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The effect of salt stress and abscisic acid on proline production, chlorophyll content and growth of *in vitro* propagated shoots of *Eucalyptus camaldulensis*

Andrew J. Woodward & Ian J. Bennett*
*School of Natural Sciences, Centre for Ecosystem Management, Edith Cowan University, Joondalup Drive, Joondalup, Western Australia 6027, Australia (*requests for offprints; Fax: +61-8-6304-5509; E-mail: i.bennett@ecu.edu.au)*

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Abstract

Three clones, selected for their variation in salt tolerance, were examined regarding their growth and physiological responses on exposure to salt (NaCl) and abscisic acid (ABA) *in vitro*. The shoot proline levels significantly increased in two salt tolerant clones when exposed to 100 mM NaCl in the shoot multiplication medium. In contrast, proline in a salt sensitive clone did not change in comparison to the control treatment. When 10 μM ABA was included in the medium all clones had an increase in proline regardless of whether they were salt tolerant or salt sensitive, linking proline production to the stress hormone ABA. Callus production was so variable that it was not possible to produce callus of consistent texture, colour and growth for all three clones. For the two clones where consistent growth was achievable, both the salt tolerant and salt sensitive clones increased proline production when exposed to salt. This response, however, was greater in the salt tolerant clone. Other parameters examined were growth (dry weight) and shoot chlorophyll content. These characteristics did not correlate with the salt tolerance of the clones, with similar weights being produced on non salt and salt media and similar chlorophyll in both salt sensitive and salt tolerant clones regardless of the medium in which they were grown. The production of proline is considered with regard to selection for differences in salt tolerance *in vitro*.

Abbreviations: ABA – abscisic acid; MS – Murashige & Skoog; NAA – naphthalene acetic acid

Introduction

Many attempts have been made to produce salt tolerant plants using tissue culture. This has included using a number of systems (i.e. callus, suspension culture and shoot culture) to screen for cells and tissues that show variation in their ability to tolerate relatively high levels of salt (NaCl) in media. Investigators have concentrated on agricultural species with some success (at least initially) in plants such as legumes (Smith and McComb, 1983; Winicov, 1991; Johnson and Smith, 1992), tomato (Rus et al., 2000), and rice (Lutts et al., 1999). Often plants regenerated from such systems fail to exhibit their salt tolerance when regenerated into whole plants or when grown in soil (Gonzales, 1994; Flowers, 2004).

Examination of salt tolerance and the production of osmolytes in tissue culture has gained attention in recent years. In particular, the role of proline in salt tolerance of agriculturally important crop plants such as alfalfa (Petrusa and Winicov,

1997), wheat (Kong et al., 2001), soybean (Liu and van Staden, 2000), rice (Basu et al., 2002), beans (Gadallah, 1999), and tobacco (Kuznetsov and Shevyakova, 1997) has been examined. Many varieties identified as salt tolerant have produced higher levels of proline (compared to salt sensitive varieties) when exposed to salt.

Response of woody species to salt exposure in tissue culture has received less attention. This area is gaining recognition due to the roles that tree species play in alleviating some soil salinity problems throughout the world. The potential of physiological responses to differentiate between salt sensitive and salt tolerant species or individuals is now being examined. Examples include poplars (*Populus euphratica* and *P. alba* cv. *Pyramidalis* × *P. tomentosa*; Watanabe et al., 2000) and grapes (Singh et al., 2000). In both cases the authors suggest that proline production might be used to identify salt tolerant varieties or clones. Eucalypts have also been examined for several aspects of salt tolerance *in vitro*. Shoot cultures of salt tolerant *Eucalyptus microcorys* were able to withstand higher levels of salinity than salt sensitive shoots, the salt tolerant shoots withstanding up to 150 mM NaCl (Chen et al., 1998; Keiper et al., 1998). Shoots exposed to levels of 150 mM NaCl produced more proline than for controls, but levels of other osmolytes (glycine betaine, choline) were not elevated. Morabito et al. (1994) examined the response of *E. microtheca* clones to salinity in tissue culture and found that survival and physiological responses were mixed, with one clone showing increased survival, while others showed less change in water potential.

Of considerable use for the development of salt tolerant eucalypts would be the availability of a technique that could identify salt tolerance within species. An examination of the physiological responses shown within a species by individual clones may provide the capacity to do this without the need to conduct extensive glasshouse and field trials (Niknam and McComb, 2000). Looking at such a response *in vitro* may also assist in providing information about the salt tolerance mechanisms, leading to further capacity to increase salt tolerance. We report here the responses of three *E. camaldulensis* Dehnh. clones, selected for varying degrees of salt tolerance, to exposure to salt in tissue culture.

