

# Developing propagation techniques for bush tomato.



Final report to the Australian Flora Foundation by

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November 2005

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AUSTRALIAN FLORA FOUNDATION - FINAL RESEARCH REPORT ON THE  
GRANT

Developing propagation techniques for bush tomato

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**ABSTRACT/SUMMARY**

*Solanum centrale* JM Black (bush tomato) (Solanaceae) commonly called bush raisin or bush tomato, or known locally as *kampurarpa*, is a small clonally spreading undershrub with yellow fruit that dry on the plant to resemble a raisin. The fruit provides excellent nourishment and is recognised as possibly the most important of all Central Australian native plant foods, and is one of the key commercially significant 'bushfood' species. One of the main obstacles to the horticultural cultivation of *S. centrale* is the low germination rate of the seed. Seed viability and the effects of heat, smoke, soaking, leaching, temperature, scarification and NaCl salinity on germination rates and percentages were investigated. Seed dormancy is imposed by the seed coat; once scarified, germination of two seed lots approached the viability levels determined through tetrazolium testing. Germination did not differ at temperatures of 12, 20 or 28°C, indicating that there is no seasonal temperature preference. Soaking and leaching promoted germination in one each out of five experiments, while heat had no effect. Smoke enhanced the total germination of three of the six provenances tested, once dormancy was broken by seed coat scarification. The seeds showed considerable salinity tolerance, germinating in solutions of up to 200mM

NaCl, although 0 and 25mM NaCl produced higher total germination

As part of this research, a plant tissue culture system was developed that stimulated multiple shoot initiation from different type of explants, resulting in multiple shoot clumps forming on various media within 6 weeks. Aseptic cultures were initiated from mature plants, using apical shoots of 5mm in length and placed on basic MS medium supplemented with 2 $\mu$ M BAP. Three types of explants were investigated: apical buds, nodal cuttings with one axillary bud per explant, and leaf segments of 10mm<sup>2</sup> including the mid vein. The formation of adventitious shoots was achieved using half MS medium with B5 vitamins and BAP, kinetin, and zeatin at concentrations of 1, 5, 10 and 25 $\mu$ M with IAA at 1 $\mu$ M. Roots formed when clumps of shootlets were transplanted to the glasshouse environment and planted in perlite:sand:peat potting mixture.

**KEYWORDS:** *Solanum centrale*, bush tomato, Solanaceae, *in vitro*, Australian native plant, edible plants.

## INTRODUCTION

The family Solanaceae includes some of the world's major food crops, such as potatoes, tomatoes, capsicums, eggplant and chillis. Some species have a high alkaloid content and are poisonous; some are used medicinally (eg *Atropa belladonna*, deadly Nightshade) or as 'recreational' narcotics eg. tobacco and *Datura* (Purdie *et al.* 1982). There are 132 endemic and 66 naturalised *Solanum* species in Australia (Purdie *et al.* 1982), a number of species of which are or were an important staple food to Central Australia's Aboriginal peoples (Latz 1995). Of the 18 species known to occur in Central Australia, 9 are edible, which include, apart from *S. centrale*, the following: *S. chippendalei*, *S. cleistogamum*, *S. coactiliferum*, *S. diversiflorum*, *S. ellipticum*, *S. esuriale*, *S. gilesii* and *S. orbiculatum* ssp.

*orbiculatum* (Latz 1995).

The bush tomato is widespread in arid areas of Western Australia, the Northern Territory and northern South Australia in sandy deserts (Purdie *et al.*, 1982). It is an often sprawling clonal, perennial herb or undershrub to 45 cm, with yellow globular fruit 10-15mm diameter, which dry to a brown colour on the plant to resemble a raisin (Purdie *et al.*, 1982). The seeds are 2-4 mm long, pale yellow or light brown. The fruit is an important staple of past and present diets of some Aboriginal communities (O'Connell *et al.*, 1983, Turner-Neale and Henderson, 1994). The plant also has spiritual significance, featuring in many Dreamtime stories and increase ceremonies (Latz, 1995).



Bush tomatoes have now become commercially significant in the native food industry, which was estimated in 1997 to have a value of \$A10-12 million

(Graham and Hart, 1997). The fruit have a strong 'sun-dried tomato' taste, and the dried, ground fruit is used as a flavouring or spice for savoury dishes and also in relishes. Little systematic research has been done to date on the edible species of Australia's very diverse flora (Ahmed and Johnson, 2000).

Some bush tomato growers have reported very poor germination, while others have achieved some success using abrasion, smoke and/or leaching/soaking to promote germination. The seed germination of many Australian species is related not only to moisture and temperature (Bell, 1999) but also to effects associated with fire, including heat (Auld and O'Connell, 1991; Keeley *et al.*, 1981), smoke (Dixon *et al.*, 1995; Kenny, 2000; Clarke *et al.*, 2000) and charcoal (Bell *et al.*, 1987). The population dynamics and productivity of the bush tomato are also reported to be highly dependent on the occurrence of fire (O'Connell *et al.*, 1983; Latz, 1995). In addition, Australian semi-arid to arid zone species are likely to show some degree of salt tolerance (Pierce-Pinto *et al.*, 1990).

## BACKGROUND

No research on the horticultural development of bush tomato has been published to date. Some bush tomato growers have reported very poor germination levels, while others have achieved some success using smoke and/or leaching/soaking to promote germination (Vic Cherikoff, Andrew Beal, Ray Rogers, pers. comm). There is also interest from the native foods industry in developing tissue culture methods for the propagation of bush tomato, in order to provide consistent quality of plant material and to allow the propagation of plants with desirable characteristics. This project therefore, has focused on the requirements for the propagation of *S. centrale*, either by

seed germination or by tissue culture.

There is generally a strong correlation between the environmental cues for germination and the ecological conditions that occur in the habitat of the plant and the seeds (Bell 1999, Bell et al. 1993). Factors affecting seed germination are discussed below, together with implications for this species.

#### Germination of *Solanum Centrale* and Related Species:

The climate in the regions where *S. centrale* grows naturally is extreme, being arid, and with high summer temperatures and low minimum temperatures at night during winter. The limited rainfall (275mm annual average) often occurs during summer thunderstorms, but can be very variable, with rain falling only in summer some years, and mainly in winter in others (O'Connell *et al.* 1983). Plants and seeds may have to endure dry periods of several years (Bell 1999). It is likely that bush tomato seed is cued to respond primarily to moisture, in order to take advantage of rainfall whenever it occurs. Fast germinability in low numbers (i.e. low proportions of the seed-bank), is a strategy noted in central Australian species, this strategy being a way of spreading risk across several rainfall events (Jurado and Westoby 1992). The population dynamics and productivity of the bush tomato are also reported to be highly dependent on the occurrence of fire (O'Connell *et al.* 1983, Latz 1995).

