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REGULAR ARTICLE

Salinity and waterlogging tolerances in three stem-succulent halophytes (*Tecticornia* species) from the margins of ephemeral salt lakes

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Abstract
Background and aims Tecticornia species are stem-succulent, perennial halophytes (sub-family Salicornioideae; Chenopodiaceae) that inhabit saline areas including the margins of ephemeral salt lakes in Australia. Based on zonation observed at salt lakes, species were hypothesised to differ in tolerances to salinity and/or waterlogging.
Methods Three *Tecticornia* species were grown in sub-irrigated or waterlogged sand culture with treatments from 10 to 800 m*M* NaCl, for 60 d in a glasshouse. Growth, tissue solutes, root porosity, root radial O_2 loss, and ethanol production, were assessed.
Results The three species were salt tolerant; at 800 m*M* NaCl shoot RGR (ash-free) was reduced by 9% in *T. indica*, 22% in *T. pergranulata* and 39% in *T. mellaria*. Na^+ and Cl^- were the predominant osmotica in succulent stem tissues. Glycinebetaine was a major organic solute. *T. pergranulata* and *T. indica* were waterlogging tolerant; shoot RGR was reduced by at most 29% irrespective of salinity. Waterlogging tolerance in *T. mellaria* was variable (shoot RGR 8%–56% of controls) and some individuals died. *T. pergranulata* formed adventitious roots with aerenchyma, but the two other species did not. Anoxic tips of lateral roots produced ethanol.
Conclusion The three *Tecticornia* species are salt tolerant. *T. pergranulata* is also waterlogging tolerant and formed adventitious roots containing aerenchyma, traits consistent with growth on mud flats of salt lakes. *T. indica* was unexpectedly tolerant of waterlogging, whereas *T. mellaria* was less tolerant. Future work is needed to evaluate tolerances of inundation (i.e. submergence) and to higher salinity treatments.

Keywords Anoxia tolerance · Adventitious roots · Aerenchyma · Chenopodiaceae · Glycinebetaine · Hypoxia · Osmotic adjustment · Salicornioideae · Salinity · Salt lake · Shoot ion concentrations · Waterlogging

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Introduction

Waterlogging is common in many of the coastal and inland habitats occupied by halophytes. Most research on halophytes has focused on salinity tolerance (reviewed by Flowers and Colmer 2008) with fewer studies of salinity combined with waterlogging

(reviewed by Colmer and Flowers 2008). Knowledge on tolerances of halophytes to both salinity and waterlogging, or even to periods of inundation, might enhance understanding of species zonation in plant communities on the margins of salt lakes, as zonation may be related to gradients in flooding (e.g. Cantero et al. 1998) and/or in soil salinity (e.g. Ungar et al. 1979). Additionally, traits associated with waterlogging tolerance, such as the formation of adventitious roots containing aerenchyma (Jackson and Drew 1984) and rates of ethanolic fermentation in root tissues (Gibbs and Greenway 2003), have only been quantified in a few halophytic species (reviewed by Colmer and Flowers 2008).

This study evaluated responses to the combined stresses of salinity and waterlogging for *Tecticornia pergranulata* (J.M.Black) K.A.Sheph. & Paul G. Wilson subsp. *pergranulata* (henceforth *T. pergranulata*), *T. indica* subsp. *bidens* (Nees) K.A.Sheph. & Paul G.Wilson (henceforth *T. indica*), and *T. mellaria* K.A.Sheph.. The genus *Tecticornia* from the subfamily Salicornioideae (Chenopodiaceae), are perennial shrubs with succulent articulated stems and comprises ~38 species, the majority being endemic to Australia (Shepherd and Wilson 2007; Western Australian Herbarium 1998–). *T. pergranulata* and *T. indica* are widely distributed in coastal and inland saline areas across Australia (Wilson 1980). *T. mellaria* is a species with a restricted known distribution of *c.* 18 km in the vicinity of salt lakes ~200 km north of Kalgoorlie in Western Australia. There is often zonation within *Tecticornia* communities on the margins of ephemeral salt lakes in the semi-arid region of Western Australia (Datson 2002). For example, on the eastern margin of Hannan Lake, *T. pergranulata* inhabits both the waterlogging prone mud flat and the more elevated gypseous dunes bordering the mud flat, whereas *T. indica* occurs only in the dunes. *T. mellaria* occurs on well-drained gypseous dunes bordering Lake Carey, or on small, elevated gypseous clay plans in the vicinity of the lake (Shepherd 2007). These field observations suggest that *T. pergranulata* may differ in tolerances to salinity and/or waterlogging, when compared with the two other *Tecticornia* species.

Previous research on *T. pergranulata* has documented this species as tolerant of salinity; at 800 m*M* NaCl shoot biomass (dry mass minus mass of Na^+, K^+ and Cl^-) was reduced by ~80% in comparison with maximum biomass at 10–200 m*M* NaCl (Short and Colmer 1999). *T. pergranulata* can also tolerate complete submergence (Pedersen et al. 2006; Colmer et al. 2009), and forms aquatic adventitious roots during prolonged partial submergence (Rich et al. 2008). Tolerances of other *Tecticornia* species to salinity or waterlogging had not been evaluated, so knowledge was lacking on the comparative physiology of these species.

By contrast with the scant knowledge on waterlogging tolerance in the genus *Tecticornia*, studies of waterlogging tolerance have been conducted on other species in the Salicornioideae, predominately on annual species from coastal salt marsh populations. As examples, dry mass of *Salicornia dolichostachya* increased by 35% when waterlogged in clay at 250 m*M* NaCl for 63 d, whereas in *Salicornia brachystachya* dry mass was reduced by 40% (Rozema et al. 1987). Similarly, RGR (based on plant fresh mass increases over 35 d) was reduced by 22% in *Salicornia dolichostachya* and 32% in *Salicornia ramosissima* when in hypoxic (O_2 at 0.5% of air-equilibrium) 200 m*M* NaCl culture solution, compared with plants in aerated solution (Schat et al. 1987). Studies of species of Salicornioideae from inland habitats are few; *Salicornia europaea* from an inland salt lake survived 84 d in waterlogged sand culture at 171 m*M* NaCl (Howes Keiffer et al. 1994).

The present study was undertaken to test the hypothesis that *T. pergranulata* (subsp. *pergranulata*), *T. indica* (subsp. *bidens*) and *T. mellaria* differ in salinity and/or waterlogging tolerance. Based on the occurrence of *T. pergranulata*, but not the two other species, on the mud flats of ephemeral salt lakes, we expected higher tolerance in *T. pergranulata* to combined waterlogging and salinity. Controlled glasshouse experiments quantified growth responses to salinity, waterlogging, and these stresses combined, and evaluated some physiological processes hypothesised to contribute to tolerance of salinity (Flowers and Colmer 2008) and waterlogging (Colmer and Voesenek 2009). Tissue solute concentrations (Na^+, Cl^-, K^+, glycinebetaine, proline, trigonelline, sucrose, glucose and fructose), expressed sap osmotic potential, formation of adventitious roots, root aerenchyma, root porosity, root radial O_2 loss, and ethanol production rates by anoxic root tips, were all assessed.

Materials and methods

Plant culture

Seeds of *T. pergranulata* (subsp. *pergranulata*) growing on the mud flat and *T. indica* (subsp. *bidens*) growing in the bordering low dunes were from Hannan Lake, 11 km south-east of Kalgoorlie (30° 51′S, 121°32′E), Western Australia. Seeds of *T. mellaria* were from plants growing on an elevated gypseous clay plan in the vicinity of Lake Carey ~200 km north of Kalgoorlie, Western Australia (precise location withheld due to conservation concerns). Seeds were collected from individual plants and stored in paper bags in a laboratory until germination within 6 months.

Seeds were washed in 0.04% $NaHClO^-$ solution for 1 min, rinsed thoroughly with deionised water, and placed on filter paper soaked with nutrient solution in Petri dishes. Petri dishes were sealed with Parafilm to prevent evaporation, and were placed in a 35/5°C 12 h light/12 h dark cabinet, a temperature regime that produced maximum germination in *T. pergranulata* (Malcolm 1964). The nutrient solution contained (m*M*): Na^+, 10; K^+, 10; NH_4^+, 0.2; Ca^{2+}, 10; Mg^{2+}, 1.0; NO_3^-, 1.4; Cl^-, 18.6; SO_4^{2-}, 10; HPO_4^{2-}, 0.5; Fe-EDTA, 0.05; $H_2BO_3^-$, 0.00625; Mn^{2+}, 0.0005; Zn^{2+}, 0.00005; Cu^{2+}, 0.000125; and Mo^{2+}, 0.000125. The pH was adjusted to 6.5 with KOH. This nutrient solution was based on that used by Short and Colmer (1999), but with concentrations of K^+, Ca^{2+}, Mg^{2+}, SO_4^{2-} and Cl^- increased on the basis of chemical analyses of soil collected from Hannan Lake (data not shown).

