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Effects of NaCl stress on proline and cation accumulation in salt sensitive and tolerant turfgrasses

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Summary Concentrations of proline, sodium and potassium in shoot tissues of five turfgrass species were measured following exposure to 170 m*M* NaCl salinity stress. Salt tolerant 'Fults' alkaligrass and 'Dawson' red fescue restricted the accumulation of Na-ions to significatnly low levels compared to the salt sensitive Kentucky bluegrasses ('Adelphi' and 'Ram I') and 'Jamestown' red fescue. Accumulation of proline began in all species within 24 h of initiation of salt stress but at a more rapid rate and higher overall concentration for 'Fults' alkaligrass. Proline levels were variable and too low in relation to sodium accumulations to have any significant osmoregulatory role in salt tolerance among all cultivars tested with the possible exception of alkaligrass.

Introduction

Differences in salt tolerance among turfgrasses has been reported in a number of screening studies based largely upon salinity induced growth reduction or relative turf quality[1,8,10,16]. Although distinct groups of relative tolerance levels have been reported among most of the widely utilized species and some cultivars[13,14], very little work has been reported regarding the possible mechanisms associated with tolerance or sensitivity to salinity stress.

Halophytic as well as glycophytic plant species adjust to high NaCl levels by lowering tissue osmotic potentials with increased uptake of solutes, primarily Na-ions and Cl-ions[6,18]. However, in less tolerant species growth is usually inhibtied due to the toxic effects of accumulated solutes despite osmotic adjustment in tissues[6,11,18]. Such results have led to the conclusion that Na-ions and Cl-ions must be preferentially compartmentalized within the vacuoles of more tolerant species while organic solutes are accumulated in the cytoplasm[18]. Sensitive sites of metabolism would, therefore, be protected from salt toxicity while osmotic imbalances between the vacuole, cytoplasm and intercellular spaces are adjusted by accumulation of organic solutes. Numerous studies have demonstrated a significant high correlation between increased salt tolerance and accumulation of various organic compounds in plant tissues[1,7,11,17].

Rapid accumulation of proline in tissues of many plant species as a response to periods of salt, drought or temperature stress has been attributed to enzyme stabilization and/or osmoregulation[6,11,18]. Accumulation of free proline has been correlated with tissue Na-ion concentration for numerous plant species which strongly suggests a possible role in osmoregulation during salt stress[1,7,11,18]. Conversely, Tal *et al.*[15] have reported that salt sensitive species of tomato accumulate more proline than tolerant species.

Solute fluctuations under high salinity levels have been measured in salt tolerant and sensitive ecotypes of creeping bentgrass (*Agrostis stolonifera* L.), a commonly utilized turfgrass[1]. The salt tolerant ecotype accumulated more proline as well as other organic solutes in response to high salinity levels. Accumulations of Na-ions and Cl-ions were accompanied by concurrent decreases in K-ions for both ecotypes. However, K-ion loss and Na-ion accumulation were greater for the sensitive ecotype. Similar Na-ion and K-ion fluctuations under saline stress were reported for bermudagrass (*Cynodon dactylon* L.)[5] and seashore paspalum (*Paspalum vaginatum* Swartz.)[4].

In a previous paper we reported the separation of turfgrass species and cultivars for salt tolerance based upon differences in growth rates over a range of NaCl concentrations[16]. The objectives of this investigation were to measure solute fluctuations within these same species and cultivars following NaCl salinization with particular emphasis on the relationships between sodium, potassium and proline.

Materials and methods

Seed of 'Fults' alkaligrass (*Puccinellia distans* L. Parl.), 'Dawson' red fescue (*Festuca rubra* L. var trichophylla Gaud.), 'Jamestown' red fescue (*Festuca rubra* L. var commutata Gaud.), 'Adelphi' and 'Ram I' Kentucky bluegrass (*Poa pratensis* L.) were germinated and cultured for 8 weeks in pots containing washed silica sand. Temperature was maintained at 21°C ± 3.0 under 1.63 Wm^{-2} of cool-white fluoresent lighting with a 12h light/dark cycle. All pots received 50 ml of half strength Hoaglands solution[9] (pH 6.2) twice each 24 h period for the first 6 weeks of culture. Saline stress was imposed during the following 11 day period by adding 170 m*M* of NaCl to the nutrient solution.

