

Growth Promotion of In Vitro Woody Plants Under Photoautotrophic Conditions

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INTRODUCTION

Demand for high quality woody transplants has been increasing worldwide in forestation and reforestation for global environment conservation and energy/food production. Transplant production by micropropagation methods is more beneficial than transplant production using seeds or vegetative methods with respect to genetic uniformity, virus-free or pathogen-free propagules, and scheduled year-round production. However, producing woody transplants using micropropagation techniques will only become cost effective when: (1) the photosynthetic ability of the transplants is fully expressed; (2) no symptoms of hyperhydricity or physiological and morphological disorders exists; (3) normal root development (formation of lateral roots and normal vascular systems) in the in vitro stage occurs; and thus, higher percent survival of transplants in the ex vitro; and (4) no acclimatization is required.

RESEARCH

Our recent research has shown that the growth of in vitro woody transplants, such as paulownia (*Paulownia fortunei*) and coffee (*Coffea arabusta*), can be greatly promoted in photoautotrophic (PA) culture (no sugar in the culture medium) as compared with that of heterotrophic culture (with sugar in the culture medium). In this paper, we also demonstrate advantages of air-porous supporting materials, such as Florialite (a mixture of vermiculite and cellulose fibers, Nisshinbo Industries Inc., Japan) and vermiculite, and a forced ventilation system applied to large culture vessels in the photoautotrophic micropropagation of these species.

Paulownia. Paulownia (*P. fortunei*) plantlets significantly showed a high potential of in vitro photoautotrophy when leafy explants were used. There were significant differences in growth of single nodal cuttings for 30 days on a half-strength MS medium with (20 g liter^{-1}) or without (0 g liter^{-1}) sucrose when PPF (photosynthetic photon flux) was $120 \mu\text{mol m}^{-2} \text{ s}^{-1}$ and a photoperiod of 16 h d^{-1} . Photoautotrophic plantlets on Day 30 of the culture period showed a significant increase in fresh weight, shoot length, root length, and multiplication rate as compared with those grown in heterotrophic conditions. The results emphasized that the in vitro plantlet could easily express its photosynthetic ability when cultured photoautotrophically. An air-porous supporting material, such as Florialite or vermiculite, obviously contributed to the increase in the nutrient uptake of in vitro plantlets due to the activity of lateral roots formed in the culture stage. Furthermore, PA plantlets continued developing better when directly transplanted to plastic bags in the greenhouse. There was a significant difference in fresh and dry weight, shoot and

root length, as well as, relative growth rate on Day 21 of the ex vitro transplants derived from PA and heterotrophic cultures.

Coffee. Growth of *C. xarabusta* plantlets in vitro under PA condition was shown in previous studies to be better than in conventional micropropagations (heterotrophic and photomixotrophic). In the present study, the photoautotrophic growth of single-node cuttings, cuttings having two opposite leaves, was promoted with a significant increase in dry weight and shoot length when Florialite was used as a supporting material. Moreover, the net photosynthetic rate of PA coffee plants was much higher under the CO₂-enriched condition (1500 μmol mol⁻¹ in the culture room) and PPF of 150 μmol m⁻² s⁻¹. These conditions resulted in a significant increase in fresh weight, shoot length, and leaf area. However, a higher PPF (250 μmol m⁻² s⁻¹) did not result in better plant growth with coffee.

The application of forced ventilation by directly pumping a gas mixture into the large culture vessels ($V = 11.14$ liters) greatly enhanced the growth of coffee plantlets with significant increases in fresh weight, leaf area, and shoot length as compared with that under natural ventilation (air diffusion through milliporous membrane on the vessel lid). The effect of ventilation systems applied during the in vitro stage was still valid on the growth of coffee transplants in the greenhouse.

These results demonstrate that the production of high quality woody transplants by PA micropropagation is possible and strongly recommended for consideration by propagators.

The Growth and Respiratory Activity of Root Systems on Pot-Grown Apple Transplants

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To determine the respiratory activity of root systems in relation to root growth during transplant production periods on apple nursery trees, a comparative study was made on apple transplants grown in field and pot conditions from June to September. The growth of root systems, expressed by number of newly generated roots, showed that white roots tended to have a peak in June and a subsequent decline by late-July with either apple transplants grown in the field or pots. After that root growth was increased in the pot-grown trees whereas the field-grown trees did not show an increase. The volume of new roots was obviously smaller in pot-grown than in field-grown apples. While the shoot growth was similar in the early period in both pot- and field-grown apple transplants, growth difference were evident by late-June; the pot-grown apple grew slowly, by contrast the field-grown apple continued to grow vigorously. In parallel with these growth states of the apple transplants, the tendency was for respiratory activities to coincide with the difference in shoot and root growth between the pot- and field-grown apple transplants. From these results, it is likely that root respiratory activity can be an indicator or a measure to understand root growth.