

Morphological and physiological changes during adventitious root formation as affected by auxin metabolism: Stimulatory effect of auxin containing seaweed extract treatment

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ABSTRACT

The formation of adventitious roots is a quantitative genetic trait regulated by both environmental (especially temperature, light, relative humidity) and endogenous factors (hormones, sugars, mineral salts and other molecules). A cutting removed from the stock plant normally undergoes various anatomical changes accompanied by alterations in metabolic activity and gene expression during the wound response and subsequent rhizogenesis. Rooting consist of different steps, each with its own hormone requirements. Over recent years, a multitude of models have been proposed to show how plant hormones and metabolites interact to control adventitious root formation and plant development. Phytohormones are directly involved in cell division and cell growth, and they indirectly interact with other hormones or metabolites. Auxin plays an essential role among phytohormones when regulating roots' development and has been shown to be intimately involved in the process of adventitious rooting. Researchers have developed different rooting treatments, either by examining the effects of auxin using short exposure to a solution with a high auxin concentration or by dipping in a rooting powder (auxin with talc). Over the past two decades, auxin-containing seaweed extracts have been recognised as promising biostimulants for adventitious root formation and plant development. The aim of this review was to summarize current knowledge associated with the anatomical and physiological aspects of adventitious root formation, and highlight any recent progress made regarding the identification of auxin-containing seaweed extracts for the control of adventitious rooting.

Key words: adventitious root formation, auxin, carbohydrates, jasmonate, phenolics, proteins, seaweed extract

Abbreviations: hpe – hours post excision; IAA – indole-3-acetic acid; IBA – indole-3-butyric acid; NAA - 1-naphthalene acetic acid; PAL - phenylalanine ammonia-lyase; TAL - tyrosine ammonia-lyase; TIBA - triiodobenzoic acid;

ADVENTITIOUS ROOTS AND THEIR FUNCTION

The rooting of stem cuttings is one of the better and economically-viable propagation methods within agriculture, horticulture, and forestry. It is governed by an array of endogenous physiological factors (Druege and Kadner 2008, Osterc and Štampar 2008). Cuttings must survive physiological stress after severance from the stock plant, and if receiving little water or nutrient uptake they can be affected by the usually-reduced stomatal conductance, until the development of the roots (Druege and Kadner 2008, Pop et al. 2011). Consequently, chlorophyll loss and an increase in the xanthophylls cycle-pool are typically associated with leaf senescence. Further severance-induced metabolic responses include increased hormone production (auxin, ethylene, jasmonate) and establishment of a sink tissue, which is characterised by decreased endogenous carbohydrate status and the translocation of nutrients to the stem base for wound healing and cell division (Kadner and Druege 2004; Rapaka et al. 2005, 2008; Druege and Kadner 2008; Ahkami et al. 2009; Misra and Misra 2010; Trueman and Richardson 2011). Noticeable progress has been made recently during research into rooting, which is not a single process but a progressive process consisting of different steps, each with its own hormone requirements (de Klerk et al. 1999, Pop et al. 2011). Physiological characterization, histological analysis and molecular research on herbaceous and woody plants have recently provided a proper tool to distinguish these phases. Ahkami et al. (2009) postulated a three-phase mechanism for the metabolic response involved during adventitious root formation (ARF) on petunia used as model herbaceous plant. (1) The 'Sink establishment phase' is characterised by jasmonate (JA) accumulation immediately after wounding, and the induction of gene coding for enzymes that degrade

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sucrose within the apoplast into hexoses. The hexoses are taken up by at least one induced monosaccharide transporter and then used for the production of energy necessary for wound-healing and cell division. (2) The 'Recovery phase' is characterized by the replenishment of resources and in the petunia it lasts up to 72 hours after excision (hpe) with the formation of meristemoids. (3) The 'Maintenance phase' is characterized by the symplastic transport of sugars translocated from the source-leaves into the stem-base. They are converted, either into intermediates directly flowing into root development or to the intermediate storage compounds starch and citrate, thereinafter used for later root formation processes.

However, there is little information on rooting process in woody plants. As yet, molecular data are lacking and physiological studies are scarce. In apple microcuttings, the timing of three phases was ascertained (De Klerk et al. 1999). During the initial 24 hpe, dedifferentiation of the cells between the vascular bundles occurs from which the root primordial originate. These cells accumulate starch during the initial 24 hours. Wounding-related compounds (breakdown products of cell membranes, jasmonic acid, cellulase, pectinase) likely play a major role in the dedifferentitation phase, probably acting with auxins and cytokinins. Later on, up to 72 or 96 h, previously activated cells become commited to the formation of root primordial by the rhizogenic action of auxin. The starch grains are degraded during the following 24 h (24-48 hpe), the first cell divisions occur 48 hpe, and by 96 hpe, meristemoids are present. The meristemoids develop into root primordial and afterwards into roots during the differentitation phase. Indirect rooting of some species, in which root initials are formed after an intermediate callus phase, is an obvious example of the occurrence of three phases with a prolonged period of dedifferentiation. The differences in auxin requirements among the three phases of adventitious rooting have important consequences for rooting of cuttings (De Klerk et al. 1999). Taken together, ARF is modulated by a combination of different pathways, including hormone biosynthesis and primary metabolism; the individual steps exert a control over ARF. In the presented review, a spatio-temporal analysis of hormones and metabolites at different developmental stages of ARF is provided with respect to specific anatomical, hormonal and biochemical changes.

