Effect of Reduction in Ammonium Nitrate of Murashige and Skoog Medium on In Vitro Rooting from the Shoots of Some Horticultural Plants©

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The formation of adventitious roots in vitro is very important for obtaining good transplants by micropropagation. Horticultural plants differ greatly in rooting ability in vitro. In some plants, the shoots are capable of rooting easily in hormone-free medium. In other plants, auxins such as naphthaleneacetic acid (NAA) or indolebutyric acid (IBA) are necessary for in vitro rooting. On the other hand, it has been our experience that a reduction in the concentration of an inorganic salt in the medium promotes the rooting from the shoots in vitro. For example, in *Anthurium* (George, 1996) and apple trees [Sriskandarajah et al.1990] a reduction in ammonium nitrate of Murashige and Skoog, (1962), (MS) medium promoted in vitro rooting from the shoots.

The present paper describes the promotion of in vitro rooting of three genera of horticultural plants, *Lupinus, Delphinium,* and *Platycodon* by a reduction in ammonium nitrate in the MS media.

With two species (*texensis* and *polyphyllus)* of *Lupinus*, shoots were obtained from cotyledonary node explants in media supplemented with benzyladenine (BA) or kinetin (KIN). In *Delphinium*, two taxa Summer Skies Group and Galahad Group, we used the adventitious shoots formed from the enlarged hypocotyls, and in *P. grandiflorus* 'Samidare-murasaki', the axillary shoots induced from nodal segments excised from the donor plants grown in vitro were used for investigation of in vitro rooting.

Complete MS medium was used for all experiments as control. In *Lupinus*, we used the MS medium with ammonium nitrate reduced to $^1/_{10}$ of full strength. The effect of NAA on in vitro rooting was also investigated. In *Delphinium*, media with ammonium nitrate reduced to $^1/_4$, $^1/_7$, and $^1/_{10}$ of MS medium were used, and the effect of NAA on in vitro rooting was investigated. In *P. grandiflorus*, a medium with ammonium nitrate reduced to $^1/_{10}$ of MS medium was used, and the effect of IBA on rooting was investigated. All media were solidified with 0.2% Gelrite and pH adjusted to 5.75. The temperature of the cultures of *Lupinus, Delphinium,* and *Platycodon* were 25, 20, and 20 ºC, respectively.

The mean value of the number of shoots formed from the cotyledonary nodes of *Lupinus texensis* amounted to 9.8 after 30 days of culture in the medium supplemented with 1 $mg \cdot L^{-1}$ BA , and that of *L. polyphyllus* was 8.3 in the same culture conditions. Thereafter, these shoots continued to increase. In vitro rooting from the shoots of the two species of *Lupinus* were increased by culturing in the medium with ammonium nitrate reduced to $^1/_{10}$ of MS medium, while rooting from the shoots in the original MS medium was very low or not observed.

When the shoots of *Delphinium* Summer Skies Group and Galahad Group were cultured in the medium with ammonium nitrate reduced to $^1/_{10}$ of MS medium, a gradual increase in rooting percentage was observed during 9 to 20 weeks after the beginning of culture. When cultured in the medium with ammonium nitrate

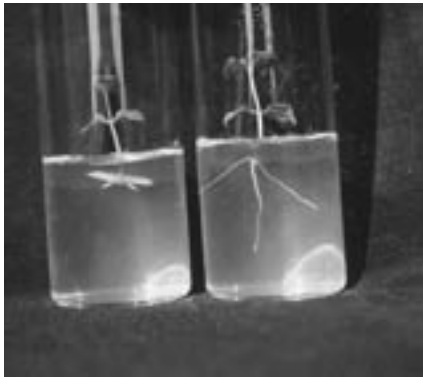

Figure 1: In vitro rooting from auxiliary shoots on a medium with 1/10 ammonium nitrate left with 1 mg·L IBA; right no IBA.

Figure 2: A regenerated plant of *Platycodon grandiflorus* growing in a greenhouse.

reduced to $^{1}/_{7}$ or $^{1}/_{4}$ of MS medium, the rooting percentage from the shoots was very low. No rooting occurred with either cultivar in the MS medium. Addition of 0.01 mg·L^{-1} NAA to the above-mentioned media was not effective in promoting the rooting from the shoots (Saitoh et al., 2004).

In the axillary shoots of *P. grandiflorus* the reduction of ammonium nitrate to $^{1}/_{10}$ of MS medium also promoted in vitro rooting. Addition of 1 mg·L^{-1} IBA to the MS medium further promoted rooting from the shoots. The rooting in the IBA-free medium with ammonium nitrate reduced to $^{1}/_{10}$ of MS medium was almost equal to that in the MS medium supplemented with 1 mg·L^{-1} IBA. In the medium with 1 mg·L^{-1} IBA there was more adventitious roots from the shoots, but they were shorter than those in IBA-free medium (Fig 1). An example of regenerated *P. grandiflorus* growing in a greenhouse is shown in Fig. 2.

When the concentration of ammonium nitrate was reduced to $^{1}/_{10}$ of MS medium, this of course reduced ammonium ion to the same ratio. Potassium nitrate was

also present as a nitrogen source in the MS medium. Therefore, in this case, the concentration of nitrate ion decreased to 53% of the original MS medium. By the reduction in ammonium nitrate to $^1/_{10}$ of MS medium, the ratio of ammonium ion to nitrate ion becomes very low based on mol concentration. Similar results have been obtained by Sriskandarajah et al. (1990). From these results, it is concluded that such a marked reduction in the concentration of ammonium ion compared with that of nitrate ion is a main factor on the enhancement of in vitro rooting of the plants in these experiments.

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Plant Tissue Culture and Plant Improvement: Some Recent Findings from Experiments with *Schlumbergera*, *Hatiora* (*Rhipsalidopsis*), and *Campanula*©

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INTRODUCTION

Plant tissue culture techniques have applications to a wide range of species and such usage has increased dramatically over the past twenty or so years. Techniques used include regeneration of cells, tissues, protoplasts, organs, embryos, ovules, microspores, anthers, etc. More recently, applications of these techniques have been extended to improve existing cultivars. Tissue culture techniques applied to plant improvement gives the opportunity to introduce genes across species. In addition, selection of somaclones through variation created by the tissue culture techniques themselves or those induced by mutagenic agents could also be used for creating new varieties.

Several ornamental plants suffer from sensitivity to exogenous ethylene, and it results in premature bud drop and early flower senescence. Anti-ethylene compounds such as silver thiosulfate, which are harmful and costly, are being used by the growers to reduce the above problems. However, the growers are very keen to reduce or