fog is reduced by increasing the time off periods and reducing the water flow. To date we have had very pleasing results, with an increase in percentage take (90% compared to 85% under mist), and less time required in the propagation house. Grafts done in mid-winter needed only 8 weeks compared with 12 weeks under mist. Grafts done in early September were virtually ready for lifting 4 weeks after grafting with developed bud initiation.

When well-callused with new growth they are lifted, untied, desuckered and potted into a PB6½, and put in a polytunnel house with high light levels and air temperatures to force new growth. They remain in the polyhouses for up to 12 weeks, being trimmed to a single leader and then tipped at 30 cm to branch. The polycover is removed and replaced with a 70% shade cover to harden-off the plants — then this is removed four weeks later.

The plants are sold from April onwards, ranging in size from 60cm to 80cm, with four to six branches on the top.

In conclusion, the main reasons for Duncan & Davies grafting are:

(1) Fuller utilisation of cutting/scion wood as we get a better "take" than with cuttings.

(2) A stronger plant is produced more quickly than by cuttings, although ultimately there is no real difference between cutting and grafted plants.

PROPAGATION OF THE N.S.W. WARATAH *(TELOPEA SPECIOSISSIMA)* BY TISSUE CULTURE

JOHN F. SEELYE

Horticultural Research Centre
Ministry of Agriculture and Fisheries
Levin

INTRODUCTION

The genus *Telopea*, in the Proteaceae family, comprises four species all indigenous to Australia. *T. speciosissima*, the New South Wales waratah, is a woody shrub which produces large red blooms in the spring.

Its cultivation in New Zealand is increasing to meet cut flower market demands. However, its potential has been limited by the variable flower quality in plants raised from seed. A few clonal selections have been made in the past and vegetatively propagated by cuttings. Others are currently being eval-

uated at this Research Centre for their cut flower qualities. Rapid propagation from the limited base stock is necessary if the local cut flower industry is to quickly realise the potential of these selections.

The procedure described here is based on studies with two *T. speciosissima* selections, clones 8 and 38. Previous micropropagation successes within Proteaceae, a family of 60 genera, have been mainly confined to the genus *Grevillea* (1,2). Shoot multiplication in *Telopea* using 6-benzyl-aminopurine (BA) in a Murashige and Skoog (MS) medium (3,4,5,6) and root development *in vitro* (5,6) have been reported.

PREPARATION AND CULTURING FACILITIES

Greenhouse-grown stock plants provided a source of explant material. The soft shoots which developed after the flowers deteriorated were removed and cut into single node segments. These were surface sterilised by dipping in ethanol, agitating for 30 min. in a 0.6% sodium hypochlorite solution and then rinsing. The segments were then surface-dried in a laminar flow hood.

The basal medium contained MS inorganics, 100 mg/l inositol, 0.4 mg/l thiamine-HCl, 30 g/l sucrose, and 7.5 g/l agar. Half strength basal medium had only the MS macro inorganic compounds reduced. The growth regulators used were indole-3-butyric acid (IBA), gibberellic acid (GA_3), and BA. The medium, with the exception of the GA_3, was adjusted to pH 5.7 and heat sterilised at 105 K Pa for 15 min. GA_3, with a similar pH, was filter-sterilized and added to the autoclaved medium; 35 ml of medium was pumped into 200 ml glass jars and closed with vented, translucent polypropylene screw lids.

The culture room temperature was 25°C ± 2°C. Cool white fluorescent tubes gave an illuminance of 2400 lux on the shelves during the 16-hr photoperiod.

SHOOT INITIATION

Single node segments were inserted into the basal medium supplemented with 0.05 mg/l IBA, 0.3 mg/l BA, and 0.1 mg/l GA_3. Removal of the outer scales from the auxillary bud hastened shoot development. Microbial contamination of some nodes necessitated repeated surface sterilising during the first few weeks.

Within one month axillary buds swelled and commenced to elongate. By 10 weeks shoots up to 20 mm long had developed.

This shoot initiation medium was also successful with 1.0 mm shoot tips aseptically dissected from the auxillary buds.