MORPHOLOGICAL CHANGES DURING ADVENTITIOUS ROOT FORMATION

On the basis of anatomical studies, the root formation phases were designated as the root initiation phase, followed by the root primordium formation phase, and then the root elongation and/or emergence phase. These anatomical events were studied precisely by Ahkami et al. (2009) on the model plant *Petunia hybrida* and by De Klerk et al. (1997, 1999) on apple microcuttings. After the severance of petunia cuttings from the stock plant, it took 72 h to initiate the earliest morphological event. This root initiation phase (Figure 1A) resulted in the occurrence of small cells with large nuclei and dense cytoplasms – both typical signs of meristematic cells. On the histological level, starch grains are degraded during the next 24 h, when the first cell divisions take place and meristemoids appear. The appearance of such meristemoids marks the transition from the root initiation phase to the root primordium formation phase (Figure 1B). This first cytological sign of ARF at 72 hpe was preceded by the RNA accumulation of the cyclin B1 gene, which might serve as a marker gene specific for the root initiation phase of ARF. During the root primordium formation phase, the first well-developed young root meristems became visible at 96 hpe (Figure 1C). At first globular structures developed root primordia with the typical dome-shape at 144 hpe, which included the meristem and behind it the first cells of the root body. The first roots with elongated cells of the elongation zone appeared at 192 hpe (Figure 1D). These structures were still inside the stem, but revealed all the morphology of a complete root, except for root hairs. They marked the transition to the root elongation phase, which resulted in the emerging of the earliest roots, visible after one additional day.

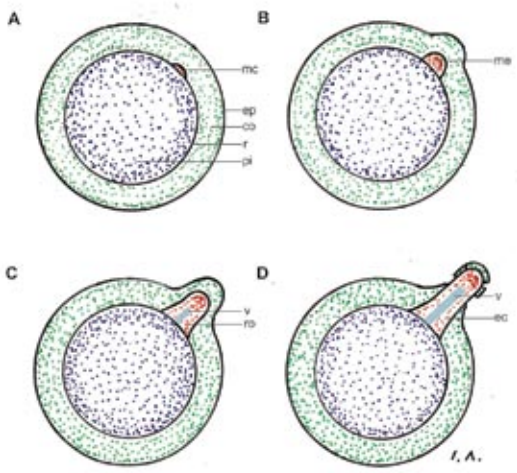

Figure 1: Anatomy of adventitious root formation (A-D) in the stem base of pelargonium (*Pelargonium peltatum*) cuttings. Typical stem anatomy consists of epidermis (ep), cortex (co), pith parenchyma (pi) and a ring of vessels (r) with outer phloem, the cambium, and the inner xylem. (A) „Root initiation phase": first meristematic cells (mc) of developing root meristems appear. (B) „Root primordium formation phase": the appearance of an meristemoid (me). (C) First differentiating root primordia with an organized meristem and a backward differentiation of cells of the root body containing root cortex (ro) and vascular bundle (v); (D) „Root elongation and/or emergence phase": emerging of the first root with vascular bundles (v) in the center surrounded by elongated cells (ec) of the elongation zone. It reaveals morphological characteristics of the complete root, except for root hairs.

HORMONE CONTROL AND METABOLIC RESPONSES INVOLVED IN ADVENTITIOUS ROOT FORMATION

Wound response and the role of jasmonates

The activation of wound-induced responses involves a complex network of signaling cascades, in which jasmonates (JA) represent the best-characterized class of signal molecules. Ahkami et al. 2009 reported that immediate after wounding, the JA content rose 12-fold and its precursor 12-oxo-phytodienoic acid (OPDA) had an approximately 4-fold accumulation, with a maximum 0.5 hpe. The JA contents returned to the basal level within 6 hpe, whereas OPDA remained at the increased level for 6 hours and dropped at 12 hpe to the basal levels. This time-course occurred locally within the stem base as well as systemically, but at lower levels, in other parts of the cuttings. Further analyses suggest that the protein constitutively present within vascular tissues has constantly high activity and is involved in the increased biosynthesis of JA, as already shown in the tomato (Hause et al. 2003, Stenzel et al. 2003). Usually the wound-induced elevation of JA levels is followed by the activation of JA-responsive genes (Wasternack and House 2002). In addition, cell wall-invertase transcript accumulation also seems to be induced by wound-induced JA within the stem base (Schaarschmidt et al. 2006). Higher levels of transcripts followed by an increased activity of cell wall invertase might then lead to a higher sink status of the stem tissue.

