How Plant Hormones Work – Auxin©

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INTRODUCTION

Plant hormones (also phytohormones) are naturally occurring organic chemicals that are active in low concentrations. The traditional definition of a hormone is that they are synthesized at one location and translocated to their site of action. However, there are some exceptions for plant hormones. The five major plant hormones are auxin, cytokinin, gibberellin, abscisic acid, and ethylene. Additional compounds considered plant hormones include brassinosteroids, jasmonic acid, salicylic acid, and polyamines. Plant hormones are important to propagation because they act endogenously to regulate plant function and can be applied to induce specific responses such as root initiation in cuttings and dormancy release in seeds (Hartmann et al., 2011).

It is beyond the scope of this paper to describe all the actions for each of the plant hormones, but because of the importance of auxin to plant propagation, it will serve as the example for hormone action. Therefore, the objective of this paper is to provide some background for the use of auxin in cutting propagation and then describe the advances in hormone action that relate to the control of adventitious root initiation.

HISTORICAL BACKGROUND

Fritz Went (1928) building on the initial research of Charles Darwin (Darwin and Darwin, 1880) and Boysen Jensen (1911) developed the first bioassay for detecting hormones in plants based on the bending of grass and oat seedlings to light. Went placed agar blocks containing suspected hormones asymmetrically on decapitated oat seedlings and measured the bending of the coleoptile. Kögl and Haagen-Smit, between 1933 and 1935, found that substances in human urine and various plant extracts that were active in Went's coleoptile bioassay. This led to the chemical isolation of "heteroauxin" [indole-3-acetic acid (IAA)] identified as the first plant hormone. Soon after this, Went (1934) developed another bioassay based on the discovery that auxin-induced adventitious roots in etiolated pea cuttings. Fischnich (1935) showed that applied IAA could induce adventitious roots to form on intact coleus (*Solenostemon*) stems. Possibly, the first specific report of IAA being used to stimulate rooting in cuttings was by Cooper (1935). He applied IAA in lanolin paste to stimulate rooting in lemon (*Citrus*), lantana (*Lantana*), and chenille plant (*Acalypha*) stem cuttings. By 1935, synthetic auxins were developed that were shown to promote rooting in cuttings (Thimann, 1935; Zimmerman and Wilcoxon, 1935). These included the familiar α-naphthaleneacetic acid (NAA) and indolebutyric acid (IBA) compounds used by modern propagators.

The potential commercial importance of auxin to cutting propagation was almost immediate. By 1935, researchers at the Boyce Thompson Institute showed the efficacy of auxin in stimulating rooting in cuttings of over 85 genera of plants, including woody plants that had proven too difficult to propagate in the past (Zimmerman, 1935). The Boyce Thompson Institute was granted a patent for use of these synthetic auxins and subsequently licensed Merck and company (New Jersey) to distribute Hormodin A for commercial application. By 1947, four commercial companies were offering synthetic auxin formulations in talc for application to cuttings. Along with Merck's Hormodin formulation, they were Quick-Root from Dow Chemical, Rootone from American Chemical Paint Co., and StimRoot from Plant Products Co. (Avery et al., 1947).

Initially cuttings were treated by prolonged (24 h) soaks in aqueous auxin solutions. Grace in 1937, developed the method of incorporating auxin in talc to deliver auxin to cuttings that would eventually become the standard commercially. The quick-dip method for treating auxin was developed by Hitchcock and Zimmerman in 1939 and later refined by Cooper in 1944. In an excellent review of research in cutting propagation by Avery

and Johnson (1947), they provide a table with references on experimental rooting for over 600 different kinds of woody plants. This provides a wonderful overview of the impact of auxin on rooting cuttings in the era prior to the use of mist by the greenhouse and nursery industry.

AUXIN BIOSYNTHESIS AND MOVEMENT

There are two biosynthetic pathways for IAA in plants (Benjamins and Scheres, 2008). Primary auxin biosynthesis is via the amino acid L-tryptophan, but IAA can also be synthesized by a tryptophan-independent pathway. Most of the IAA in plant tissue is in the conjugated form using both amino acids and sugars for conjugation. Primary sites of auxin biosynthesis include root and shoot meristems, young leaf primordia, vascular tissue, and reproductive organs including developing seeds.

