed by cell division and differentiation of the new cell clusters into dome-shaped root primordia. The final expression phase be-gins with the differentiation of primordia into the complete root body, with differentiated vascular bundles connected to the vas-cular cylinder of the stem, followed by the emergence of roots. that auxin is an effective inducer of AR formation (Pacurar *et al*., 2014). Polar auxin transport (PAT) plays a crucial role in controlling the level of indole-3-acetic acid (IAA), which is the major active auxin, and is of highly dynamic nature. The regu-lation of PAT involves auxin influx transporters of the AUXIN1 (AUX) and LIKE-AUX1 (LAX) types, efflux carrier proteins of the ATP-binding cassette (ABC) and PIN-FORMED (PIN) families, and PINOID family kinases that control the intracellular localization of PINs (Bennett *et al.*, 2014; Geisler *et al.*, 2017). Studies on petunia (*Petunia hybrida*) cuttings revealed early IAA accumulation in the stem base as dependent on PAT and essential for subsequent AR formation (Ahkami *et al*., 2013), and highlighted the excision-induced transcriptional fine-tuning of the auxin transport machinery that involved auxin transporters as well as PINOID kinases (Druege *et al.*, 2014). Reviewing these findings in context with other related studies, Druege *et al*. (2016) postulated a model where PAT and cutting off from the basipetal auxin drain are considered as important principles generating early accumulation of IAA in the rooting zone. Further being linked to wound-induced biosynthesis of jasmonic acid (JA) and ethylene (ET), IAA accumulation was suggested to trigger self-regulatory canalization and maximiza-tion to responding target cells, there inducing the programme of AR formation.The important roles of PAT and auxin allocation to particular cells as principles of AR induction and subsequent AR differen-tiation were highlighted in arabidopsis (*Arabidopsis thaliana*) by tissue-specific monitoring of molecular factors that control auxin homeostasis and by functional analysis of target genes in mutants. In the hypocotyls of de-rooted seedlings, early auxin maxima were identified via pGH3-2:GUS in pericycle cells as sites of subsequent AR primordium formation, whereas AR formation was reduced by mutations of PIN1, PIN3, PIN7 and ABCB19 (Sukumar *et al.*, 2013). In isolated TCLs and intact hypocotyls, a local auxin maximum is first initiated in the root founder cells and thereafter directed to the tip of the developing AR meristems (Della Rovere *et al.*, 2013). The DR5-reported maximum of auxin perception follows a co-ordinated expres-sion of LAX3 and of PIN1, while the signals are reinforced by exogenous auxin (Della Rovere *et al*., 2013).

Under low auxin levels, specific auxin/IAA (Aux/IAA) proteins recruit TOPLESS (TPL) to exert their repressive function on specific AUXIN RESPONSE FACTORS (ARFs), which are transcriptional regulators of auxin-responsive genes. IAA directly binds to the TRANSPORT INHIBITOR RESPONSE 1/AUXIN-SIGNALLING F-BOX (TIR1/AFB) component of the SKP/CULLIN/F-BOX (SCF)–TIR1/AFB complex and to Aux/IAA repressor proteins. This allows the ubiquitination and subsequent proteasomal degradation of Aux/IAA proteins so that the ARFs are released from repression. Aux/IAA proteins further provide cross-nodes to other plant hormones such as CKs, ET, JA and brassinosteroids (reviewed in Druege *et al*., 2016). In petunia cuttings, genes of the AIL and GRAS families, such as PLT-, SHR- and SCR-like TF genes, are upregulated during AR for-mation (Bombarely *et al.*, 2016).

The findings further strongly suggest the downstream involvement of TFs of the families of GRAS, AP2/ERF (in particular PLT) and WOX (in particular WOX11 and WOX5) and indicate

an important role for auxin-mediated GH3 regulation in adjusting the IAA pool to the different requirements of AR induction and AR differentiation (Figure 8).