AUXIN BIOSYNTHESIS DURING ADVENTITIOUS ROOT FORMATION

Auxins are a group of tryptophan-derived signals that are involved in most aspects of plant development (Woodward and Bartel 2005). Auxin is mainly formed in young leaves and stem-tips, and is then transported to the roots, both in the phloem and by a special polar mechanism. Auxins play a major role in controlling the growth and development of plants, the early stages of embryogenesis, the organization of apical meristem (phyllotaxy) and the branching of the plant's aerial parts (apical dominance), formation of the main root, and lateral and adventitious root initiation (Went and Thimann 1937). Furthermore, it elicits those responses throughout the plant required for the function of developing leaves and roots. Auxins are also involved in gravitropism and phototropism (Kepinski and Leyser 2005). Auxin has a central role in shoot/root relation, correlating the presence and development of leaves with root initiation. In addition, auxin induces the differentiation of vascular tissues, it inhibits or induces the differentiation of branches and prevents the abscission of leaves (Sachs 2005, Pop et al. 2011). High photosynthesis could be coupled with auxin synthesis, thus enhancing root formation. High ion and water absorptions could be coupled with high auxin catabolism, thus enhancing leaf development (Sachs 2005). These multiple effects across the plant result from its control of cell division, cell elongation, and certain stages of differentiation. On the cellular level, the response to auxin includes a rapid initial cell-growth response that may involve auxin-induced changes in pH, calcium and gene expression (Davies 2004, Pop et al. 2011).

Rooting-phases have different auxin requirements. There is always a temporary increase in the endogenous level of free indol-3-acetic acid (IAA) during the inductive phase (corresponding to a minimal level of peroxidase/oxidase activity). The inductive phase is the auxin sensitive, when the plants are responsive to exogenous auxin application. It is followed by the auxin insensitive phase (initiation phase) characterized by a decrease in IAA levels to a minimum and high peroxidase and oxidase activity (Nag et al. 2001, Faivre-Rampant et al. 2002). The IAA oxidation at this stage of rooting (initiation phase) seems to be related to the auxin response. Oxidation products of IAA may promote root formation, especially when linked to the phenolic compounds present (Günes 2000). During the root expressive phase, IAA is again needed to promote the growth of root's initials (Štefančič et al. 2007).

The importance of auxin during the production of lateral or adventitious roots was demonstrated with several 'gain-of-function' as well as 'loss-of-function' *iaa* mutations (Fukaki et al. 2002, Rogg et al. 2001, Tatematsu et al. 2004). In *Arabidopsis*, the *superroot* (*sur1* and *sur2*) mutants, accumulate IAA, developing numerous adventitious roots on the hypocotyl and cuttings of different organs in the case of *sur1* (Delarue et al. 1998). Triiodobenzoic acid (TIBA), an auxin polar transport inhibitor, applied to the top of the hypocotyls, lowered the rate of root formation (Fabijan et al. 1981). Rice mutants affected in the expression of the *PIN-FORMED (OsPIN1)* gene, potentially involved in auxin polar transport, are affected in adventitious root emergence and tillering confirming that the auxin concentrations and distributions within the different tissues are essential (Xu et al. 2005).

New insight has been provided over recent years, into the interaction between auxin and other hormones. Auxin and ethylene are often described as activators, whilst cytokinins and gibberellins are seen as inhibitors of adventitious root formation, even when some positive effects have been observed. 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). The auxin and ethylene relations, during root development, has been shown by a number of isolated mutants that have resistance to both hormones (Müller et al. 1998, Pan et al. 2002). Studies have emphasized that polyamines play a role during adventitious rooting (Biondi et al. 1990, Hausman et al. 1994, Heloir et al. 1996) and a possible interrelation between polyamines and auxin-controlling rooting induction has been suggested (Hausman et al. 1995).

In addition over recent years, new insight into the interaction between auxin and carbohydrates, and their regulation of ARF, has been provided (Haissig 1989, Roitsch 1999, Druege and Kadner 2008, Agullo-Anton et al. 2011). It has been reported that auxin stimulates the mobilization of carbohydrates in leaves and the upper stem, and increases the translocation of assimilates towards the rooting zone.

