J. Trop. Agric. and Fd. Sc. 32(1)(2004): 81–84

Application of in vitro techniques in species conservation and micropropagation of *Didymocarpus platypus*

(Aplikasi teknik in vitro dalam pemuliharaan dan pembiakan mikro spesies *Didymocarpus platypus*)

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Key words: plant conservation, micropropagation, *Didymocarpus platypus*

Abstract

Seed and leaf tissues were successfully used in the propagation of *Didymocarpus platypus*, a potential ornamental and endangered species. MS media supplemented with 0.1 mg/litre 2,4-D and 1 mg/litre BAP enhanced shoot multiplication. Rooting was achieved on MS medium with low concentrations of IBA (0.1–1 mg/litre) or without IBA. Important features of the in vitro derived plants observed were the shorter stature and bushy growth, which are generally desirable for potted ornamental plants. These results are, therefore, very encouraging not only from the perspective of conservation but also in the creation of plants with better morphological traits.

Introduction

Didymocarpus platypus, a small shrub of the family Gesneriaceae is a rare and unique plant that has potential as potted ornamental. The plant has a short, woody stem and beautiful velvety leaves arranged in a rosette at the top and occasionally crowned with purple-white flowers *(Plate 1)*. This species was found in a hilly area in the state of Pahang and is fast disappearing. Recent visits showed that it was getting very difficult to see it in its natural habitat.

The studies reported herein were aimed at attempting the conservation of this species, of which none has been reported, so far. As seeds were very tiny and did not

Plate 1. ***Didymocarpus platypus*** *in its natural habitat*

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seem to germinate easily in nature, studies were conducted to germinate them in vitro. Further efforts were also carried out using leaf tissues to study the possibility for micropropagation. In vitro propagation using leaf tissues has been successful with many plant species such as orchids (Fu-Fan 1979; Tanaka and Sakanishi 1980; Rungruchkanont 2002), African violet (Norris and Smith 1981), apple (Welander 1988) and *Brassicas* (Dunwell 1981).

Materials and methods

Seed culture

Mature seed pods were obtained from a plant in Pahang. Pods were sliced open and the tiny seeds were immersed for 30 s in a 70% Ethanol solution, followed by 10 min in a 10% Clorox solution. They were then, rinsed three times with sterile distilled water and plated on a solidified MS (Murashige and Skoog 1962) medium. Cultures were kept on growth racks at 25–27 °C with an illumination of about 3 000 lux provided by fluorescent lights, 12 h/day. Seedlings were transferred to a solidified MS medium supplemented with 0.5 mg/litre IBA and 0.3% activated charcoal for rooting prior to planting in the nursery.

Leaf culture

Young leaves were obtained from a one-year-old in vitro germinated seedling plant, growing in the nursery. Leaves were sterilized as mentioned above for pods; cut into 5 mm x 5 mm segments and placed on a solidified MS medium supplemented with 0.1 mg/litre 2,4-D and 0.1 mg/litre BAP. When shoot masses began to form on the segments, tissues were sub cultured onto MS medium supplemented with 0.1 mg/litre 2,4-D and 1 mg/litre BAP for further multiplication. Shoot elongation was done on plain solidified MS medium. For rooting of these shoots, a solidified MS medium supplemented with IBA at 0, 0.1, 1 and 10 mg/litre were used. Culture conditions were the same as that for seed culture.

Plantlet establishment

Well-rooted seedlings were planted in Jiffy-7 pellets and grown under 80% shade with mist irrigation in the nursery. Subsequently, they were transferred to 20-cm pots containing 3:2:1 soil mixture to be grown until maturity.

Results and discussion

Seed culture

About 20% of the seeds germinated after 6 weeks in culture on MS medium. After 2 months, these seedlings developed into clumps of shoots *(Plate 2)*. Six months after culture, various stages of growth were observed within each clump, from newly developed shoots to shoots about 3 cm in height. The taller shoots were individually separated and successfully rooted on MS medium supplemented with 0.5 mg/litre IBA and 0.3% activated charcoal *(Plate 3)*.

One hundred of these plantlets were transferred to the soil and a survival rate of about 40% was achieved. This shows that the acclimatization procedure needs to be improved to obtain a higher transplanting rate. Observation made on 6-month-old plants showed variability in terms of growth rate. However, all plants seem to have short stems and were growing in clumps *(Plate 4)*, as opposed to the mother plant, which was taller and grew as a solitary unit. Similarly, tissue culture techniques have been reported to produce plants with a more compact form and enhanced branching, traits beneficial in some ornamentals (Capellades Queralt et al. 1991), as in this case.