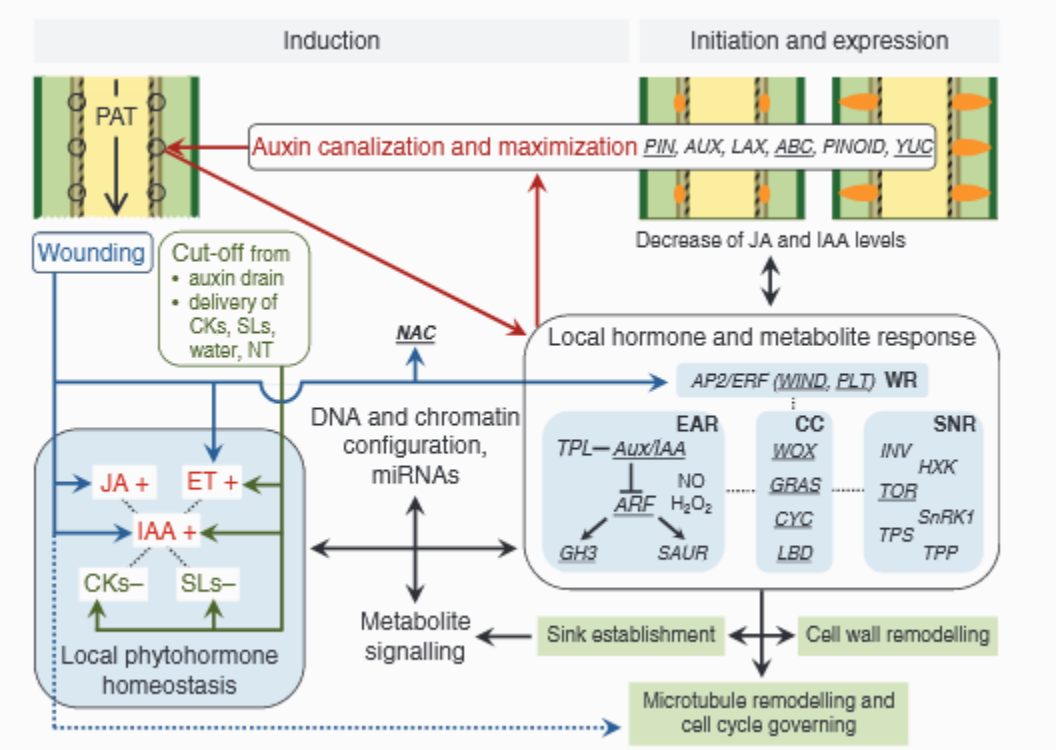

Figure 8: Model for physiological changes in rooting of cuttings (from Druege *et al*., 2019).

GENETIC ASPECTS

The cyclin Cyc B2 may prove an important marker of cell division since it was expressed early in the pericycle in cells that will develop into lateral roots (Beeckman *et al*., 2001) and adventitious roots (Akhami *et al*.,2009) , but the gene itself was not expressed in primary roots (Porcedu *et al*.,1999). Brinker *et al*.(2004) also identified the upregulation of a gene closely related to gene from Arabidopsis AG01, that was first identified as essential for normal leaf development (Bohmert *et al*., 1998). AGO1 mutants (ago1) were defective in light-regulated hypocotyls elongation and adventitious rooting, indicating that a properly functioning of AGO1 was necessary for auxin homeostasis and for processes specific to rooting of cuttings (Sorin *et al*.,2005). AGO1 was a critical element in micro-RNA mediated regulation of gene silencing, because AGO1 was a principal component of RNA-induced silencing complex (Hammond *et al*., 2001) . AGO1 has been shown to regulate rooting of cuttings by influencing the expression of ARF17 and through ARF17, GH3 genes (Sorin *et al*., 2005). GH3 can be regulated by both light and auxin (Hsieh *et al*., 2000) , and accumulation of GH3 is positively related to rooting, possibly that GH3 can adenylate auxins.

