Growth and development of *Lotus* and *Trifolium* species under saline and waterlogging conditions

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Abstract

Saline and waterlogging conditions can be detrimental to the growth of pasture plants. The aim of this study was to compare the tolerance of two new pasture species, *Lotus tenuis* and *Trifolium tumens*, to comparable traditional species, *L. corniculatus*, *L. pedunculatus*, *T. repens*, and *T. fragiferum*. Plant growth was studied under four treatments; control (non-saline, non-waterlogging), saline (150mM), waterlogging, and saline with waterlogging. The waterlogging treatment was imposed by placing the pots in plastic troughs where the water level was maintained at 2 cm below the rim. Root and shoot dry matter yield, and root length were measured 27 days after emergence. There was no interaction ($P<0.05$) between salinity and waterlogging. Waterlogging had a negative effect on root length for all species, but not on shoot or root dry matter. Of the *Lotus* species, the four accessions of *L. tenuis* were not affected by salinity, whereas salinity reduced shoot dry matter yield in the accessions of *L. corniculatus*, *L. pedunculatus* and both *Trifolium* species. Although there was a 31% reduction in *L. corniculatus* shoot dry matter yield with the salinity treatment, it was the highest yielding species for this treatment. Salinity reduced root dry matter yield in *T. tumens* and *T. repens*, further confirming their sensitivity to salinity. The results confirm that *L. tenuis* has tolerance of saline and waterlogging conditions where it has potential to be a useful species.

Key Words

Trifolium tumens, Lotus tenuis, Lotus corniculatus, Lotus pedunculatus

Introduction

Salinity is an ever increasing problem in Australia, with over 2 million hectares of agricultural land reported as being affected by salinity (Australian Bureau of Statistics 2002). In addition to this problem, plants can be exposed to waterlogging during certain times of the year. The combined effect of salinity and waterlogging can lead to extremely poor productivity from pasture legumes. Traditional clover varieties such as *Trifolium fragiferum* (strawberry clover) and *Trifolium repens* (white clover) are resistant to waterlogging (Gibberd et al. 2001). *T. fragiferum* shows moderate salinity tolerance (Rumbaugh et al. 1993), whereas *T. repens* is susceptible to salinity (Rogers et al. 1997).

There are several published papers pertaining to the waterlogging tolerance of the three species of *Lotus* that were grown in this study, (Gibberd et al. 2001; Striker et al. 2005) but there has been less research on the combined effects of salinity and waterlogging (Teakle et al. 2006). As a newly domesticated species, there has also been very little work done on *Trifolium tumens*, and no indication in the literature of its salinity or waterlogging tolerance. The objective of this project was to determine if there is an interaction between salinity and waterlogging for a range of clover and lotus species.

Methods

Seed was supplied by the TIAR genetic resources collection and Tasglobal Seeds and consisted of three *Lotus* and three *Trifolium* species. The *Lotus* species included *L. corniculatus* (Tas 2948), *L. pedunculatus* (Tas 280), and *L. tenuis* (Tas 2527, Tas 2828, Tas 2849, Tas 2526). The *Trifolium* species were *T. repens* (Tas 2861), *T. fragiferum* (Palestine) and a new cultivar of *T. tumens* (Permatas). The seeds were all scarified to counter hard seededness, then placed on filter paper in petri dishes with distilled water and germinated in a 21^{o}C room for three days. Six germinated seeds were planted into 100mm pots containing a 30 percent perlite and 70 percent sterilized sand mix along with 5 ml of Osmocote slow release fertiliser (Scotts Australia 2009) on top of the growing medium. Plants were grown under overhead irrigation for 28 days and a full strength Hoagland nutrient solution was applied to all pots three times per week.

The experiment was set up in a randomized complete block design with nine species/accessions, four salinity and waterlogging treatments and four replicates. Each experimental unit consisted of one pot with four seedlings of a single species/accession. The treatments included salinity at 150 mM, waterlogging, 150 mM salinity and waterlogging combined, and a control. The waterlogging treatment was imposed for the duration of the trial by placing the pots in plastic troughs. A gravity-fed irrigation system with an overflow pipe in each trough was used to maintain the water level at approximately 2 cm below the rim. All troughs were drained and replenished halfway through the trial to eliminate the potential of nutrients, especially salts, concentrating in the water supply. After 27 days of imposing the treatments the plants were harvested and analyzed for shoot and root dry matter (DM) yield, and root length. All experiments were analysed using PROC GLM in SAS, v. 9.1 (SAS Institute, 2003) and Fisher's LSD was used to test the differences ($P \leq 0.05$) among means.

Results

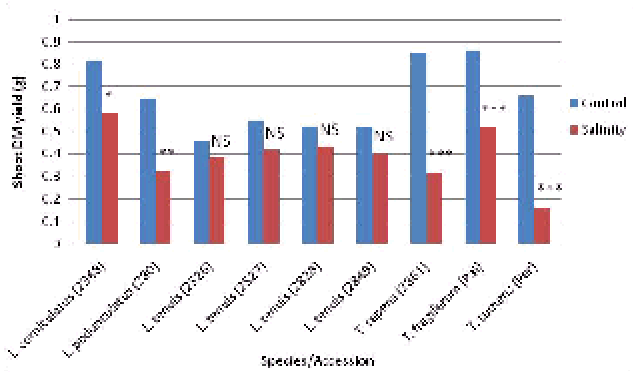
There was a significant effect of waterlogging on the root length, but not on shoot or root weight (Table 1). The mean root length decreased from 176 mm in the control treatment to 158 mm in the waterlogged treatment.