Materials and methods

Shoot cultures

Shoot material was obtained from cultures established from a programme investigating the selection and salt tolerance of *Eucalyptus camadulensis* Dehnh. (Bell et al., 1994). The cultures had been stabilised (McCown, 2000) through continuous subculture for at least 18 months before these experiments were conducted and three clones were used; 919 (salt sensitive), 66 (salt tolerant) and 502 (salt tolerant). Shoots were grown on a medium modified from Bennett and McComb (1982) that contained Murashige and Skoog (MS; 1962) nutrients, vitamins and inositol, 0.1 μM naphthalene acetic acid (NAA), 2.5 μM benzyl aminopurine, 2% sucrose, 2.5 g l^{-1} agar, 2.5 g l^{-1} gelrite and the pH adjusted to 5.8 before autoclaving. The cultures were maintained in a photoperiod of 16-h light (90 μmol m^{-2} s^{-1}) and 8-h dark at 24 ± 1 °C. For experimental treatments, even sized shoot clumps approximately 2 cm in height were transferred to the treatment media and maintained for 4 weeks in the above environmental conditions.

Callus cultures were initiated from leaf explants taken from the *in vitro* grown shoots. Callus was induced on a medium with the same composition as for shoot cultures but with the plant growth regulators changed to 5 μM NAA and 5 μM 2,4-dichlorophenoxyacetic acid. These cultures were maintained in complete darkness at 24 ± 1 °C.

Experimental treatments

To examine proline production over a subculture period (28 days), shoots of the three clones were grown on media containing different levels of NaCl. Initially this tested media containing 50 and 100 mM NaCl with media containing no salt (except that which exists in MS) used as a control. Subsequently, two clones (502 and 919) were grown on the same media with the additional parameters of chlorophyll content and biomass (dry weight) also determined. Lastly, a comparison between the three clones in proline production, chlorophyll content and biomass was conducted by growing shoots on a control medium (no additional NaCl) and a medium containing 100 mM NaCl. For the

initial experiment proline was determined weekly for 28 days. For the subsequent two experiments proline was measured weekly for 28 days and chlorophyll and biomass measured when the experiments were terminated at 28 days.

The effect of NaCl and abscisic acid (ABA) on proline production was investigated. The level of 100 mM NaCl was used as the experimental variable to investigate the effect of salt, 10 μM ABA was the concentration used to determine the effect of this hormone, with media containing no salt or ABA used as a control. All three clones were used; 502, 066 and 919. Proline was determined weekly for 28 days and chlorophyll content and dry weight were determined after four weeks.

Proline production in callus was examined by growing callus from two clones (919 and 66) on media containing 50 and 100 mM NaCl and a medium containing no salt as the control. Each callus piece was approximately 25 mm^2 and was selected from stock for consistency in appearance and texture. Despite numerous attempts it was not possible to obtain such consistent callus from clone 502.

Proline measurement

Proline content was determined according to the method of Bates et al. (1973). Proline was extracted from approximately 0.5 g of shoot or callus by grinding in 10 ml of 3% sulfosalicylic acid. Two millilitres of extract was reacted with 2 ml acid-ninhydrin and 2 ml of glacial acetic acid for 75 min at 100 °C. An ice bath was used to terminate the reaction. The reaction mixture was extracted with 4 ml of toluene and vortexed. Absorbance of the toluene layer was read in a spectrophotometer at 520 nm and proline concentration determined from a standard curve. Proline shoot content was calculated by: (μg proline in extract/115.5)/g sample = μmoles proline g^{-1} fwt.

Chlorophyll measurement

Chlorophyll content was determined using methods developed by Moran and Porath (1980). Whole shoot clumps were soaked in 5 ml of dimethyl formamide for 24 h (in the dark) and absorbance (ABS) read at 664 and 647 nm to provide total chlorophyll ml^{-1} and shoot chlorophyll content calculated by: $((ABS_{664} \times 7.04) + (ABS_{647} \times 20.27)) \times 5$/sample weight (g) = μg chlorophyll g^{-1} fwt.

Statistical analysis

Statistical analysis was conducted by ANOVA using SPSS (version 11). The effect of treatment × clone was tested by two way ANOVA for proline, chlorophyll content and shoot biomass (dry weight). Where there was a significant clonal effect individual clones were tested using one-way ANOVA and Tukey's multiple range test was used to determine differences between treatments within clones. Where variances between treatments were significantly different using Levene's test ($p = 0.05$) a natural log transformation was performed. Replicates for proline measurement were from 6 to 8 (per week) and 8 for chlorophyll and biomass determination.

Results

NaCl in the medium

There was neither an increase in shoot proline levels nor a difference between clones in proline concentration for the first 3 weeks of culture (Figure 1). However, after 4 weeks the proline significantly increased, with values varying for each clone. Clone 919 had between 3.2 ± 0.4 and 2.3 ± 0.3 μmol proline g^{-1} fresh weight (μmol pro g^{-1} fwt), clone 502 had between 1.4 ± 0.2 and 4.6 ± 0.4 μmol pro g^{-1} fwt and clone 66 had between 2.0 ± 0.4 and 4.3 ± 0.5 μmol pro g^{-1} fwt. The trend of a significant increase in proline accumulation in week 4 (Figure 1), and sometimes at week 3, was observed in all subsequent experiments.