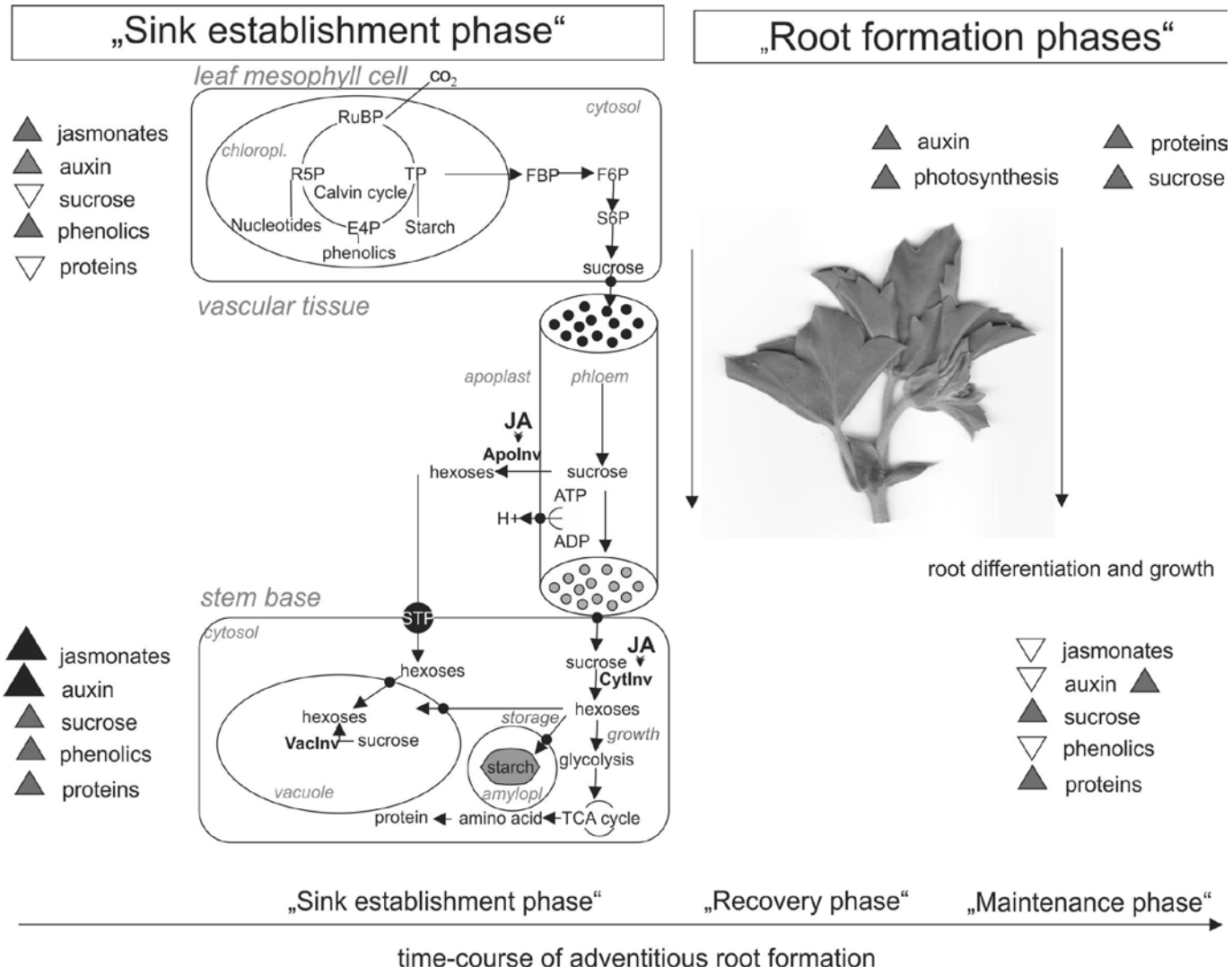

Figure 2: Schematic presentation of the metabolic response during adventitious root formation (ARF) based on model plants Petunia hybrida and Pelargonium peltatum (modified according to Ahkami et al. 2009, Urbanek Krajnc et al. 2012). Assimilates are produced in source tissues and translocated towards the cutting base to establish a sink organ. Wounding induces the accumulation of jasmonates (JA), which further induce the cell wall invertase (apoInv) transcript accumulation leading to cleavage of sucrose and transport of hexoses into the cell by monosaccharide transporter STP. Two days after excision the recovery starts followed by the maintenance phase characterised by symplastic transport of sugars from source leaves into the stem base and used either for energy production or accumulated in the vacuole. RuBP – ribulose-1,5-bisphosphate, TP – triose phosphate, E4P – erythrose-4-phosphate, R5P – ribulose-5-phosphate, FBP – fructose-1,6-bisphosphat, F6P – fructose-6-phosphate, S6P – sucrose-6-phosphate, apoInv – apoplastic invertase, cytInv – cytoplasmatic invertase, vacInv – vacuolar invertase, STP - monosaccharide transporter, TCA cycle – tricarboxylic acid cycle

THE IMPORTANCE OF CARBOHYDRATES IN ROOTING OF CUTTINGS

Cell division and cell enlargement during ARF require high input of energy and carbon skeletons. A major carbon source is sucrose, which is formed in photosynthetically active tissues and translocated towards the sink-parts of the plant. Sucrose can be used after cleavage into hexoses as a direct carbon source or is converted in to storage compounds such as starch (Figure 2). Moreover, there are increasing indications that sugars have a regulatory role in ARF (Takahashi et al. 2003, Gibson 2005).

In the stem-bases of petunia cuttings (Ahkami et al. 2009), the levels of soluble and insoluble sugars start to accumulate at 24 hpe, despite high metabolic activity from 12 to 24 hpe onwards. However, the most pronounced increase in sugar levels was documented during the later stages of ARF. A continuous increase of sucrose within source leaves can be observed from 144 hpe onwards, probably owing to an

increased photosynthetic capacity. This sucrose can be translocated towards the stem base for further metabolism. The basipetal translocated sucrose is, however, not only used for delivering energy for differentiation (cell division and cell enlargement), but considerable portion of the sucrose is converted into starch, which probably acts as the major carbon source when the adventitious roots are growing. As shown for *Pinus radiata*, sucrose applied to a growing-medium leads to higher levels of sugars and starch in the rooting regions of the hypocotyl cuttings, and enhances root formation (Li and Leung 2000). The analyses of Ahkami et al. (2009) indicated that starch can be synthesized and stored in different cell types in order to meet their demand for increased metabolic activity when the adventitious roots emerge (Ahkami et al. 2009).

