Research Updates

Effect of NH_4NO_3 Level on Philodendron Root Development in Vitro

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Additional index words. tissue culture, propagation

Summary. Extensive root development was observed on Stage 2 Red Emerald philodendron shoots grown on standard multiplication medium consisting of Murashige and Skoog salts and vitamins with 6-benzylaminopurine (BA, 0.2 mg liter⁻¹). Root development was suppressed significantly when the level of NH_4NO_3 was doubled from standard levels (1x, 1650 mg liter⁻¹) to 3300 mg liter⁻¹ (2x). A higher level of NH_4NO_3 (3x, 4950 mg liter⁻¹) was detrimental to shoot growth and proliferation. This information may be useful for commercial propagators who wish to suppress root development at the shoot multiplication stage.

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Micropropagation techniques are used widely in commercial nurseries for propagating ornamental species. Philodendron varieties often are propagated in vitro. The rapid multiplication protocol involves three major stages (Debergh and Maene, 1981; Murashige, 1974): 1) establishment of cultures in vitro, 2) their maintenance and increase, and 3) preparation of propagules for establishment in soil.

Shoot multiplication in the second stage is crucial to production. High multiplication rates often are associated with the use of cytokinin in the medium. In the multiplication stage some varieties of philodendron, such as Red Emerald, tend to produce excessive roots at the expense of shoot proliferation. This creates problems for the propagator. This study describes a method to suppress excess root development in Red Emerald philodendron.

Materials and Methods

Shoot cultures. Stage-2 tissue culture plantlets of Red Emerald philodendron (supplied by Hartman's Plants, Homestead, Fla.) were used in this study.

Experiment 1. Shoots were separated from proliferating cultures with a piece of the parental base still attached. All roots and visible root initials were cut from the base. Each clump then consisted of a single shoot, 10 to 12 mm wide, and its base. Three clumps were planted in 5 × 10-cm jars capped with clear plastic cap (Magenta Corp.). Each jar contained 30 ml of one or two of the following media: Both media were based on Murashige and Skoog (1962) high mineral salts and vitamins supplemented with 6-benzylaminopurine (BA, 0.2 mg liter⁻¹),

sucrose (30 g-liter⁻¹), and agar (6.0 g liter⁻¹). Medium 1 contained full-strength NH_4NO_3 (1650 mg liter⁻¹) and medium 2 contained double-strength NH_4NO_3 (3300 mg liter⁻¹). All media were adjusted to pH 5.7 with KOH prior to autoclaving for 15 min at 121C and 1.1 kg cm⁻².

Experiment 2. Shoots were separated into single shoots without a parental base. All visible roots and root initials were removed carefully from the proximal end. Three shoots then were placed in 5 × 7-cm jars that contained 30 ml of one of the following three media: Media 1 and 2 were the same as described for Expt. 1. Medium 3 contained three times the full strength of NH_4NO_3 (4950 mg liter⁻¹). All media were prepared and sterilized as described earlier.

Cultures were maintained and grown for 4 weeks at 22 to 24C under cool-white fluorescent tubes (60 μmol m⁻² s⁻¹) with a 16-h photoperiod. Each jar was a replicate and there were at least 15 replicates for each treatment. At the end of the incubation, number of roots, total length of roots, fresh weight of roots, number of root initials, and number of new shoots per plant were recorded.

A general analysis of variance (ANOVA) was conducted for all variables using the SAS program (SAS Institute, 1985). For quantitative factors, the ANOVA was followed by mean separation using LSD procedures. Percentage data were transformed to arcsin values prior to analysis. The results are presented in a nontransformed format.

Results

Experiment 1. The clumps of philodendron cultured in medium 1 produced numerous roots, most of which grew aerially. The number of roots per clump and their average length and fresh weight on medium 1 all were significantly higher than clumps grown in medium 2 (Table 1). The extensive root system produced in medium 1 was matted at the base of the clump. There was no significant difference in the number of shoots produced on either media. On average, 1.5 shoots per clump were observed after 4 weeks of culturing.

Experiment 2. The production of roots by single shoots in media 1 and 2 was similar to that observed in clumps in Expt. 1 (Table 2), except that shoots with parental bases produced many

Table 1. The effect of NH_4NO_3 concentration on root formation by single stage-2 shoots of *philodendron* grown on Murashige and Skoog medium (Expt. 1) with a piece of parental base attached.

MS + NH_4NO_3 (mg·liter ⁻¹)	Avg no. roots/plant	Avg root length (mm)	Avg fresh wt of roots/plant (mg)	No. shoots/ plant
1650	11.2	17.4	0.22	1.4
3300	3.1	7.9	0.03	1.6
LSD _{0.05}	1.9	2.0	0.04	NS

Table 2. The effect of NH_4NO_3 concentration on root formation by single stage-2 shoots of *philodendron* grown on Murashige and Skoog medium (Expt. 2) without a piece of parental base attached.

MS + NH_4NO_3 (mg·liter ⁻¹)	Avg no. roots/plant	Avg root length (mm)	Avg fresh wt of roots/plant (mg)	No. shoots/ plant	No. root initials/ plant
1650	2.1	36.8	0.3	0.6	0.8
3300	0.8	9.4	0.04	1.1	0.8
4950	0.1	1.1	0.003	0.4	0.7
LSD _{0.05}	0.7	8.4	0.3	0.5	NS

more roots than single shoots (Tables 1 and 2). Tripling the concentration of NH_4NO_3 in the medium did not cause a significant change in root number, length, or fresh weight compared to medium 2. However, there was a significant reduction in the number of shoots per plant in medium 3 compared to medium 2 (Table 2). Shoots in medium 3 also showed some yellowing and loss of vigor. Shoots cultured in all three media produced similar numbers of root initials (Table 2).

Discussion

Philodendron is known to have preformed root initials (Debergh and Maene, 1981), the development of which is favored by tissue culture conditions. Some varieties are noted for producing excessive roots that suppress growth and shoot proliferation (S.S., personal observation). Roots grow in all directions and those that grow aerially tend to occupy much of the space in the culture vessel. Excessive rooting not only has a negative

effect on shoot production, but the cultures become less productive and separating plants or microcuttings become laborious and costly.

This study has established that root development and growth can be suppressed by increasing the concentration of NH_4NO_3 in the medium. Exogenous macronutrients are known to regulate organogenesis in several plants (Gertsson, 1988; Moe and Anderson, 1988; Nemeth, 1986). Adventitious root formation was shown to be enhanced in difficult-to-root apple cultivars by reduction or complete omission of NH_4NO_3 in the medium (Sriskandarajah et al., 1990). In this study, satisfactory suppression of root formation was achieved by doubling the NH_4NO_3 concentration. The higher levels (3×) produced no further significant benefits, but were detrimental in terms of vigor loss and shoot yellowing. We made no attempt to balance our media osmotically.

Although we were able to suppress root development by increasing the NH_4NO_3 level of our medium, our

shoot proliferation rates were not satisfactory on any of the media tested. Proliferation rates may be improved by manipulation of the ratio of auxins and cytokinins and other medium constituents in vitro.

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Acknowledgements

This research was supported in part by funds provided by the Univ. of Illinois Experiment Station.