Combined Proceedings IPPS 68:432-440. 2018.

The Key Role of The Physiological and Developmental Conditions of Donor Plants in Adventitious Root Formation

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Keywords: Cuttings, auxin, rejuvenation, etiolation, sensitivity, miRNA.

Abstract

In many genotypes, satisfactory adventitious rooting of cuttings is achieved by a treatment with auxin. There has been no essential improvement of this treatment ever since its invention in the 1930s. To achieve rooting in otherwise recalcitrant genotypes, a donor-plant pretreatment may be the way out.

INTRODUCTION

Vegetative propagation depends on the ability of the cuttings to form roots, a process referred to as adventitious root (AR) formation. Despite considerable progress in understanding mechanisms underlying AR formation, the firstly discovered (in the 1930s) method to achieve AR formation, a treatment with auxin, is still the only commonly used way to induce AR formation (De Klerk et al. 1999) and no other generally usable rooting treatments have been developed even though many genotypes are recalcitrant in rooting. There is, however, an alternative way to improve rooting, *viz.*, a pretreatment of donor plants (Massoumi et al. 2017b). In the present article, we review the recent findings on the effect of the three major donor plant pretreatments: rejuvenation, etiolation and flooding. Such pretreatments are in particular relevant for micropropagation as they can be applied more easily in vitro as compared to ex vitro.

Rejuvenation

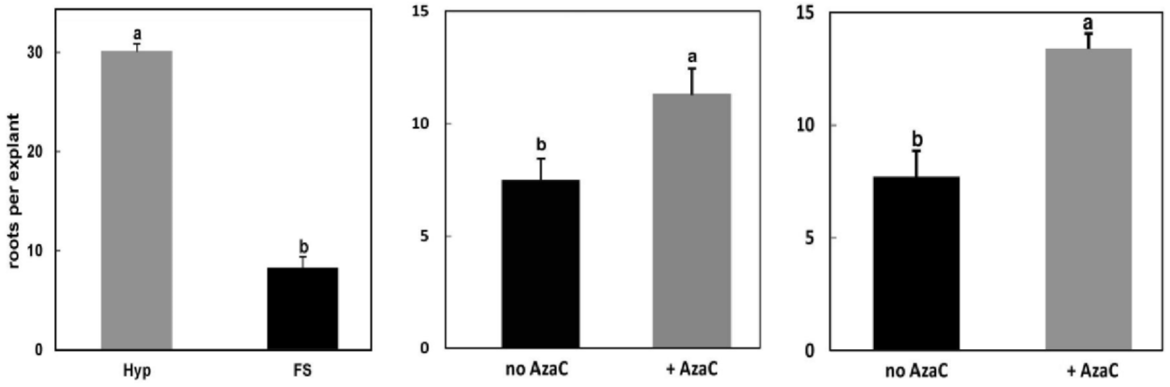
In plants, three different types of aging have been defined: chronological, ontogenetic and physiological aging (Wendling et al. 2014a). Ontogenetic aging refers to the transition to the next developmental stage (from juvenile to adult) and has been extensively studied as it is of high practical importance for both breeders and plant propagators. Plant breeders are interested in shortening juvenile stage to be able to evaluate the flowering characteristics of new cultivars as early as possible and consequently to shorten the breeding cycle. Plant propagators, on the other hand, are interested to extend the juvenile stage as juvenile donor plants are more capable of rooting and have a higher multiplication rate. The length of the juvenile stage may be a few days but also several years depending on the species (Poethig, 1990). In herbaceous species the length of juvenile stage is shorter and the morphological and physiological changes associated with the phase transition are less distinct.

Reduced AR formation potential upon maturation has been reported in many plant species (Diaz-Sala et al. 2002; Rasmussen et al. 2015; Massoumi et al. 2017a). Maturation is, however, a reversible process: adult plants may be rejuvenated and become again able to form ARs *e.g.,* in apple (De Klerk and Ter Brugge 1992). Wendling et al. (2014b) have reviewed different rejuvenation techniques, *viz.,* repeated sub-culturing of *in vitro* grown plants, repeated *ex vitro* pruning as well as sequential grafting of adult scions onto juvenile rootstocks to rejuvenate the mature plant materials (Wendling et al. 2014b).