In agreement with the low sucrose content, directly after excision (»sink establishment phase«), at the cutting base the cell wall invertase activity was increased during the first hours after excising, and decreased again to the basal level before root formation. By contrast, the activities of vacuolar and cytosolic invertases decreased continuously to a low level (Ahkami et al. 2009). Cell wall invertase is not only a key enzyme of the apoplastic phloem unloading of transported sucrose but also links phytohormone action with primary metabolism (Roitsch and González 2004). Being present within the apoplast, it can establish the sink function of a certain tissue and thus provide a mechanism for flexible and appropriate adjustment to a wide range of internal and external stimuli (Roitsch et al. 2003). The metabolic activity through which the hexoses from the basipetal transported sucrose can be catabolized leads to successive increases in both, phosphofructokinase (PFK) and glucose-6-phosphate dehydrogenase (Glc6P DH) activities, and a decrease in the cytosolic fructose-1,6-bisphosphatase activity. These events strongly suggest that the catabolism of glucose occurs in parallel within the pentose phosphate pathway and by glycolysis. This yields ATP as energy-source and amino acids for protein synthesis (Ahkami et al. 2009).

The importance of carbohydrates in ARF of pelargonium cuttings and the effects of carbohydrate shortage were studied by Druege and Kadner (2008). Especially, rooting of dark-stored pelargonium cuttings can be restricted by carbohydrate shortage resulting from the interplay between depleted carbohydrate reserves and weak photosynthesis under the low light conditions in the winter's in Central Europe. Druege and Kadner 2008 assumed that considerably reduced air temperature during rooting increased the current availability of carbohydrates, thereby improving survival and root formation. Higher sugar levels during rooting, mediated by low air temperature (10 °C, root zone temperature: 20 °C) were positively correlated with reduced leaf senescence, a higher survival rate, and a higher number of roots. The authors thus provided new prospects for the control of air temperature during the rooting of cuttings that exhibit low current photosynthesis.

AMINO ACIDS AND PROTEIN METABOLISM DURING ADVENTITIOUS ROOT FORMATION

In most of the plant systems analyzed, the most abundant amino acids in vascular tissues were glutamate, glutamine and, in some cases, asparagine (Urquhart and Joy 1982, Schobert and Komor 1989, Lohaus and Moellers 2000). Analysis of the petunia stems before excision indicated that mainly glutamine and asparagine were synthesized in the source-tissues and transported downwards to the sink-tissues (Ahkami et al. 2009). Since glutamine biosynthesis is the key step of nitrate assimilation in plants (Joy 1988) and supplies nitrogen for amino acid synthesis, the basipetal translocation of glutamine is an important process to meet the demands of the cells during root-formation. Levels of glutamine and asparagine remained low at the later stages. This indicated a high turnover of the translocated amino acids into others that appear to be essential for accelerated protein synthesis during ARF (Druege and Kadner 2008, Ahkami et al. 2009). A similar response was also determined for proteins in the case of pelargonium cuttings. In the presented experiment, a transient decline in total protein values was monitored twenty-three days after the severance of pelargonium cuttings (Urbanek Krajnc et al. 2012). However, this decline can not only be linked to the establishment of a sink-tissue and adequate mobilisation of primary metabolites to the region of root regeneration. Recent analyses of tomatos suggested that the proteins and amino acids constitutively presented in vascular tissues are involved in the increased biosynthesis of jasmonates, the best-characterised class of signal molecules involved in wound-induced responses (Hause et al. 2003). Jasmonate accumulation induces genes coding for invertases that degrade sucrose within the apoplast to hexoses used for the production of energy necessary for wound healing and cell devision. In the petunia stems' cuttings, the strong depletion of total amino acids between 6 and 48 hours after excision was measured and, thereafter, a recovery reaching only 50 % of the initial value was observed (Ahkami et al. 2009). These same authors confirmed a change of carbon to nitrogen ratio in petunias towards a threefold increase of carbon against nitrogen, beginning 48 hours post excision. The analysis of petunia stems pre-excision confirmed that mainly glutamine and asparagine were synthetised in source tissues and transported downwards to the sink tissues. Since glutamine biosynthesis is the key step of nitrate assimilation in plants and supplies nitrogen for amino acid synthesis (Taiz and Zeiger 2010), a basipetal translocation of glutamine is an important process for meeting the demands of the cells during root formation. These early events in ARF described by different authors would explain the observed depletion of protein contents within pelargonium cuttings 23 days post severance. Later on (36 dps), a strong increase was determined in the protein levels of pelargonium leaves (Urbanek Krajnc et al. 2012), which would be parallel to an increase in the carbohydrates due to high photosynthesis rates.