PROLIFERATION

Initiated shoots were removed, subcultured by cutting into segments, and placed on the proliferation medium comprising half strength basal medium with 0.05 mg/l IBA, 0.3 mg/l BA, and 2.0 mg/l GA_3. After six to eight weeks multiple shoots 5 to 15 mm long developed. These were removed from the basal tissue, and again cut into segments and placed onto fresh proliferation medium. By the fourth subculture, the time between subculturing could be reduced by two weeks while still maintaining a six-fold proliferation rate.

Increasing the BA levels from 0.3 to 2.0 mg/l without the presence of GA_3, resulted in a corresponding increase in the subsequent formation of fused or fasciated shoots. This was overcome by using lower levels of BA and by adding 1.0 to 3.0 mg/l GA_3. This resulted in a significant increase in the number of shoots forming from buds (Table 1), although on a medium containing 3.0 mg/l GA_3 some shoots became excessively etiolated. This elongation effect was suppressed by the higher BA levels.

Table 1. Effect of GA_3 concentrations in the proliferation medium on the mean number of visible buds and/or shoots present eight weeks after placing subcultured tissue on the media *T speciosissima*, clone 8

GA_3 rate (mg/l)	Shoots and buds	Shoots only	Shoots > 10 mm
0	2.18 a[1]	1 38 a	0.20 a
0.3	2.96 ab	2 18 ab	0.55 ab
1 0	3 56 bc	3.00 b	1 15 b
3 0	4.64 c	4 34 c	2.39 c

[1]Numbers with the same letters are not significantly different at the 5% level

With clone 8, 1.0 or 3.0 mg/l of GA_3, with 0.05 mg/l IBA and 0.3 mg/l BA, significantly increased the total number of visible buds and shoots present compared to similar media without GA_3 (Table 1). A similar but less significant trend was observed wtih the other clone. There were no significant differences in shoot proliferation rates between the two clones growing on media containing GA_3 in the range 0.3 to 3.0 mg/l.

The proliferation medium described here, containing 0.05 mg/l IBA, 0.3 mg/l BA, and 2.0 mg/l GA_3 gave adequate multiplication, consistent shoot size, and minimal fasciation (Figure 1a).