Researchers have attempted to decipher mechanisms underlying phase change and its effects on adventitious rooting. They first tried to link the difference in rooting response of juvenile and adult plant materials with morphological and anatomical differences. For instance, Ballester et al. (1999) studied the rooting process in juvenile and mature chestnut (*Castanea sativa*) shoots. However, they observed no difference in anatomical characteristics between these shoots. Later, biochemical and physiological features, especially with respect to phytohormones, became the center of attention. Although auxin is the central player in the induction of roots, the phytohormone does not seem to be the limiting factor during the maturation-related decline in rooting potential. It has been shown in *Pinus sylvestris* or *Pinus taeda* that neither auxin uptake and metabolism nor its transport correlate with the differences in the extent of the formation of ARs (Diaz-Sala et al. 1996).

At the molecular level, however, the difference between juvenile and adult tissues became clear. This concerns differences in methylation status of DNA and expression of microRNAs (miRNAs). In most cases, transition from juvenile to adult coincides with DNA hypermethylation (increased methylation) (Valledor et al. 2007). Changes in the methylation status of DNA affect gene expression. In particular, a gene that is methylated is silenced and cannot be transcribed (Grant-Downton and Dickinson 2005). This may be a reason for the maturation-related decline of rooting response observed in woody and herbaceous plant species. We have shown in *Arabidopsis* that juvenile plant material produce significantly more ARs than adult material (hypocotyl vs. flower stem explants, Fig. 1, left panel). In addition, juvenile plant material had lower (ca. 12 vs. 5%) DNA methylation status (Massoumi et al. 2017a). To promote rooting of adult plant materials we did apply 5-azacytidine (a drug that reduces methylation status of DNA). When applied during seed germination or rooting treatment (Fig1. Right panel), 5-azacytidine (AzaC) increased rooting of flower stem explants and not that of hypocotyl tissues indicating that maturation-related loss in rooting response is caused by increased DNA methylation and can be reversed when hypomethylating compound like 5-azacytidine is applied (Massoumi et al. 2017a). To promote rooting of adult plant materials we did apply 5-azacytidine (a drug that reduces methylation status of DNA). When applied during seed germination or rooting treatment (Fig1. Right panel), 5-azacytidine (AzaC) increased rooting of flower stem explants and not that of hypocotyl tissues indicating that maturation-related loss in rooting response is caused by increased DNA methylation and can be reversed when hypomethylating compound like 5-azacytidine is applied (Massoumi et al. 2017a).

Figure 1. **Left panel**: The formation of adventitious roots from juvenile (Hyp; hypocotyl) and adult (FS; flower stem) tissues of *Arabidopsis* cut from plants that had been treated with 30 µM of the IAA. Hypocotyl segments were taken from 12d-old seedlings and flower stem segments were taken from 5w-old plants (lower 1,5cm of the stem). **Middle panel**: Rooting of *Arabidopsis* FS segments when AzaC (10 µM) was added during the seed germination (5 weeks) then treated with IAA (30 µM).**Right panel**: Rooting of *Arabidopsis* FS segments when AzaC (10 µM) was added during the rooting treatment with IAA (30 µM).



Means across replicates are presented with SE. Different letters represent means that are significantly different at $P < 0.05$. note that different scales are used in different figures. Derived from Massoumi et al. (2017a).