Seedlings (shoots 5–10 mm) were transplanted into 70 mm tall plastic pots containing 100 g of washed silica sand. Pots were in trays containing nutrient solution maintained at 50 mm below the sand surface, and were watered daily with nutrient solution. Seedlings were maintained in these conditions for 60 d, then a selection of seedlings with similar shoot heights were transplanted into 150 mm diameter, 450 mm high PVC pots containing 10 kg of washed silica sand over a 50 mm gravel base (two seedlings from the same parent plant per pot), to establish 180 pots. A drainage outlet was located 5 mm from the base of each pot. Pots were sub-irrigated by placing each pot in a 70 mm high black plastic bucket to maintain solution levels at 360 mm below the sand surface. A black plastic sleeve was fitted around each pot to cover the top of the bucket, to prevent growth of algae in the buckets. Pots were watered every 2nd day from the top with 1.5 l of nutrient solution (at 'field capacity' the pots held approximately 1.0 l), and every 7 d solution in the buckets was emptied and replaced. After 30 d, seedlings were thinned to 1 per pot, and 132 pots with seedlings of similar size were selected for the experiment.

The experimental design was three species ×5 NaCl treatments ×2 water treatments ×4 replicates. Four different parent plants of each species had provided seeds to raise seedlings for each of the four replicates. An initial harvest of four replicates per species was taken, then salinity treatments of 10, 200, 400, 600 and 800 m*M* NaCl were imposed in steps of 100 m*M* per d. During the step up period, all pots were flushed daily with treatment solution and the buckets were also emptied and filled with treatment solution. The lower salinity treatments continued to be flushed during the time taken to reach the highest treatment, and then four pots of each species at each NaCl treatment level were waterlogged by partially submerging each pot in saline nutrient solution (10, 200, 400, 600 or 800 m*M* NaCl, as appropriate) so that solution entered via the basal drainage hole and rose to the sand surface. For each waterlogged pot, a transparent plastic tube was attached to the drainage outlet and held vertically by being attached to the pot to prevent drainage. Solution in waterlogged pots was maintained at 10–20 mm above the sand surface by daily addition of deionised water. Solutions were replaced every 7 d by allowing the pot to drain for 5 min and then re-waterlogging (using the procedure described above) with nutrient solution that had been flushed with N_2 gas to purge away O_2. The O_2 concentration in solution in the pots remained below 0.003 m*M*, the detection limit of the O_2 electrode used. Every second day, sub-irrigated pots were flushed from the top with 1.5 l of nutrient solution, to maintain sand water content and salinity levels. Twice weekly the solution in the buckets was also replaced with fresh treatment solution. The electrical conductivity of solution in the waterlogged pots, and in the buckets of the sub-irrigated pots, varied by less than 10% from the target levels between solution changes.

Pots were completely randomised on benches in a naturally-lit glasshouse (months of March and April, Perth, Western Australia), and were re-assigned

random positions weekly. The mean (± s.e.) maximum and minimum air temperatures during the treatment period were 26±3°C and 17±2°C. Treatments were maintained for 60 d after waterlogging was imposed.

Measurements of growth, shoot water content, and root porosity

Fresh mass, dry mass and ash content of roots and shoots were measured in an initial harvest taken immediately prior to imposition of salinity treatments and at the conclusion of the 60 d treatment period. Ash content was determined using a muffle furnace at 600°C for 6 h. Relative growth rate (RGR) was calculated on the basis of ash-free dry mass. Shoot water content was expressed as ml g^{-1} ash-free dry mass.

The final harvest was undertaken over 4 d, with one replicate of all species and treatment combinations harvested each d. Shoots were rinsed with deionised water and blotted dry. For each plant, the three youngest expanding segments at the tips of five branches were sampled and immediately weighed; two were placed in an air-tight vial and three wrapped in aluminium foil, and both samples plunged into liquid N_2, and then stored at −80°C. All other expanding succulent shoot tissues (first 3–5 stem segments) were then collected, the remaining shoot tissues were divided into 'succulent' or 'woody' tissues, and all were weighed and then oven-dried at 70°C.

Roots were washed free of sand using tap-water, the length of the longest lateral root measured, and the appearance of the root system noted. *T. pergranulata* grown in waterlogged treatments formed adventitious roots; the length of the longest adventitious root was measured, then roots were separated into adventitious or primary root system (tap-root and laterals).

Root porosity (% gas volume root volume^{-1}) was measured for lateral roots of the primary root system of all three species, and for adventitious roots of *T. pergranulata*. Roots were cut into 50 mm segments, and a sub-sample of 1–2 g fresh mass was used, following the method of Raskin (1983) with equations as modified by Thomson et al. (1990). Roots were then oven-dried at 70°C.

Analyses of tissue solutes

The osmotic potential of sap expressed from expanding succulent shoot tissues was measured using a freezing point depression osmometer (Fiske Associates, Model One-Ten, Massachusetts, USA). Samples that had been frozen in air-tight vials were thawed in the vials, crushed in a stainless steel press to extrude sap, and 10 μl of sap was immediately analysed.

Expanding succulent shoot tissues that had been wrapped in aluminium foil and frozen were lyophilised and then ground using a ball and mill grinder. Approximately 0.1 g of the lyophilised ground tissue was extracted twice in 3 ml of ice cold 5% (v/v) perchloric acid using the procedure described by Fan et al. (1993). Extracts were filtered (0.22 μm) prior to injection into a HPLC system. The HPLC system (Waters Corporation, Milford, MA, USA) consisted of a 600E pump, 717 auto-sampler, 996 photodiode array detector and Millenium Chromatography Manager software. The system was equipped with a Sugar-Pak column (300 mm length×6.5 mm i.d., Waters Corporation, Milford, MA, USA) at 90°C. The methods were as described by Naidu (1998), but with the Ca-EDTA concentration in the mobile phase increased from 5 to 7.5 mg l^{-1}. Recoveries of glycinebetaine, proline, trigonelline, sucrose, glucose and fructose from spiked samples of *Tecticornia* shoot tissue were >90%. No adjustments were made to the data presented.

Na^+, K^+ and Cl^- were measured in each category of shoot tissues. Na^+ and K^+ were extracted from tissues in 0.5 m*M* HCl shaken for 2 d (Hunt 1982). Na^+ and K^+ in dilutions of the tissue extracts were measured using a flame photometer (Corning, Model 410, Essex, UK). Cl^- was extracted from tissues in water at 70°C for 3 h, and then shaken for 2 d. Cl^- in dilutions of the tissue extracts was measured using a chloridometer (Buchler Instruments, Model 4-2000, New Jersey, USA). Analyses were verified by a plant standard (State Chemistry Laboratory, Victoria, Australia), which gave values of 95%–100% for the expected Na^+, K^+ and Cl^- concentrations. No adjustments were made to the data presented.

Radial O_2 loss and proportional cross-sectional area of aerenchyma in adventitious roots

Three additional pots of each species were waterlogged and grown for 60 d at 10 m*M* NaCl at the same time as the main experiment, to provide plants for radial O_2 loss (ROL) measurements. Only *T. pergranulata* formed adventitious roots, so ROL

measurements were taken only for this species. The average length of the adventitious roots measured was 118 mm±28.

At sampling, pots were submerged in large tubs of de-oxygenated nutrient solution, and sand was gently washed from the roots. Plants were inserted into a polystyrene holder individually cut to fit the base of each plant, which was then fitted on a Perspex container (100 mm×100 mm×170 mm; w × b × h), filled with a de-oxygenated solution containing 0.1% (w/v) agar and (m*M*) Na^+, 10; Cl^-, 15; K^+, 5; Ca^{2+}, 0.5; and SO_4^{2-}, 0.5. The root system was immersed in this solution, while the shoot remained in air, all in a 20°C controlled-temperature room. A root-sleeving O_2 electrode (height 5 mm, internal diameter 2.25 mm, fitted with guides) was placed around an adventitious root and ROL measured (Armstrong and Wright 1975; Armstrong 1994) at 40, 30, 20, 10 and 5 mm behind the root tip; lateral roots prevented movement of the electrode to more basal positions. Cross sections were taken, using a hand-held razor blade, at 80, 50, 30, 20, 10 and 5 mm behind the root tip. Sections were photographed and the proportional area of aerenchyma in each cross section was determined using an image analysis program (Scion Image, Scion Corporation, Maryland, USA).