ROLE OF PHENOLICS DURING ADVENTITIOUS ROOT FORMATION

Severance from the stock plant, induces the biosynthesis of phenolic compounds, which is why many authors have detected an increased phenolic content level during the first days after establishing the cuttings (Osterc et al. 2004, Quaddoury and Amssa 2004, Štefančič et al. 2007, Osterc and Štampar 2008). Several results have indicated that some phenolic compounds (chlorogenic acid, epicatechin, caffeic acid, catechol, gallic acid, ferulic acid) act as rooting co-factors during the root formation process, especially as protectors of IAA against oxidation. On the other hand, the presense of specific phenolic compounds (sinapinic acid, vanillic acid, cinnamic acid) have a negative impact on ARF during the vegetative propagation of cuttings (Osterc et al. 2004, Trobec et al. 2005). Focusing on IAA protectors, two modes of action are ascribed: they might act as competing oxidation substrates for IAA-oxydase instead of IAA, or more likely they may function as free radical scavengers that drive the peroxidase-catalysed reaction (Trobec et al. 2005; Osterc et al. 2007, 2008; Štefančič et al. 2007; Osterc and Štampar 2008). Higher amounts of these phenolic substances mean more IAA in this way. Thus fluctuation of phenol contents during adventitious rhizogenesis undergoes a time-course variation paralleling that of free IAA, which is generally credited as the trigger for root initiation, and the physiological stages of rooting are correlated with changes in endogenous auxin concentrations (Bartel et al. 2001, Faivre-Rampant et al. 2002, Pop et al. 2011).

In the presented study, an increased accumulation of total phenolics three weeks after the establishment of pelargonium cuttings coincided with a decrease in total protein levels (Urbanek Krajnc et al. 2012). These results are in agreement with current knowledge that the synthesis of phenolics is coupled with amino acid metabolism and controlled by enzymes, including **phenylalanine** ammonia-lyase (PAL) and tyrosine ammonia-lyase (TAL). These enzymes act through the deamination of phenylalanine and tyrosine to yield t-cynnamic and p-coumaric acids, respectively, with the liberation of ammonia (Taiz and Zeiger 2010).

Thirty six days after severance, a slight decline in total phenolics was measured in pelargonium cuttings in comparison to the previous sampling (Urbanek Krajnc et al. 2012). A similar time-course of changes in phenolic contents has also been described by other authors (Trobec et al. 2005, Štefančič et al. 2007, Osterc and Štampar 2008). These authors first recorded increases in certain phenolic compounds in the leaves of chestnut cuttings, and the basal portions of cherry cuttings during rooting, later on the concentrations of monophenols rapidly decreased, especially sinapinic acid and vanillic acid, to which a negative influence on rooting is ascribed. Štefančič et al. (2007) reported that the fluctuation of phenolic content undergoes a time-course variation that parallels that of free IAA. A time-course analysis of single phenolic compounds and enzymes involved in the phenylpropanoid pathway would provide a better understanding of the interactions amongst indole-3-butyric acid (IBA) and phenolics during adventitous root formation in the future.

THE SIGNIFICANCE OF ENDOGENOUS AND EXOGENOUS AUXIN IN CONTROL OVER ADVENTITIOUS ROOT FORMATION

Auxin is one of the major endogenous hormones involved in the process of adventitious rooting and the physiological stages of rooting are correlated with changes in endogenous auxin concentrations. Plants produce IAA in the shoot apices and in young leaves. After detachment of the shoot, basipetal polar transport of auxin contributes to auxin accumulation in the stem base and the rise of free auxin in the basal stem very probably contributes to the early events of adventitious root formation (Ahkami et al. 2009). However, for successful rooting in difficult-to-root plant species, exogenous application of auxin can contribute significantly to the plant's auxin-pool and promote adventitious root formation (Pop et al. 2011). IAA was the first used to stimulate rooting of cuttings (Cooper 1935) and soon afterwards other auxins that also promoted rooting, IBA and NAA (1-naphthalene acetic acid) were synthesized chemically and were considered even more effective (Zimmerman and Wilcoxon 1935). Talc powder was introduced as a carrier for auxin (Grace 1937).

Nowadays IBA is used for rooting during commercial operations, followed by IAA and NAA, and the chemical analogues synthesized and examined for auxin-like activities. Auxin mostly enters cuttings 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 to auxin over a prolonged period, but in other studies cuttings have been exposed to auxin over short periods (Diaz-Sala et al. 1996, Liu and Reid 1992) It was recognised, that an optimal auxin concentration for one of the three phases may be supraoptimal or suboptimal for the next. Over recent years, a multitude of models have been proposed for showing how auxin interacts for controlling adventitious root formation (ARF) and plant development (Sachs 2005, Jaillas and Chory 2010, Agullo-Anton et al. 2011, Pop et al. 2011). Although roots may be induced by auxin, it must be taken into a account that wounding is usually required to achieve rooting and it was suggested that WRCs (wounding-related compounds) play a major role during the de-differentiation phase (de Klerk et al. 1999).

When applying exogenous IBA on cuttings, the endogenous auxin concentration reaches a peak after wounding, thus coinciding with the initation of the rooting process. Interaction between endogenous IAA and exogenous IBA during ARF has been suggested and the performance of IBA versus IAA explained regarding several possibilities: higher stability, differences in metabolism, differences in transport, and IBA as a slow release source of IAA. Biochemical studies in numerous plants, and genetic studies of *Arabidopsis* IBA-mutants, indicate that IBA acts primarily via its conversion to IAA, which occurs through a mechanism similar to peroxisomal fatty acid b-oxidation; however, some evidence suggests that IBA acts as an auxin on its own (Pop et al. 2011).