Both SCL and SHR act within the first 24h after auxin treatment during dedifferentiation but before cell division. It is noted that SCL was induced by exogenous auxin, but expression of SHR was auxin independent (Gutierrrez *et al*., 2009). The SHR/SCR pathway apparently regulates root pattern promotion independently that of AGO1 pathway (Shunsuke *et al*., 2009). Hasbun *et al*. (2007) found that aging implied a progressive increase of methylated 5-deoxycytidines but it is not very much clear. Methylation of an allele of the purple-plant1 gene called PI-Blotched increased during the juvenile-to-adult transition, was maximal in mature leaves. Irish and McMurray (2006) investigated the same gene using an in vitro system, and found that rejuvenated shoot apices were hypomethylated, indicating the reversion of phase

is due to loss of methylation. Studies suggest a converse regulatory relationship between micro RNAs MIR156 and MIR172. The level of MIR156 was high and level of MIR172 was low during juvenile phase, but was vice versa during mature phase (Chuck *et al*.,2009). The GH3 class of auxin early response genes was probably regulated by ARF17, ARF17 was however appears to be negatively regulated by micro RNAs MIR 160 and MIR 167 in concert with AGO1, as mentioned. These two micro RNAs also positively regulates ARF6 and ARF8 (Gutierrez *et al*., 2008) , thus setting up a system where ARF17 and ARF 6/8 are maintained in a dynamic balance auxin homeostasis to determine the cell status. Mutations in two other ARFs via. NPH4/ARF7 and ARF19, also led to loss of rooting (Wilmoth *et al*.,2013) light can regulate ARF 6/8 and other ARFs at both transcriptional and post transcriptional level by affecting the maturation of MIR160 and MIR 167 (Gutierrez *et al*., 2008). Micro RNA MIR 164 responds to auxin induction and participates in the regulation of NAM/ATAF/CUC (NAC) domain transcription factor proteins.

By using a microarray of 2,178 cDNAs, Lindroth *et al*. (2001a,b) identified 220 genes that were differentially expressed during root development, with most of the genes (121) differentially expressed within 3 days of wounding and initial auxin treatment. Transcriptional profiling of the first 3 days after auxin treatment showed an increase in expression of genes for protein synthesis and decrease in genes for protein degradation. The reverse happens when the root is formed. They found that on ATP-binding cassette (ABC) transporter, a gene typically repressed by auxin, was upregulated when the meristem was formed. Kohler *et al*. (2003) generated 7,013 ESTs from the adventitious roots of hybrid cottonwood at different stages of process suggesting that aquaporins and transporters were differentially expressed in the process of rooting of cuttings.