Ethanol production by excised tips of lateral roots, at 10 or 400 mM NaCl

A second experiment was conducted with plants raised in nutrient solution culture, so that roots could be sampled for measurements of ethanol production. Seeds were germinated as described above. Seedlings were transplanted into trays containing potting mix and watered daily with nutrient solution (same composition as in the sand culture experiment) for 30 d. A selection of seedlings with similar shoot heights were then washed free of potting mix and transplanted into 4.5 l plastic pots containing aerated nutrient solution (composition as given above for the sand culture experiment), in a controlled-environment room (25/10°C d/night, 12 h photoperiod, 375–500 μmol quanta m^{-2} s^{-1}, PAR). Each pot contained four seedlings from the same parent plant held at the root-shoot junction by a polyethylene plug fitted into the lid. Six pots of each species (i.e. two from each parent plant) were established. The pots and lids were wrapped in aluminium foil to prevent light entry. Plants were grown for 30 d, with nutrient solution renewed weekly. Pots were thinned to the three most uniform plants per pot, just prior to imposing NaCl treatments.

The experimental design was three species ×2 salinity treatments (10 or 400 m*M* NaCl) ×3 replicates. The 400 m*M* NaCl treatment was imposed in daily steps of 100 m*M*. Treatments lasted 21 d, with solutions replaced weekly. Pots were completely randomised, and were re-assigned random positions weekly.

After 21 d of NaCl treatments, pots were refreshed with nutrient solutions (10 or 400 m*M* NaCl) that also contained 20 m*M* glucose and 10 mg L^{-1} carbenicillin. A hypoxic pre-treatment of 0.020 m*M* dissolved O_2 was imposed for 6 h by flushing solutions with an air:N_2 gas mixture. Shoots were enclosed in a plastic bag flushed with N_2 gas, to prevent internal O_2 movement from atmosphere to roots during the hypoxic pre-treatment. After the pre-treatment, lateral root tips (10 mm) were excised, tips from each pot provided one replicate. Excised tips were 'healed' for 5 h in hypoxic (0.020 m*M* O_2) nutrient solution (10 or 400 m*M* NaCl) that also contained 20 m*M* glucose and 10 mg L^{-1} carbenicillin. Root tips were then incubated in anoxic nutrient solution (10 or 400 mM^{-3} NaCl) that also contained 20 m*M* glucose and 10 mg L^{-1} carbenicillin, in sealed glass vials for 2 h as described in McDonald et al. (2001). Ethanol in the incubation solution and neutralised perchloric acid extracts of the root tips was assayed using the enzymatic technique of Beutler (1983). Recovery of ethanol from the incubation solution was 93%, and ethanol in the tissues was <5% of that in the medium. Ethanol in blank solutions from vials incubated without root tips was below detection.

Statistical analyses

Analyses were conducted using the Genstat (version 6) statistical package (Lawes Agricultural Trust, IACR-Rothamsted, 2002).

Data from the sand culture experiment were analysed by one and two way ANOVA, and trends in variate response to increases in external NaCl concentration were determined by including linear and quadratic polynomial contrasts in the ANOVA (Keppel 1973, 1991). R^2 was used to determine the proportions of variation among means accounted for by linear and

quadratic trend components (e.g. $R^2_{linear} = SS_{linear}/SS_{treatment}$; $R^2_{quad} = SS_{quadratic}/SS_{treatment}$) (Keppel 1991). For *T. pergranulata* and *T. indica*, two-way ANOVA with polynomial contrasts were conducted to determine variate responses to the water level (i.e. water-level treatment, WT) and NaCl treatments (5 NaCl treatment levels ×2 water-level treatments). When the *F*-value of the interaction term was significant in a two-way ANOVA, variate responses to NaCl in sub-irrigated or waterlogged treatments were then determined by one-way ANOVA with polynomial contrasts. For *T. mellaria*, two-way ANOVA could not be conducted due to the death of eight plants in waterlogged treatments. Variate response of *T. mellaria* in sub-irrigated treatments, and for plants that survived waterlogging, were determined by one-way ANOVA with polynomial contrasts (ANOVA was conducted with unequal sample sizes for *T. mellaria* that survived waterlogging). *P* values for ANOVAs, and results of trend analysis, are presented in the Appendix (Table 3).

The data analyses were modified in several instances. (i) As *T. pergranulata* formed adventitious roots in waterlogged treatments, an additional two-way ANOVA was conducted for this species in waterlogged treatments to compare the RGR of the primary root system (tap-root + laterals) to the RGR of the entire root system (i.e. tap-root + laterals + adventitious roots). (ii) For root porosity data from waterlogged *T. pergranulata*, an additional two-way ANOVA was conducted to compare the porosity of adventitious and lateral roots. For the two smaller experiments on: (i) ROL and aerenchyma in adventitious roots and (ii) ethanol production rates, data were analysed using one-way ANOVA.

Results

Effects of salinity and waterlogging on growth (ash-free dry mass)

In sub-irrigated conditions, shoot RGRs of the three species responded to increasing NaCl treatments with curvilinear trends, and shoot RGRs were highest at 10–400 m*M* NaCl in *T. pergranulata*, 200 m*M* in *T. mellaria*, but at 400–600 m*M* NaCl in *T. indica* (Fig. 1a). At 800 m*M* NaCl (the highest NaCl treatment in the experiment), shoot RGRs in sub-irrigated plants had declined from maximal rates by

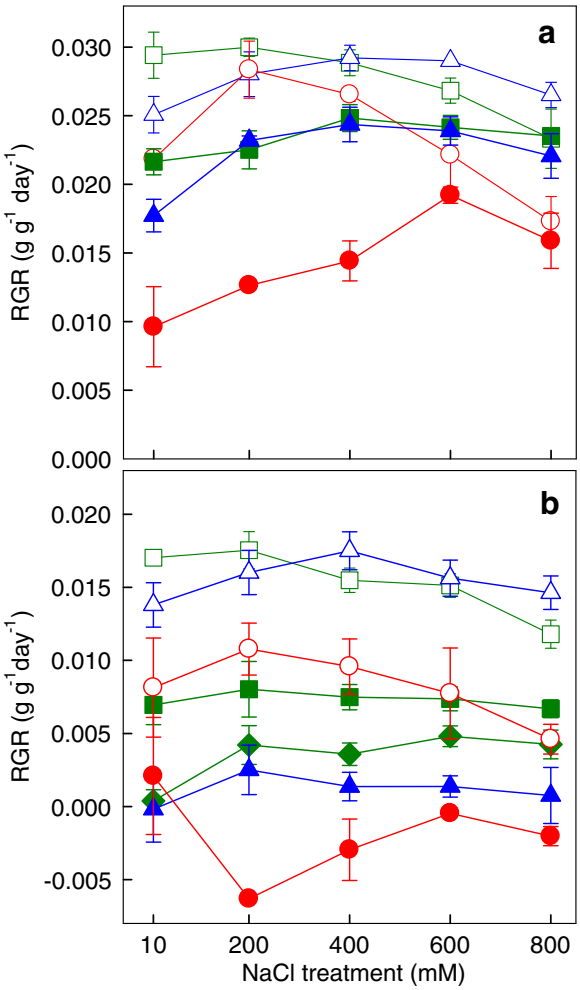


Fig. 1 Relative growth rate (RGR) of **a** shoots and **b** roots of *Tecticornia pergranulata*, *T. indica* and *T. mellaria*, in sub-irrigated or waterlogged sand culture at 10–800 m*M* NaCl for 60 d. RGR was calculated using ash free dry mass. Values are means of four replicates ± s.e., except for waterlogged *T. mellaria*, where mortality reduced live replicates at 10, 200, 600 and 800 m*M* NaCl to 2, 1, 3 and 2, respectively. Initial ash free dry mass (g) of shoots and roots, respectively, were: *T. pergranulata,* 2.6, 1.2; *T. indica*, 1.1, 0.59; *T. mellaria*, 1.6, 0.64. Closed symbols, waterlogged; open symbols, sub-irrigated: ■, □, *T. pergranulata*; ▲, △, *T. indica*; ●, ○, *T. mellaria*. In (**b**), the same symbols were used, except, ◆, waterlogged *T. pergranulata* primary root system (tap-root+ lateral roots); ■, waterlogged *T. pergranulata* primary root system+adventitious roots

22% in *T. pergranulata*, 9% in *T. indica*, and 39% in *T. mellaria*. Previous work on *T. pergranulata* in drained sand culture (Short and Colmer 1999) reported a larger reduction in growth at 800 m*M* NaCl than observed in the present study. This

difference may be due to: (i) sub-irrigated treatments used in the present study rather than drained treatments, and (ii) higher concentrations of macronutrients in the nutrient solution used in the present study, such as K^+ (17-fold higher), Ca^{2+} (5-fold higher) and HPO_4^{2-} (2.5-fold higher), than used by Short and Colmer (1999).