The related species, *Solanum laciniatum* (southern kangaroo apple), also edible, is a weedy shrub which grows in southern Australia, and north to Dorrigo NSW, in a variety of locations, from coastal dunes, to damp forest valleys and on rocky slopes. The seed of this species germinates readily at 25°C with an eight hour photoperiod (Lush *et al.* 1980), with an average seed

viability of 80%. In this case, pre-chilling advanced germination by 36 hours and washing advanced germination by 5 hours. In the project reported here, the effects on germination of scarification of the seed coat, soaking, leaching, heat, smoke and NaCl treatments, were examined in *S. centrale*, at a range of temperatures.

To elucidate the responses of seeds of this species to such factors, three sets of experiments were conducted, using seed obtained from a total of six provenances from around Australia. The seedlots were obtained from a variety of sources; commercial native edible plant suppliers, commercial seed collectors and the Alice Springs Desert Park. The sets of experiments are presented in sequence within the sections below.

#### Tissue Culture Propagation

Tissue culture is increasingly used for the commercial propagation of plants, especially in species where seed germination is difficult or where uniform features or disease free stock is required. Tissue culture protocols have been developed for only a few Australian native edible plants. Several tissue culture protocols have been developed for the aniseed myrtle, and the essential oils were found to be produced by the foliage *in vitro* (van Ritten 1997). Tissue culture propagation protocols for the quandong (*Santalum acuminatum*), and also for the closely related *Santalum lanceolatum* (plum bush), were developed by Barlass *et al.* (1981). Williams *et al.* (1985) have propagated the native mint *Prostanthera rotundifolia in vitro*. Johnson (1996) has reviewed the achievements in the field of micropropagation of Australian native plants.

## RESEARCH METHODOLOGY

### (i) Seed germination

*Series 1: Exploring viability, and effects of temperature and pre-treatments* Seed: Lots 1 and 2 (Table 1) were used in this set of experiments. All seed was stored at ambient temperatures. Lot 1 seed had been extracted by the suppliers by processing the fruit through a hammer mill, which had resulted in some broken seeds which were discarded. It is considered that hammer milling has the additional effect of abrading the seed coat. Seeds of lot 2 were dissected from the fruit by hand (as were those of lots 3, 5 and 6; lot 4 was extracted by drying and crushing the fruit).

**Table 1.** Details of seedlots used in germination trials

Lot	Origin	Date collected	Seeds removed from fruit
<b>First series of experiments</b>			
1	Northern Territory	1996	Hammer milled
2	Alice Springs, Northern Territory	August 1996	Dissected by hand
<b>Second and third series of experiments</b>			
3	South Australia, commercially grown (SA)	Autumn 2001	Dissected by hand
4	Newman, East Pilbara, Western Australia (WA)	Dec. 1998	Dissected by hand
5	48km S of Alice Springs, Northern Territory (Alice Springs Desert Park)	August 2000	Fruit dried and crushed
6	Utopia, Northern Territory	Nov. 2000	Dissected by hand



*Seed viability:* This was determined using tetrazolium staining. For lot 1 (hammer-milled), the test was carried out with three replicates of 25 seeds. Seeds were soaked in distilled water for 24h before testing, and then placed on moist filter paper in petri dishes and soaked with a 1% 2,3,5- triphenyl tetrazolium chloride solution. The petri dishes were covered with foil to ensure darkness, and placed in an incubator at 37°C until staining was evident (after 13h). For lot 2 (with intact seed coats), a further test was carried out, again with three replicates of 25 seeds. In this case, seeds were soaked, seed coats removed and seeds stained as above. The petri dishes were incubated at 37°C until staining was evident (after 20 h). In all cases, embryos that stained red or pink were scored as viable, while embryos that remained unstained or that had large unstained areas were scored as non-viable. Viability percentages were then determined.

*Seed germination pre-treatments:* Four experiments were conducted, as described below, with 4 replicates of 25 seeds for each treatment, including an untreated control.

(i). *Heat, soak, leach:* Nine treatments were applied, as follows. Seeds were heated for 10 minutes in an oven pre-heated to 40, 60, 80, 100, or 120°C. For the soaking treatments, each replicate was soaked in 10 ml tap water for either 12 or 24 h. The water was not aerated during soaking treatments. For the leaching treatments, each replicate of seeds was placed in a 1-micron sieve, with tap water constantly leaching through, for either 12 or 24 h.

(ii). *Soaking range finding:* Since soaking was a successful pre-treatment, a subsequent, more detailed range-finding test was carried out on lot 1 only. Replicate batches were soaked in 10 ml distilled water for 0, 1, 4, 7 or 12h.

(iii). *Order of smoking and soaking*: Smoke was applied using the method of Kenny (2000). Dried leaf litter material (leaves, twigs and bark) was burned in a bee keeper's burner. The smoke was channeled through a length of hose (to cool it) into a chamber containing the seeds. Smoke was applied for 15 minutes per replicate. Three treatments were used to determine whether a combination of soaking and smoking the seeds had a greater beneficial effect than soaking alone, and whether the order of smoking and soaking produced any difference in response. The first two treatments were: smoke 15mins then soak 7h, and soak 7h then smoke 15mins. The third treatment was the soaking of seeds in 10ml distilled water for 7h per replicate, this length of treatment having produced the best results in the soaking range-finding experiment.

(iv). *Temperature*: The experiments investigating the effects of the order of smoking and soaking were repeated at three constant temperatures: 12°C, 20°C, and 28°C, which are the mean seasonal temperatures of winter, spring/autumn and summer respectively, in Alice Springs (Jurado and Westoby, 1992). The experiments were carried out in the dark in growth cabinets (Environ Air, SRJ Cabinets, Australia).

*Scarification of seed coat*: A pilot trial was carried out on lot 2, in which seeds were nicked slightly with a scalpel to break the seed coat. The nicked seeds were then subjected to the smoking and soaking treatments described above.

## **Series 2: Comparison among provenances of effects of scarification, smoking and soaking**

*Scarification of the seed coat*: Since from Series 1 it was shown that attempts to germinate seed with intact coats were unsuccessful and that nicked seeds,

subjected to smoking and soaking, had quadrupled germination, controls with both intact and nicked seeds were tested along with the smoking and soaking treatments of nicked seeds in this set of experiments. As mentioned earlier, in this Series, seedlots 3-6 were used. Experiments were carried out in the growth cabinets, in the dark, at 20°C.

### **Series 3: Effects of NaCl salinity**

Seeds from seedlots 3-6 were nicked and smoked as described in Series 2, and were then subjected to a range of salinity treatments as follows: 0, 25, 50, 100 and 200 mM NaCl, with electrical conductivities of 0.01, 2.58, 4.34, 9.22, and 16.02 dS/m respectively. The salinity treatments were applied by wetting the filter paper within the petri dish with the solution at the beginning of the experiment. The petri dishes were wrapped in plastic film and the filter paper remained moist for the duration of the experiment. The experiments were again carried out in the growth cabinets in the dark at 20°C.