Samples for proline and mineral analysis were taken 1, 3, 5, 7, 9 and 11 days after initiation of salt stress. At each sampling date 3 pots of each species along with controls were removed and plants were harvested by excising all shoots just below the base of the crown. All harvested samples were immediately frozen in liquid nitrogen and stored at − 11°C until analyzed.

Proline extractions were made using a modificaiton of the method outlined by Bates *et al.*[3] with approximately 0.5 g fresh weight of tissue. Each sample was homogenized in 10 ml of 3% aqueous sulfosalicylic acid followed by agitation for 1 h prior to filtration through #2 Whatman filter paper. After filtration 2 ml of extract from each sample was reacted with 2 ml of acid ninhydrin and 2 ml of glacial acetic acid followed by 1 h of heating at 100°C in an enclosed water bath. Samples were then quickly cooled by immersion in an ice bath and total proline was determined spectrophotometrically at 520 nm.

Approximately 0.5 g of oven dried tissue (70°C for 2 days) of each sample was dry ashed at 500°C for 6 h prior to flame photometric analysis for sodium and potassium. Actual Na-ion, K-ion and proline tissue accumulation levels were determined by subtracting mean control data from NaCl treated data for all cultivars during the entire experimental period.

Results and discussion

In a previous study of relative growth rates, alkalinegrass and 'Dawson' red fescue were shown to be more salt resistant than other red fescue cultivars with Kentucky bluegrass cultivars having the least tolerance[16]. In the present study, differences between cultivars over the imposed NaCl stress period were highly significant for Na-ion, K-ion and proline tissue concentrations and accumulation rates (Table 1). These results support the previous ranking study of these species and cultivars[16].

Table 1. Analysis of variance with single degree of freedom comparisons for each cultivar over time for salinity-induced changes in proline, sodium and potassium

		Mean Squares (× 10^3)		
Source	Df	Proline	Sodium	Potassium
Cultivars	4	21.2**	208.8**	4.3**
Adelphi	5	25.2**	137.5**	30.5**
Linear	1	120.4**	629.0**	101.5**
Quadratic	1	2.7*	23.3**	36.4**
Ram I	5	10.9**	115.2**	22.9**
Linear	1	48.8**	558.5**	100.9**
Quadratic	1	0.9*	9.8*	4.8*
Jamestown	5	9.7**	126.6**	24.4**
Linear	1	41.5**	611.8**	78.1**
Quadratic	1	2.6*	4.2^{NS}	39.2**
Dawson	5	14.2**	51.9**	23.1**
Linear	1	64.7**	244.8**	101.5**
Quadratic	1	1.5^{NS}	4.9^{NS}	7.0**
Fults (Alkaligrass)	5	15.5**	112.6**	21.5**
Linear	1	76.3**	53.9**	80.4**
Quadratic	1	0.1^{NS}	0.01^{NS}	22.5**
Error	60	0.5	1.5	0.9

*,**,NS Significant at the 5% level and 1% level, and not significant respectively.