T. pergranulata and *T. indica* were both relatively tolerant of saline waterlogged treatments, with reductions in shoot RGR of less than 29% compared with plants in saline sub-irrigated treatments (Fig. 1a). In *T. pergranulata*, waterlogging reduced shoot RGR by 26% at 10 m*M* NaCl, whereas at 800 m*M* NaCl there was no reduction ($P<0.05$ NaCl × WT interaction). Waterlogging reduced shoot RGR of *T. indica* by 17%–29% at 10–800 m*M* NaCl ($P<0.001$). In *T. mellaria*, eight plants died after 35–55 d of waterlogging; each *T. mellaria* parent used as a seed source produced progeny that died and deaths occurred across NaCl treatments (see caption of Fig. 1). All subsequent data for waterlogged *T. mellaria* are presented only for the plants that survived. Shoots did grow for the 12 individuals that survived, albeit with reductions in RGR of 8%–56% compared with plants in saline sub-irrigated treatments (Fig. 1a).

Root RGR in sub-irrigated *T. pergranulata* was highest at 10–200 m*M* NaCl, and was reduced by 30% at 800 m*M* NaCl (Fig. 1b). There was no reduction in root RGR of sub-irrigated *T. indica* at 10–800 m*M* NaCl, whereas it had declined at 800 m*M* in *T. mellaria*. Root RGR was reduced by waterlogging in the three species (Fig. 1b). Waterlogging at 10–800 m*M* NaCl reduced root RGRs of *T. indica* by 84%–100% ($P<0.001$) and of *T. mellaria* by 26%–100% (in these species root RGRs were not different to zero; ±0.0035 and 0.0037, respectively, for 95% confidence intervals). *T. pergranulata* formed adventitious roots in waterlogged conditions, whereas these roots were not formed by *T. indica* or *T. mellaria*. In *T. pergranulata* waterlogged with 10–800 m*M* NaCl, RGRs of the entire root system (i.e. tap-root+laterals+adventitious) were reduced by 43%–59% ($P<0.001$), whereas RGRs of the primary root system (i.e. tap-root+laterals only) were reduced by 64%–98% ($P<0.001$). Nevertheless, the *T. pergranulata* primary root system maintained growth at 200–800 m*M* NaCl (±0.002 for 95% confidence intervals), in contrast to *T. indica* and *T. mellaria*.

Increasing external NaCl, both in sub-irrigated or waterlogged conditions, caused an increase in shoot ash content in the three species (Fig. 2). Waterlogging reduced shoot ash content in the three species (Fig. 2), by 8%–32% in *T. pergranulata* ($P<0.001$), 9%–33% in *T. indica* ($P<0.001$), and 11%–30% in *T. mellaria*. The mass of Na^+, Cl^- plus K^+ comprised a large proportion of the shoot ash content; 85%–91% in plants grown in sub-irrigated treatments, and 66%–92% in waterlogged treatments. Data on the tissue concentrations of these ions are presented below.

Osmotic potential of sap from expanding shoot tissues

In sub-irrigated conditions, the osmotic potential of sap (π_{sap}) of expanding shoot tissues of the three species became more negative as external NaCl concentration increased (Fig. 3a). Declines in π_{sap} as external NaCl concentration increased were due predominantly to solute accumulation, as tissue water contents generally increased, or remained stable, in the three species (Fig. 3b). There were two exceptions to this generalisation: (i) in sub-irrigated *T. mellaria*

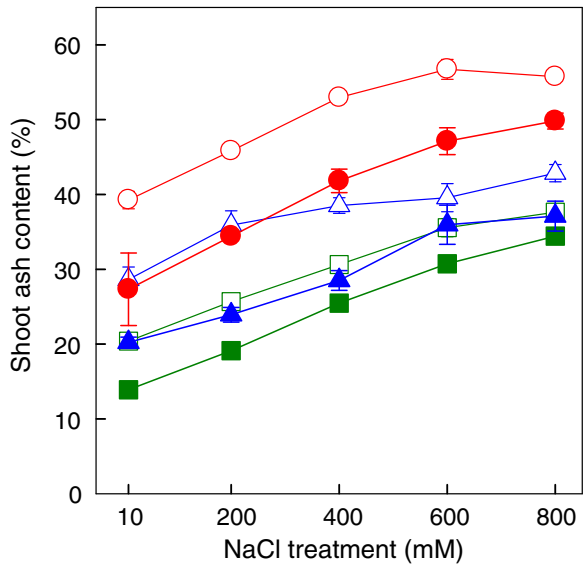


Fig. 2 Shoot (all succulent and woody tissue) ash content (% by dry mass) of *Tecticornia pergranulata*, *T. indica* and *T. mellaria*, grown in sub-irrigated or waterlogged sand culture at 10–800 m*M* NaCl for 60 d. Values are means of four replicates ± s.e., except for waterlogged *T. mellaria*, where mortality reduced live replicates at 10, 200, 600 and 800 m*M* NaCl to 2, 1, 3 and 2, respectively. Closed symbols, waterlogged; open symbols, sub-irrigated. ■, □, *T. pergranulata*; ▲, △, *T. indica*; ●, ○, *T. mellaria*

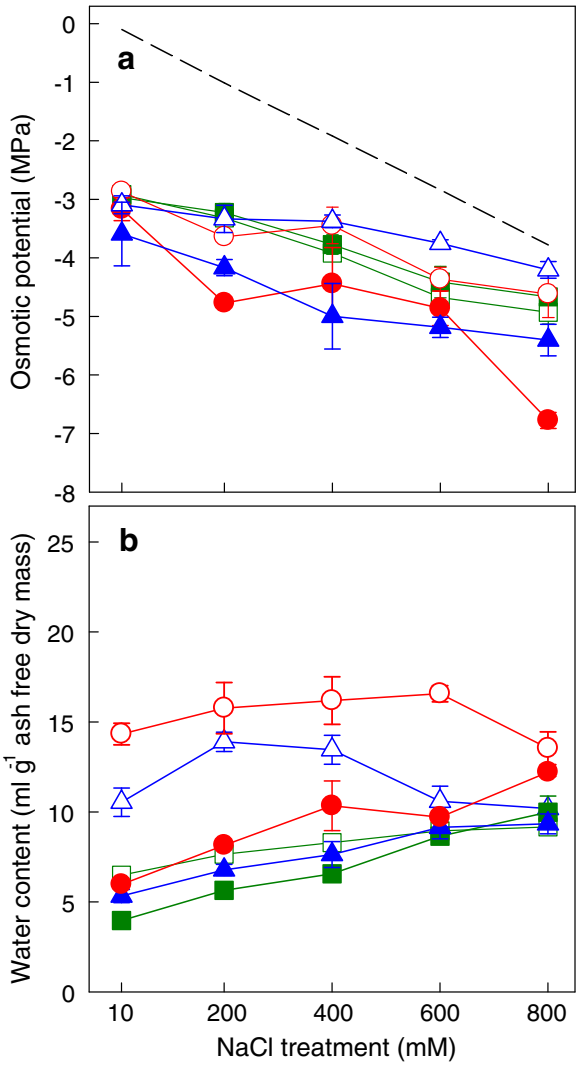


Fig. 3 **a** Osmotic potential of sap, and **b** water content based on ash free dry mass, of expanding shoot tissues of *Tecticornia pergranulata*, *T. indica* and *T. mellaria*, grown in sub-irrigated or waterlogged sand culture at 10–800 m*M* NaCl for 60 d. Values are means of four replicates ± s.e., except for waterlogged *T. mellaria*, where mortality reduced live replicates at 10, 200, 600 and 800 m*M* NaCl to 2, 1, 3 and 2, respectively. Closed symbols, waterlogged; open symbols, sub-irrigated. ■, □, *T. pergranulata*; ▲, △, *T. indica*; ●, ○, *T. mellaria*. In (**a**) the dashed line = osmotic potential of external solution