Auxin movement from cell to cell requires efflux carriers located on the plant membrane (Peer et al., 2011; Woodward and Bartel, 2005). They control polar auxin movement from plant tips (distal ends) to their base (proximal end). Auxin movement and the subsequent polar gradient established between cells is important for normal plant development. The auxin source in untreated stem cuttings is the shoot tips and polar movement of this auxin allows accumulation at the base of the cutting where rooting occurs.

Indole-3-acetic acid degrades in the light and exogenously applied IAA is quickly degraded by the enzyme IAA-oxidase. Synthetic auxins are less susceptible to enzyme degradation and are therefore used more often for commercial applications. The most often used auxins are derivatives of indole-3-butyric acid (IBA) and α-naphthalene acetic acid (NAA). Auxins are not readily dissolved in water and must be dissolved in a solvent (ethanol, DMSO) or a base (1N NaOH) before being quickly added to water. Potassium salts of IBA and NAA (K-IBA, K-NAA) are auxin formulations that are water soluble and available commercially. Auxin application for cuttings can be at the base of the cutting as a low concentration dilute soak or higher concentration quick dip. Alternatively, auxin can be foliar applied as a spray (Hartmann et al., 2011).

AUXIN-INDUCED ROOT FORMATION

As described previously, auxin can induce adventitious rooting in cuttings in a wide distribution of species. However, there are cuttings from some plant species that do not respond to auxin to promote adventitious rooting. These cuttings are termed difficult-to-root and auxin non-responsive. For many years, researchers have been searching for biochemical substances that in addition to auxin would promote rooting in this group of cuttings. This refers to a theory of root induction that ascribes to a strictly biochemical basis for rooting. This theory implies that there are root-promoting and root-inhibiting substances produced in plants and their interaction is thought to control rooting. Therefore, difficult-to-root cuttings either lack the appropriate root-promoting substances or are high in root-inhibiting substances. This theory has a long historical foundation, but has yet to yield important information on these elusive compounds.

The initial concept for substances initiating root formation in plants comes from the pioneering German plant physiologist, Julius Sachs in the1880s. He felt that there were specific chemical plant morphogens responsible for directing growth and development including root formation (Sachs, 1880). Fritz Went in 1938, postulated that root forming substances made in the leaves moved to the base of cuttings and were involved in adventitious root formation. He termed this substance(s) rhizocaline.

In an elegant study in 1946, Van Overbeek et al. reported that grafts between easy and difficult-to-root cultivars of rose-of-Sharon (*Hibiscus rosa-sinensis*) resulted in a graft transmission of a substance from the easy-to-root cultivar that improved rooting in the difficult-to-root cultivar. In 1959, Charles Hess detected “rooting cofactors” from extracts of the juvenile form of English ivy (*Hedera helix*) as indicated by increased root formation in the mung bean (*Phaseolus aureus*) bioassay. The chemical nature of rhizocaline has never been elucidated and the physiological relevance of graft

transmissible rooting factors and rooting cofactors has been challenged (Hartmann et al., 2011). It appears that there is a fundamental difference between a cell's competency to respond to auxin between easy and difficult-to-root phases of a plant's life cycle that requires gene expression experiments to elucidate. However, the importance of buds and leaves for rooting in some species and other interesting cofactor studies still hint at the existence of the illusive rhizocaline.

MOLECULAR BASIS FOR ROOTING

There are few studies that describe auxin-induced gene activity during critical phases of the adventitious rooting process. However, it is clear from global gene expression experiments that there is a suite of new gene expression that is induced during the root initiation process (Brinker et al., 2004; Wei et al., 2013). Considerable progress has been made in the past 5 years on the molecular perception and gene activation following auxin treatment.