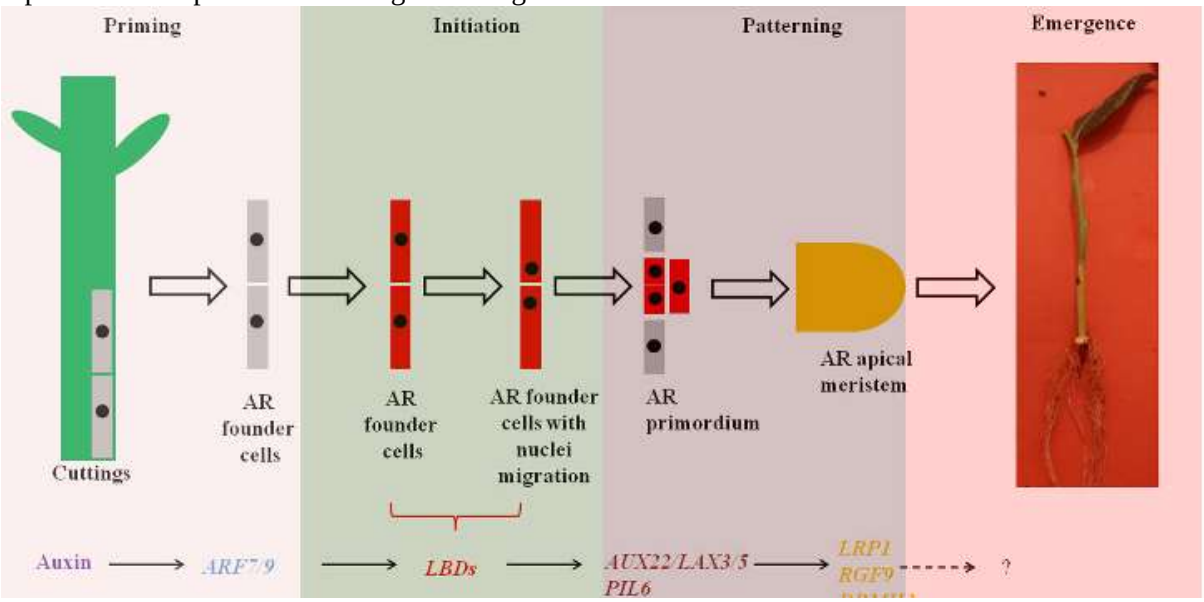

Figure 9. Assumed gene networks that regulate AR formation in blueberry green cuttings. Note: AR, adventitious root; *ARF7/9*, *Auxin responsive factors 7/9*; *LBDs*, Lateral organ boundaries domain; *AUX22*, *Auxin induced protein 22*; *PIL6*, *PIN-LIKE 6*; *LRP1*, *Lateral root primordium 1*; *RGF9*, *root meristem growth factor 9*; and *DRMH3*, *Dormancy-associated protein homologue 3(from* An *et al*.,2020)

According to the known regulatory networks reported previously for root formation in *Arabidopsis* and other plant species, the regulatory pathway that controls blueberry AR formation was derived. It was speculated that auxin would induce the expression of auxin responsive factors *ARF7/9* to perceive auxin signalling, whereas *ARF7/9* directly or indirectly affected the downstream target *LBDs* genes to establish AR founder cells with nuclei migration. Then, auxin polar carriers, including influx carriers *AUX22* or

LAX3/5 and efflux carriers *PIL6s*, would be upregulated to facilitate the establishment of auxin asymmetric distribution, which includes AR primordium formation. Finally, the AR primordium transforms to the AR apical meristem and outgrowth from the cuttings under the effect of *LRP1*, *RGF9*, *DRHM3* and other genes (Figure 9).

CONCLUSION

Auxin action is still unclear .There are many gaps in our knowledge and deep understanding, though much advancement had been made so far. Besides thorough genetic and molecular studies of the process involved, system biology and modeling approaches are necessary for deepening our understanding of auxin action and regulatory networks in rooting of stem cuttings.

REFERENCES

Ahkami AH, Lischowski S, Haensch KT, Porfirova S, Hofmann J, Rolletschek H, Melzer M, Franken P, Hause B, Druege V and Hajirezaei MR (2009). Molecular physiology of adventitious root formation in *Petunia* hybrid cuttings: Involvement of wound response and primary metabolism, *New Phytol*ogy **181** 613-625.

Ahkami AH, Melzer M, Ghaffari MR. *et al. (*2013). Distribution of indole-3-acetic acid in *Petunia hybrida* shoot tip cuttings and relationship between auxin transport, carbohydrate metabolism and adventitious root formation. *Planta* **238** 499–517.

Alsup CM, Cole JC and Claypool PL (2004). Stem cuttings from Caddo sugar maple trees differ in their rooting potential.*Acta Horticulture* **630** 263-269.

Altamura MM. (1996). Root histogenesis in herbaceous and woody explants cultured in vitro. A critical review. *Agronomie* 16 589–602.

Amissah JN and Bassuk N (2007). Effect of light and cutting age on rooting in *Quercus bicolor*,*Quercus robur* and *Quercus macrocrpa* cuttings. *Comb.Proceedings of International Plant Propagation Society* **57** 286-292.

An H, Zhang J, Xu F, Jiang S and Zhang X (2020). Transcriptomic profiling and discovery of key genes involved in adventitious root formation from green cuttings of high bush blueberry (*Vaccinium corymbosum* L). *BMC Plant Biology* 20 182.

Barbez E, Kubes M, Rolcik J, Beziat C, Pencik A, Wang B, Rosquete MR, Zhu J, Dobrev PT, Lee Y, Zazimalova E, Petrasek J, Geisler M, Friml J and Kleine-Vehn J (2012). A novel putative auxin carrier family regulates intracellular auxin homeostasis in plants. *Nature* **485** 119-122.

Barnes HW.(2002). Comperative studies on the rooting of *Betula* species. *Comb.Proceedings of International Plant Propagation Society* **52** 543-547.

Barnes HW (2003). Rooting potential of *Fagus grandifolia* cuttings.*Comb.Proceedings of International Plant Propagation Society* **53** 375-376.

Beeckman T, Burssens S and Inze D (2001). The pericell-cycle in *Arabidopsis*. *Journal of Experimental Botany* 52 403-411.