There was no difference in proline content between the control and the 50 or 100 mM salt treatments for the salt sensitive clone (919; Figure 1a). However, for the two salt tolerant clones, shoots from both the 50 and 100 mM salt treatments had significantly higher amounts of proline than the control treatment after 4 weeks. Clone 502 produced 1.4 ± 0.2 in the control treatment and 3.6 ± 0.7 and 4.6 ± 0.4 μmol pro g^{-1} fwt in the 50 and 100 mM salt treatments respectively (Figure 1b). Similarly, clone 66 produced 2.0 ± 0.4 in the control treatment and 3.7 ± 0.3 and 4.3 ± 0.5 μmol pro g^{-1} fwt at the 50 and 100 mM salt treatments, respectively (Figure 1c).

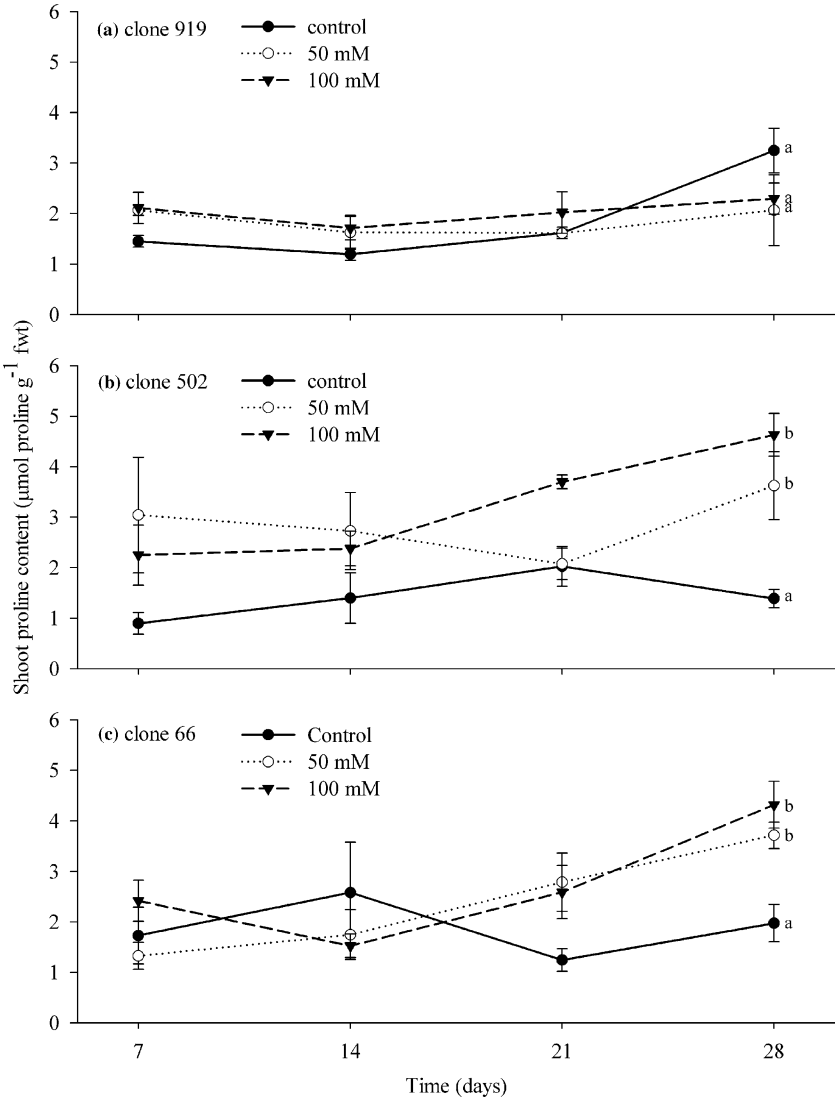

Figure 1. The effect of 50 and 100 mM NaCl on shoot proline accumulation over 28 days for (*a*) one salt sensitive clone 919 and two salt tolerant clones, (*b*) 502 and (*c*) 66 of *E. camaldulensis* in tissue culture. Vertical bars are standard errors. Values at week four followed by the same letter are not statistically different from each other ($p < 0.05$).

In each of the subsequent experiments there was a significant difference in proline production between the clones. Clone 919 consistently produced more proline with time with a significant difference between weeks 1–3 and week 4 (between 2.3 ± 0.7 and 3.4 ± 0.6 µmol pro g^{-1} fwt). There was, however, no difference due to the salt treatments with shoots grown on the control medium producing 3.4 ± 0.6, on 50 mM NaCl producing 2.3 ± 0.7 and on the 100 mM NaCl treatment producing 2.4 ± 0.4 µmol pro g^{-1} fwt (Figure 2a). When only two levels of salt were examined there was a reduction in proline content in shoots growing on the 100 mM NaCl medium (Figure 3a). For clone 502, there was consistently an increase in proline shoot content (2–2.9 times compared to the control) when exposed to NaCl in the medium. This varied slightly with NaCl concentration where in some trials the 50 mM NaCl treatment produced a significant increase in proline (Figures 1b and 3a) while in others it produced levels of proline intermediate of the control and the 100 mM NaCl treatment (Figure 2a). Clone 66 had significantly higher levels of proline