Table 1. The significance of treatments on shoot DM yield, root DM yield and the longest root length.

Treatment	Shoot DM yield (g)	Root DM yield (g)	Root Length (mm)
Salinity (S)	***	***	***
Waterlogging (W)	NS	NS	***
Accession (A)	***	***	***
S x W	NS	NS	NS
S x A	**	*	**
W x A	NS	NS	NS
S x W x A	NS	NS	NS

NS, Not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

For all measured variables there was a significant interaction between salinity and accession (Table 1). The interactive effect of salinity and accession on shoot DM yield is shown in Figure 1. For shoot DM yield of *L. tenuis* accessions there was no significant difference between the control and salinity treatments and they were the lowest yielding accessions under the control treatment. *L. corniculatus* had the third highest shoot DM yield under the control treatment. For all species except *L. tenuis* there was a significant decline in shoot DM yield under saline conditions. The shoot DM yield of *L. pedunculatus* under saline conditions was about half that of the control. Shoot DM yield of all the *Trifolium* cultivars was greatly affected by the salinity treatments imposed, particularly *T. tumens* which experienced a decrease of 75%.



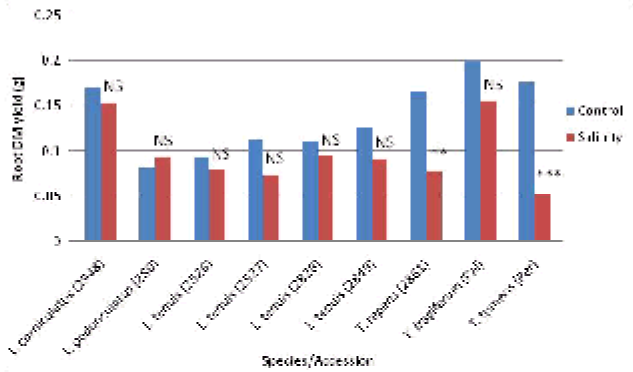


Figure 1. The interaction of salinity and cultivar on shoot dry matter after 27 days of imposed stress. Indicated significance levels are the comparison between the control and salinity treatment for each cultivar (NS, Not significant; * P <0.05; ** P<0.01; *** P<0.001)

Figure 2. The interaction of salinity and cultivar on root dry matter after 27 days of imposed stress. Indicated significance levels are the comparison between the control and salinity treatment for each cultivar (NS, Not significant; * P <0.05; ** P<0.01; *** P<0.001)

The interaction of salinity and cultivar on root DM yield is shown in Figure 2. The results were similar to shoot DM yield with no effect of salinity on root DM yield for *L. tenuis* accessions, *L. corniculatus*, *L. pedunculatus* or *T. fragiferum*. Root DM yield under the salinity treatment was reduced in *T. repens* by approximately 50% and in *T. tumens* by approximately 70%.

There was no significant effect of salinity on root length for *L. pedunculatus, L. tenuis* or *T. fragiferum;* whereas the root length of *L. corniculatus, T. repens* and *T. tumens* was slightly reduced by the salinity treatment (Figure 3).

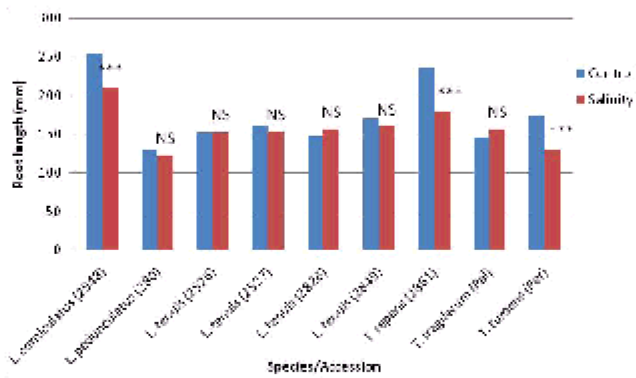


Figure 3. The interaction of salinity and cultivar on the longest root length after 27 days of imposed stress. Indicated significance levels are the comparison between the control and salinity treatment for each cultivar

(NS, Not significant; * P <0.05; ** P<0.01; *** P<0.001)

Discussion and Conclusion

The results confirm that the effect of salinity differs between species. *L. tenuis* was least affected by salinity with no effect on shoot dry matter yield, root dry matter yield, or shoot length. This result relates well with conclusions made by Teakle et al. (2006) that showed that *L. tenuis* is highly salt tolerant. *L. corniculatus* was less salt tolerant than *L. tenuis* but more tolerant than *L. pedunculatus.* The commercially available *Trifolium* species performed as described in previous research under saline conditions (Rumbaugh et al. 1993; Rogers et al. 1997). The growth in plants of *T. repens* were significantly affected by salinity, while only the shoot dry matter yield of *T. fragiferum* was affected. For *T. tumens*, shoot DM yield, root DM yield, and root length were all significantly affected by salinity, and thus it can be concluded that the species is not salt tolerant.

There was no interaction between waterlogging and salinity, a result which is contrary to (Teakle et al. 2006) who documented that *L. tenuis* had a greater tolerance to the combined effects of waterlogging and salinity when compared to *L. corniculatus*.

Future research opportunities would benefit by using different levels of salinity to determine the boundaries of salt tolerance for the different species and by including, waterlogging-sensitive “check” species such as lucerne (Real et al. 2008), to provide a comparison with the experimental species. In addition, when studying perennial species such as *Lotus*, it may be necessary to impose the waterlogging treatment for longer periods (greater than 27 days) in order to determine genotypic differences in growth.

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