Bennett T, Hines G and Leyser O (2014). Canalization: what the flux? *Trends in Genetics* **30** 41–48.

Bohmert K, Camus I, Bellini C, Bouchez D, Caboche M and Benning C (1998). AG01 defines a novel locus of Arabidopsis controlling leaf development. *EMBO J*.17:170-180.

Bombarely A, Moser M, Amrad A, *et al.(* 2016). Insight into the evolution of the *Solanaceae* from the parental genomes of *Petunia hybrida*. *Nature Plants* **2** 16074.

Brinker M, Van Zyl L, Liu W, Craig D, Sederoff RR, Clapham DH and von Arnold S (2004). Microarray analysis of gene expression during adventitious root development in *Pinus contorta*. *Plant Physiology* **135** 1526-1539.

Chatterjee BK and Mukherjee SK (1980). Effect of different media on rooting of cuttings of jackfruit (*Artocarpus heterophyllus* Lam). *Indian Journal of Horticulture* **37** 360-363.
Chuck G, Candela H and Hake S (2009). Big impacts by small RNAs in plant development. *Current Opinion Plant Biol*ogy **12** 81-86.
Couvillon GA (1985). Propagation and performance of inexpensive peach trees from cuttings for high density plantings. *Acta Horticulture* **173** 271-281.
da Costa CT, de Almeida MR, Ruedell CM, Schwambach J, Maraschin FS and Fett-Neto AG (2013). When stress and development go hand in hand: main hormonal controls of adventitious rooting in cuttings. *Frontiers in Plant Science* **4** 133.
De Klerk GJ, Van der Krieken W and De Jong JC (1999). The formation of adventitious roots : new concepts, new possibilities. *In Vitro Cell Development Biology Plant* **35** 189-199**.**
Della Rovere F, Fattorini L, D'Angeli S, Veloccia A, Falasca G and Altamura MM (2013). Auxin and cytokinin control formation of the quiescent centre in the adventitious root apex of Arabidopsis. *Annals of Botany* **112** 1395–1407.
Druege U, Franken P, Lischewski S, *et al.(* 2014). Transcriptomic analysis reveals ethylene as stimulator and auxin as regulator of adventitious root formation
in petunia cuttings. *Frontiers in Plant Science* **5** 494. doi: 10.3389/fpls.2014.00494
Druege U, Franken P and Hajirezaei M-R (2016). Plant hormone homeostasis, signaling and function during adventitious root formation in cuttings.
Frontiers in Plant Science **7** 381.
Druege U, Hilo A, Perez-Perez JM, Klopotek Y, Acosta M, Shahinnia F, *et al.* (2019). Molecular and physiological control of adventitious rooting in cuttings: phytohormone action meets resource allocation. *Annals of Botany* **123** 929–949
Dvin SR, Moghadam EG, Nevestani E and Mokhtarian A (2011). Studies on rooting of Azayesh apple cuttings using indolebutyric acid and media.*Asian Journal of Appied Science* DOI:10.3923/ajaps.2011
Geisler M, Aryal B, di Donato M and Hao PC (2017). A critical view on ABC
transporters and their interacting partners in auxin transport. *Plant & Cell*
Physiology **58** 1601–1614.
Guerrero JR, Garrido G, Acosta M and Sanchez-Bravo J (1999). Influence of 2,3,5-triiodobenzoic acid and 1-N-nathylphthalamic acid on indoleacetic acid transport in carnation cuttings : relationship with rooting. *Journal of Plant Growth Regulation* **18** 185-190.
Gur A, Altman A, Stern R and Wolowitz B (1986). Improving rooting and survival of softwood peach cuttings. *Scientific Horticulture* **30** 97-118.
Gutierrez L, Bussell J, Pacurar D and Bellini C (2008). Feedback loops between micro RNAs MIR160, MIR167 and their targeted Auxin Response Factors control adventitious rooting in Arabidopsis hypocotyle. p.51. In Book of Abstracts, 5th *International Symposium* On Adventitious Root Formation, June 16-20, 2008, Madrid, Spain.
Hammond SM, Boettcher S, Caudy AA, Kobayashi R and Hannon GJ (2001). Argonante2, a link between genetic and biochemical analysis of RNAi. *Science* **293** 1146-1150.
Hartmann HT, Kester DE, Davies Jr. FT and Geneve RL.(2002). Plant Propagation : Principles and Practices, 7th Ed. Prentice Hall, Englewood Cliffs, NJ.
Hasbun R, Valledor L, Snatamaria E, Canal MJ, Rodriguez R and Berdasco M (2007). Dynamics of DNA methylation in chestnut trees development. p. 563-566. In : K. Hammer (ed). *Proceedings* 27th IHCS-S1 *Plant Genetics Research, Acta Horticulture* 760.
Henrique A, Campinhos EN, Ono EO and de Pinho SZ (2006). Effect of plant growth regulators in rooting of *Pinus* cuttings. *Brazilian Archives of Biological Technology* **49** 189-196.
Henry PH and Preece JE (1997). Production and rooting of shoots generated from dormant stem sections of maple species.*Horticulture Science **32*** 1274-1275.