Leaf culture

Interestingly, in the seed culture study, some of the leaves that were in contact with the tissue culture medium developed tiny shoots on their surfaces, and each of these grew into new clumps of shoots. This indicated that clonal propagation using vegetative tissues such as the leaf was quite easily achieved with this species. Following this observation, young leaves from 4-month-old

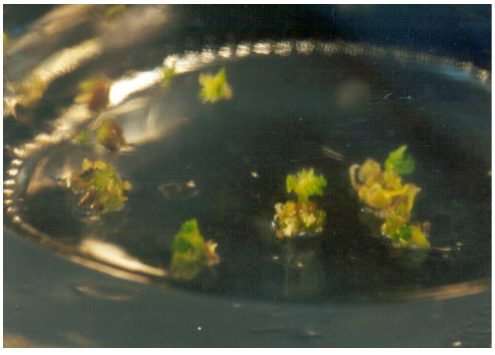

*Plate 2. Two-month-old **Didymocarpus platypus** seedlings*

Plate 3. Shoots growing on rooting medium

*Plate 4. **Didymocarpus platypus** plants – 6 months after planting to soil*

nursery-grown plants were used to initiate new cultures.

It was observed that bud development occurred directly on all of the leaf segments after one month in culture on a solidified MS medium supplemented with 0.1 mg/litre 2,4-D and 0.1 mg/litre BAP *(Plate 5)*. Upon

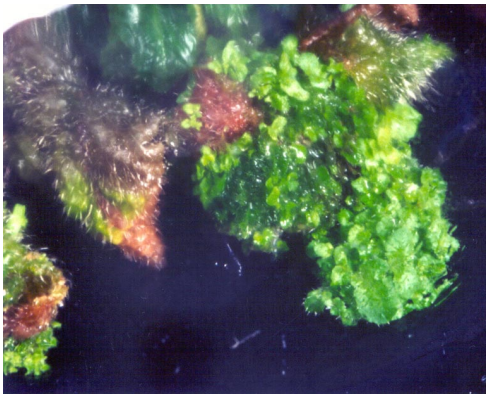

Plate 5. Adventitious shoots forming on leaf explants

transfer of the tissues to MS medium supplemented with 0.1 mg/litre 2,4-D and 1 mg/litre BAP, shoot multiplication was enhanced. This could be due to the higher BAP concentration in the medium, as was shown in other culture systems such as the *Cedrus* (Piola and Rohr 1996). Cytokinins in general, are capable of initiating multicellular meristematic regions capable of differentiation into organized structures (Lowe et al. 1996).

Almost all of the shoots rooted on medium without plant growth regulators and media supplemented with 0.1 and 1 mg/litre of IBA. However, addition of IBA in the media enhanced root growth and thus, was desirable. At the higher IBA level of 10 mg/litre, rooting was suppressed.

The above results may also be used in in vitro conservation approach which is very attractive compared to field gene banks. The latter is usually faced with serious problems such as exposure to attacks by pests and diseases, and natural hazards, besides its requirement for land and high maintenance costs (Withers and Engels 1990).

Plantlets obtained have been planted in soil and were growing quite well in the nursery in Serdang, despite their origin in the higher elevation. Being a flowering ornamental plant, observations were also being made to determine its capability of flowering in the lowland environment.

Conclusion

The conservation of *Didymocarpus platypus* can be easily achieved using in vitro methods employing both somatic (leaf) and zygotic tissues. Important features of the in vitro derived plants observed were the shorter stature and bushy growth, which are generally desirable for potted ornamental plants. These results are very encouraging not only from the perspective of conservation but also in the creation of plants with better morphological traits.

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Abstrak

Biji benih dan tisu daun berjaya digunakan dalam penghasilan bahan tanaman *Didymocarpus platypus*, satu spesies tanaman yang berpotensi sebagai tanaman hiasan tetapi diancam kepupusan. Medium MS yang ditambah dengan 0.1 mg/liter 2, 4-D dan 1 mg/liter BAP dapat merangsang pembiakan tunas. Pengakaran tunas ini pula diperoleh dengan menggunakan medium MS yang mengandungi kepekatan IBA yang rendah (0.1–1 mg/liter) serta medium MS tanpa IBA. Tanaman yang terhasil lebih rendah serta padat, dan merupakan ciri-ciri penting bagi tanaman hiasan pasu. Oleh itu, hasil kajian ini bukan saja berfaedah dalam pemuliharaan spesies berkenaan tetapi juga dalam penghasilan tanaman yang mempunyai morfologi atau ciri-ciri fizikal yang lebih baik.

Accepted for publication on 10 October 2003