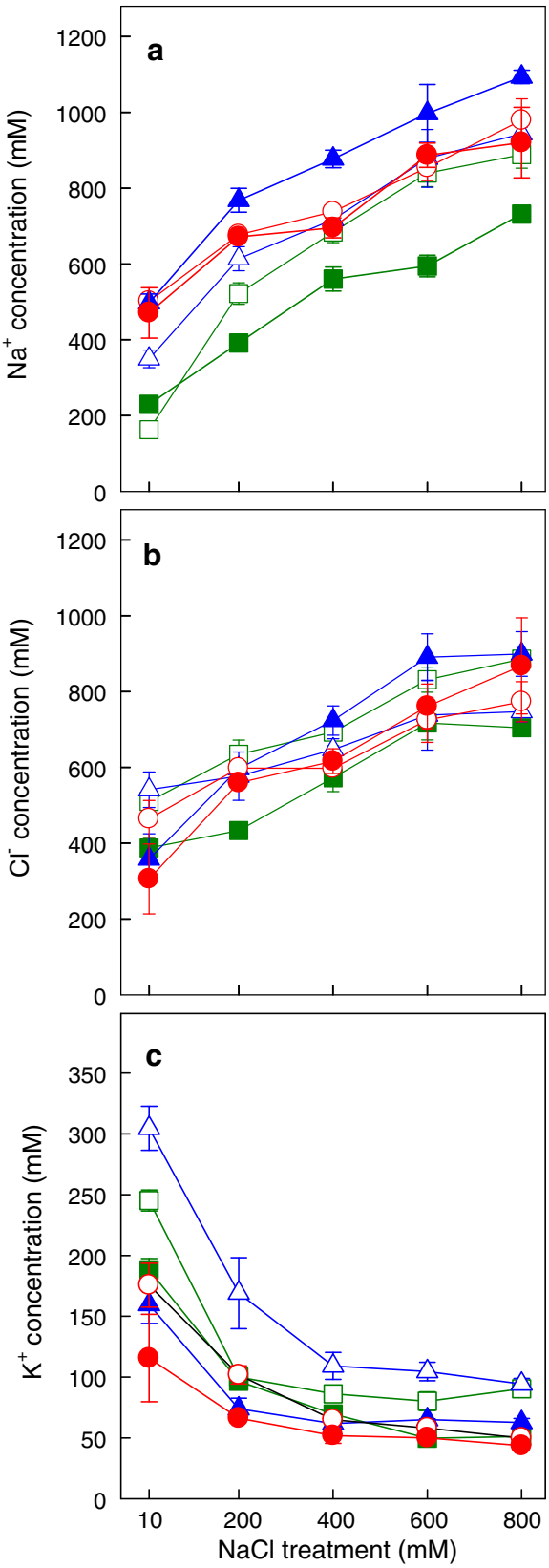
water content declined by 19% between plants at 600 and 800 m*M* NaCl ($P<0.001$); (ii) in sub-irrigated *T. indica*, water content declined by 21% between plants at 400 and 600 m*M* NaCl ($P<0.001$) (Fig. 3b). Compared with the change in external osmotic potential ($\Delta\pi_{external}$) of −3.7 MPa as NaCl treatments increased from 10 to 800 m*M*, the $\Delta\pi_{sap}$ were: *T. pergranulata* −2.0 MPa (sub-irrigated) and −1.7 MPa (waterlogged), *T. indica* −1.1 MPa (sub-irrigated) and −1.8 MPa (waterlogged), and *T. mellaria* −1.8 MPa (sub-irrigated) and −3.6 MPa (waterlogged) ($P<0.001$). Waterlogging did not significantly affect π_{sap} in *T. pergranulata*, whereas in waterlogged *T. indica* π_{sap} was 16%–48% more negative, and in waterlogged *T. mellaria* it was 10%–47% more negative.

The contributions of Na^+, Cl^-, K^+ and glycinebetaine to π_{sap} of expanding shoot tissues were calculated from the concentrations of these solutes presented in Figs. 4 and 5. The π of a solute was calculated as: $\pi = -nRT/V$, where n is the number of solute molecules; R, the universal gas constant; T, temperature in °K; and V, volume in l. Osmotic coefficients of Na^+, Cl^- and K^+ were assumed to equal 0.92, based on NaCl solutions at 25°C (Lang 1967). The average contributions of each solute to the π_{sap} of expanding shoot tissues of the three species, at 10 and 800 m*M* NaCl respectively, were; Na^+, 27%±5 and 42%±4; Cl^-, 32%±4 and 37%±2; K^+, 15%±3 and 3%±1; and glycinebetaine, 7%±1 and 5%±1. The average combined contributions of these solutes to π_{sap} of expanding shoot tissues of the three species were 81%±6 at 10 m*M* NaCl and 87%±7 at 800 m*M* NaCl.

Na^+, Cl^- and K^+ concentrations in expanding succulent shoot tissues

In sub-irrigated and in waterlogged conditions, increasing external NaCl concentration caused an increase in Na^+ concentrations in expanding shoot tissues in the three species (Fig. 4a). In comparison with plants grown at 10 m*M* NaCl, at 800 m*M* NaCl tissue Na^+ concentrations in sub-irrigated and waterlogged plants, respectively, were 3.2- and 5.5-fold higher in *T. pergranulata*, 2.2- and 2.7-fold higher in *T. indica*, and in both situations 2-fold higher in *T. mellaria* (Fig. 4a). In comparison with sub-irrigated plants,

Fig. 4 Concentrations of Na^+, Cl^- and K^+ on a tissue water basis in expanding succulent shoot tissues of *Tecticornia pergranulata*, *T. indica* and *T. mellaria*, grown in sub-irrigated or waterlogged sand culture at 10–800 m*M* NaCl for 60 d. Values are means of four replicates ± s.e., except for waterlogged *T. mellaria*, where mortality reduced live replicates at 10, 200, 600 and 800 m*M* NaCl to 2, 1, 3 and 2, respectively. Closed symbols, waterlogged; open symbols, sub-irrigated. ■, □, *T. pergranulata*; ▲, △, *T. indica*; ●, ○, *T. mellaria*



waterlogging reduced Na^+ concentrations in *T. pergranulata* by 18%–29% at 200–800 m*M* NaCl ($P<0.001$). By contrast, in *T. indica* waterlogging increased Na^+ concentrations by 16%–42% at 10–800 m*M* NaCl ($P<0.001$), whereas in *T. mellaria* tissue Na^+ concentrations were similar in sub-irrigated and waterlogged treatments (Fig. 4a).

In sub-irrigated and in waterlogged conditions, increasing external NaCl concentration caused an increase in Cl^- concentrations in expanding shoot tissues in the three species (Fig. 4b). In comparison with plants grown at 10 m*M* NaCl, at 800 m*M* NaCl tissue Cl^- concentrations in sub-irrigated and waterlogged plants, respectively, were: 1.7- and 1.8-fold higher in *T. pergranulata*, 1.4- and 2.5-fold higher in *T. indica*, and 1.7- and 2.8-fold higher in *T. mellaria*. In comparison with sub-irrigated plants, waterlogging reduced Cl^- concentrations in *T. pergranulata* by 14%–32% at 10–800 m*M* NaCl ($P<0.001$). By contrast, in *T. indica* waterlogging reduced Cl^- concentrations at 10 m*M* NaCl by 34%, whereas at 800 m*M* NaCl tissue Cl^- concentrations were increased by 20% ($P<0.05$ NaCl × WT interaction). In *T. mellaria*, shoot tissue Cl^- concentrations were generally similar in sub-irrigated and waterlogged treatments.

Tissue K^+ concentrations in the three *Tecticornia* species grown at 10 m*M* NaCl were about 2.0-fold higher than those in plants grown at 200 m*M* NaCl, and 2.9-fold higher than those in plants grown at 400–800 m*M* NaCl (Fig. 4c; $P<0.001$). The high tissue K^+ concentrations in plants grown at 10 m*M* NaCl were considered to be due to an osmotic requirement for cations (Flowers et al. 1977; Glenn and O'Leary 1984) rather than as a consequence of a specific metabolic requirement for K^+. Therefore, the declines in tissue K^+ as external NaCl increased from 10 to 200 m*M* may be attributed primarily to the increased availability of Na^+ for use as osmotica, rather than as an adverse effect of salinity on K^+ net uptake. It was therefore considered more informative to statistically analyse trends in tissue K^+ for plants at 200–800 m*M* NaCl, where Na^+ is likely to be the major cation utilised as osmotica.

As external NaCl concentrations increased from 200 to 800 m*M* NaCl, tissue K^+ concentrations did not change in sub-irrigated *T. pergranulata*, waterlogged *T. indica* or waterlogged *T. mellaria*, whereas K^+ concentrations declined by 47% in waterlogged *T.*

pergranulata, by 44% in sub-irrigated *T. indica*, and by 51% in sub-irrigated *T. mellaria*. In comparison with sub-irrigated plants at 200–800 m*M* NaCl, waterlogging reduced K^+ concentrations in expanding shoot tissues by 3%–43% in *T. pergranulata*, by 34%–56% in *T. indica*, and by 13%–35% in *T. mellaria*. Therefore, tissue Na^+:K^+ in waterlogged plants generally increased in comparison with sub-irrigated treatments, by up to 1.9-fold in *T. pergranulata*, up to 2.8-fold in *T. indica*, and up to 1.6-fold in *T. mellaria* (calculated from data in Fig. 4a and c).