#### *Trial procedure*

For all trials, seeds were placed uniformly on one layer of Whatman's No. 1 filter paper within 9 cm plastic petri dishes. Petri dishes were moistened with distilled water as necessary (except in the salinity experiment). The dishes were placed in the dark in a custom built germination cabinet designed to protect seeds from changes in temperature and humidity, in a controlled temperature laboratory – 20°C, unless otherwise specified. Dishes were checked for germination every 2-3 days for at least 28 days. Once germination was recorded (radicle emergence), the germinated seeds were removed. Mouldy seeds were removed immediately and recorded as dead. A solution of a fungicide (0.5 g/L Benlate®; methyl benzimidazole carbamate) was occasionally used if seeds became mouldy.

## **(ii) Plant Tissue Culture**

### **Establishment of aseptic cultures**

Ten week old seedlings of *S. centrale* were obtained from Australian Native Produce Industries, South Australia and were potted up (potting mix pH 6.6) and preconditioned in the glasshouse for a further two months prior to culture initiation. Cuttings 2 cm long were then excised from the plants and sterilised after a thorough wash of explants under running water with soap, by immersing cuttings in 0.5-1.0% (v/v) sodium hypochlorite solution for 15-20 minutes. This was followed by three rinses in sterile distilled water.

Apical shoots of 5 mm in length were dissected from the cuttings and placed on basic MS medium (Murashige and Skoog 1962) with 2  $\mu$ M N6-benzylaminopurine (BAP), supplemented with 3% (w/v) sucrose, and solidified with Gelcarin at 0.2% (w/v). The pH was adjusted to 5.6 prior to autoclaving. This is a standard medium used in the University of Technology Sydney laboratory for the first four weeks after the culture initiation of investigated plant species. Cultures were maintained under light at 50  $\mu$ mol m<sup>-2</sup>s<sup>-1</sup>, radiation provided by white fluorescent tubes for 16 h per day. The room temperature was kept at 25°C $\pm$ 1°C.

### **Plant media and explant type for callus and bud initiation**

Plantlets regenerated from the apical shoots were used in further investigations into callus and multiple shoot regeneration. Three types of explants were investigated: apical buds, nodal cuttings with one axillary bud per explant and leaf segments of 10 mm<sup>2</sup> including the mid vein. Explants were cultured on half MS (Murashige and Skoog 1962) with B5 vitamins (BI /Thiamine HCl 33.24  $\mu$ mol/L, B5/Nicotinic acid 2.29  $\mu$ mol/L, B6/Pyridoxine HCl

4.86  $\mu\text{mol/L}$  and Inositol 555.06  $\mu\text{mol/L}$ ) (Gamborg *et al.* 1968) with BAP at 2, 5 and 10  $\mu\text{M}$ , each with indole-3-acetic acid (IAA) at 1 $\mu\text{M}$ , referred to as media 1, 2 and 3 respectively. The standard laboratory medium used to establish aseptic cultures (basic MS medium with 2 $\mu\text{M}$  BAP) was used as medium 4 as a type of control.

Media were solidified with Gelcarin at 0.2% (w/v) and pH of 5.6. A replicate consisted of five shoots grown in a 200 ml jar containing 50 ml of media, and there were six replicates per media treatment. There were 30 replicates of each type of explant on each of the four media. Observations on growth were noted after three weeks, and total fresh weight, fresh weight of callus, length of the longest shoot and number of longest shoots and roots were recorded after seven weeks.

### **Cytokinin range finding study for shoot and root regeneration**

To investigate further the shoot and root regeneration, a second experiment was conducted. In this experiment only apical shoots were cultured, which had been harvested from the previous experiment. The medium used was half MS medium with B5 vitamins, IAA at 1 $\mu\text{M}$  and the following 15 cytokinin treatments: 0, 1, 5, 10 and 25  $\mu\text{M}$  BAP; 0, 1, 5, 10, 25  $\mu\text{M}$  kinetin; and 0, 1, 5, 10, 25  $\mu\text{M}$  zeatin. As previously, the media were solidified with Gelcarin at 0.2% (w/v) and pH was 5.6 prior to autoclaving. The incubation conditions were as described for the previous experiment. The total fresh weight, number of shoots, shoot length and number of roots were recorded after eight weeks.

### **Acclimatisation and *ex vitro* planting**

Plantlets from the cytokinin range finding study were washed to remove excess agar and dipped in Rovral fungicide 500 mg AI/200ml (50% iprodione)

if roots were present and dipped in Rootex powder (indole butyric acid 3000 mg/kg) if no roots were present. Explants were then planted in a perlite:sand:peat mix (1:1:1 v/v) and placed in a fog tent in the glasshouse.

### **Statistical analysis**

Plant tissue culture data were analysed by Kruskal-Wallis ANOVA and differences between treatments were determined by Dunn's Multiple Comparison Test (GraphPad Software, 1993). Total germination percentages were calculated, and speed of germination was defined as total germination in the first three days of each experiment. Analysis of variance (ANOVA) was used to assess the statistical significance of treatment differences (*StatSoft*, 1996). Alpha was set at 0.05. When ANOVAs indicated significant treatment effects, least significant difference (LSD) pairwise comparisons were conducted.

## **RESULTS**

### **(i) Seed germination**

#### **Series 1: Exploring viability, and effects of temperature and pre-treatments**

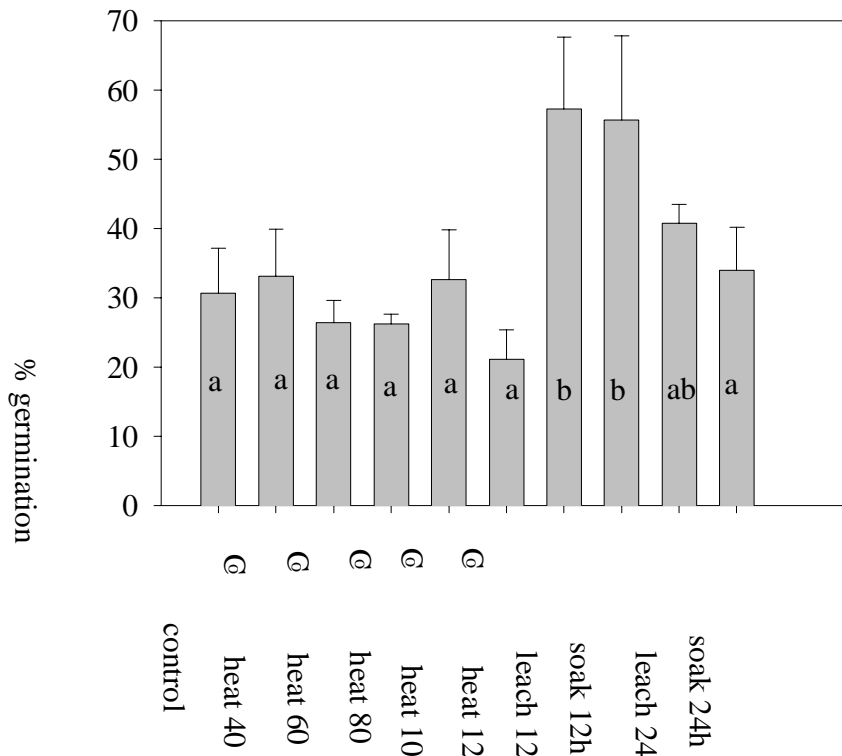
*Seed viability:* The lot 1 (hammer-milled) seed showed a similar viability to germination (Table 2). In contrast, lot 2 seed (dissected from fruit) had a much higher viability than actual germination, whether the seed coat was left intact or nicked. It was found in the pilot study, however, that with nicking the germination was approximately four times as high as it was with the seed coat intact, resulting in a mean germination of 33% ( $\pm 1.5$ ).

