

The Influence of Exogenous Abscisic Acid and Carbon Dioxide Enrichment on the Rooting of an Australian Ornamental Plant

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INTRODUCTION

Factors which affect the capability of cuttings to form roots are of great interest to plant propagators and nursery people since a significant proportion of ornamental plants are propagated this way. Plant physiologists also find the events leading to the formation of adventitious roots of interest as there is considerable evidence that the process is under hormonal control. The dramatic effects on root formation of application of synthetic auxins may be taken as evidence for the involvement of naturally occurring auxins in root initiation and development. However, attempts to relate changes in endogenous auxins in cuttings to their ability to form roots has not produced a clear picture and have been summarised by Gasper and Hofinger (1988). For example, Wu and Barnes (1981) found that there was no difference in the endogenous auxin content of two *Rhododendron* cultivars, one of which was difficult to root and the other easy. Tréfois and Brunner (1982) showed that there was no effect of applied auxin on cuttings from a number of *Prunus* species when endogenous auxin content was low but when endogenous auxin content was high at the time the cuttings were prepared a positive relationship could be demonstrated between rooting success and auxin content. This suggests that some other unknown factor is influencing both rooting performance and endogenous auxin level. Gasper and Hofinger (1985) also summarise evidence from the work of Blakesley et al. (1985) which demonstrated a good relationship between auxin concentration and rooting success in *Cotinus coggygria* cuttings. Furthermore, substances such as triiodo benzoic acid (TIBA), which are known to interfere with the transport of auxins from the apex to the basal end of the cutting, also inhibit rooting (Davis and Sankhla, 1988). The precise role of auxins in the induction of adventitious root formation is far from clear, but the weight of evidence suggests that a high auxin content at the time cuttings are taken, which is probably a reflection of the vigour and physiological state of the stock plant, is beneficial to subsequent rooting and that exogenous auxin application at this time may also be beneficial.

Although auxins such as indole-3-butyric acid (IBA) are widely used to promote rooting of cuttings, other plant hormones are also known to influence the formation of adventitious roots. Gibberellins are generally thought to inhibit rooting (Hartmann and Kester, 1983) and synthetic substances which are known to inhibit gibberellin synthesis may have a beneficial effect on rooting (Davis and Sankhla, 1988).

Absciscic acid (ABA) is a naturally occurring plant hormone also known to oppose the effects of gibberellins (Lin and Ho, 1986) and there are a number of reported instances where ABA application has stimulated root formation on cuttings. Davis and Sankhla (1988) summarise these reports but also draw attention to evidence that ABA may have no effect or even inhibit rooting. Since one of the major roles of ABA in plants is to control stomatal aperture, and hence transpirational water loss (Raschke, 1979), we have investigated the possibility that any beneficial effect of ABA in rooting stimulation is through its effect on stomatal behaviour and hence water status of the cutting. Our test system was the Australian ornamental plant *Chamelaucium* 'Lady Stephanie' [*C. uncinatum* (Schauer) × *C. floriferum* (MS)] and rooting performance was studied at ambient levels of CO₂ and in a CO₂-enriched (750 to 800 ppm) environment. This approach was adopted because of the desire to also assess the effects of carbohydrate status on rooting and the known interactive effects of ABA and CO₂ whereby high CO₂ intensifies the effects of applied or endogenous ABA (Raschke, 1975).

MATERIALS AND METHODS

Plant material, propagation medium, CO₂ enrichment methods, and methods of starch analysis have been described previously (Grant et al., 1992). Briefly, terminal cuttings 50 to 75 mm long were taken from *Chamelaucium* 'Lady Stephanie' plants growing in pots in a glasshouse. The basal 20 mm of the cuttings were dipped in IBA solution (1 g litre⁻¹ in 50% ethanol) for 20 sec and then placed in a mixture of peat, perlite, and coarse sand in plastic propagation trays. Three trays, each containing about 100 cuttings, were placed in each of two polythene tunnels. One was maintained at approximately 750 ppm CO₂ [range 700 to 900 ppm] and other at ambient CO₂ (approximately 350 ppm). Humidity was maintained at >95% by sonic fogging nozzles using deionised water. The trays were randomised on the benches frequently to avoid position effects. Rooting success was estimated by removal of 10 randomly selected cuttings from each tray at each sampling time. In some experiments cuttings were sprayed with ABA (10 mg litre⁻¹) to runoff every second day. Control cuttings were sprayed with water.

Water potential of cuttings was measured with a pressure bomb. For starch determination, cuttings were oven dried at 80°C to constant weight and ground to a fine powder. Samples of this powder and a series of starch standards were hydrolysed with amyloglucosidase and the liberated glucose assayed in terms of changed absorbance at 340 nm following incubation with hexokinase, ATP, NADP, and glucose-6-phosphate dehydrogenase. Absciscic acid was determined according to Loveys and van Dijk (1988). Cuttings were washed with deionised water, surface dried, weighed and frozen at -20°C until required. Frozen material was extracted for 5 min in boiling water. After cooling and addition of an internal standard of (±)²H₃-ABA, the tissue was homogenised, centrifuged, and the supernatant extracted at pH 2.5 with ethyl acetate. This acidic extract was dried purified by reverse-phase HPLC and a fraction containing ABA was methylated with ethereal diazomethane. Quantitative analysis was carried out by gas chromatography/mass spectrometry using stable isotope dilution analysis.