Organic solute concentrations in expanding shoot tissues

In sub-irrigated conditions, glycinebetaine concentrations in expanding shoot tissues of the three species responded to increasing external NaCl concentrations with curvilinear trends (Fig. 5). In comparison with plants at 10 m*M* NaCl, glycinebetaine in plants at 800 m*M* NaCl was higher by 23% (*T. pergranulata*), 7% (*T. indica*) and 89% (*T. mellaria*). Waterlogging influenced shoot glycinebetaine in different ways for the three species. In waterlogged *T. pergranulata* glycinebetaine levels were 24%–34% higher at 10–200 m*M* NaCl, and 26%–35% lower at 600–800 m*M* NaCl, compared to sub-irrigated plants ($P<0.01$ NaCl × WT interaction). In waterlogged *T. indica*, glycinebetaine was similar to that in sub-irrigated plants at 10–200 m*M* NaCl, and 15%–34% lower at 400–800 m*M* NaCl ($P<0.05$ NaCl × WT interaction). In waterlogged *T. mellaria* glycinebetaine concentrations were 2-fold higher at 10 m*M* NaCl, and were similar to those in sub-irrigated plants at 800 m*M* NaCl.

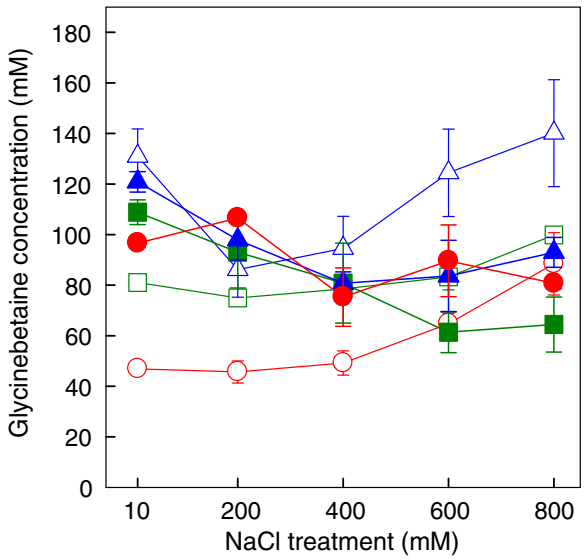


Fig. 5 Glycinebetaine concentrations on a tissue water basis in expanding succulent shoot tissue of *Tecticornia pergranulata*, *T. indica* and *T. mellaria*, grown in sub-irrigated or waterlogged sand culture at 10–800 m*M* NaCl for 60 d. Values are means of four replicates ± s.e., except for waterlogged *T. mellaria*, where mortality reduced live replicates at 10, 200, 600 and 800 m*M* NaCl to 2, 1, 3 and 2, respectively. Closed symbols, waterlogged; open symbols, sub-irrigated. ■, □, *T. pergranulata*; ▲, △, *T. indica*; ●, ○, *T. mellaria*

Concentrations of proline, trigonelline, sucrose, glucose, and fructose were also determined in the expanding shoot tissues. Proline was below detection limits of 3 μmol g^{-1} dry mass, and trigonelline was 0.24–3.25 μmol g^{-1} dry mass (0.05–0.56 m*M* on a tissue water basis), for the three species (data not shown). For the three sugars, the combined levels of sucrose, glucose, and fructose (m*M*) in plants in sub-irrigated conditions, at 10 and 800 m*M* NaCl, respectively, were (means ± standard errors): *T. pergranulata*, 77±13 and 28±2; *T. indica*, 57±10 and 65±24; *T. mellaria*, 48±9 and 108±24. In waterlogged conditions, the concentrations (m*M*) of these three sugars combined, at 10 and 800 m*M* NaCl, respectively, were: *T. pergranulata*, 74±7 and 44±9; *T. indica*, 90±21 and 38±13; *T. mellaria* (note only single samples available), 110 and 19.

Adventitious roots formed in response to waterlogging only in *T. pergranulata*

T. pergranulata formed adventitious roots when waterlogged; these roots were not formed by either *T. indica* or *T. mellaria*. Adventitious roots grew predominantly from shoot nodes of horizontal stems that were submerged or in contact with surface water, and some also grew from near the tap-root/shoot junction. As external NaCl concentration increased from 10 to 800 m*M* NaCl, adventitious root ash-free dry mass of waterlogged *T. pergranulata* declined by 60% (Table 1). There was no significant effect of NaCl on adventitious root number or the length of the longest adventitious roots.

Adventitious roots of *T. pergranulata* contained aerenchyma (Fig. 6), that on average occupied 11%± 0.8 of the root cross-sectional area at positions measured from 10–80 mm behind the root tip (Table 2). ROL from adventitious roots of *T. pergranulata* in an O_2-free medium did not differ along the

Table 1 Parameters of adventitious roots of *T. pergranulata* in waterlogged sand culture at 10–800 m*M* NaCl for 60 d

NaCl (m*M*)	Adventitious root ash free dry mass (g)	Adventitious root mass as % of total root ash free dry mass	Longest adventitious root length (mm)	Number of adventitious roots per plant	Adventitious root porosity (% gas volume root volume^{-1})
10	0.79±0.15	39±4	113±8	51±10	11.4±0.5
200	0.57±0.14	25±3	117±15	38±9	9.7±1.7
400	0.53±0.06	25±2	126±10	45±4	10.1±0.5
600	0.36±0.10	17±4	118±7	42±6	9.5±0.9
800	0.32±0.09	16±5	88±15	40±12	10.0±1.4
P value	(*P*<0.01)	(*P*<0.001)	(*P*=0.22)	(*P*=0.84)	(*P*=0.96)

Values are means of four replicates ± s.e

most apical 40 mm (laterals prevented measurements further than 40 mm behind the root tip), and averaged 210 nmol m^{-2} s^{-1} (Table 2).

The porosity of adventitious roots of waterlogged *T. pergranulata* was 10%±0.5 for plants grown at 10–800 m*M* NaCl (Table 1), and was approximately 3-fold higher than the lateral root porosity of this species (*P*<0.001). NaCl and waterlogging treatments had no effect on lateral root porosity of *T. pergranulata* (average 3.2%±0.4), *T. indica* (average 2.3%±0.3), nor *T. mellaria* (average 2.9%±0.4).

Ethanol production rates in lateral root tips

In plants grown at 10 m*M* NaCl, rates of ethanol production in excised 10 mm lateral root tips in anoxia were (μmol g^{-1} fresh mass h^{-1}): *T. pergranulata*, 8.7±0.66, *T. indica*, 10.4±0.12, and *T. mellaria*, 8.7±0.5; these mean values were not significantly different (*P*=0.06). There were insufficient lateral root tips in *T. indica* and *T. mellaria* grown at 400 m*M* NaCl to measure rates of ethanol production, so measurements were only taken for *T. pergranulata*. The rate for *T. pergranulata* of 9.6±1.4 μmol g^{-1} fresh mass h^{-1} at 400 m*M* NaCl did not differ from that at 10 m*M* NaCl (*P*=0.6).

Fig. 6 Cross section at 30 mm behind the root tip of an adventitious root of *Tecticornia pergranulata*, grown in waterlogged sand culture with 10 m*M* NaCl. Root diameter is 0.69 mm

Discussion

Here we first summarise our key findings, and then discuss in the sections below physiological

Table 2 Proportional cross sectional area of aerenchyma in adventitious roots, and radial O_2 loss (ROL) (nmol m^{-2} s^{-1}) from adventitious roots when in an O_2-free medium, of *T. pergranulata* grown in waterlogged sand culture at 10 m*M* NaCl for 60 d

Distance behind root tip (mm)	Cross-sectional area of aerenchyma (%)	Radial O_2 loss (nmol m^{-2} s^{-1})
5	n.a.	200±3
10	7.5±1.6	192±11
20	10.4±0.3	199±18
30	11.5±1.2	243±25
40	n.a.	217±18
50	11.5±2.0	n.a.
80	12.4±2.0	n.a.
P value	(*P*=0.25)	(*P*=0.53)

Average root length was 118 mm±28. Values are means of three replicates ± s.e. ROL was only taken to 40 mm behind the root tip, as lateral roots prevented the movement of the electrode to more basal positions. n.a. = not available

aspects of waterlogging and salinity tolerance in the three *Tecticornia* species, with comparisons made also to other halophytes and wetland plants. *T. pergranulata*, *T. indica* and *T. mellaria* were all salt tolerant, RGR was highest at 200–400 m*M* NaCl, and even at 800 m*M* NaCl shoot RGRs were only reduced by 9%–39%. Differences in waterlogging tolerance were apparent. *T. pergranulata* and *T. indica* were both tolerant of waterlogging with reductions in shoot RGR of less than 29%. By contrast, eight *T. mellaria* individuals died when waterlogged, and shoot RGR was reduced by 8%–56% in the plants that survived. For all three species, root RGR was less affected by salinity than shoot RGR, but waterlogging had severe adverse effects on root growth. *T. pergranulata* produced aerenchymatous adventitious roots in response to waterlogging, whereas the two other species did not develop these additional roots.