Hsieh HL, Okamoto H, Wang M, Ang LH, Matsui M, Goodan H and Deng XW (2000). FIN219,an auxin regulated gene, defines a link between phytochrome A and the downstream regulator COP1 in light control of Arabidopsis development.*Gene Dev*elopment **14** 1958-1970.

Husen A.(2008). Clonal propagation of *Dalbergia sissoo* Roxb and associated metabolic changes during adventitious root primordium development. *New Forestry* **36** 13-27.

Hutchison KW, Singer PB, McInnis S, Diaz-Sala C and Greenwood MS (1999). Expansins are conserved in conifers and expressed in hypocotyls in response to exogenous auxin. *Plant Physiology* **120** 827-831.

Ikeuchi M, Ogawa Y, Iwase A and Sugimoto K (2016). Plant regeneration: cellular origins and molecular mechanisms. *Development* **143** 1442–1451.

Irish EE and McMurray D (2006). Rejuvenation by shoot apex culture recapitulates the developmental increase of methylation at the maize gene PL-Blotched. *Plant Molecular Biology* **60** 747-758.

Ivanika J and Pastyrick L (1978). The utilization of 3-indolebutyric acid in rooting hardwood cutting of fruit trees. *Acta Horticulture* **80** 83-85

Jutta Ludwig-Müller (2011). Auxin conjugates: their role for plant development and in the evolution of land plants Journal of Experimental Botany, 62(6), 1757–1773.

Karakurt H, Aslantas R, Ozakan G and Guleryuz M (2009). Effects of indole 3-butyric acid(IBA),plant growth promoting rhizobacteria(PGPR) and carbohydrates on rooting of hardwood cuttings of MM106 apple root stock. *African Journal Agricultural Research* **4** 60-64.

Kareem A, Manan A, Saeed S, Rehman SU, Shahzad U and Nafees M (2016). Effect of different concentrations of IBA on rooting of Guava *Pisidium guajava* L.in low tunnel under shady situation.*JournalAgriculture,Environment and International Development* **110** 197-203

Kester DE, Gradziel TM and Grasselly C (1990). Almonds (*Prunus*). *Acta Horticultur*e **290** 699-758.

Kohler A, Delaruelle C, Martin D, Encelot N and Martin F.(2003). The poplar root transcriptome : analysis of 7000 exprssed sequence tags. *FEBS Letter* **542** 37-41.

Laplaze L, Benkova E, Casimiro I, Maes L, Vanneste S, Swarup R, Weijers D, Calvo V, Parizot B, Herrera-Rodriguez MB, Offringa R, Graham N, Doumas P, Friml J, Bogusz D, Beeckman T and Bennett M (2007). Cytokinins act directly on lateral root founder cells to inhibit root initiation. *Plant Cell* **19** 3889-3900.

Lindroth A, Kvarnheden A and von Arnold S (2001a). Isolation of a PSTAIRE CDC2 cDNA from *Pinus contorta* and its expression during adventitious root development. *Plant Physiology Biochemistry* **39** 107-114.