Inorganic solute levels

Sodium and potassium ion concentrations of controls for each cultivar were stable and did not vary to any significant degree over the entire experimental period. Sodium ion levels for all control treatments never exceeded 8.0 mg g^{-1} tissue dry wt while K-ion control levels remained above 30.0 mg g^{-1}. In general, levels of sodium in shoot tissues increased while potassium levels decreased during the imposed

NaCl stress period for all cultivars (Fig. 1–3). Sodium accumulation coinciding with loss or restriction of K-ion uptake during salinity stress has been reported for *Sorghum bicolor*[17], *Agrostisstolonifera*[1], *Cynodon dactylon*[5], *Paspalum vaginatum*[4] and various pasture species[12]. During the entire stress period alkaligrass and 'Dawson' red fescue accumulated significantly less sodium than the less tolerant 'Jamestown' red fescue and the Kentucky bluegrasses ('Adelphi' and 'Ram I'). The Na-ion/K-ion ratios for the more salt tolerant alakaligrass and 'Dawson' red fescue ranged between 0.1 and 1.9 and 0.2 and 3.5 respectively over the entire experimental period (Fig. 1 and 2). In contrast, Na-ion/K-ion ratios ranged higher for 'Jamestown' (0.1–5.3); 'Ram I' (0.2–4.8), and 'Adelphi' (0.2–8.2) (Fig. 2 and 3). The more rapid accumulation of sodium in the less tolerant 'Jamestown', 'Ram I' and 'Adelphi' yielded Na-ion/K-ion ratios above 1.0 within 5 days of salt treatment. The Na-ion/K-ion ratios for alkaligrass and 'Dawson' remained below 1.0 for at least 9 days of the experimental period. The maintenance of lower Na-ion/K-ion ratios in the more tolerant cultivars is most likely a result of their ability to restrict Na-ion accumulations to comparatively low levels since K-ion levels between all cultivars varied little over time.

Proline accumulation

Proline concentration for all cultivars increased over the 11 day NaCl stress period (Fig. 1–3). Proline concentration for each cultivar was atleast 2 to 3 times the level of controls after day 1 and ranged between 6 and 15 times higher than controls on day 11. Throughout the stress period proline content was significantly higher for alkaligrass

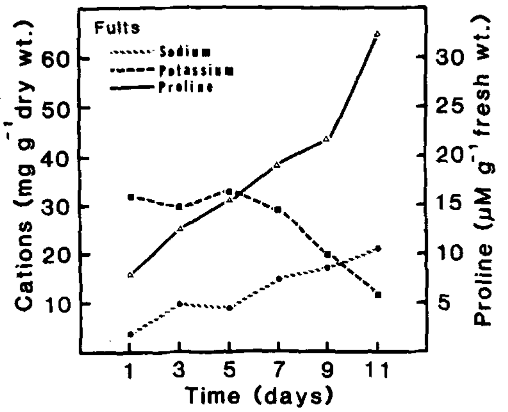

Fig. 1. Changes in proline, sodium and potassium levels in salt-tolerant 'Fults' alkalaigrass subjected to 170 m*M* NaCl salinity stress.

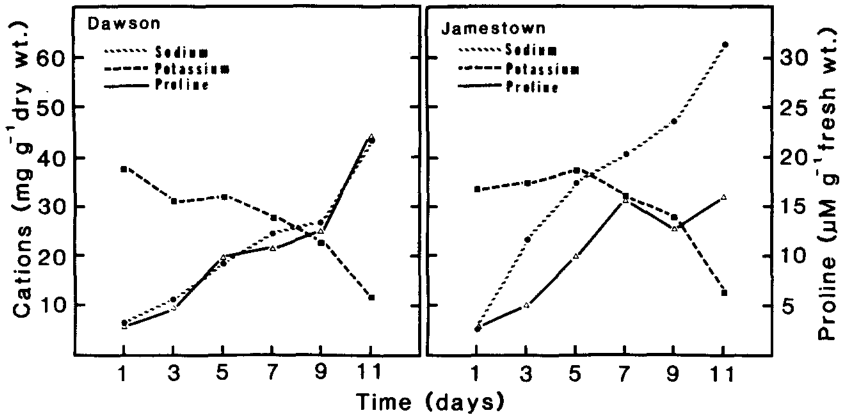

Fig. 2. Changes in proline, sodium and potassium levels in salt-tolerant 'Dawson' and salt-sensitive 'Jamestown' red fescues subjected to 170 m*M* NaCl salinity stress.