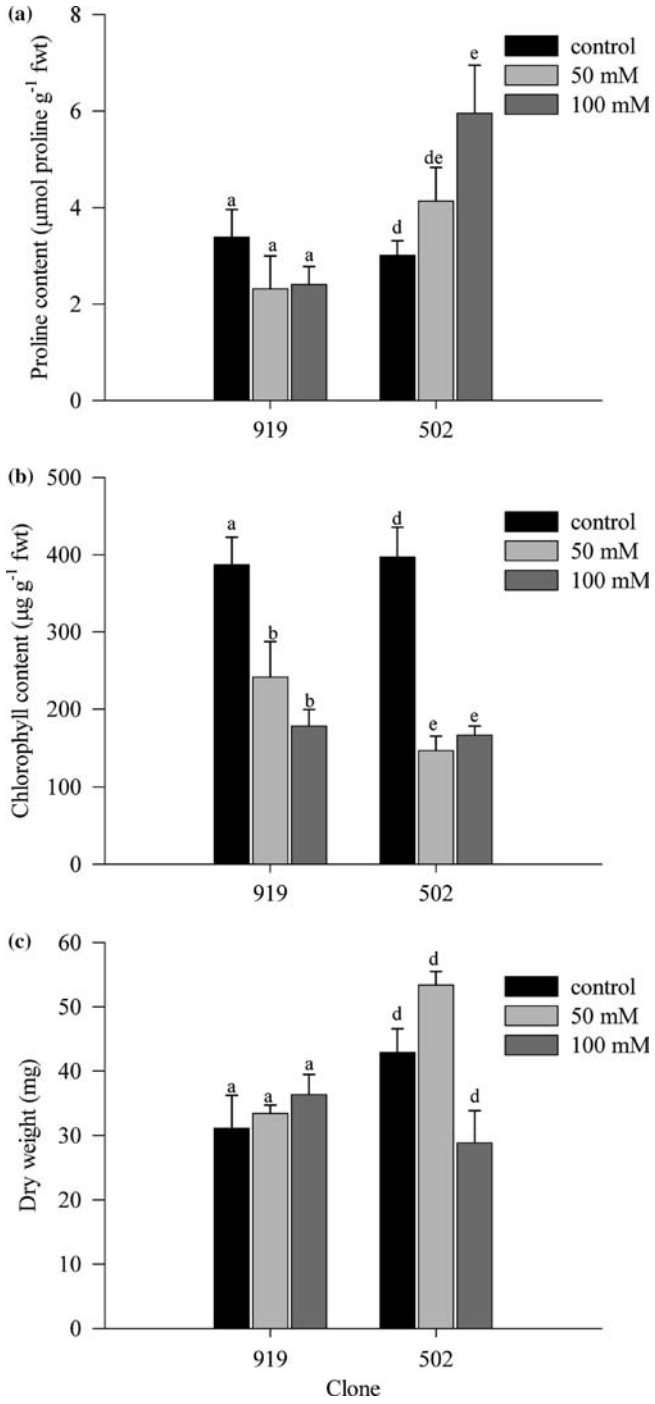

Figure 2. The effect of 50 and 100 mM NaCl on (*a*) shoot proline accumulation, (*b*) shoot chlorophyll content and (*c*) shoot dry weight for two clones, 502 (salt tolerant) and 919 (salt sensitive), of *E. camaldulensis* in tissue culture. Vertical bars are standard errors. Values within clones with the same letters are not statistically different from each other ($p < 0.05$).

on the control media than either clone 919 or 502. As with clone 502, this clone produced higher shoot proline content when exposed to salt. The level of increase, however, was not as great as for clone 502 and ranged from 1.4 (Figure 1c) to 1.8 (Figure 3a) times increase in shoot proline content.