**Table 2.** The viability of *Solanum centrale* seeds compared to the germination of the seeds in the treatments with the highest germination in germination trials. The figures are percentages, and are the means of 3 replicates for viability, and 4 replicates for germination,  $\pm$  sem

Seed preparation	Viability (%)	Germination (%)	
Lot 1, run 1	62 $\pm$ 7.3	57 $\pm$ 10 (leach 12h)	
Lot 1, run 2	55 $\pm$ 4.7	57 $\pm$ 10 (leach 12h)	
Lot 2	53 $\pm$ 7.3	9 $\pm$ 2 (leach then smoke)	33 $\pm$ 1.51 (seed coat nicked)

*Seed germination pre-treatments*

*Heat, soak, leach:* For lot 1, there were no significant differences between any heat treatment and the control (31%) (Figure 1). Germination percentages for leaching (57%) and soaking (56%) for 12 h were significantly higher than either the control or the heat treatments and soaking for 24 h (34%). However, the results of the 12h treatments were not significantly different from seeds leached for 24h (41%). Germination occurred more quickly in the seeds leached for either 12 or 24 h, or soaked for 12 h, than for all other treatments: 44% in the first three days for leach 12 h; 32% for soak 12 h and for leach 24 h. However, soaking for 24 h produced only 22% germination in the first three days, which was not significantly different from the control rate of 17%.



**Figure 1.** The effect of heat, leaching and soaking on the total % germination of lot 1 *Solanum centrale* seeds (n = 4,  $\pm$  SE). Values with the same letter are not significantly different ( $p < 0.05$ ) based on the LSD test.

Lot 2 had much lower germination percentages than lot 1, the control and heat treatments producing no germination (except in the 120°C treatment, where one seed germinated out of 100). The wetting treatments produced a slight response. The 24 h leaching gave the highest germination rate, of 8%. This was significantly higher than the rate produced by leaching for 12 h and soaking for 24 h (3%), however it was not significantly different from the rate produced by soaking 12 h (4%). These treatments had no significant effect on the speed of germination.



*Soaking range finding:* For lot 1, 4, 7 and 12 h soaking gave significantly higher germination than the control (36%). Soaking for 7 h gave significantly higher germination (60%) than the control and 1 h treatments (38%), but was not significantly different from the 4h (52%) and 12h (51%) treatments. The treatments had no significant effect on the speed of germination.

*Order of smoking and soaking:* In this experiment, no treatment, including smoke alone, produced any significant differences from the control germination of 25%. No treatment had a significant effect on the speed of germination. Surprisingly, even soaking did not promote germination for this batch of seeds, as it had done in the heat, soak, leach and soak range finding experiments above. For lot 2, there was no germination (except for one seed in the control; data not shown).

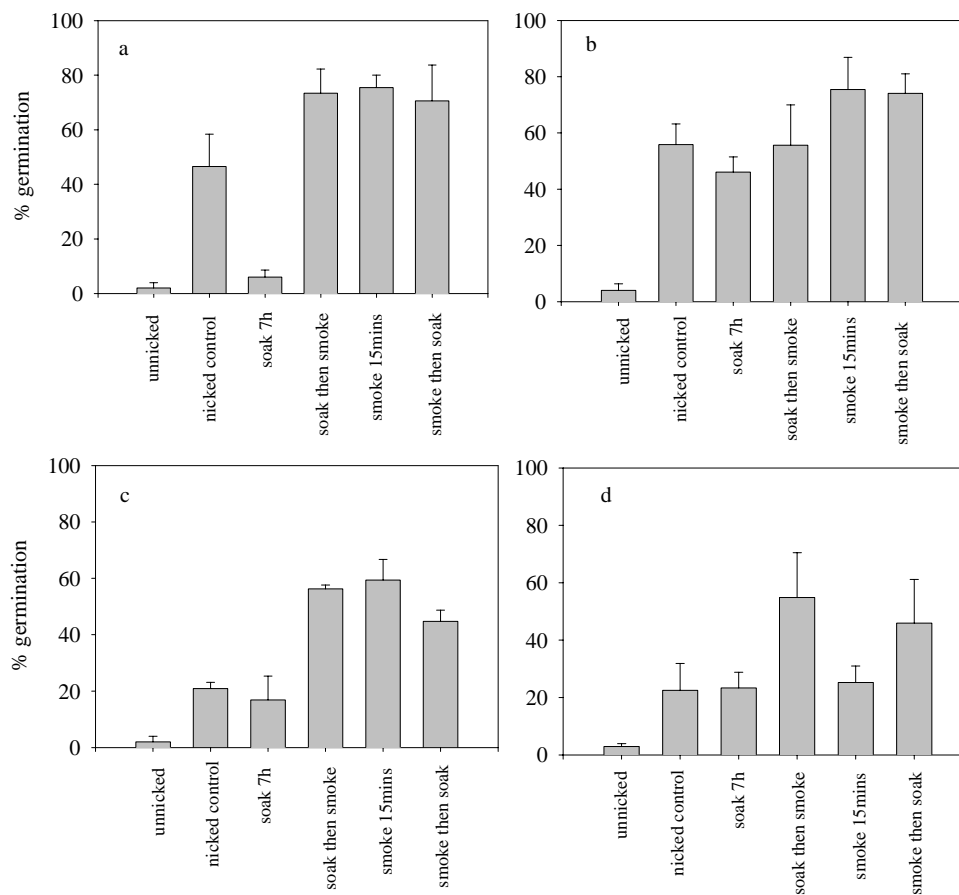
*Temperature:* Different temperatures produced no significant effect on germination in lot 1, all treatments and temperatures producing an average germination of 38%. As in the experiment on the order of smoking and soaking, soaking alone did not promote germination. Temperature alone also had no significant effect on germination in lot 2, but there was a significant treatment effect, with a significant interaction between treatment and temperature. That is, leaching for 24 h followed by smoking for 15 minutes, significantly enhanced germination of seeds at the higher temperatures trialed, namely 20°C (9%) and 28°C (7%).