A common mechanism for auxin-induced gene activity is outlined in Figure 1 (Chapman and Estelle, 2009). It is common for auxin-inducible genes to be repressed (prevented from action) by the protein-protein interaction the Aux/IAA repressor molecule acting on the Auxin Response Factor (ARF) positioned in the promoter region of an auxin-inducible gene (Fig. 1A). In order to activate the gene, the repressor molecule (Aux/IAA) must be removed from its interaction with ARF. In order for this to occur, auxin (IAA) must interact with its receptor complex (Fig. 1B). The auxin receptor is the F-box protein TIR1 located in the cell's nucleus. There are several proteins that associate with TIR1 to form a receptor complex. The job of the receptor complex is to locate Aux/IAA and to attach several ubiquitin molecules to the protein (Fig. 1C). This poly-ubiquitination targets Aux/IAA for proteolytic destruction. Once Aux/IAA is removed from association with ARF, it acts to initiate gene transcription of the auxin-inducible gene.

It is evident from this discussion that Auxin Response Factors are important for initiating an auxin response. Recently, the ARFs important for adventitious root formation have been found in *Arabidopsis*. They include ARF6, ARF8, and ARF16. Additional research has shown that the level of these ARFs is under translational control by microRNAs. A microRNA (Fig. 2A) is a small regulatory RNA consisting of 22 nucleotides (Meng et al., 2011). The sequence of these nucleotides related to auxin-induced gene expression corresponds to a section of the coding region of the mRNA for ARF. If a specific microRNA corresponding to an ARF is produced, it will prevent or considerably reduce the production of the ARF protein (Fig. 2B). In *Arabidopsis*, the microRNAs responsible for ARF levels are miRNA 160 and miRNA 167 (Gutierrez et al., 2009).

Therefore, it is becoming evident that for a cutting (at least in *Arabidopsis*) to be competent to form adventitious roots, there must be available auxin to interact with its receptor complex as well as the production of specific Auxin Response Factors to initiate gene expression. Although, this research is yet to be accomplished, it is logical to hypothesize that one possible reason difficult-to-root, non-auxin responsive cuttings fail to root is that they fail to produce appropriate ARFs. It is also logical to assume that the deficiency in ARF production is likely due in part to gene silencing by microRNAs.