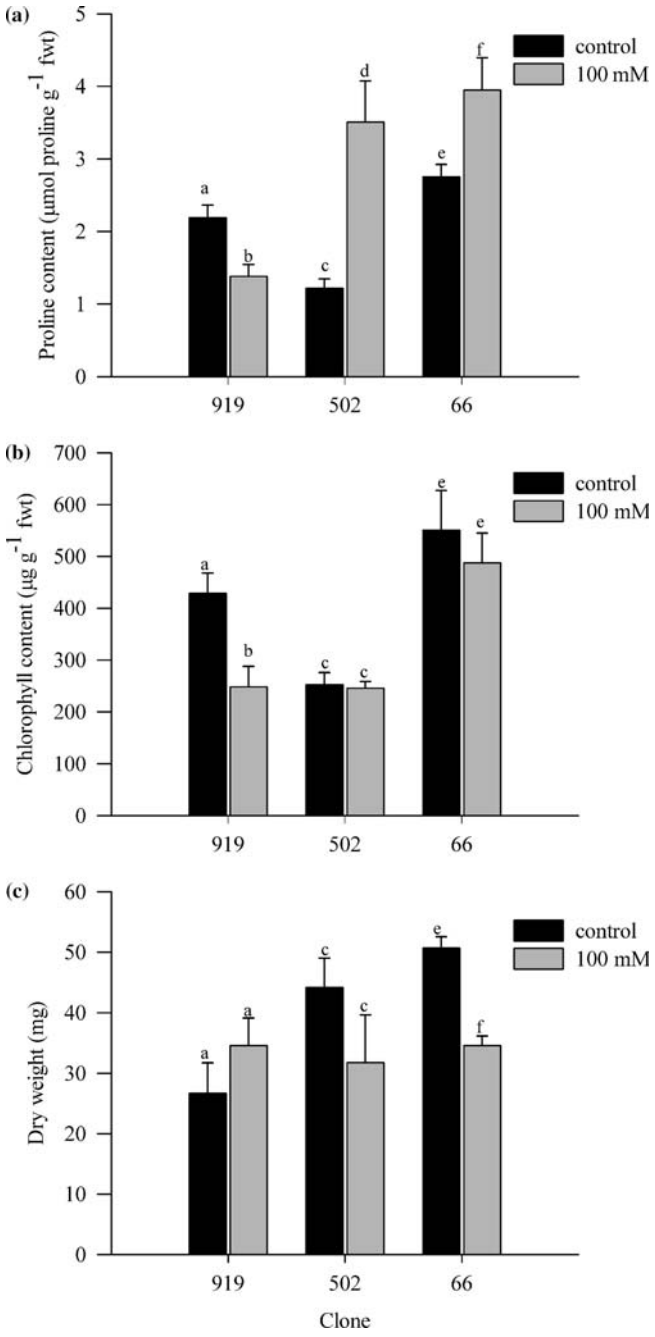

Figure 3. The effect of 100 mM NaCl on (*a*) shoot proline accumulation, (*b*) shoot chlorophyll content and (*c*) shoot dry weights for three clones, 66 and 502 (salt tolerant) and 919 (salt sensitive), of *E. camaldulensis* in tissue culture. Vertical bars are standard errors. Values within clones with the same letters are not statistically different from each other ($p < 0.05$).

Shoot chlorophyll content varied considerably between different experiments. Initially the 2 clones tested showed a decrease in chlorophyll related to the presence of NaCl in the medium (Figure 2b). In subsequent trials the two salt tolerant clones showed no effect of NaCl on chlorophyll content while there was a significant reduction in the levels in the salt sensitive clone

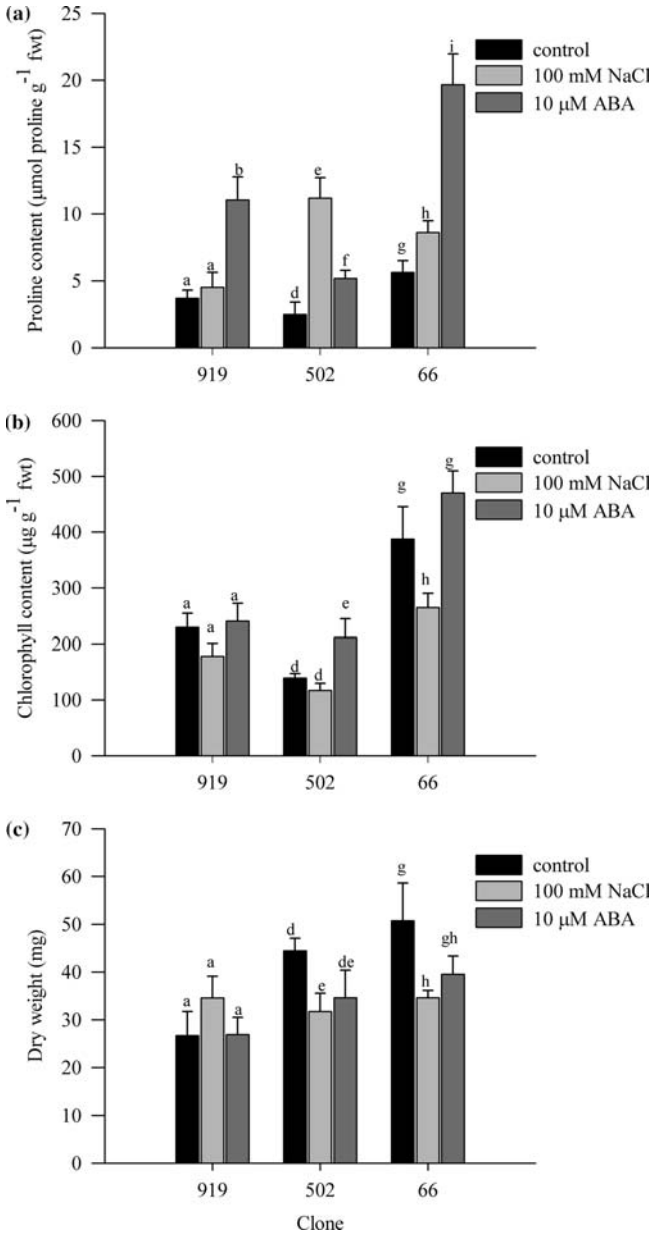

Figure 4. The effect of 10 µM ABA and 100 mM NaCl on (*a*) shoot proline accumulation, (*b*) shoot chlorophyll content and (*c*) shoot dry weight for three clones, 502 and 66 (salt tolerant) and 919 (salt sensitive), of *E. camaldulensis* in tissue culture. Vertical bars are standard errors. Values within clones with the same letters are not statistically different from each other ($p < 0.05$).

from 429 ± 39 µg chl g^{-1} fwt on the control medium to 248 ± 39 µg chl g^{-1} fwt on the 100 mM NaCl medium (Figure 3b).