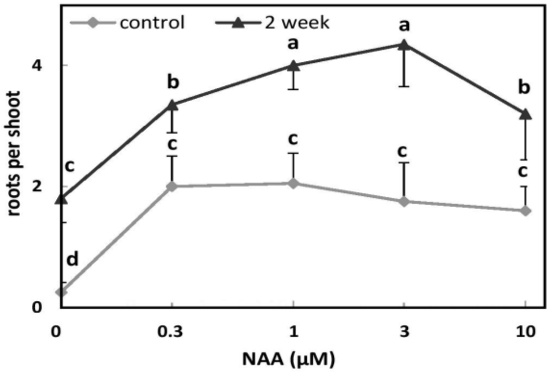
Another striking difference between juvenile and adult plant materials is the level of miRNA156 (Wu and Poethig 2006). MiR156 level is high in the juvenile phase, whereas its expression decreases during vegetative phase change in different plant species, *e.g.*, *Arabidopsis*, maize, *Acacia*, *Eucalyptus*, *Hedera* and *Quercus* (Wu and Poethig 2006; Chuck et al. 2007; Wang et al. 2011). External factors have been shown to influence the level of miR156 in the plants. For example, low sugar brought about by leaf detachment or reduced photosynthesis increase the level of miR156 (Yang et al. 2013). Overexpression of miR156 (by genetic engineering) delays the transition to the adult phase (Wu and Poethig 2006; Chuck et al. 2007). Recently, Yu *et* al. (2015a) showed that *Arabidopsis* plants over-expressing miR156 produce more lateral roots than plants overexpressing its target mimic, MIM156 (the activity of miR156 is blocked), indicating a role for miR156 in lateral root development. We have recently showed that maturation-related decline in adventitious rooting is under the control of miR156. Overexpression of miR156, increases the capacity of *Arabidopsis* adult tissues to form ARs (Massoumi et al. 2017a). Xu et al. (2017) have found similar results in apple. They showed that semi-lignified leafy cuttings from juvenile phase and rejuvenated apple tree (*Malus xiaojinensis*) show higher expression of miR156 which is necessary for auxin-induced AR formation. It seems, therefore, that miRNA156 plays a role in many plant species controlling vegetative phase change as well as regulating AR formation capacity of the cuttings. Any horticultural practice that leads to an increase in the level of miR156 can restore juvenile characteristics and AR formation. In the next two sections, we will discuss the relation between environmental factors, miR156 and AR formation capacity.

As noted before, it has been found in the model plant *Arabidopsis* that a high endogenous sugar concentration is related with a transition to the adult stage. In agreement with this, in lily regenerated in tissue culture far more adult plants occur when a high level of sucrose was added to the medium. The effect of sucrose on the rootability of woody plants has not yet been examined.

Etiolation

Light stands out amongst the environmental factors that shape plant development. It has always been considered as an important parameter in vegetative propagation practices when optimizing conditions for rooting of cuttings. Different aspects of light, *viz.*, light quality, intensity and duration are shown to influence the rooting of cuttings (Daud et al. 2013; Fett-Neto et al. 2001). Such studies have highlighted possible synergistic or antagonistic effects of light with plant growth regulators such as auxin and cytokinins (Fett-Neto *et al.*, 2001; Wynne and McDonald, 2002) suggesting the involvement of photoreceptors in the regulation of AR development. Other research has focused on the effect of darkness, referred to as etiolation (development of a plant or plant part in the absence of light), on improving the rooting of cuttings (Klopotek et al. 2010; Massoumi et al. 2017b). Similarly, we have found that etiolation pretreatment in apple microshoots multiplied *in vitro* promotes adventitious rooting (Fig. 2).

Figure 2. Rooting response of apple micro-cuttings excised from etiolated (for 2 weeks during multiplication) and control donor plants when NAA was used as auxin.



Means across replicates are presented with SE. note that different scales are used in A and B. Means followed by the same letter are not statistically different at $P = 0.05$.
Massoumi and De Klerk; unpublished observations.

Researchers have attempted to explain this effect of light. It has been found that various anatomical, physiological and molecular changes are associated with enhanced rooting efficiency in etiolated stem tissues (Haissig and Davis 1994; Sorin *et al.* 2005). However, there are some reports that there is no correlation between anatomical differences caused by etiolation and the doubling of root number. For example, Takahashi et al. (2003) investigated the rooting of hypocotyl in *Arabidopsis hy4* mutant (elongation growth of hypocotyls occurred in the light as well as in darkness). They observed that despite the hypocotyls were of sufficient length, no ARs were induced under long day conditions indicating that elongated hypocotyl is not of primary importance.

Evaluations concerning changes in endogenous IAA levels in cuttings have given conflicting results. Kawase and Matsui (1980) concluded that etiolation did not affect IAA content in hypocotyls of *Phaseolus vulgaris* L. and still others (Agulló-Antón et al. 2011; Fett-Neto et al. 2001) observed an increase of IAA in etiolated stem parts. Maynard (1991) showed that banding of stem base (covering the base of cuttings with black plastic) increases sensitivity of the cells to applied auxin. Additionally, light would affect the level of endogenous auxin either by influencing its transport or its metabolism into conjugates or via photo-oxidation (Normanly et al. 2004; Naqvi and Gordon 1967).

