POSTER SESSIONS

Involvement of Cytokinins in Tissue Proliferation of *Rhododendron* 'Montego'

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Tissue proliferation (TP) of rhododendrons is an unusual morphological variant characterized by tumorous growths at the crown with or without abnormal shoots. In many cultivars, TP occurs in plants produced by tissue culture within 1 to 3 years after initial propagation. However, in *Rhododendron* 'Montego', TP occurs in tissue culture as shoots [TP(+)] that produce nodal tumors with many compressed lateral shoots and small leaves, unlike tissue-cultured normal [TP(-)] shoots. Tumors develop from abnormal axillary buds with a bulbous structure that forms at the base of the growing axillary shoot. Growth of the tumor is dominated either by continued growth of the single shoot or by proliferation of the bulbous structure with many de novo meristems on the tumor surface.

Another difference between TP(+) and TP(-) shoots is their plant hormone requirement for in vitro growth. Tissue proliferation(+) shoot cultures appear to be habituated since they grow and multiply rapidly on hormone-free medium. Tissue proliferation(-) shoot cultures, however, require the cytokinin isopentenyladenine (iP) for multiplication. When the growth of TP(-) axillary buds was compared to the growth of TP tumors on woody plant medium with iP (WP-iP) or without iP (WP-None), TP(+) tumors cultured on WP-None had the highest growth rate. Growth of these TP(+) tumors was characterized by the formation of TP(+) shoots. Although iP was necessary for growth to occur [as TP(-) shoots] from TP(-) axillary buds, TP(+) tumors cultured on WP-iP had a lower growth rate than the tumors cultured on WP-None. The high growth rate of TP tumors on WP-None indicated that tumors, like TP(+) shoots, are habituated. The slower growth rate of TP(+) tumors cultured on WP-iP suggested that exogenous cytokinin creates supraoptimal levels which inhibit growth.

The effect of exogenous cytokinin on the formation of TP(+) shoots from TP(-) tissues was also studied. Since abnormal shoots are proposed to be more readily formed from *de novo* buds than from organized axillary buds, we studied the ability of TP(-) leaves to produce adventitious TP(+) shoots. Mature TP(-) leaf blades were cultured for 10 weeks on adventitious shoot regeneration (ASR) medium with 3 cytokinin treatments (WP medium with 1 mM indolebutyric acid and 15, 30, or 60 mM iP). After transfer to WP-None medium, leaf explants were evaluated for death and for abnormal, TP(+), and TP(-) shoots present 5 and 10 weeks later. Abnormal shoots had unusual leaf morphology and/or were freely branching but did not have nodal tumors. When the level of iP was increased from 15 to 30 mM, more TP(-) leaf explants survived, but the number of leaves with TP(+) and abnormal shoots increased significantly after transfer to WP-None medium. In the case of 30 and 60 mM iP, leaf explants generated more abnormal shoots after 5 weeks. These results

not only suggest that exogenous iP induces the adventitious formation of TP(+) and abnormal shoots, but also that the cytokinin affects shoot morphology long after transfer to hormone-free medium. Above 30 mM iP, no increase in leaf death or leaf explants with TP(+) shoots was observed.

Because exogenous iP influences the growth of TP tumors and induces adventitious TP(+) shoots to develop, the uptake and metabolism of exogenous iP was studied by using radioactively-labeled iP ([3H]iP) as a tracer. The [3H]iP (specific activity 6.6 × 104 dpm $pmol^{-1}$) was combined with cold iP to provide a final concentration of 10 mM iP in liquid WP medium. Excised TP(-) and TP(+) shoot tips were placed separately in the medium, and the percent uptake of [3H]iP was calculated from counted aliquots of the medium over 7 days of culture. After 7 days, cytokinins were extracted and prepared for HPLC. Samples were analyzed for cytokinin and adenine nucleotides by anion exchange HPLC. Fractions corresponding to free base and conjugated cytokinins were collected, and prepared for analysis by reverse-phase HPLC. Radioactive peaks were quantified by an IN/US on-line liquid scintillation counter. Our results showed that the percent uptake of [3H]iP increased greatly between 1 and 3 days of culture, with 85% to 90% uptake by the 7th day of culture. Analysis after extraction did not show significant differences in the uptake of iP between TP(-) and TP(+) shoots. The cytokinin metabolites adenosine 5' monophosphate, a glucoside conjugate of iP (IP9G), and iP were identified in both TP(+) and TP(-) shoots. However, only iP levels were significantly different between the two shoot types. In both shoots, greater than 50% of the iP was conjugated to IP9G, resulting in inactivation. Overall, TP(+) shoots appear to metabolize iP faster than TP(-) shoots by 7 days of culture.

The differences in the growth of TP(-) of TP(+) tissues in response to exogenous iP and differences in the metabolism of iP between TP(-) of TP(+) shoots suggest that changes in endogenous cytokinins could be producing the tumorous morphology in *R.* 'Montego'. Currently, we are measuring endogenous cytokinins in TP(-) and TP(+) tissues using an ELISA method.

Long-Term Inhibition of Stem Elongation of *Rhododendron* and *Kalmia* by Triazole Growth Retardants

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That a growth retardant chemical, in combination with a cold- and day-length forcing treatment, could induced *Rhododendron* to flower a year after propagation, was first shown by Stuart (1960). The triazole growth regulators more effectively reduce stem elongation than the chemicals used previously (Davis et al., 1988). Two of these, paclobutrazol and uniconazole, promoted flowering of field-grown *Rhododendron* and *Kalmia* (Gent, 1995a, Ranney et al., 1994; Wilkinson and Richards, 1991), where other chemicals had inconsistent effects. However, paclobutrazol inhibited stem elongation a year after application when applied to *Rhododendron* (Ranney et al., 1994; Wilkinson and Richards, 1991) and *Vitis* (Reynolds and Wardle, 1990), and for 2 years when applied to *Malus* (Williams 1984). This