There was no significant difference in dry weight between clones (Figures 2c and 3c). Shoots of clone 66 on control media weighed significantly more than for salt treatments. There was no effect of the salt treatment on either clones 502 or 919 (Figures 2c and 3c).

ABA and NaCl in the medium

Shoots of the salt sensitive clone (919) grown on control medium (3.7 ± 0.6 µmol pro g^{-1} fwt)

had no difference in proline level when compared to shoots grown on media containing 100 mM NaCl (4.5 ± 1.2 μmol pro g^{-1} fwt). However, proline did increase when shoots were grown on 10 μM ABA medium (11.1 ± 1.7 μmol pro g^{-1} fwt; Figure 4a). Shoots of both the salt tolerant clones produced more proline when grown on 10 μM ABA and 100 mM NaCl. Shoots of clone 502 produced twice the amount of proline on 10 μM ABA (5.2 ± 0.6 μmol pro g^{-1} fwt) and four times the amount on 100 mM NaCl (11.2 ± 1.5 μmol pro g^{-1} fwt) medium than they did when grown on control medium (2.5 ± 0.9 μmol pro g^{-1} fwt). The response in clone 66 was similar. This clone had a greater increase in proline on the ABA medium (4 times; 19.7 ± 2.3 μmol pro g^{-1} fwt) but less than twice as much on 100 mM NaCl (8.6 ± 0.9 μmol pro g^{-1} fwt) than for shoots on the control medium (5.6 ± 0.9 μmol pro g^{-1} fwt).

The media treatments did not affect the chlorophyll content of the salt sensitive clone (919) (Figure 4b). Shoots of the salt tolerant clone 502 contained significantly more chlorophyll when grown on 10 μM ABA (212 ± 33 μg chl g^{-1} fwt) than they did when grown on control medium (139 ± 8 μg chl g^{-1} fwt). However, there was no effect of 100 mM NaCl on chlorophyll content

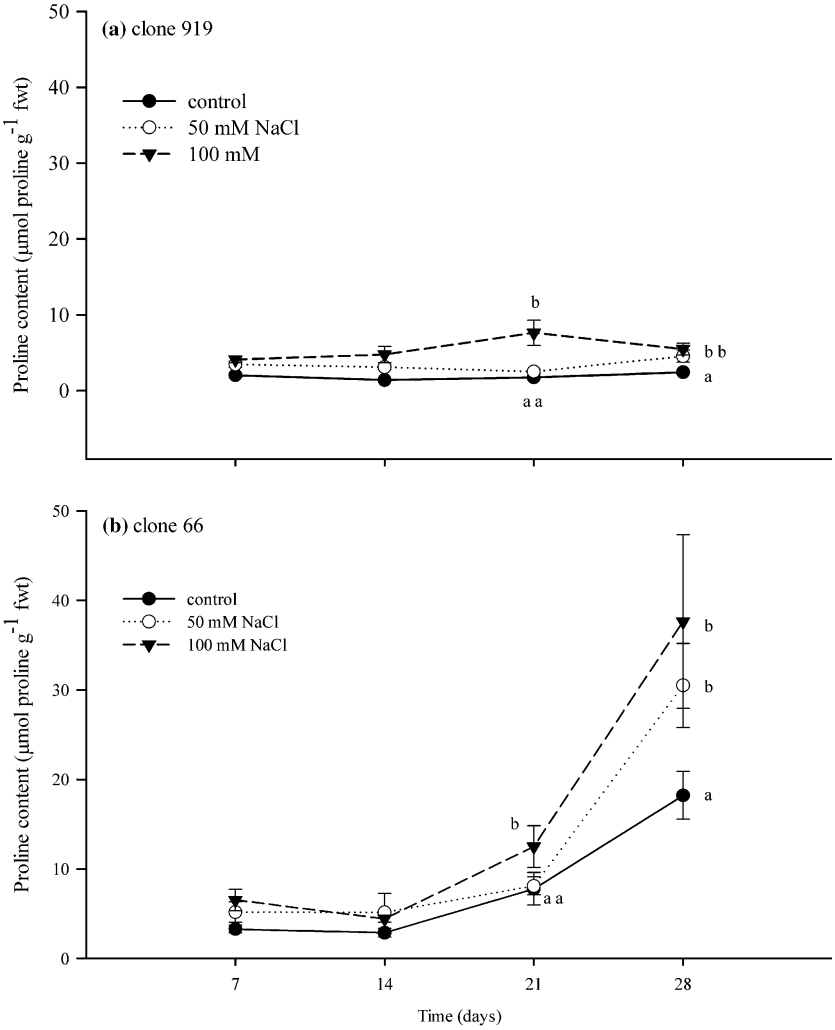

Figure 5. The effect of salinity on callus proline production for two clones (*a*) 919 (salt sensitive) and (*b*) 66 (salt tolerant). Vertical bars are standard errors. Values at weeks three and four followed by the same letter are not statistically different from each other ($p < 0.05$).

