Preliminary assessment reveals tolerance to salinity and waterlogging (and these stresses combined) in Tedera (Bituminaria bituminosa var. albomarginata)

Teakle N.L., Real D.

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Preliminary assessment reveals tolerance to salinity and waterlogging (and these stresses combined) in Tedera (*Bituminaria bituminosa* var. *albomarginata*)

N.L. Teakle* and D. Real**

*Centre for Ecohydrology and School of Plant Biology, The University of Western Australia,
35 Stirling Hwy, Crawley WA 6009 (Australia)
**Department of Agriculture and Food Western Australia, South Perth WA 6151 (Australia); Future Farm Industries CRC and School of Plant Biology, The University of Western Australia,
35 Stirling Hwy, Crawley WA 6009 (Australia)
e-mail: dreal@agric.wa.gov.au

Abstract. Tedera *(Bituminaria bituminosa* var. *albomarginata*) is a promising herbaceous new drought tolerant perennial forage legume for southern Australia. In valley floors or high rainfall areas of southern Australia it is common for species to be exposed to transient waterlogging and/or salinity stresses. Tedera's tolerance to these stresses is unknown. We are presenting the results of a hydroponic experiment in which four accessions were grown in an aerated nutrient solution for 20 days and then exposed to the following treatments: (i) stagnant, non-saline; (ii) aerated, 200 mM NaCl; (iii) stagnant, 200 mM NaCl; and (iv) aerated, non-saline control. After 27 days treatment, biomass of shoots and roots were measured and root morphology studied. Two of the accessions had the same shoot and root biomass in stagnant and control treatments, indicating good waterlogging tolerance. New lateral roots with aerenchyma were formed in the stagnant treatments, a typical response of waterlogging tolerant species. Both the aerated and stagnant saline treatments had significant reductions in shoot and root biomass; however, some plants were still green with no dead leaves and producing new shoots. These preliminary results are encouraging that there is variability within Tedera to select elite plants with enhanced tolerance to transient waterlogging and salinity.

Keywords. Tedera – Salinity – Waterlogging – Aerenchyma.

Évaluation préliminaire révélant la tolérance à la salinité et à l'engorgement (et à ces stress combinés) chez Tedera (*Bituminaria bituminosa *var.* albomarginata*)

Résumé. *Tedera (*Bituminaria bituminosa *var.* albomarginata*) est une légumineuse pérenne herbacée très résistante à la sécheresse et prometteuse pour être utilisée dans le sud de l'Australie. Dans les vallées ou les zones à haute pluviosité en Australie, il est fréquent que les espèces aient à tolérer des périodes d'engorgement d'eau et/ou de salinité. La tolérance de Tedera à ces conditions adverses est inconnue. Dans ce travail nous présentons les résultats d'une expérience d'hydroponie au cours de laquelle quatre accessions de Tedera ont grandi pendant 20 jours dans une solution nutritive aérée et ont ensuite été soumises à un des traitements suivants : (i) engorgement d'eau, non saline ; (ii) aération, 200 mM NaCl ; (iii) engorgement d'eau, 200 mM NaCl ; et (iv) aération et non salin (témoin). Après 27 jours d'exposition aux différents traitements, on a pesé les parties aériennes et racinaires et on a étudié la morphologie racinaire. Deux des accessions du traitement d'engorgement ont eu le même poids de la partie aérienne et racinaire que le témoin, ce qui indique une bonne tolérance à l'engorgement. Les plantes engorgées ont formé de nouvelles racines latérales avec aérenchyme, ceci étant une réponse typique des plantes tolérantes à l'engorgement. Les traitements salins avec ou sans engorgement ont donné des réductions significatives de poids de la partie aérienne et racinaire, cependant certaines plantes sont restées vertes et ont continué de grandir. Ces résultats préliminaires sont très encourageants dans le sens qu'il existe une variabilité chez Tedera pour sélectionner des plantes présentant une bonne adaptation à l'engorgement transitoire et à la salinité.*