Apart from a change in auxin level, biosynthesis of cytokinins (Agulló-Antón et al. 2011; Bollmark and Eliasson 1990), ethylene (Cao et al. 1999), flavonoids (Buer and Muday 2004), strigolactone (Massoumi et al. 2017b) and carbohydrates (Husen 2008; Klopotek et al. 2010; Massoumi et al. 2017b) have also been reported to be affected in response to different light intensities.

It has also been proposed that increased AR formation of cuttings by lower irradiation (shading, etiolation) is the result of arresting/ or reversing of ontogenetic aging (Husen 2008; Husen and Pal 2003). Massoumi et al. (2017b) applied etiolation as a donor plant pretreatment to *in vitro* grown *Arabidopsis* seedlings. They reported an increased rooting response despite a reduction in endogenous sugar levels. The authors have speculated that reduced endogenous sugar level increases the level of miR156 as had been reported by (Yang et al. 2013) and this in response promotes juvenile characteristics.

Flooding

Soil water is another environmental factor which causes stresses such as drought or waterlogging to affect plant characteristics (Promkhambut et al. 2011). Roots are most sensitive to flooding and the first to suffer from oxygen shortage. Plants use several mechanisms to maintain root function through an improved oxygen supply during flooding. Formation of internal gas channels (aerenchyma) (Colmer and Voesenek 2009), establishment of a lateral diffusion barrier to minimize radial oxygen loss from flooded roots to the soil (Bramley et al. 2010), as well as initiating organogenesis are adaptive mechanisms that have been substantially addressed (Maurenza et al. 2012; McDonald and Visser 2003; Vidoz et al. 2010; Zhou et al. 2012). The latter refer to the formation of ARs to replace the original root system.

In many plant species, *e.g.,* rice, maize, *Rumex* and *Arabidopsis,* it has been shown that ethylene accumulation occurs in the submerged tissues (Geisler-Lee et al. 2010; Peng et al. 2005; Rieu et al. 2005; Van Der Straeten et al. 2001). The role of ethylene in controlling AR formation is shown to be species specific (Vidoz et al. 2010). For instance, in *Rumex palustris* Sm., ethylene increases auxin sensitivity and leads to the production of ARs (Visser et al. 1996). In deepwater rice, however, ethylene causes the death of the epidermal cells that cover the root tip and thereby facilitates the emergence of pre-formed ARs (Mergemann and Sauter 2000). In addition, ethylene may affect auxin transport, resulting in its accumulation at the stem base of flooded plants (Grichko and Glick 2001).

Apart from the positive influence of flooding on AR formation when the root system is still present, flooding has also been reported to have similar positive effects in cuttings. Shibuya et al. (2013 and 2014) reported that soaking the basal cuttings of Carolina poplar (*Populus canadensis*

Moench.) and Japanese cedar (*Cryptomeria japonica* D. Don) in warm water at a controlled low-air-temperature improves early initiation and development of ARs. Massoumi et al. (2017b) applied flooding to *in vitro* grown *Arabidopsis* seedlings and in response rooting of excised stem segments increased. At the anatomical level, a massive formation of secondary phloem (the tissue close to which ARs roots are induced) was observed in flooded seedlings. Additionally, increased rooting response in flooded donor plants was associated with decreased endogenous sugar levels similar to what had been reported in etiolated seedlings (Massoumi et al. 2017b). Possibly, the decreased sugar level promoted juvenile characteristics via increasing the level of miR156 (sugar negatively affects miR156 level).

Final remarks

In horticultural practice, AR formation is highly important considering that seventy percent of the propagation systems depends on successful rooting of cuttings. Despite numerous research on understanding the underlying mechanisms of AR formation, treatment with auxin seems to be the common way. However, there is an alternative, pretreatment of donor plants to increase response of cuttings to applied auxins. In this review, we have discussed information about the effect of three different donor plants' pretreatments, *viz.*, rejuvenation, etiolation and flooding, on the capacity of the cuttings to root. These pretreatments affect the physiological and biochemical conditions of donor plants in a way that rooting is promoted and can be used as efficient ways to increase AR. This holds in particular for commercial micropropagation since donor plants can be relatively easily treated.

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