(118 ± 13 μg chl g^{-1} fwt) for this clone. Shoots of the other salt tolerant clone (66) contained significantly less chlorophyll when grown on salt medium (265 ± 25 μg chl g^{-1} fwt) than the control (387 ± 58 μg chl g^{-1} fwt), but there was no effect of 10 μM ABA on chlorophyll content (470 ± 40 μg chl g^{-1} fwt).

There was no effect of either 10 μM ABA or 100 mM NaCl on dry weights of the salt sensitive clone (919) (Figure 4c). Dry weights for shoots of the salt tolerant clone 502 were significantly less when grown on 100 mM NaCl than on the control medium and there was no effect of 10 μM ABA on dry weight for this clone. There was a similar effect for the other salt tolerant clone (66) with shoots of this clone also weighing significantly less on 100 mM NaCl than on the control, and no effect of 10 μM ABA on dry weight.

NaCl and callus proline production

There was a significant difference in proline production between the two clones, with callus of clone 66, producing more proline than clone 919 (Figure 5). For clone 919 more proline was produced in the 100 mM NaCl treatment than the control and the 50 mM NaCl treatment after 21 days (Figure 5a). By 28 days, callus grown on salt media (50 mM 4.5 ± 0.8 μmol pro g^{-1} fwt; 100 mM 5.5 ± 0.8 μmol pro g^{-1} fwt) contained significantly more proline than the control (2.4 ± 0.1 μmol pro g^{-1} fwt). There was no significant increase in the amount of proline produced from 7 to 28 days. Similar trends were seen for clone 66, where there was a difference in proline levels developing after 21 days (Figure 5b). By 28 days this difference was more pronounced, callus on 100 mM (37.6 ± 9.7 μmol pro g^{-1} fwt) contained significantly more proline than the control (18.2 ± 2.7 μmol pro g^{-1} fwt), but similar amounts to the 50 mM NaCl treatment (30.5 ± 4.7 μmol pro g^{-1} fwt). For this clone callus at 28 days produced significantly more proline than for the other three sampling periods.

Discussion

Growth parameters

The different growth parameters used clearly had varying capacities to differentiate between the salt tolerant and salt sensitive clones. While observational differences were apparent in the salt sensitive clone as the leaves showed browning symptoms as cultures progressed, these did not always transfer to a quantitative difference in chlorophyll content. Other reports examining the effects of salts in media on tissues *in vitro* suggest that chlorophyll is an appropriate measure (Singh et al., 2000; Santos et al., 2001), its inconsistency in these examples suggests it may not be an appropriate measure by itself. The variation did not only occur within the salt sensitive clone, but also in the salt tolerant clones and the timing of measurement may be important. Clearly the differences repeatedly seen in proline production from 21 to 28 days show that this may be the time period over which the shoots become stressed due to exhaustion of available resources within these closed systems. Measurement of chlorophyll over the culture period, as was done with proline, may provide a better picture of what might be happening.

Similarly, there was no clear differentiation through measurement of biomass (dry weight). There was a significant reduction in dry weight for the salt tolerant clones in some trials, but this was not consistent. The salt sensitive clone always showed an apparent increase in dry weight, but this was never significant. If there is some part of the salt response mechanism missing in the salt sensitive plant, it would explain why there was no reduction in growth, while there was for the salt tolerant. It would be useful to see what happened with this clone over a longer period, as it appears that it has continued to grow regardless of the salt in the medium, and may suffer high mortality after longer exposure to salt (Chen et al., 1998; Rus et al., 2000).

Proline in shoot cultures

A number of roles have been proposed for proline in salinity tolerance (Hare et al., 1998). One possibility is that it acts as a store of energy that can be rapidly broken down and used when the plant is relieved of stress. Another is that it acts as an osmolyte and reduces the osmotic potential of the cell, thus reducing toxic ion uptake (Hare et al., 1998). In this case, the latter is more likely, with the salt tolerant plants not only producing more proline when stressed, but also having (in most

cases) no significant drop in the chlorophyll content. This indicates that the increase in proline is reducing the physiologically detrimental effects of the salt (Delauney and Verma, 1993; Hare et al., 1998).

As well as differentiating between salt tolerant and salt sensitive clones, there is also a clear difference between the two salt tolerant clones even though both clones always produced significantly more proline on salt media than for the control. Where the two clones were grown together, clone 66 had a higher background level of proline but a lower increase when exposed to 100 mM NaCl; 1.4–1.8 times. Clone 502 had lower background levels and generally a greater increase in proline when exposed to salt (2–3.2 ×). Morabito et al. (1994) had a similar finding with *E. microtheca*; clones selected for their salt tolerance using conventional means produced varied amounts of proline when salt stressed. It is possible that the level of salt in the medium (100 mM) was not sufficient to produce such an increase in proline for clone 66, but was enough for clone 502. This could indicate that other physiological aspects of clone 502 are reducing the effect that the salt has on the clone's physiology, and that it did not need to produce higher levels of proline. If a clone has a higher background (i.e. not stressed) then this could be an indicator of natural tolerance. Clone 66, for example, showed a salt tolerant response by producing more proline when grown on salt media, and is more tolerant than clone 502 because it did not need to increase proline content as much. Further testing of these clones is required to determine whether it is a higher background level of proline or the ability to produce more proline when stressed that is associated with higher salt tolerance. In addition, given the different clonal responses, an examination of many clones is warranted. We are currently screening seedlings under glasshouse conditions to obtain such clones. Proline as an indicator of tolerance may provide a marker that allows better differentiation between varieties or clones (Niknam and McComb, 2000; Flowers, 2004).