Mots-clés. *Tedera – Salinité – Engorgement – Aérenchyme.*

I – Introduction

Tedera *(Bituminaria bituminosa* var. *albomarginata*) is a drought tolerant perennial legume used as forage for goats in the Canary Islands (Ventura *et al.*, 2009). This species is regarded as a promising new option for drought-prone areas of southern Australia due to its ability to maintain green foliage over summer and withstand heavy grazing (Real *et al.*, 2009). However, in valley floors or high rainfall areas of southern Australia it is common for species to also be exposed to transient waterlogging and/or salinity stresses. Waterlogging frequently occurs in saline areas, reducing aeration of the soil and hence O_2 movement to plant roots (Rengasamy, 2006). Salinity and waterlogging stresses interact adversely to reduce pasture production, as very few species of agricultural relevance can tolerate the combination of salinity and waterlogging (Bennet *et al.*, 2009). The tolerance of Tedera to these stresses is unknown, and its ability to tolerate salinity and waterlogging will impact on the area of agricultural land suitable for Tedera production. The aim of this experiment was to evaluate the tolerance of four Tedera accessions to salinity and waterlogging (and these stresses combined).

II – Materials and methods

Four accessions of Tedera (designated as T3, T5, T6 and T9) were grown in a naturally lit glasshouse in root cooling tanks from August to September in Perth, Western Australia. Seeds were imbibed in 0.5 mM $CaSO_4$ and then carefully transferred to a mesh screen over aerated 10% concentration nutrient solution and kept in darkness for 3 d. The full strength nutrient solution consisted of macronutrients (mM): 0.5 KH_2PO_4, 3.0 KNO_3, 4.0 $Ca(NO_3)_2$, 1.0 $MgSO_4$; and micronutrients (µM): 37.5 $FeNa_3EDTA$, 23.0 H_3BO_3, 4.5 $MnCl_2$, 4.0 $ZnSO_4$, 1.5 $CuSO_4$ and 0.05 MoO_3. Solution pH was buffered with 2.5 mM MES [2-(N-Morpholino) ethanesulfonic acid] adjusted with KOH to 6.3. After 3 d, seedlings were transferred to 25% nutrient solution and exposed to light. After a further 4 d, individual seedlings were transplanted to 50% nutrient solution and another 7 d later the solution was changed to 100% concentration. Nutrient solutions were renewed weekly and topped up with deionised water as required. There were three replicates and each accession was represented by two seedlings per experimental unit.

Four treatments were imposed 20 d after imbibition and these were: aerated control (0.1 mM NaCl); saline treatment (aerated, 200 mM NaCl); stagnant non-saline treatment (non-aerated, 0.1 mM NaCl) and the combination of stagnant-plus-NaCl (non-aerated, 200 mM NaCl). NaCl was added in daily 50 mM increments until the final concentration of 200 mM. Hypoxia was imposed 24 h later in all pots assigned to stagnant and stagnant-plus-NaCl treatments, by bubbling with N_2 gas until the dissolved O_2 level was less than approximately 10% of air-saturated solution. The following day, the nutrient solution in these pots was changed to a stagnant deoxygenated (i.e. anoxic) 0.1% (w/v) agar solution to simulate waterlogging (Wiengweera *et al.*, 1997). Plants were harvested 27 d after treatments were initiated. Shoots were separated from roots and stem bases gently rinsed in DI water and blotted dry. Fresh weights of root and shoot tissues were recorded. A young lateral root close to the shoot base was removed and cross sections taken to examine aerenchyma. Shoot and roots were oven-dried for 3 d at 70℃ and dry weights were recorded.