The applied auxins are reported to lower the IAA oxidation, and this might reduce the consumption of phenolic antioxidants, which play a very important role as protectors of the IAA against oxidation (Volpert et al. 1995, Krylov et al. 1995, De Klerk et al. 1999)

Štefančič et al. (2007) investigated the influence of exogenous indole-3-acetic acid (IAA) and indole-3-butyric acid (IBA) on changes in the internal levels of IAA, indole-3-acetylaspartic acid (IAAsp) and antioxidant phenolics (chlorogenic acid and epicatechin) during the first 5 days of adventitious root formation in leafy cuttings of the cherry rootstock 'GiSelA 5'. The highest free and conjugated IAA accumulations in the cutting bases were observed in the IAA treated cuttings, but that did not promote the percentage of cuttings rooted. IBA gave the best propagation results, as 80% of cuttings formed roots during the first month of rooting. This indicates the influence of IBA on early root development, independently of IAA (Štefančič et al. 2007). Similarly, it was also reported for *Malus* microcuttings. 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 Kriken et al. 1992). Many other investigations have shown that IBA (indole-3-butyric acid) has a greater ability to promote adventitious root formation in comparison to IAA (Spethmann and Hamzah, 1988; Riov, 1993; De Klerk et al., 1999; Ludwig-Müller, 2000). It is more stable and less sensitive to the auxin degrading enzymes (Nordström et al. 1991; Epstein and Ludwig-Müller, 1993; Riov 1993). IAA is rapidly metabolized by the peroxidase, acting as an IAA-oxidase, with the strongest activity during the root initiation phase (Caboni et al. 1997, Nag et al. 2001). IAA is partially protected against oxidation when transformed into one of its conjugated forms, from amongst which IAAsp (indole-3-acetylaspartic acid) is the more abundant in various plant species (Norcini and Heuser 1988, Nordström et al. 1991). Rapid conjugation also prevents the overaccumulation of IAA within the tissue, which is a more common occurrence in exogenous auxin applications (Faivre-Rampant et al. 2002). When hydrolyzed, IAAsp can serve as a slowly released source of free auxin during the advanced stages of the rooting process (Epstein and Ludwig-Müller 1993; Riov 1993). IBA conjugates are an even better source of free auxin, because they are more resistant to the in vivo degradation, whilst IAAsp is subjected to oxidation into biologically inactive products (Riov 1993).

Several other positive physiological effects of IBA treatment have been recognised over recent years. It has been demonstrated that exogenous IBA, when applied to the rooting zone, activates the sugar metabolism that releases energy and provides carbon skeletons for the synthesis of other essential compounds such as proteins (Agullo-Anton et al. 2011). Furthermore, IBA is reported to increase the rate of ethylene biosynthesis, and an auxin-ethylene relation during root development has been shown by the number of isolated mutants that have resistance to both hormones (Sachs 2005, Werner et al. 2008, Perilli et al. 2010, Pop et al. 2011).

Several authors reported that the stimulation of ARF in cuttings by exogenous indole-3-acetic acid coincided with increased sugar availability at the site of root primordial development. It was discovered that exogenous auxin applied to the rooting zone stimulates the mobilization of carbohydrates in leaves and the upper stem and increases the translocation of assimilates towards the rooting zone in order to release energy, and provides carbon skeletons for the syntheses of other essential compounds such as proteins (Haissig 1989). Auxin application to the stem base of carnation cuttings raised sugar levels within the same tissues during rooting and counteracted the transient sugar depletion in leaves (Agullo-Anton et al. 2011).

SEAWEEDS ARE AN IMPORTANT SOURCE OF HORMONES

Seaweeds are an important source of plant growth regulators, together with organic osmolites, amino acids, mineral nutrients, vitamins, and vitamin precursors. Over recent years, the use of natural seaweed as a plant growth stimulant has allowed for its substitution in place of conventional synthetic growth regulators and fertilizers (Sahoo 2000, Khan et al. 2009). In particular, Kelpak® [Kelpak® Kelp Products (Pty) Ltd] contains 11 mg/L IBA, and 0.031 mg /L kinetin, as well as a high amount of amino acids, vitamins, and mineral nutrients. This biostimulant is prepared by a cell burst process from brown algae *Ecklonia maxima (*Osbecki) Papenfuss, located in the cold waters along the Atlantic coast of Southern Africa. Recent reports have shown that low levels of *Ecklonia maxima* concentrate, when applied as a foliar spray or root drench, increases seedlings' qualities and survival, improves root-growth, vegetative and reproductive growth, flowering, fruit production, and the yields of many crop plants and vegetables (Crouch and van Staden 1993, 1994; van Staden et al. 1994; Kowalski et al. 1999; Arthur et al. 2003; Khan et al. 2009). Whilst there is a growing acceptance that seaweed liquid fertilizers (SLF) are tools when addressing the need for improved current crop production practices for long-term sustainability, the margin for beneficial action regarding Kelpak® during the vegetative propagation of ornamental cuttings, was found to be rather narrow. Urbanek Krajnc et al. (2012) performed a time-course analysis of the photosynthetic pigment, phenolic, and protein contents within the leaf tissues of *Pelargonium peltatum* 'Ville de Paris Red' from the initial cuttings to market maturity, in regard to brown algae [*Ecklonia maxima (*Osbeck) Papenfuss] extract treatment (Kelpak®). A positive impact by the 0.5% and 1% Kelpak® treatment on the protein accumulation within the leaves of pelargoniums was monitored. Elevated protein contents within the Kelpak®treated samples could be attributed to a high concentration of IBA within the Kelpak® extract, since it is well-known that IBA has a positive-effect on those enzymes involved in nitrogen assimilation (Kaur et al. 2002, Hayat et al. 2009). Beside the exogenous IBA, kinetin, and other seaweed components, especially nitrogen, amino acids, and vitamins, may have a synergistic impact on protein accumulation within Kelpak®treated cuttings. The results are in accordance with other seaweed treatment experiments. For example, Beckett et al. (1994) indicated increased levels of nitrogen within the leaves of nutrient-stressed tepary beans after treatment with Kelpak®. Furthermore, increased