Lindroth A, Saarikoski P, Flygh G, Clapham D, Gronross R, Thelander M, Ronne H and von Arnold S (2001b). Two S-adenosylnethionine synthetase-encoding genes differentially expressed during adventitious root development in *Pinus contorta*. *Plant Molecular Biol*ogy **46** 335-346.

Macdonald B (1986). Practical woody plant propagation for nursery growers. Vol.1. Timber Press, Portland, OR.

Mano Y and Nemoto K (2012). The pathway of auxin biosynthesis in plants. *Journal of Experimental Bot*any **63** 2853-2872.

Nag S, Saha K and Choudhuri MA (2001). Role of auxin and polyamines in adventitious root formation in relation to changes in compounds involved in rooting. . *Journal of Plant Growth Regul*ation **20** 182–194

Naija S, Elloumi N, Ammar S, Kevers C and Dommes J (2009). Involvement of polyamines in the adventitious rooting micropropagated shoots of the apple rootstock MM106. *In vitro Cell Development Biology Plant* **45** 83-91.

Normanly J, Cohen JD and Fink GR (1993). *Arabidopsis thaliana* auxotrophs reveal a tryptophan-independent biosynthetic pathway for indole-3-acetic acid. *Proceedings National Academy of Sc*ience USA **90** 10355-10359.

Nemoto K, Hara M, Suzuki M, Seki H, Muranaka T and Mano Y (2009). The NtAMI1 gene functions in cell division of tobacco BY-2 cells in the presence of indole-3-acetamide. *FEBS Letter* **583** 487-492.

Normanly J (2010). Approaching cellular and molecular resolution of auxin biosynthesis and metabolism. *Cold Spring Harbour Perspectives of Biology* **2** 0001594.

Novak O, Henykova E, Sairanen I, Kowalczyk M, Pospisil T and Ljung K (2012). Tissue-specific profiling of the *Arabidopsis thaliana* auxin metabolome. *Plant Journal* **72** 523-536.

Ouyang J, Shao X and Li J (2000). Indole-3-glycerol phosphate a branch point of idole-3-acetic acid biosynthesis in the tryptophan biosynthetic pathway in *Arabidopsis thaliana. Plant Journal* **24** 327-334.

Pacurar DI, Perrone I and Bellini C (2014). Auxin is a central player in the hormone cross-talks that control adventitious rooting. *Physiologia Plantarum* **151** 83–96.

Pagnussat GC, Lanteri ML and Lamattina L (2003). Nitric oxide and cyclic GMP are messangers in the IAA induced adventitious rooting process. *Plant Physiology* **132** 1241-1248.

Pijut PM, Woeste KE and Michler CH (2011). Promotion of adventitious root formation of difficult to root hardwood tree species. *Horticulture Review* **38** 213-251.

Pijut PM and Moore MJ (2002). Early season softwood cuttings effective for vegetative propagation of *Juglans cinerea*. *Horticulture Science* **37** 697-700.

Pijut PM.(2004). Vegetative propagation of butternut (*Juglans cinerea*): Field resuts.p.37-44 In:Michler,C.H.et al.(eds)Black walnut in a new century.Proc.6th Walnut Council Research Symposium.Gen.Tech.Rep.NC-243,St.Paul,MN.

Pijut PM and Espinos C (2004). Development of a rooted cutting propagation method for *Prunus serotina*. *Comb.Proceedings International Plant Propagation Soc*iety **54** 513-515.

Porceddu A, Reale L, Lanfaloni L, Moretti C, Sorbolini S, Tedeschini E, Ferranti F and Pezzotti M (1999). Cloning and expression analysis of a *Petunia* hybrid flower specific mitotic – like cyclin. *FEBS Letter* **462** : 211-215

Preece JE, Ledbetter DI and Zaczek JJ (2001). Rooting softwood cuttings collected from forced large stems of oak leaf hydrangea and American chestnut.*Comb.Proceedings International Plant Propagation Society* **51** 267-270.

Rosquete MR, Barbez E and Kleine-Vehn J (2012). Cellular auxin homeostasis: gatekeeping is housekeeping. *Molecular Plant* **5** 772-786.