Waterlogging tolerance

T. pergranulata formed adventitious roots when grown in waterlogged sand culture, and these roots were not formed in either *T. indica* or *T. mellaria*. It is unclear whether the formation of adventitious roots as a response to waterlogging is widespread in the Salicornioideae, although in a survey of the 15 Salicornioideae species that occur in southern Africa, utilising 500 specimens collected from a variety of field locations, five species were characterised by the formation of adventitious roots (O'Callaghan 1992). Adventitious root formation has also been observed in *Sarcocornia fruticosa* (syn. *Arthrocnemum fruticosum*) (SaadEddin and Doddema 1986). Adventitious roots contribute to waterlogging tolerance, and while many wetland species form these roots constitutively, both wetland and non-wetland species may form more adventitious roots when the soil becomes waterlogged (Jackson and Drew 1984). As one example, the numbers of adventitious roots formed in response to waterlogging has been associated with tolerance of different *Rumex* species (Laan et al. 1989; Blom et al. 1994). Thus, formation of aerenchymatous (see next paragraph) adventitious roots presumably contributed to waterlogging tolerance in *T. pergranulata*.

The adventitious roots of *T. pergranulata* contained aerenchyma, resulting in a porosity of 10%±0.5, at least 3-fold higher than porosity in lateral roots of this species and in those of *T. indica* and *T. mellaria* in waterlogged sand culture. Root porosity of 10% is low by comparison with many wetland species (40 species: 1%–53%, with an average of 22%±2; Justin and Armstrong 1987). The aerenchyma in adventitious roots of *T. pergranulata* was functional as demonstrated by ROL just behind the tips of roots in an O_2-free medium with shoots in air. The ROL profile along the roots was flat, indicating development of a partial barrier to ROL, as reported for other wetland species (e.g. *Rumex. scleratus*, *Caltha palustris* and *Rumex palustris*; Visser et al. 2000). Internal O_2 diffusion to the apex determines root penetration into anaerobic substrates (Armstrong 1979). The maximum length of 126 mm for the main axis of adventitious roots of *T. pergranulata* in waterlogged conditions is within the range of 85–196 mm in eight wetland species with root porosity of 9.4%–15.8% recorded by Justin and Armstrong (1987).

The potential importance of adventitious root formation by *T. pergranulata* is further emphasised by the marked reductions in lateral root RGR during waterlogging (64%–98% in *T. pergranulata*) and complete cessation of growth of lateral roots in *T. indica* and *T. mellaria*. Adventitious root formation in *T. pergranulata* resulted in the RGR of the total root system being reduced by 43%–59%, rather than completely inhibited as occurred in the other two species. Interestingly, although lateral root growth ceased in *T. indica* and *T. mellaria*, lateral roots must have maintained at least partial functioning as some shoot growth continued. Lateral roots of the three *Tecticornia* species had low porosity (2.3%–3.2%), so any internal O_2 movement would only have entered the upper parts (cf. Armstrong 1979). The woody portion of the tap root contains aerenchyma in several *Salicornia* species (de Fraine 1912) and in *Sarcocornia fruticosa* (syn. *Arthrocnemum fruticosum*) (SaadEddin and Doddema 1986), so the woody portion of the tap root in the three species of *Tecticornia* (50–100 mm in length), may have facilitated the diffusion of O_2 to lateral roots (porosity of the tap root was not assessed in the present study). Justin and Armstrong (1987) noted

that four wetland species with root porosity <4.4% had shallow roots (<10 mm) possibly accessing O_2 in the surface layers of flooded soil. However, the longest lateral roots of the three *Tecticornia* species were 380 mm below the sand surface (these roots presumably formed prior to imposing the waterlogging treatment), and lateral roots did not proliferate in the upper layers of waterlogged pots.

Lateral roots of the three *Tecticornia* species potentially utilised ethanolic fermentation to survive waterlogging; rates of ethanol production were 8.7–10.4 μmol g^{-1} fresh mass h^{-1} in 10 mm root tips when in anoxia at 25°C. Ethanolic fermentation rates in the three species of *Tecticornia* were relatively high in comparison with rates observed in other species, such as in 10 mm root tips of *Lophopyrum elongatum* at 2.84 μmol g^{-1} fresh mass h^{-1} (at 20°C, McDonald et al. 2001), and rates in 20–40 mm root tips of five wetland species removed from waterlogged soil ranged from 0.84 μmol g^{-1} fresh mass h^{-1} in *Poa trivalis* to 2.65 μmol g^{-1} fresh mass h^{-1} in *Filipendula ulmaria* (at 25°C, Smith et al. 1986), although the larger proportion of fully expanded cells in these larger tips (i.e. less cytoplasmic volume per unit fresh mass) would likely have lowered rates expressed on a fresh mass basis (cf. Gibbs and Greenway 2003). The rates in the three *Tecticornia* species were, however, similar to those in hypoxically pre-treated 5 mm root tips of wheat (9.8 μmol g^{-1} fresh mass h^{-1} at 25°C) and 10 mm tips of maize roots (9.2 μmol g^{-1} fresh mass h^{-1} at 25°C), both known only to survive anoxia for days, not weeks (Waters et al. 1991; Hole et al. 1992). So, although ethanolic fermentation presumably contributes to anoxia tolerance, other metabolic traits are also required (Gibbs and Greenway 2003), so further studies of lateral root physiology and survival during waterlogging are needed for *Tecticornia* species.

Salt tolerance

The highest shoot RGRs of 0.028–0.030 $g g^{-1}$ d^{-1} (ash-free dry mass) of the three *Tecticornia* species were relatively low in comparison with other dicotyledenous halophytes (see below); extending the conclusion of Short and Colmer (1999) that *T. pergranulata* is a relatively slow-growing halophyte. For comparison, shoot RGRs of 10 dicotyledenous 'euhalophytes' grown in drained sand culture for 35 d were 0.039–0.081 $g g^{-1}$ d^{-1}, in treatments of 180–360 m*M* NaCl that resulted in most growth (Glenn and O'Leary 1984). At 720 m*M* NaCl, RGRs of these plants had declined by 15%–55% compared with the optimal rates (Glenn and O'Leary 1984), a similar range of reductions as measured for the three *Tecticornia* species in the present study. Ash-free dry mass, or ethanol-insoluble dry mass, were recommended as being the most appropriate measures of growth in dicotyledenous halophytes as the influence of high tissue ion concentrations is eliminated (Greenway and Munns 1983). Shoot ash contents of 44%–66%, similar to the highest values measured in the three species of *Tecticornia* in the present study, have previously been reported in *Salicornia bigelovii* (Ayala and O'Leary 1995), *Salicornia europaea* (Guy et al. 1984) and *Suaeda fruticosa* (Khan et al. 2000).

The regulation of tissue ions is essential to ensuring that tissue water potential is maintained below the external medium, and also to prevent excessive accumulation of ions that may damage metabolism (Flowers et al. 1986; Flowers and Colmer 2008). The concentrations of Na^+ and Cl^- in expanding shoot tissues, required to maintain π_{sap} more negative than the external solution, were well regulated in the three *Tecticornia* species; at 800 m*M* NaCl, Na^+ and Cl^- in expanding shoot tissues were 88%–137% of the concentrations in the external solution. The majority of Na^+ and Cl^- was presumably in vacuoles, while the cytoplasm would have been osmotically balanced with K^+ and 'compatible' organic solutes (reviewed by Flowers et al. 1977; Flowers and Colmer 2008).

Glycinebetaine appears to be a major compatible solute in species from the Chenopodiaceae. In 23 Chenopodiaceae species collected from the field, or grown in controlled conditions, proline concentrations were <7.5% of those of glycinebetaine (Cavalieri and Huang 1979; Storey and Wyn Jones 1979; Gorham et al. 1980; Briens and Larher 1982; Poljakoff-Mayber et al. 1987; Pujol et al. 2001). Glycinebetaine concentrations on a tissue water basis in expanding shoot tissues of the three *Tecticornia* species grown at 10–800 m*M* NaCl were 46–140 m*M* (0.37–0.90 mmol g^{-1} dry mass). These shoot glycinebetaine concentrations in *Tecticornia* were similar to those in *Halocnemum strobilaceum*

(32–91 m*M*) in sub-irrigated sand culture at 0–680 m*M* NaCl for 180 d (Pujol et al. 2001; calculated from their data) and *Salicornia europaea* (39–80 m*M*) in solution culture at ~44–~1,400 m*M* NaCl for 35 d (Guy et al. 1984; calculated from their data). In addition to glycinebetaine, the combined concentrations of sucrose, glucose and fructose in expanding shoot tissues of the three *Tecticornia* species ranged from 28 to 108 m*M*, being 28%± 3%–127%±24 of the glycinebetaine concentrations. Support for the quantitative significance of glycinebetaine, K^+ and sugars measured in the three *Tecticornia* species at 800 m*M* NaCl, as likely cytoplasmic osmotica, is demonstrated by the simplistic assumptions that: (i) cytoplasmic osmotic volume was 10% of the vacuolar osmotic volume, and (ii) glycinebetaine, K^+ and sugars were in the cytoplasm, and Na^+ and Cl^- were in vacuoles; then, glycinebetaine would comprise 18%–33%, K^+ 10%–22%, and sugars 9%–62%, of the cytoplasmic π necessary to balance Na^+ and Cl^- in the vacuole.

