Micropropagation of *Nerium oleander*

Jennifer L. Oliphant
Cyclone Flora, 2/4 Fleet Street, Devonport Auckland 9

***Nerium oleander* 'Petite Salmon' was successfully established in vitro from shoot tips taken from spring growth. Multiplication was achieved on Murashige and Skoog (MS) medium with 3 mg/l BAP. A prerooting treatment was used with MS medium and either 0.5 or 1.0 mg/l BAP. Rooting occurred on half-strength MS medium with 1 mg/l IBA. Plantlets flowered 7 months after deflasking.**

INTRODUCTION

The bacterium *Pseudomonas savastanoi* attacks the stems, foliage, and inflorescences of *Nerium oleander*. Brown lesions form on the leaves, and the stems blacken and die back. Because of the bacterial infection, it can be difficult to obtain clean plant material for cuttings. Also, the time of the year for taking cuttings is restricted to the summer months (January and February) with rooting occurring in March. Micropropagation is a useful method for the production of stock plants free of disease for the general cutting-grown production plants. In New Zealand, if the plantlets are deflasked in July and August, this tissue culture technique can produce a bushy plant suitable for taking cuttings in November-December.

MATERIALS AND METHODS

Several *N. oleander* 'Petite Salmon' plants, 30 to 40 cm high, were held in containers and sprayed with a fungicide mixture. Shoots 2 to 3 cm in length were cut from the new spring growth. These explants were disinfested with a 3-sec dip in ethyl alcohol, a 20-min wash in 0.6% sodium hypochlorite, and followed by three washes in sterile distilled water.

The basic medium trialed for initiation and shoot multiplication was full strength Murashige and Skoog (MS) minerals with Linsmaier and Skoog vitamins, 30 g/l sucrose, 7 g/l Davis agar with the pH adjusted to 5.5 (Table 1). The cytokinin benzylaminopurine (BAP) was added at 0.1 to 5.0 mg/l. Media were sterilised under pressure at 121°C for 20 min. Plant pieces were subcultured at 4-week intervals and grown in a culture room at a temperature of 25°C, photoperiod of 16 h, and light intensity of 2,000 lux. The medium used for root formation was either liquid or solid half-strength MS medium with 1 mg/l indolebutyric acid (IBA) (Table 1). The growth retardant paclobutrazol at 1 mg/l active ingredient was added to the rooting medium in an attempt to increase the survival rate after deflasking.

RESULTS

After disinfestation the explants grew new axillary shoots. These shoots were cut and subcultured onto a range of media. The most successful medium for shoot production was full strength MS with 3 mg/l BAP. Shoots gave a 3-fold multiplication rate while maintaining a small leaf size which made dissection easier.

Changing the hormone strength to 0.5 or 1 mg/l BAP for 2 weeks encouraged more upright growth and was an excellent prerooting treatment. Roots formed within 2 weeks, when the shoots were transferred to a half strength MS medium with 1 mg/l IBA, or when this rooting medium was added in liquid form at the prerooting stage. When paclobutrazol (1 mg/l) was included in the liquid rooting medium the shoots produced fewer roots which were shorter and thicker. The rooted plantlets were transferred to a 2 peat : 1 bark : 1 pumice sand (by volume) mixture in a humidity tent moistened by a fog system. The survival rate varied between 50% and 70% and was better when deflasking took place in the spring months from Sep.-Nov. The addition of paclobutrazol at 1 mg/l did not alter the rooting percentage in vitro or the survival rate at deflasking. The plantlets grew straight upwards without branching until, after 3 months, axillary bud break occurred and the plants bushed out. This could be accelerated by pinching out the shoot tips. Plants flowered after 7 months although it was better to encourage vegetative growth at this stage.

DISCUSSION

In the U.S.A. *N. oleander* cultivars have been micropropagated for some time at Monrovia Nurseries. At the Arslev Research Station in Denmark this species is micropropagated to produce stock plants for the general cutting-grown production of pot plants. In New Zealand, if the plantlets are deflasked in July and August, this tissue culture technique can produce a bushy plant suitable for taking many cuttings in Nov.-Dec. This will ensure good survival of the rooted cuttings through the following winter and freedom from the troublesome oleander knot.

Table 1. Media recommended for the micropropagation of *Nerium oleander*.

Murashige and Skoog (MS) full-strength mineral medium supplemented as follows:	myo-inositol	100	mg/l
	thiamine HCl	0.4	mg/l
	sucrose	30	g/l
	Davis agar	7	g/l
	pH	5.5	
shoot multiplication:	BAP	3.0	mg/l
prerooting medium:	BAP	0.5 to 1.0	mg/l
root elongation:	MS half-strength mineral medium supplemented as above, with 20 g sucrose and 1.0 mg/l IBA. This medium could be used either solid or in liquid form as an addition to the prerooting medium.		

LITERATURE CITED

Murashige, T. and **P. Skoog**. 1962. Revised media for rapid growth and bioassays with tobacco tissue culture. Physiol. Plant. 15:473-97.

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