RESULTS

The water potential of the cuttings fell dramatically during the first few days in the propagation environment, despite the maintenance of high relative humidity. However, as callus formation at the base of the cuttings commenced water potentials gradually recovered. This recovery was more rapid in cuttings exposed to elevated CO_2 . Soon after roots first appeared at about day 17 water potentials stabilised between -0.6 and -0.4 MPa. In another experiment some of the cuttings were sprayed with ABA (10 mg litre⁻¹). The water potentials of the control cuttings were similar to those noted in the previous experiment but ABA-sprayed cuttings had higher water potentials. The effect of ABA was most marked at the elevated level of CO_2 .

One of the major factors likely to influence the physiological state and vigour of stock plants and cuttings taken from them is the current photosynthesis and reserves of carbohydrate which may be mobilised to provide the substrates necessary for root formation. We, therefore, monitored the accumulation of starch in *Chamelaucium* cuttings at ambient and elevated CO_2 with and without ABA treatment. During the first 15 days of the experiment, before there were any visible roots, starch levels increased by a factor of two or three in all treatments. The increase was greatest in cuttings with no ABA at elevated CO_2 . ABA tended to reduce starch accumulation at both ambient and elevated CO_2 . There was little further increase in starch in cuttings which developed roots but accumulation continued if roots were not present.

Roots first appeared at between 15 and 20 days after cuttings first entered the propagation environment. In the experiments reported here there was little difference in rooting performance at ambient and elevated CO_2 . However, ABA had a marked positive effect on root formation but again there was no additional effect due to exposure to high CO_2 .

ABA was measured in unsprayed cuttings at ambient and elevated CO_2 . During the first 8 days ABA concentrations were elevated when compared with the concentration at the time of removal but as root formation occurred the concentration in the cuttings exposed to ambient CO_2 fell. The fall was less marked in cuttings exposed to elevated CO_2 . In another experiment ABA was measured in cuttings which had been sprayed with ABA. As expected, the ABA treatment resulted in high levels of the compound remaining in the plant tissues. The concentration was highly variable even though care was taken to remove ABA remaining on the tissue surface by washing and by sampling on alternate days to spraying. On average, the concentration of ABA in the tissues was about ten-fold higher than the endogenous level and higher ABA levels remained in the cuttings maintained at elevated CO_2 .

DISCUSSION

Despite the maintenance of high ambient relative humidity and optimum water availability in the rooting medium, water potentials fell during the first few days after cutting removal. This is not surprising since considerable water potential gradients exist between tissue and atmosphere, even in the high humidity conditions maintained in the polythene tunnels. Recovery in water potential was accelerated in the environment enriched with CO_2 , probably due to reduced stomatal conductance which is known to occur when ambient CO_2 is raised (Wong, 1980). It has been shown previously that *Chamelaucium* cuttings transpire less

water under conditions of elevated CO₂ (Grant et al., 1992). Callus and root formation, which would allow more efficient water uptake from the rooting medium, did not appear to be advanced by the exposure to elevated CO₂.

As the water potential of the cuttings fell during the first few days in the propagation environment there would have been an accompanying fall in tissue turgor. This fall in turgor would have been the stimulus for the synthesis of ABA in the cuttings and it is this increased ABA which sensitises the stomata to CO₂, resulting in reduced transpiration (Raschke, 1975). It is interesting that endogenous ABA levels and ABA resulting from exogenous treatment remained higher in the cuttings when they were exposed to elevated CO₂. This may have been due to modified patterns of breakdown or to the presence of more root apices able to actively synthesise new ABA. In either case the result would have been a tighter control over transpirational water loss for the cuttings in the high CO₂ environment.

In the experiments reported here the dominant effect on rooting was the presence of ABA but in previously reported experiments (Grant et al., 1992) CO₂ enrichment was shown to be of benefit in stimulating rooting of *Chamelaucium* cuttings. However, the water potentials during these earlier experiments were considerably lower, as was the proportion of cuttings forming roots in ambient CO₂ conditions. Since water status of cuttings is known to be of critical importance for the successful production of adventitious roots (Loach and Whalley, 1978), it seems reasonable to conclude that it is this factor which is of prime importance and that ABA and CO₂ are having their effect through their ability to increase water potentials.

Carbohydrate production and availability during propagation is generally considered to be necessary for the provision of energy and carbon skeletons for the production of new tissues (Veierskov, 1988). Starch reserves at the time the cuttings were taken were low and in all treatments there was a marked accumulation of starch during the course of the experiment but this was reduced in the presence of ABA, especially at ambient CO₂. There was thus a negative correlation between rooting performance and starch accumulation, suggesting that carbohydrate accumulation was of secondary importance to the water relations issues discussed above. Davis (1988) has summarised the evidence for the evidence for the requirement for carbohydrate and concluded that current photosynthesis does positively influence the rooting success of most leafy cuttings but that other factors such as water status or auxin availability may be of overriding importance. Our results support this view.

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