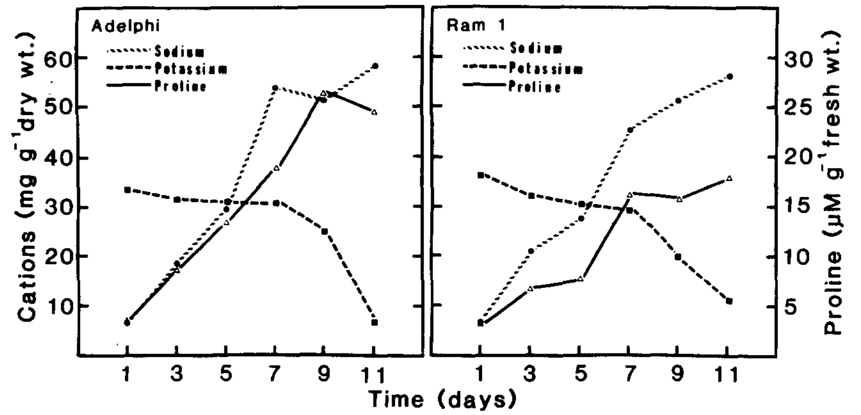

Fig. 3. Changes in proline, sodium and potassium levels in salt-sensitive 'Adelphi' and 'Ram I' Kentucky bluegrasses subjected to 170 m*M* NaCl salinity stress.

compared to the fescues and 'Ram I' Kentucky bluegrass. 'Adelphi' Kentucky bluegrass is known to have the least salt tolerance among those grasses tested but proline levels were similar to those of alkaligrass. However, the accumulation rate and total level of Na-ion were much more rapid and significantly higher for 'Adelphi' than for alkaligrass which may account for such a high proline content. In general, the least-tolerant grasses ('Adelphi', 'Ram I' and 'Jamestown') accumulated at least twice and and much as 4 times more Na-ions than the comparatively tolerant 'Dawson' red fescue and alkaligrass. Proline levels in 'Ram I' and 'Jamestown' were, however, much lower than 'Adelphi', alkaligrass and

'Dawson' indicating significant variability for proline accumulation between cultivars.

The osmoregulatory effect of proline was estimated by converting units of sodium data reported in this study as $mg\,g^{-1}$ dry weight to $\mu M\,g^{-1}$ fresh weight assuming a constant tissue water content of 90%. such a comparative estimate was considered very conservative since water content of salt tolerant and sensitive grasses usually range between 88 and 65% with higher salinity levels inducing lower water contents[1]. The Sodium/proline ratio for alkaligrass over the entire stress period never exceeded 2.9. Proline levels for all grasses tested increased in response to Na-ion accumulation. Alkaligrass, however, accumulated the highest level of proline while greatly restricting Na-ion accumulation compared with the other cultivars tested. In contrast, sodium/proline ratios for the remaining species and cultivars tested ranged between 7.8 and 17.1. Proline accumulation may be an important factor involved with osmoregulation only in alkaligrass in view of such a comparatively low sodium/proline ratio. Similar conclusions were reported in a study comparing salt tolerant and sensitive ecotypes of *Agrostis stolonifera* L., a widely utilized turfgrass species[1]. However, overall proline levels and accumulation rates were highly variable between all grasses tested in this study and, as such, are not indicative of relative salt tolerance levels. The results of this study indicate that increased salt tolerance relies strongly upon the ability of a grass to restrict and/or regulate the accumulation of Na-ions. With the exception of alkaligrass, proline accumulations were variable and too low in relation to Na-ion levels to have any significant effect on osmoregulation in these turfgrasses. Proline may, however, be complimentary to other organic compounds such as glycine-betaine and glycerol which are known to accumulate in numerous salt-stressed plants[6,7,11]. Further research is necessary to evaluate the osmoregulatory roles of these as well as other possible organic compounds in salt-stressed turfgrasses.

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