A marked increase in proline content in the 4th week was observed in most trials, and for all clones. While proline production is most frequently linked to drought and salt stress, it is well recognised that it is also produced in response to various other stress factors such as temperature (Kuznetsov and Shevyakova, 1997; Hare et al., 1998). A tissue culture vessel is a sealed system, and must be subcultured on a regular basis due to the build up of toxic compounds in the medium and a reduction in availability of sucrose and other nutrients (George, 1993). This build-up may have been responsible for the increase seen after 4 weeks. It may also be a useful tool in assisting in explaining the role that proline may have under stress conditions. The shoots were not water stressed or salt stressed (in control containers) yet still produced extra proline as the culture period extended. Closer examination could be useful in differentiating between responses that are clearly linked to salt and water stress and those that relate to other stresses where proline production is induced. The salt sensitive clone(s) may be most useful here. It can produce proline, what is the trigger?

ABA on proline production

It has been proposed that ABA is the hormone responsible for inducing proline production in stressed plants (Jia et al., 2002; Makela et al., 2003) and the effect of the exogenous application of ABA on proline production has been examined (Yang et al., 2000). Other studies have also looked at the application of substances to whole plants to confer salt tolerance. For example, Shalata and Neumann (2001) found that ascorbic acid added to the root medium of tomato seedlings increased a plant's ability to tolerate saline conditions. This response to exogenous application may have been useful in differentiating between salt sensitive and salt tolerant species or individuals. However, this is clearly not possible with the plants used in this work. The salt sensitive, clone 919, responded in the same way as the salt tolerant clones with the application of ABA.

There is both direct and indirect evidence linking endogenous ABA production to proline production. Indirect evidence shows elevated levels of both ABA and proline in stressed plants. Peuke et al. (2002), investigating drought tolerance in beech ecotypes, found elevated levels of proline and ABA in leaves of stressed plants, but not in controls. Similarly, Gómez-Cadenas et al. (1998) found that citrus seedlings had elevated levels of ABA in roots and leaves, and proline in leaves, when subjected to 200 mM

NaCl. Direct evidence has been shown in canola leaf discs; not only was ABA involved in osmo-induced proline accumulation, it was also involved in the mobilisation of proline once the stress was alleviated (Trotel-Aziz et al., 2003). Proline synthesis in this system relies on increased transcription of the Δ^1-pyrroline-5-carboxylate synthetase and prevention of its degradation requires inactivation of the proline dehydrogenase enzyme (Trotel-Aziz et al., 2003). If similar mechanisms are present in eucalypts then it is likely that they are incomplete in the salt sensitive clone used in this work.

The increased production of proline in response to exogenous application of ABA by plants may indicate that the mechanism involved with salinity tolerance is first linked to the production (or lack) of ABA. With ABA the likely hormone responsible for triggering increased proline production, perhaps there is no increase in production of this triggering substance in plants considered to be salt sensitive (e.g. clone 919). Further investigation into endogenous ABA levels in eucalypts, and its involvement in the induction of proline synthesis may provide a better understanding of the responses of these plants (eucalypts) on exposure to salt, and therefore a capacity to differentiate in their salt tolerance.

Callus

The recalcitrant nature of eucalypts in producing callus of consistent texture, colour and form (McComb et al., 1996) made this component of the work difficult to make reasonable comparisons. The results obtained, however, were encouraging as the salt tolerant clone (66) produced greater amounts of proline. Callus of the salt sensitive clone (919) grown on salt media also produced more proline, but the levels were not as great as those observed for the salt tolerant clone. The proline response observed for these two clones in callus culture is different from that observed when grown in shoot culture. The cause of this response is uncertain, but could be due to the relatively short amount of time for which the callus cultures had been established (Rus et al., 2000). Perhaps the culture conditions were suboptimal for callus growth and thus, continuously stressed as evidenced in the shoot cultures after 3–4 weeks. This is contrary to reports for species such as *Mesembryanthemum crystallinum* where cells of this species showed a salt response similar to that of the whole plant (Vera-Estrella et al., 1999).

Practical implications

An increase in proline on exposure to salt clearly can be used to distinguish between salt tolerant and salt sensitive clones. Programmes looking at using many clones (particularly those growing plants in tissue culture) might use proline production to rank clones. There is a further requirement to know how differences in response between tolerant clones should be interpreted. Are the most tolerant clones those that produce the most proline? At what level of NaCl would tolerant clones be expected to produce more proline? Does a more tolerant clone require higher concentrations of salt to produce a proline response? In addition, this system can be used to further examine the role proline plays in plant stress. Even salt sensitive clones produce higher amounts of proline under certain conditions. It may be possible to separate some of the protective roles attributed to proline in salt and drought stress, where neither is present.

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