*Scarification:* A pilot experiment of nicking the seed coat significantly promoted germination in lot 2 at 20°C (the only temperature tried for this treatment), raising the mean germination to 33% ( $\pm 1.5$ ) (Table 2).

## **Series 2: Comparison among provenances of effects of scarification, smoking and soaking**

In all seedlots, the scarified seed showed significantly higher germination than unscarified seeds (Figure 2). The combined average germination of the unnicked controls was only 3% ( $\pm 0.9$ ), whereas the overall germination for nicked controls was 36% ( $\pm 5.4$ ). Overall, the seed from the two Northern Territory provenances (lots 5 and 6, Alice Springs Desert Park and Utopia) had significantly lower germination ( $33\% \pm 4.8$  and  $29\% \pm 5.1$ ) than overall germination in seed from South Australia and Western Australia (lots 3 and 4) ( $46\% \pm 7.1$  and  $52\% \pm 5.9$ ).

Apart from lot 4, from Western Australia, the seedlots in all the smoke treatments (smoke, soak/smoke, smoke/soak), showed significant further increases in germination over the control scarified seeds. There were no significant differences among the 3 smoke treatments in any of the seedlots. However, for lot 3 (South Australia) and lot 5 (Alice Springs Desert Park), the soaking treatment produced a significant decrease in germination, compared with the control scarified seed.



**Figure 2.** The effect of smoke (15 mins), soaking (7 h), smoking then soaking, and soaking then smoking on the total % germination of nicked *Solanum centrale* seeds (a) Lot 3, from SA (b) Lot 4, from WA (c) Lot 5, from Alice Springs Desert Park (d) Lot 6, from Utopia. Values are the means of 4 replicates, bars are standard errors.

Taken together, the results from these two series of experiments clearly indicate, first, that nicking (or perhaps some alternative means of rupturing of the seed coat) is necessary to produce commercial rates of germination in this species; and secondly, that then applying smoke or soaking is likely to bring optimal results, depending on the provenance, and age, of the seeds.

### **Series 3: Effects of NaCl salinity**

To consider general trends, data for all seedlots were initially analysed together. It was found that salinity had a significant effect on germination, the 0 and 25 mM NaCl treatments having significantly higher germination percentages ( $57\% \pm 7.1$  and  $49\% \pm 6.2$  respectively) than those at higher concentrations. The germination in the 200 mM NaCl treatment was significantly lower than all other treatments ( $27\% \pm 4.5$ ). As in Series 2, among the four seedlots, lot 3 from South Australia gave a significantly higher germination than all the others ( $70\% \pm 4.7$  compared to an average of 35% for the other seedlots).

The results from individual seedlots showed interesting provenance variation, with only lot 3, from South Australia, and lot 5, from Alice Springs Desert Park, showing significant differences among salinity treatments (figure 3). For lot 3, 0 and 25 mM NaCl produced significantly higher total germination. The total germination rate for the South Australia controls ( $95\% \pm 2.9$ ) was much higher than the rate achieved in the equivalent treatment in the scarification trial ( $76\% \pm 4.6$ ). Conversely, seed lot 4 from WA reached 75% ( $\pm 11.4$ ) germination in series 2, compared to less than 56% ( $\pm 6.4$ ) in this experiment.

The total germination for the 100 mM NaCl treatment was high, at 74% ( $\pm 6.7$ ).

Germination was not delayed in any NaCl treatment.

For lot 5, total germination was significantly higher in 0, 25 and 50 mM NaCl than at higher salinities. Germination in these two highest NaCl treatments was delayed.

There were no significant differences in total germination due to increasing salinity for seeds from lots 4 and 6 (from Western Australia and Utopia,

Northern Territory respectively), although again, the germination percentage in 200 mM NaCl was in both cases the lowest recorded, i.e. this trend still appears. Salinity did not delay germination in any treatment for lot 4, though with lot 6 there was delayed germination in the 50 and 200 mM NaCl treatments (Ahmed, 2001).

## **(ii) Plant tissue culture**

### **Plant media and explant type for callus and bud initiation**

Callus and shoots regenerated on the three explant tissue types and the four types of media (Table 3). After three weeks it was noted that leaf segments in medium 1 were shooting well, and that apical and axillary buds in medium 1 were 2-4 cm and a few had developed multiple shoots. In medium 2, apical and axillary shoots were small, with roots forming, and some of the leaf segments were starting to shoot from callus and roots were forming. All material on medium 3 was very small and the leaf segments had little or no callus. Material on medium 4 performed much better than medium 3. Some of the apical and axillary buds were 2-4 cm long. There was no callus on leaf segments.

**Table 3** The effect of explant source on the morphogenetic response of apical buds, nodes with axillary buds and leaf segments of *Solanum centrale* cultured *in vitro* on four different media.

Explant type	Total fresh weight				Callus weight			
	Medium				Medium			
	1	2	3	4	1	2	3	4
Apical bud	0.65	0.23	0.23	0.38	0.71	0.52	0.22	0.4
Node with axillary bud	0.63	0.2	0.26	0.4	1.32*	0.87	0.2	0.37
Leaf segment	0.84*	0.23	0.11	0.1	0.92	0.5	0.03	0
	Number of shoots				Number of roots			
	Medium				Medium			
	1	2	3	4	1	2	3	4
Apical bud	3.53*	1	2	2.6	0	1	0	2
Node with axillary bud	2	1	1	1.5	0	1	0	0
Leaf segment	3.67*	0.33	0.03	0	0	1	0	0

Medium 1-3 –  $\frac{1}{2}$ MS (Murashige and Skoog 1962) with B5 vitamins (Gamborg *et al* 1968) with BAP at 2, 5, 10  $\mu$ M and IAA at 1  $\mu$ M

Medium 4 – basic MS (Murashige and Skoog 1962) medium with 2  $\mu$ M BAP.