Santos MQC, Lemos EEP, Salvador TL, Rezende LP, LimaSalvador T, Silva JW, Barros PG and Campos RS.(2011). Rootings of cuttings of soursop (*Annona muricata*)Giant of Alagoas.2011 DOI:10.17660/*Acta Horticculture* 2011.923.36

Sato Y, Sugiyama M, Gorecki RJ, Fukuda H and Komamine A (1993). Interrelationship between lignin deposition and the activities of peroxidase isoenzymes in differentiating tracheary elements of *Zinnia. Planta* **189** 584–589.

Sedira M, Welander M and Geier T (2007). Influence of IBA and aphidicolin on DNA synthesis and adventitious root regeneration from *Malus* 'Jork 9" stem discs. *Plant Cell Report* **26** 539-545.

Shunsuke M, Hashimoto T and Nakajima K (2009). ARGO NAUTE 1 acts in Arabidopsis root radial pattern formation independently of the SHR/SCR pathway. *Plant Cell Physiology* **50** : 626-634.

Sorin C, Bussell JD, Camus I, Ljung K, Kowalczyk K, Geiss G, Mckhann H, Garcion C, Vaucheret H, Sandberg G and Bellini C (2005). Auxin and light control of adventitious rooting in *Arabidopsis* require ARGONAUTE. *Plant Cell* 17 1343-1359.

Starder LC and Bartel B (2011). Transport and metabolism of the endogenous auxin precursor indole-3-butyric acid. *Molecular Plant* **4** 477-486.

Sugawara S, Hishiyama S, Jikumaru Y, Hanada A, Nishimura T, Kashiba T, Zhao Y, Kamiya Y and Kasahara H (2009). Biochemical analyses of indole-3-acetaldoxime-dependent auxin biosynthesis in *Arabidopsis .Proceedings National Academy of Science* USA **106** 5430-5435.

Sukumar P, Maloney GS and Muday GK (2013). Localized induction of the ATPbinding cassette B19 auxin transporter enhances adventitious root formation
in *Arabidopsis*. *Plant Physiology* **162** 1392–1405.
Swamy SL, Puri S and Kanwar K (2002a). Prpagation of *Robinia pseudoacacia* Linn and *Grewia optiva* Drummond from rooted stem cuttings.*Agroforest system* **56** 231-237.
Swamy SL, Puri S and Singh AK (2002b). Effect of auxins(IBA and NAA) and season on rooting of juvenile and mature hardwood cuttings of *Robinia pseudoacacia* and *Grewia optiva*. *New Forestry* **23** 143-157.
Swarup R and Peret B (2012). AUX/LAX family of auxin influx carriers – an overview. *Frontiers of Plant Science* **3** 225.
Tsipouridis C, Thomidis T and Bladenopoulou S (2006). Seasonal variation in sprouting of GF677 peach almond hybrid root cuttings. *NewZealand Journal of Crop Horticulture Scienc*e **34** 45-50.
Tsipouridis C, Thomidis T and Isaakidis A (2003). Rooting of peach hardwood and semihardwood cuttings. *Australian Journal Experimental Argriculture* **43** 1363-1368.
Tworkoski T and Takeda F (2007). Rooting response of shoot cuttings from three peach growth habits. *Sci*entific *Horticultur*e **115** 98-100.
Wilmoth JC, Want S, Tiwari SB, Atul AD, Hagen G, GuilfoyleTJ, Alonso JM, Ecker JR and Reed JW (2013). NPH4/ARF7 and ARF19 expression. *Plant signaling Behavior* 8.
Woodward AW and Bartel B (2005). Auxin : regulation, action and interaction. *Annuals of Botany* **95** 707-735.
Wright AD, Sampson MB, Neuffer MG, Michalczuk L, Solvin JP and Cohen JD (1991). Indole-3-acetic acid biosynthesis in the mutant-maize orange pericarp, a tryptophan auxotroph. *Science* **254** 989-1000.
Zaczek JJ, Heuser CW and Steiner KC (1997). Effect of Shade levels and IBA during the rooting of eight tree taxa.*Journal of Environment and Horticulture* **15** 56-60
Zaczek JJ, Heuser CW and Steiner KC (2000). Timing of low solar irradiance affects *Quercus* and *Acer* propagation.*Comb*.*Proceedings International Plant Propagation Society* **50** 434-441.