Interactions of waterlogging and salinity

When saline and waterlogged conditions occur simultaneously, energy deficits in poorly aerated roots may lead to increased shoot Na^+ and Cl^- concentrations in non-halophytes (Barrett-Lennard 2003), although this usually does not occur in wetland halophytes (Colmer and Flowers 2008). Energy deficits in poorly aerated roots can also lead to reduced uptake of nutrients, including K^+ (Jackson and Drew 1984; Colmer and Greenway 2011). In the present study, expanding shoot tissue Na^+, Cl^- and K^+ concentrations in waterlogged conditions at 800 m*M* NaCl, as a percentage of sub-irrigated concentrations, were respectively 82%, 80% and 57% in *T. pergranulata*, 116%, 120% and 66% in *T. indica*, and 94%, 112% and 87% in *T. mellaria*. Thus, the three species of *Tecticornia* grown in waterlogged treatments at 800 m*M* NaCl maintained regulation of Na^+ and Cl^- net uptake, but the capacity for K^+ net uptake was reduced. Similarly for the coastal marsh halophytes *Salicornia dolichostachya* and *Salicornia ramosissima* in hypoxic solution (O_2 was 0.5% or 5% of air-equilibrium) with 150–200 m*M* NaCl, shoot Na^+ and Cl^- generally did not change, but shoot K^+ was reduced by up to 42% (Schat et al. 1987). These results for species in the Salicornioideae from waterlogging-prone areas, contrast with the more severe effects of 14 d root-zone O_2 deficiency on the halophyte *Atriplex amnicola* (Chenopodiaceae) at 400 m*M* NaCl, for which shoot Na^+ and Cl^- concentrations increased by 59% and 100%, attributed to a breakdown in active 'exclusion' mechanisms as a consequence of root death in hypoxia (Galloway and Davidson 1993).

Conclusions

T. pergranulata, *T. indica* and *T. mellaria* were all salt tolerant; these three species each utilised Na^+ and Cl^- as osmotica in shoot tissues and also contained relatively high concentrations of the 'compatible' solute glycinebetaine. *T. pergranulata* and *T. indica* were both tolerant of waterlogging, even when salinity varied across a wide range (10–800 m*M* NaCl), whereas waterlogging tolerance in *T. mellaria* was variable, with death of some individuals. Waterlogging tolerance of *T. indica* and *T. mellaria* presumably depends upon lateral roots maintaining at least partial function when waterlogged, whereas *T. pergranulata* formed adventitious roots with aerenchyma. Adventitious roots containing aerenchyma should aid tolerance of prolonged waterlogging and therefore these roots likely contribute to the ability of *T. pergranulata* to grow on mud flats, in contrast to *T. indica* and *T. mellaria* that generally occupy low dunes on the outer margins of salt lakes. An understanding of species zonation at salt lakes will require further work to investigate tolerance of *Tecticornia* species to inundation resulting in submergence (submergence tolerance of *T. pergranulata* was demonstrated in Pedersen et al. 2006; Colmer et al. 2009), and responses to higher salinities and to periods of waterlogging >60 d tested in the present study.

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Appendix

Table 3 *P* values and trends in variate responses determined via analysis of variance (one- and two-way, as appropriate), for *Tecticornia pergranulata*, *T. indica* and *T. mellaria*, grown in sub-irrigated or waterlogged sand culture at 10–800 m*M*NaCl for 60 d. Water-level treatments (WT) were sub-irrigated (SI) or waterlogging (WL)

	P values and trends for 2-way ANOVA, response of each species to NaCl treatment and Water-level treatment (WT)				*P* values and trends for 1-way ANOVA, response of each species to NaCl treatment in either Sub-irrigated (SI) or Waterlogged (WL) treatments		
	2 way interaction (NaCl ×WT)	Main Effect (NaCl)	Main Effect (WT)	Trend and R^2 values		(NaCl)	Trend and R^2 values
Shoot RGR							
T. pergranulata	<0.05	0.08	<0.001	na	SI	<0.05	lin (79%) & quad (19%)
					WL	0.21	na
T. indica	0.72	<0.001	<0.001	lin (22%) & quad (76%)	SI	na	na
					WL	na	na
T. mellaria	na	na	na	na	SI	<0.001	lin (31%) & quad (61%)
					WL	0.06	na
Root RGR							
T. pergranulata	<0.05	<0.05	<0.001	na	SI	<0.001	lin (81%)
					WL	0.94	na
T. pergranulata (primary vs primary + adv.)	0.34	0.17	<0.001	no trend		na	na
T. indica	0.91	0.38	<0.001	no trend	SI	na	na
					WL	na	na
T. mellaria	na	na	na	na	SI	0.47	na
					WL	0.37	na
Osmotic potential							
T. pergranulata	0.86	<0.001	0.18	lin (97%)	SI	na	na
					WL	na	na
T. indica	0.32	<0.001	<0.001	lin (99%)	SI	na	na
					WL	na	na
T. mellaria	na	na	na	na	SI	<0.001	lin (88%)
					WL	<0.001	lin (81%)
Water content							
T. pergranulata	<0.001	<0.001	<0.001	na	SI	<0.001	lin (94%)
					WL	<0.001	lin (99%)
T. indica	<0.01	<0.001	<0.001	na	SI	<0.001	quad (55%)
					WL	<0.001	lin (96%)
T. mellaria	na	na	na	na	SI	<0.05	quad (87%)
					WL	<0.05	lin (89%)
Ash content							
T. pergranulata	0.14	<0.001	< 0.001	lin (99%) & quad (1%)	SI	na	na
					WL	na	na
T. indica	0.11	<0.001	< 0.001	lin (98%)	SI	na	na
					WL	na	na
T. mellaria	na	na	na	na	SI	<0.001	lin (88%) & quad (1%)
					WL	<0.01	lin (86%)

Table 3 (continued)

	P values and trends for 2-way ANOVA, response of each species to NaCl treatment and Water-level treatment (WT)				*P* values and trends for 1-way ANOVA, response of each species to NaCl treatment in either Sub-irrigated (SI) or Waterlogged (WL) treatments		
	2 way interaction (NaCl ×WT)	Main Effect (NaCl)	Main Effect (WT)	Trend and R^2 values		(NaCl)	Trend and R^2 values
Tissue Na^+							
T. pergranulata	<0.001	<0.001	<0.001	na	SI	<0.001	lin (91%) & quad (8%)
					WL	<0.001	lin (96%)
T. indica	0.99	<0.001	<0.001	lin (95%) & quad (4%)	SI	na	na
					WL	na	na
T. mellaria	na	na	na	na	SI	<0.001	lin (98%)
					WL	<0.01	lin (94%)
Tissue Cl^-							
T. pergranulata	0.48	<0.001	<0.001	lin (97%)	SI	na	na
					WL	na	na
T. indica	<0.05	<0.001	0.22		SI	<0.01	lin (95%)
					WL	<0.001	lin (93%) & quad (6%)
T.mellaria	na	na	na	na	SI	<0.001	lin (94%)
					WL	<0.001	lin (97%)
Tissue K^+							
T. pergranulata	<0.05	<0.001	<0.001		SI	0.1	na
					WL	<0.001	lin (85%) & quad (14%)
T. indica	<0.05	<0.001	<0.01		SI	<0.05	lin (76%)
					WL	0.32	na
T. mellaria	na	na	na	na	SI	<0.001	lin (84%) & quad (13%)
					WL	0.35	na
Tissue GB							
T. pergranulata	<0.01	0.15	0.74		SI	<0.01	lin (57%) & quad (4%)
					WL	<0.05	lin (92%)
T. indica	<0.05	<0.05	<0.05		SI	<0.05	quad (65%)
					WL	<0.05	lin (48%) & quad (50%)
T. mellaria	na	na	na	na	SI	<0.05	lin (79%) & quad (21%)
					WL	<0.6	na
Root porosity							
T. pergranulata (laterals only)	0.64	0.9	0.09		SI	na	na
T. pergranulata (adv. vs lateral)	0.57	0.96	<0.001	no trend			
T. indica	0.9	0.18	0.51		SI	na	na
					WL	na	na
T. mellaria	na	na	na	na	SI	0.6	na
					WL	0.07	na

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