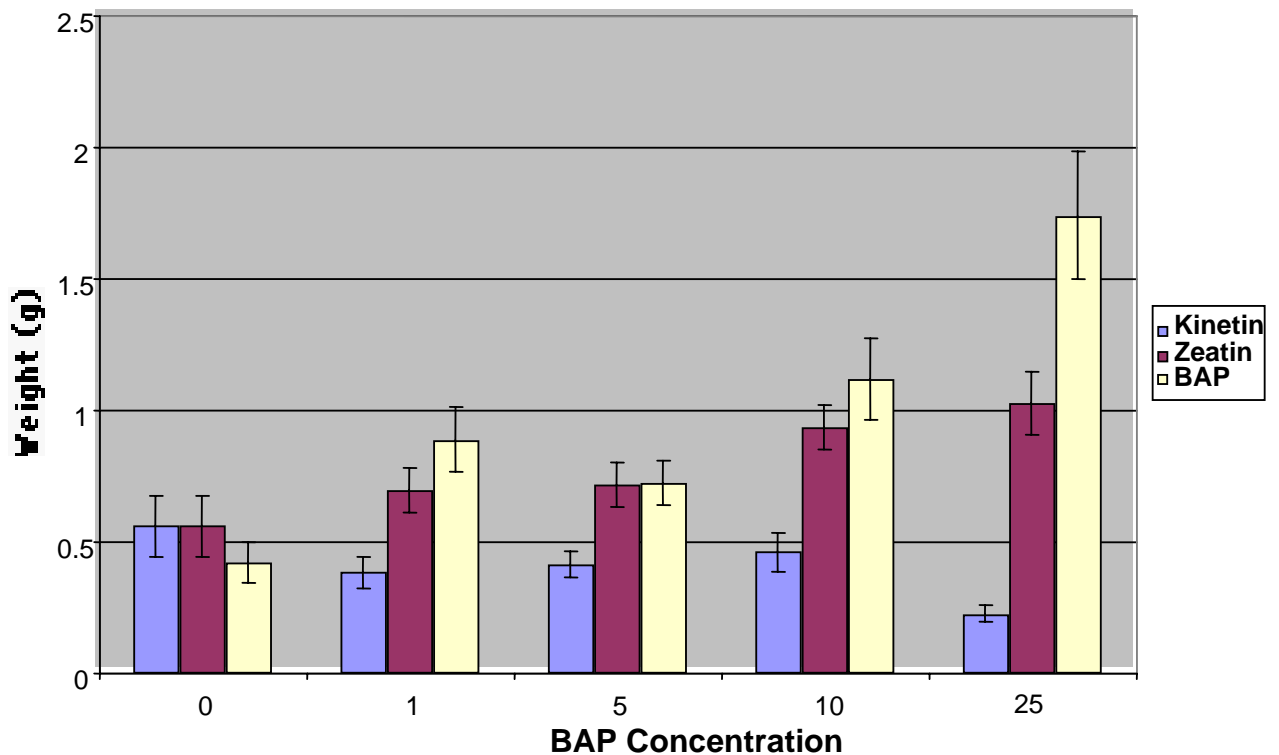
\*significantly different ( $p < 0.01$ )

At harvest after 7 weeks, there were significant differences ( $p < 0.01$ ) between media for all plant growth measurements ie. fresh weight shoots, fresh weight callus, length of the longest shoot, number of shoots and number of roots (Table 3). Medium 2 was found to be best for producing roots and medium 1 significantly better for all other growth measurements. Of the explant types, the callus with the highest fresh weight was formed on the nodal explants with the axillary bud. The number of shoots for apical bud culture was significantly higher and leaf explant material produced significantly shorter shoots than material from apical and axillary buds. There were significant interactions between explant type and media for all growth measurements except shoot length and number of roots.

## Multiple shoot regeneration and rooting

### *Fresh weight*

Increasing the concentration of zeatin and BAP improved the fresh weight of explants (Fig. 3). BAP at 25  $\mu\text{M}$  with IAA at 1  $\mu\text{M}$  produced larger explants than those on 0, 1 and 5  $\mu\text{M}$  BAP and 1  $\mu\text{M}$  IAA ( $P < 0.001$ ). The fresh weight was significantly higher at concentrations of 10  $\mu\text{M}$  and 25  $\mu\text{M}$  zeatin than at lower concentrations ( $P < 0.001$ ).



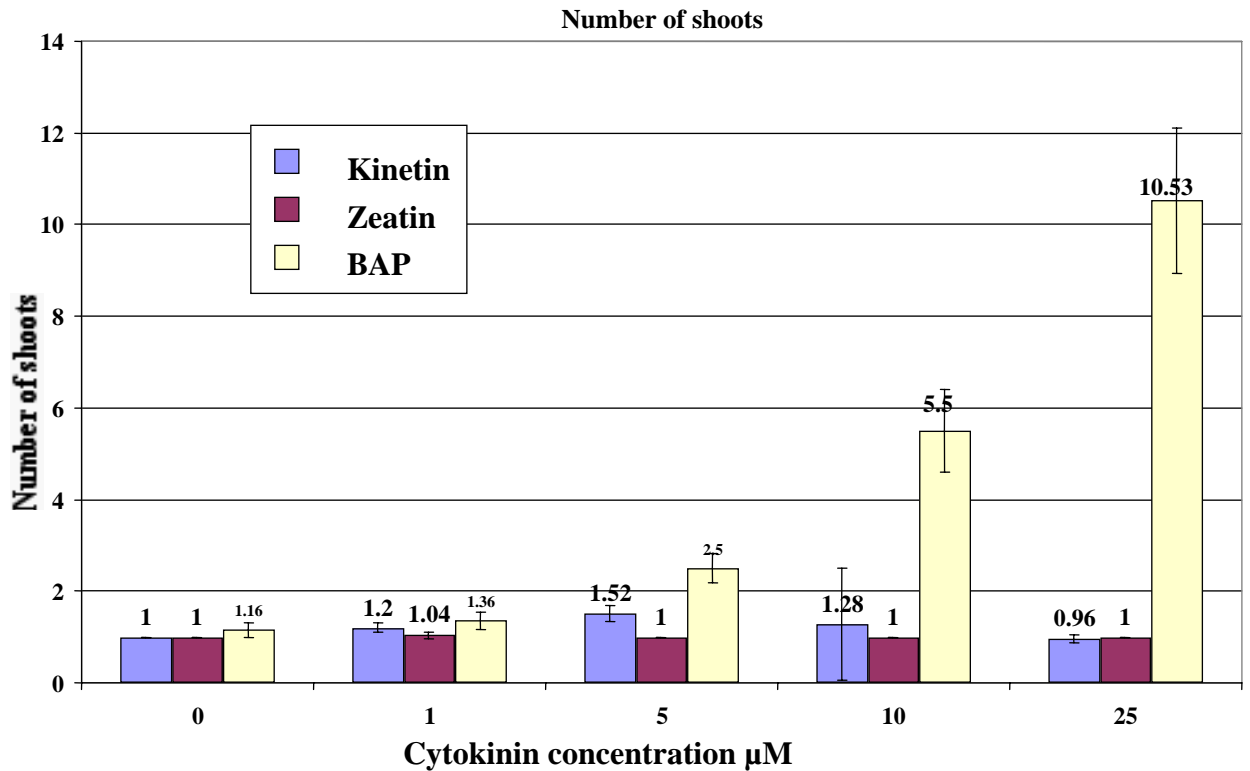
**Figure 3.** Total fresh weight of apical shoot explants, harvested from the previous experiment, and exposed to various cytokinin regimes. Recorded after eight weeks.

### Number of roots

Some roots formed on the basic half MS medium (control) and on all concentrations of zeatin in combination with IAA. Roots also formed on the lowest concentrations of kinetin and BAP.