total protein values were also reported in *Arachys hypogaea* by Sridhar and Rengasamy (2010) after treatment with brown algae *Sargassum wightii* extract.

Kelpak® treatment was also found to significantly affect the concentration of phenolic compounds within the pelargonium leaves (Urbanek Krajnc et al. 2012), thus the involvement of IBA in phenylpropanoid pathways was hypothesized. Similarly, Fan et al. (2011) reported that root treatment with brown algae (*Ascophyllum nodosum*) extract elicits the phenylpropanoid and flavonoid pathways in spinach, but the mechanism remains unexplained.

In our previous study (Urbanek Krajnc et al. 2012), we also demonstrated that Kelpak® treatment of *Pelargonium peltatum* Ville de Paris Red' cuttings can maintain the functionally active green leaves due to increased photosynthetic pigment accumulation, thus improving the 'whole plant' functional integrity following the insertions of cuttings into the soil. Maintaining the functionally active green leaves during root induction is crucial, since root formation relies on an adequate supply of carbohydrates from the source leaves to the region of root regeneration (Druege and Kadner 2008). Carbohydrate depletion is compensated for by current photosynthesis (Haissig 1989, Rapaka et al. 2005, Ahkami et al. 2009, Agullo-Anton et al. 2011). However, the excised cuttings of most plant species, including control pelargonium, show low photosynthetic rates, a decrease in chlorophyll contents and an increase in β-carotene and lutein unless first roots are formed, which could be explained by lower water potential due to severance of cuttings (Druege and Kadner 2008, Urbanek Krajnc et al. 2012). In contrast, Kelpak® treated pelargonium cuttings showed higher pigment concentrations when compared to the controls, reflecting a readjustment of the photosynthetic carbon metabolism. Urbanek Krajnc et al. (2012) assumed that the presence of IBA in the Kelpak® extract may increase chlorophyll concentration in leaves of cuttings. It was reported previously that IBA treatments cause an enhancement of chlorophyll contents within different stem cuttings (Mustafa 1996, Kaur et al. 2002). Furthermore, seaweed products are known to enhance plant chlorophyll content and reduce leaf senescence as a result of a reduction in chlorophyll degradation, which might be caused in part by betaines within the seaweed extract (Khan et al. 2009). It has been reported recently that SLF prepared from different brown algae species has a beneficial effect on photosynthetic pigments' contents. The application of SLF derived from the brown algae *Ascophyllum nodosum* in combination with standard fertilization, increased the leaf chlorophyll content, photosynthesis, and transpiration rates in tomato, cucumber, dwarf French beans, wheat, barley,maize, apples, grapes, and strawberries (Blunden 1991, Whapham et al. 1993, Blunden et al. 1997, Spinelli et al. 2010). Thirumaran et al. (2009) reported that, a SLF prepared from the brown algae *Rosenvingea intricata*, increased the total chlorophyll and carotenoids in *Cyamopsis tetragonoloba*. Furthermore, Sridhar and Rengasamy (2010) reported that groundnut treated with *Sargassum wightii* extract showed enhanced concentrations of photosynthetic pigments in leaves.

Nethertheless, the biostimulatory effect of Kelpak® treatment on primary and secondary metabolism of pelargonium cuttings was reflected in significantly increased root fresh mass, shoot/root ratio, and maximum number of leaves (Urbanek Krajnc et al. 2012). To sum up, higher photosynthetic pigment concentrations in cuttings treated with auxin-containg SLF, significantly contribute to increased photosynthetic activity and enhanced production of photoassimilates. Consequently, higher sugar levels during rooting are positively correlated with less expressed leaf senescence, higher survival rate, increased root formation and more efficient nutrients' uptake of cuttings.

CONCLUSIONS

In the presented review, causal interactions between auxins and specific primary and secondary metabolites were analysed during the process of ARF. It has been shown how the applications of different auxin sources or compounds inhibiting basal auxin transport modulate different metabolites during ARF. Taken together, exogenous auxin application promotes ARF in several ways. It has a direct influence on root induction at the stem bases of cuttings, as well as a stimulatory effect on sink activity and the translocation of assimilates towards the stem base. Auxin further inhibits leaf senescence, increases the chlorophyll contents and the photosynthesis in leaves, and accounts for the plasticity of shoot/root relations.

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