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Adventitious root formation in *Anacardium occidentale* L. in response to phytohormones and removal of roots

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Abstract

Despite advances in tissue culture techniques, propagation by leafy, softwood cuttings is the preferred, practical system for vegetative reproduction of many tree and shrub species. Species are frequently defined as 'difficult'- or 'easy-to-root' when propagated by conventional cuttings. Speed of rooting is often linked with ease of propagation, and slow-to-root species may be 'difficult' precisely because tissues deteriorate prior to the formation of adventitious roots. Even when roots form, limited development of these may impair the establishment of a cutting.

In this study we used softwood cuttings of cashew (*Anacardium occidentale*), a species considered as 'difficult-to-root'. We aimed to test the hypothesis that speed, and extent of early rooting, is critical in determining success with this species; and that the potential to form adventitious roots will decrease with time in the propagation environment. Using two genotypes, initial rooting rates were examined in the presence or absence of exogenous auxin. In cuttings that formed adventitious roots, either entire roots or root tips were removed, to determine if further root formation/development was feasible. To investigate if subsequent root responses were linked to phytohormone action, a number of cuttings were also treated with either exogenous auxin (indole-3-butyric acid—IBA) or cytokinin (zeatin).

Despite the reputation of *Anacardium* as being 'difficult-to-root', we found high rooting rates in two genotypes (AC 10 and CCP 1001). Removing adventitious roots from cuttings and returning them to the propagation environment, resulted in subsequent re-rooting. Indeed, individual cuttings could develop new adventitious roots on four to five separate occasions over a 9 week period. Data showed that rooting potential increased, not decreased with time in the propagation environment and that cutting viability was unaffected. Root expression was faster (8–15 days) after the removal of previous roots compared to when the cuttings were first stuck (21 days). Exposing cuttings to IBA at the time of preparation, improved initial rooting in AC 10, but not in CCP 1001. Application of IBA once roots had formed had little effect on subsequent development, but zeatin reduced root length and promoted root number and dry matter accumulation. These results challenge our hypothesis, and indicate that rooting potential remains high in *Anacardium*. The precise mechanisms that regulate the number of adventitious roots expressed, remain to be determined. Nevertheless, results indicate that rooting potential can be high in 'difficult-to-root' species, and suggest that providing supportive environments is the key to expressing this potential.

Keywords: Cashew; Cuttings; Adventitious roots; Injury; Auxin; Cytokinin

1. Introduction

Clonal propagation of many tree and shrub species is effective through 'leafy softwood' cuttings, however, a number of important genera remain difficult to propagate and establish using this technique. The limited potential to form adventitious roots may in part result from inappropriate time of propagation (via stock plant effects, Cameron et al., 2001, 2003) or the physiological state of tissues (Hackett, 1988; Ermel et al., 2000; Reineke et al., 2002). Some species may be slow to form adventitious roots and the cutting may fail prior to the formation of functional roots (Rose and Pellett, 1994; Stankova and Panetsos, 1997; Voyiatzi et al., 2002). Indeed, species where roots emerge rapidly are often categorised as 'easy-to-root' (e.g. 12 days, *Euphorbia pulcherrima*, Wilkerson et al., 2005) in contrast to those more 'difficult-to-root' ones that require longer periods before emergence (e.g. 35 days, *Ilex paraguariensis*, Tarrago et al., 2005). There is a perception that leafy 'softwood' cuttings need to form adventitious roots quickly, otherwise tissues will become dysfunctional through either prolonged exposure to sub-optimal environments (Howard and Harrison-Murray, 1995; Aiello and Graves,

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1998) or pathogen activity associated with such environments (e.g. Littlejohn and Gertse, 2001). In slow-to-root species, reasons for cutting failure are frequently associated with tissue dehydration (Grange and Loach, 1983), loss or an inability to photosynthesis new carbohydrates (Reuveni and Raviv, 1981; Del Rio et al., 1991) and possibly a limited response to exogenous auxins (Aminah, 2003). Providing environmental conditions that both minimise water stress and provide light for photosynthesis appears to be critical, and the use of sub-optimal propagation environments can often explain failure to root (Howard and Harrison-Murray, 1995). For those species where success from conventional cuttings remains elusive or inconsistent, tissue culture, grafting or air-layering may be employed. These themselves, however, do not necessarily guarantee success and even when proven useful can be prohibitive due to; expense, the amount of labour required or low reproduction rates. Improving propagation success through the use of conventional cuttings therefore remains a key objective for nurserymen, farmers and foresters worldwide.