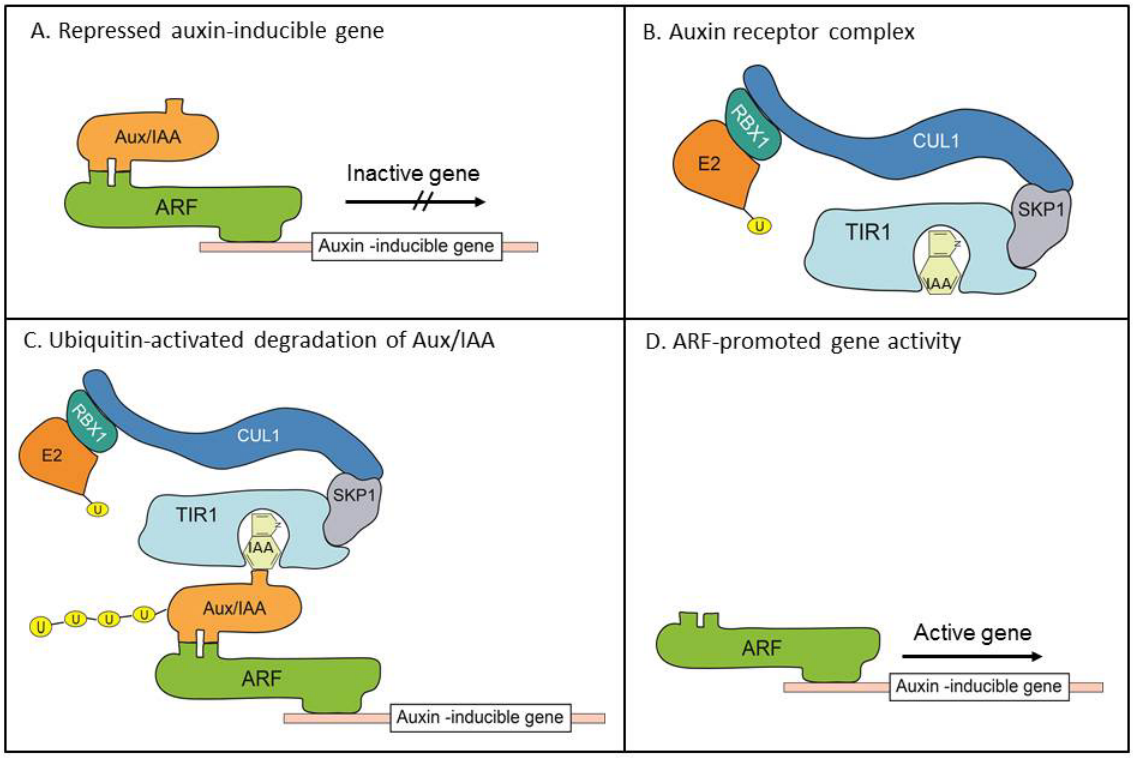

Fig. 1. Steps in auxin perception and gene activation. ARF is an auxin response factor; Aux/IAA is a transcription factor repressor; TIR1 is the auxin receptor.

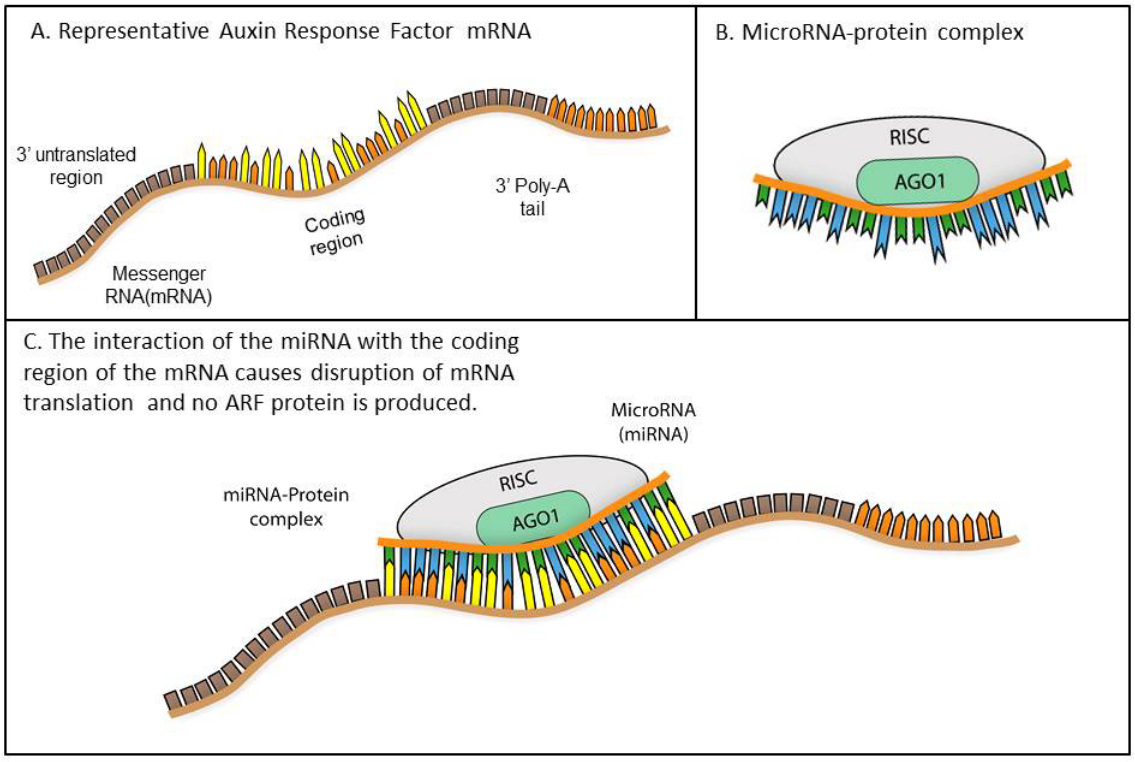

Fig. 2. Representation of translational control of an Auxin Response Factor production by microRNA.

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