### Shoot proliferation

The best multiplication was achieved by supplementing the medium with 25 $\mu$ M BAP and 1 $\mu$ M IAA ( $p < 0.001$ ) (Fig. 4). There were significant differences ( $p < 0.001$ ) between kinetin and BAP and zeatin and BAP at 2, 5, 10 and 25 $\mu$ M with 1 $\mu$ M IAA.

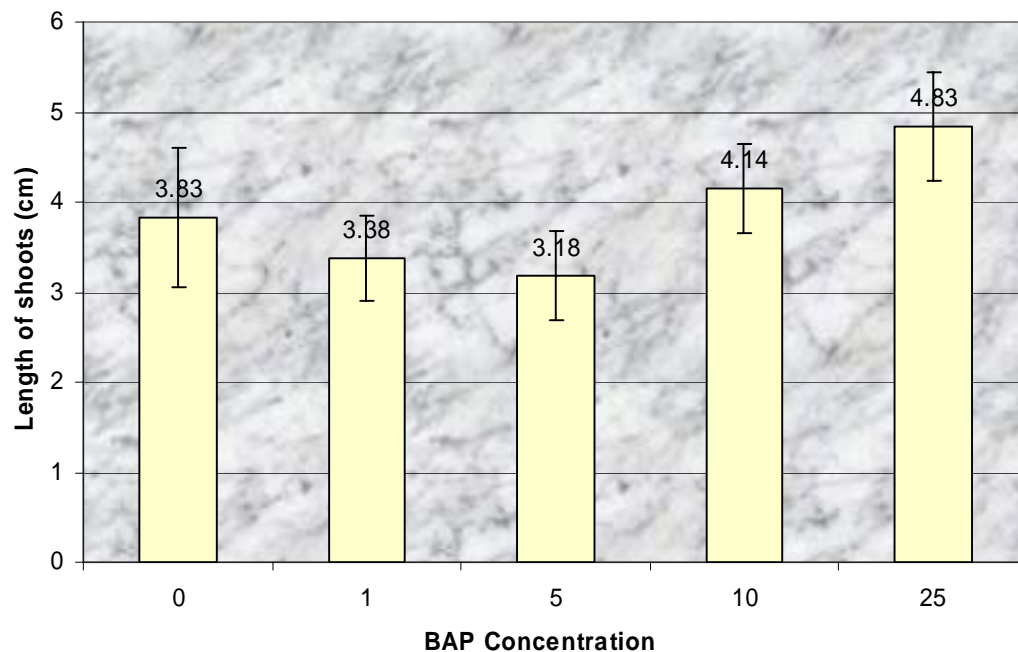


**Figure 4.** Total number of shoots from apical shoot explants exposed to various cytokinin regimes. Recorded after eight weeks.



### *Length of plantlets*

There was a tendency for height to decrease with an increased concentration of zeatin and kinetin (Fig. 5). However plantlets grown on media with different concentrations of BAP showed no significant difference in shoot length on the trialed media ( $p < 0.001$ ), and they were about 5 cm long on the medium with 25  $\mu\text{M}$  BAP.



**Figure 5.** Mean shoot length of apical shoot explants, harvested from the previous experiment, and exposed to various cytokinin regimes. Recorded after eight weeks.

### **Acclimatisation and *ex vitro* planting**

There was a 75% survival rate after six weeks when plantlets were transplanted to glasshouse conditions. Roots formed on clumps of shootlets

that had no roots when first transplanted.

## DISCUSSION/CONCLUSIONS

### (i) Seed germination

#### *Viability*

Viability and germination percentages were fairly similar for lot 1, which had already abraded seed coats. However the colour intensity of the staining was highly variable, and it is recommended that refinements in technique be made when future physiological studies are conducted. For seeds in lot 2, with intact seed coats, the high viability compared to actual germination indicated the existence of dormancy.

#### *Dormancy and scarification*

The very marked results of the various nicking experiments reported here, indicate that primary dormancy of *S. centrale* seed is imposed by the seed coat. In general, the seed coat can be a physical barrier to water penetration, or it can contain germination inhibitors (Kelly *et al.*, 1992). In nature, seed coat-imposed dormancy could be broken by rupture of the seed coat caused by: changes in temperature and humidity, seeds moving along the soil surface and being abraded against the soil particles (Mott and Groves, 1981), passing through an animal's digestive system, or removal of inhibitors through leaching (Jurado and Westoby, 1992). Anecdotal evidence suggests that bush tomatoes occur more frequently in areas where the soil has been disturbed, such as roadsides, where abrasion is more likely than in other parts of the terrain. Germination of the seeds of the related *Solanum luteum* was significantly improved

after ingestion by birds, electron microscopy revealing abrasion of the seed coat (Barnea *et al.*, 1990). Physical scarification of *Emmenanthe penduliflora*, a native of the California chaparral, induced 100% germination, but the effect was not due to penetration of a water barrier, or to enhanced oxygen uptake or to wound responses (Keeley and Fotheringham, 1998).

#### *Leaching / Soaking*

Germination of *S. centrale* was found to be promoted by leaching (12 or 24 h) or, in one experiment, soaking (4, 7 or 12 h). Germination occurred more quickly for the seeds leached for 12 and 24 h, and soaked for 12 h than for all other treatments. Soaking and leaching are thought to promote germination either because they remove inhibitors from the seed coat (Kelly *et al.*, 1992), or because the moisture initiates metabolic processes (Bewley and Black, 1978). Washing has been shown to advance germination by 5h in the related species *Solanum laciniatum* (Lush *et al.*, 1980). Ahmad *et al.* (1998) found that soaking for 12 h promoted germination of 10 wheat varieties, but that soaking beyond 12 h did not increase germination, and beyond 21 h germination was significantly reduced.

Soaking did not promote germination of seeds in some of the experiments, and in the scarification trials, soaking significantly reduced total germination for two out of four seedlots. Soaking for 24 hours produced lower germination than soaking for 12 hours. This is due to likely damage to the seed or seed membrane, caused by potential physiological mechanisms, such as ethanol toxicity, oxygen deprivation, and carbon dioxide accumulation (Wuebker *et al.*, 2001).

### *Heat*

Heat treatments were found to have no effect on the germination of bush tomato. Heat shock tends to promote germination in species with seed-coat imposed dormancy, most commonly reported with the water-impermeable seeds of Fabaceae (eg. Auld and O'Connell, 1991). Mortality will also occur at high temperatures in species, especially in seeds without the protection of a hard seed coat (Bell *et al.*, 1987). However, high temperatures of 120°C did not kill *S. centrale* seeds in the Series 1 experiment on heat, soaking and leaching.

### *Smoke*

Smoke enhanced the total germination of three of the six seedlots tested. A positive germination response to smoke appears to be a common phenomenon in the fire-adapted Australian flora. Almost half of the 94 native Western Australian species tested by Dixon *et al.* (1995) responded to the application of smoke. However, three of the six seedlots did not respond to smoke, which again is in line with the results of Dixon *et al.* (1995), who found that seeds of different age or provenance have a different response to smoke.