III – Results and discussion

After 27 d of salt and stagnant treatments, all Tedera accessions were alive. Due to poor germination in the hydroponics, we were not able to evaluate T6 in the stagnant saline treatment. Accessions T5 and T9 had good waterlogging tolerance, as shoot mass in stagnant treatment was similar to control (Table 1). Tolerance to the stagnant treatment was associated with the formation of new lateral roots containing aerenchyma, a typical response of waterlogging tolerant species (Justin and Armstrong, 1987). Salt treatment significantly reduced the biomass of all accessions, although variability in response to salinity was observed. T5 was the most tolerant, with shoot and root dry mass relative to control 57% and 81% respectively (Table 1). The least salt tolerant accession was T6, with a relative shoot and root dry mass of only 30% and 20% respectively. All three accessions evaluated under the combined stagnant and saline treatment survived, although it is unlikely T9 would have survived much longer than the 27 d treatment period. However, some plants were still green with no dead leaves and producing new shoots. The most tolerant accession was again T5 (shoot biomass 33% of control), although there was still a significant adverse interaction between salinity and waterlogging for this accession as the combination of stresses reduced biomass by more than the sum of the individual stresses (Table 1). The adverse interaction is likely caused by increased uptake of Na^+ and Cl^- to the shoot when salinity is combined with root-zone O_2 deficiency (Barrett-Lennard, 2003).

Table 1. Shoot and root dry mass relative to control for Tedera accessions under stagnant and saline treatments. Treatments were imposed for 27 d and were initiated 20 d after seeds were imbibed. Values are means of 3 replicates. Control values were (shoot, root g/plant): T3 – 0.9, 0.3; T5 – 0.4, 0.1; T6 – 0.6, 0.3; T9 – 0.7, 0.2

Treatment	Plant #	Shoot mass (% of control)	Root DW (% of control)	Aerenchyma present
Stagnant, non-saline	T3	42	31	No
	T5	110	122	Yes
	T6	58	51	Yes
	T9	83	79	Yes
Aerated, 200 mM NaCl	T3	30	24	n.d.
	T5	57	81	n.d.
	T6	30	20	n.d.
	T9	47	47	n.d.
Stagnant, 200 mM NaCl	T3	25	16	n.d.
	T5	33	29	n.d.
	T6†	–	–	n.d.
	T9	19	15	n.d.

†T6 plants were not grown in stagnant plus saline treatment; n.d.: not demonstrated.

IV – Conclusions

These preliminary results are encouraging that there is variability within Tedera to select elite plants with enhanced tolerance to transient waterlogging and salinity. Future work is needed to evaluate the tolerance of Tedera over a range of salt concentrations and recovery from waterlogging. The results of this study clearly show that Tedera does possess some tolerance to both salinity and waterlogging, which suggests the area of agricultural land suitable for Tedera production is larger than initially expected.

References

Barrett-Lennard E.G., 2003. The interaction between waterlogging and salinity in higher plants: Causes, consequences and implications. In: *Plant and Soil*, 253, p. 35-54.

Bennett S.J., Barrett-Lennard E.G. and Colmer T.D., 2009. Salinity and waterlogging as constraints to saltland pasture production: A review. In: *Agriculture, Ecosystems & Environment*, 129, p. 349-360.

Justin S.H.F.W. and Armstrong W., 1987. The anatomical characteristics of roots and plant response to soil flooding. In: *New Phytologist*, 106, p. 465-495.

Real D., Correal E., Méndez P., Santos A., Ríos Ruiz S., Sternberg M., Dini-Papanastasi O., Pecetti L. and Tava A., 2009*.* *Bituminaria bituminosa* C.H. Stirton (synonym: *Psoralea bituminosa* L.). In: *Grassland Species*. FAO. http://www.fao.org/ag/AGP/AGPC/doc/GBASE/new_species/tedera/bitbit.htm).

Rengasamy P., 2006. World salinisation with emphasis on Australia. In: *Journal of Experimental Botany*, 57, p. 1017-1023.

Ventura M.R., Castanon J.I.R. and Mendez P., 2009. Effect of season on tedera (*Bituminaria bituminosa*) intake by goats. In: *Animal Feed Science and Technology*, 153, p. 314-319.

Wiengweera A., Greenway H. and Thomson C.J., 1997. The use of agar nutrient solution to simulate lack of convection in waterlogged soils. In: *Annals of Botany*, 80, p. 115-123.