The precise determination of 'rooting success' is controversial (Wilson and Struve, 2003), with reports suggesting it can be based on percentage of the cutting population that form roots, the numbers of adventitious roots per cutting, the number of total roots per cutting (where the numbers may include secondary and tertiary roots) or even the speed in which cuttings root. In addition, rooting success does not necessarily correlate with propagation success as the number of cuttings that eventually establish may vary considerably from those that initially formed roots (Owen et al., 2001; Griffin and Schroeder, 2004). Indeed, it is possible for a cutting to form only a single adventitious root (and constitute success), promote numerous lateral root branches from this first root, yet, finally fail due to the original root being damaged (e.g. at transplanting, Billingsley, 2003). Therefore, it is evident that the rooting of cuttings is a dynamic event and that relationships need to be established that take account of both adventitious root formation and subsequent cutting development.

This research explores the rooting potential in a 'difficult-to-root' species (Cashew—*Anacardium occidentale* L.). Due to difficulties in rooting of cuttings (Rao, 1985; Duarte et al., 1992), vegetative propagation of superior clones has relied on techniques such as air-layering, grafting (Damodaran, 1985) and tissue culture (Mneney and Mantell, 2002). Previous research demonstrates, however, that rooting of stem cuttings is possible, although success often correlates with; more elaborate preparation techniques such as etiolation or shoot ringing (Rao et al., 1988), the provision of contact polythene or mist to minimise desiccation (Rao et al., 1990; Sen et al., 1991) and in at least one occasion, the provision of a well-aerated rooting medium (Coester and Ohler, 1976). The fact that tissues form roots readily *in vitro*, and can do so under certain circumstances *in vivo*, suggests that failure in *Anacardium* may relate to a slow root formation process and the loss of cutting viability prior to root emergence. This loss of viability being accelerated under sub-optimal propagation environments. Therefore, in addition to improving propagation of this species from a practical viewpoint, the work aimed to elucidate the relationship between propagation duration/environment and rooting potential (both in terms of number of adventitious roots but also subsequent root development). Indeed, we tested the hypothesis that speed of adventitious rooting is critical to the success of propagation and that the potential to form adventitious roots would decrease with time in the propagation environment. This was evaluated by the repeated removal of any adventitious roots that formed over a 9 week period. Subsequently, we wished to examine the extent to which any new root development was regulated by phytohormone action, via removing root tips (e.g. a possible source of endogenous auxins and cytokinins) or exogenously adding these compounds.

2. Materials and methods

2.1. Plant material and cutting preparation

Stockplants of cashew (*A. occidentale* L.) were raised from seed sown in 1999 and grown on in glasshouses at minimum air temperature of 20 °C and natural photoperiods at the University of Reading, UK. Seed was collected from two selected genotypes, based on agronomic characteristics; CCP 1001 with 'dwarfing' habit and non-vigorous growth and precocious flowering/fruiting characteristics and AC 10 with a more vigorous growth habit. Stock material was pruned (removing 50% of growth) and re-potted on an annual basis to promote new shoot growth suitable for the selection of softwood leafy cuttings. Cuttings were harvested when stock material was approximately 4–5 years old. Although stock material was considered chronologically young, flower formation was common and tissues selected for propagation were phenotypically mature.

Apical cuttings, approximately 100 mm long by 5–8 mm wide (calliper) and comprising three to four nodes were used throughout. Leaves were retained on the cuttings, with the exception of the basal node where they were removed. Cuttings were selected from stock plants and within each genotype randomised to avoid any bias associated with individual mother-plants. Depending on treatment, cuttings would be dipped in 6.2 mM indole-3-butyric acid (IBA), 0.03 mM zeatin (Z) in aqueous acetone solution, or a combination of both for 5 s. Chemicals were dissolved in 10 ml acetone and made up to the appropriate concentration by adding distilled water. Control cuttings were dipped in a 5% (v/v) acetone aqueous solution. Choice of IBA and zeatin concentrations reflected positive responses on root generation in preliminary experiments. Cuttings were then inserted two per 90 mm diameter pots containing perlite (0.5–2.0 mm, grade) and placed into a polythene enclosed mist system (to maintain both high humidity and leaf cooling, Harrison-Murray and Howard, 1992) for periods up to 63 days. Mist application was controlled via an electronic leaf (Mist Irrigation System Controls, Ringwood, UK) and relative humidity maintained at >97% (temperature and humidity were monitored by data-loggers, TGX-3580, Gemini Data Loggers, Chichester, UK). The mist