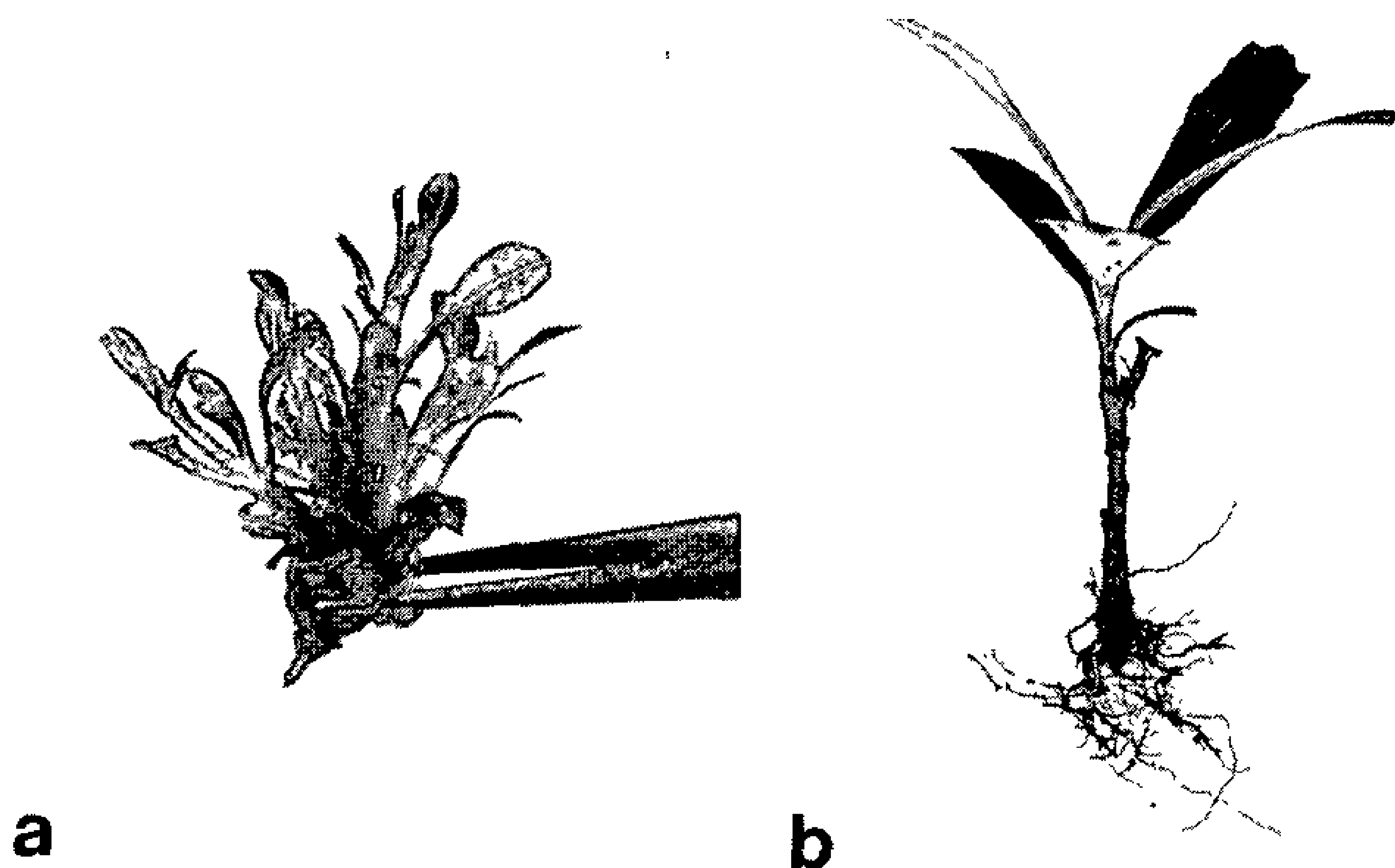

Figure 1. (a) *Telopea* shoot development *in vitro* after six weeks on proliferation medium containing 2.0 mg/l GA_3.
(b) *Telopea* plant 4 months out of culture

ROOT DEVELOPMENT

Attempts to produce rooted plants *in vitro* with IBA or NAA up to 1.0 mg/l incorporated in the basal medium were unsuccessful resulting, after six weeks, in excessive callus growth and no roots, although roots did appear many months after shoots were placed on the half strength basal medium.

When individual shoots from the proliferation medium had basal ends dipped for a few seconds in filter-sterilised 500 or 1000 mg/l IBA solitions and were placed back on to half strength basal medium, roots appeared after two weeks.

When these plantlets were moved into conventional greenhouse propagation facilities with open mist and basal heat four weeks after root initiation, they collapsed and eventually died. However, good survival was obtained when plantlets with root initials or very short roots were inserted into trays of a porous, nutrient-free, pumice medium. The trays were placed on dampened capillary matting with basal heat, and enclosed in a high humidity polythene tent. A commercial liquid-feed formulation was applied weekly. When, after six to eight weeks, roots elongated and new leaves formed, the humidity was gradually reduced. Plants were eventually potted into a standard mix with equal volumes of pumice, bark, and peat, with added nutrients in preparation for field growth (Figure 1b). Incorporating soil from mature, field-grown *Telopea* plants into this mix appeared beneficial to plant growth, suggesting a symbiotic association with micro-organisms.

Because of the importance of having a cost-effective commercial tissue culture method, with as few steps as possible, attempts are being made to establish plantlets out of culture using micro-cuttings directly from the proliferation medium. Using the pumice mix and high humidity tent, shoots dipped in 500 to 1500 mg/l IBA at exflasking had initially a lower survival rate than those undipped. Although some root development did occur, survival rates were greatly improved if IBA treatments were delayed for at least two weeks after exflasking. Root development also occurred in 20% of the plantlets which had not been dipped in IBA, indicating the IBA in the tissue culture medium may be contributing to this.

Studies based on the foregoing are continuing.

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VEGETATIVE PROPAGATION AND DEVELOPMENT OF *SOPHORA MICROPHYLLA* AIT.

STEPHEN M. BUTCHER and SHEILA M.N. WOOD

Horticultural Research Centre
Research Division
Ministry of Agriculture and Fisheries
Levin

INTRODUCTION

The genus *Sophora* is in the family Papilionaceae, and consists of about 30 species of temperate and subtropical trees and shrubs (1) of wide distribution. Three species are found in New Zealand, *S. tetraptera*, *S. prostrata*, and *S. microphylla*.