O'Connell *et al.* (1983) reported that bush tomato populations were dependent on fire (also noted in Latz, 1995). The report was based on the observation that a colony of 100 plants was bearing an average of 80 large fruit per plant one year after a fire, compared with only 10 plants remaining, bearing an average of 20 fruit each, three years after the fire. Unfortunately, pre-fire data were not recorded. If fire does promote growth of *S. centrale*, the results show that it may be because of smoke promoting seed germination.

### *Temperature*

Temperatures within the normal seasonal range for this species had no significant effect on the speed of germination or final percentages, indicating that this species can germinate year round, possibly by responding to moisture cues. This is in line with the findings of Jurado and Westoby (1992) who reported that 30% of the central Australian desert species they studied had no seasonal (temperature related) germination tendency. In contrast, however, they found in the same study that the related species, *Solanum quadriloculatum*, had an optimum germination temperature of 28°C. It might be useful to investigate whether alternating temperatures can help promote germination in bush tomato, as it does for *Spinifex hirsutus* Labill. (Harty and McDonald, 1972) and two other *Solanum* species (International Seed Testing Association, 1985).

### *Speed of germination*

In almost all cases, germination began to occur shortly after contact with water, and in one case germination was observed after 5 h. Fast germinability in low numbers is a strategy noted in central Australian species, as a way of spreading risk across several rainfall events (Jurado and Westoby, 1992).

### *Salinity*

Bush tomato seeds showed considerable salinity tolerance, some germination occurring in solutions of up to 200 mM NaCl. However, overall, 0 and 25 mM NaCl produced the highest total germination percentages and 200 mM NaCl the lowest. The inhibitory effects of salinity on germination are due to osmotic effects and in some cases to chloride toxicity, or a combination of both (Bell, 1999). Some halophytic species can germinate at very high salinities, for example, *Juncus maritimus* can germinate in salt solutions of 600 mM NaCl

(Rozema, 1975) and *Suaeda moquinii* at 1000 mM NaCl (Ajmal Khan *et al.*, 2001), and higher ranges remain to be tested for *S. centrale*. Similar results to those presented here have been found for other members of the Solanaceae, with eggplant (*Solanum melongena*) (Chartzoulakis and Loupassaki, 1997) and capsicum (*Capsicum annuum*) (Chartzoulakis and Klapaki, 2000) germinating in salinities up to 150 mM NaCl, though germination was reduced at this level.

There were different responses to salinity between provenances, and it is possible that is related to the differences in prevailing soil salinities at the collection sites. Seeds of some *Eucalyptus* and *Melaleuca* species from populations that grew in relatively saline soil (2.6 mS/m) had higher salt tolerance than those from low or non-saline soils (van der Moezel and Bell, 1987). However, Pearce-Pinto *et al.* (1990) found no relationship between salinity tolerance at germination and the level of topsoil salinity at the seed collection site of Western Australian *Eucalyptus* species. These authors also found that there was no relationship between salinity tolerance at germination and salinity tolerance at the seedling stage. It cannot be automatically extrapolated from the results presented here that *S. centrale* would be salt tolerant during later growth stages; further work is needed on the field salinities encountered by this species, and its salinity tolerance at all stages of growth.

#### *Significance of findings*

From the results of this investigation, it can be concluded that the primary dormancy of bush tomato seed is imposed by the seed coat. The scarification trials produced strong evidence that this is due to physical means (seed coat impermeable to water). Once the seed coat is partially or totally removed, germination levels can approach maximal viability determined through

tetrazolium testing. The commercial practice of hammer milling bush tomato fruit to remove the seed is to some extent beneficial, in that it abrades the seed coat, however, it also causes destruction of some of the seed stock. Scarification can also be achieved by mechanical scarifiers, by immersion in boiling water (Australian Tree Seed Centre and Mortlock, 1999) or using acid (Keeley and Fotheringham, 1998).

Germination can occur equally well over a wide range of temperatures, while heat shock application had no effect. Soaking or leaching promoted germination in two out of five experiments. Smoke enhanced the total germination in three of the six seedlots tested. Growers should test seedlots for a smoke response before proceeding with smoke treatments.

The seeds of all seedlots tested showed significant salinity tolerance, germinating in solutions of up to 200 mM NaCl; however 0 and 25 mM NaCl produced the highest total germination percentages. The species therefore has potential for development as a crop that can be grown with soils or waters of higher than ideal salinities. However more trials at different stages of the life cycle are required before this possibility can be confirmed.

#### **(ii) Plant Tissue culture**

While the aim of this investigation was to develop micropropagation procedures for *S. centrale*, the longer term aim is to enable the selection of clones based on desired characteristics. The results obtained are encouraging; since the first experiment to determine the plant media and explant type for callus and bud initiation showed that callus and shoots could regenerate on all the three explant types and four types of media trailed. This suggests that this species is amenable to manipulation by tissue culture,

which could be used to accelerate commercial cultivar development of promising lines. Half strength MS (Murashige and Skoog 1962) with B5 vitamins (Gamborg *et al.* 1968) with BAP at 5  $\mu$ M and IAA at 1  $\mu$ M was best for producing roots and the same base medium with 2  $\mu$ M BAP was significantly better for all other growth measurements. Since the number of shoots from apical bud culture was significantly higher than with other explant types, this material was chosen for the cytokinin range finding trial.

The results are in line with the results of previous studies on this family. Investigations conducted on a range of *Lycopersicon* species, have shown regeneration capacity in explants derived from both seedling germination tissues (hypocotyls and cotyledons) (Javahar *et al.* 1997, 1998) and leaf mesophyll (Pindel *et al.* 1998, Chandra *et al.* 1995). In another study, tomato cultivars 'Potentat' and 'Rutgers', and wild tomatoes *Lycopersicon peruvianum*, *L. peruvianum* var. *dentatum* and *L. glandulosum* regenerated from leaf fragments taken from plants raised in a glasshouse (Lech *et al.* 1996). Callus tissue was found to form readily from stem and leaf explants of aseptically germinated seedlings of *Solanum aviculare* and *S. lacinatedum* (Macek 1989) and from leaf and nodal segments of older plants of these species.

### **In summary**

This study shows the good regeneration capacity of *S. centrale* and hence the promising potential for the use of *in vitro* technology in the development of this species. Growers can now access new useable germination and tissue culture techniques for the production of bush tomato plants and the development of improved varieties. For example, the variation between populations in fruit flavour and sweetness can now be exploited by tissue culture technology. If growers choose to use micropropagation, they will be able to select which plants



to clone, based on characteristics such as flavour, colour, fruit size and seed/flesh ratio, and in due course other qualities, such as flower/fruit abundance, cultivation requirements, post-harvest handling and the like.

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