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Comparative studies on growth and physiological responses to saline and alkaline stresses of Foxtail millet (*Setaria italica* L.) and Proso millet (*Panicum miliaceum* L.)

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Abstract

Soil salinization and alkalization are widespread environmental problems. To compare the growth and physiological responses under saline stress (SS) and alkaline stress (AS), Foxtail millet (*Setaria italic L*) and Proso millet (*Panicum miliaceum L*) were tested under saline and alkaline conditions by mixing the two neutral salts (NaCl and Na_2SO_4) and the two alkaline salts ($NaHCO_3$ and Na_2CO_3). Compared to SS, AS inhibited in greater extent plant dry weight, relative growth rate (RGR), net assimilation rate (NAR), leaf area ratio (LAR) and relative water content (RWC) and the rates of reduction were more pronounced in Foxtail millet, indicating that tolerance to AS was higher in Proso millet than in Foxtail millet. Sodium concentration in leaves was enhanced at double by AS than in SS. Foxtail millet accumulated more Na in the leaves and stem but the roots of Proso millet contained a higher concentration of Na, indicating that Proso millet inhibited the transportation of Na from roots to shoots which resulted in less of a decrease in plant growth. The N content markedly decreased under AS in leaves, stems and roots of both species. The proline content in leaves increased under both treatments and it was higher in SS than in AS. These results suggest that the potential extent of stress-induced injuries was higher in Foxtail millet than in Proso millet and thus Proso millet may have a higher capacity to tolerate saline and alkaline conditions, especially the more deleterious alkaline condition.

Keywords: Alkaline stress, Nitrogen, *Panicum miliaceum*, Proline, *Setaria italica*, Saline stress.
Abbreviations: AS-alkaline stress; LAR-leaf area ratio; NAR-net assimilation rate; Pn-photosynthesis; RGR-relative growth rate; RWC-relative water content; SS-saline stress.

Introduction

Environmental stresses adversely affect the growth and productivity of plants, particularly those which are sensitive to salinity and sodicity (alkalinity). These stresses cause severe changes in the growth, physiology and metabolism of plants thus threatening the cultivation of plants around the globe (Lunde et al., 2007). According to an estimate, the world's land surface occupies about $13.2x10^9$ ha, no more than $7x10^9$ ha are potentially arable, and only $1.5x10^9$ ha are currently cultivated. Of the cultivated area, about $0.34x10^9$ ha (23%) are saline and another $0.56x10^9$ ha (37%) are sodic (Tanji, 1990). The loss of potentially cultivable land is likely to increase over the next 20 years and threats the world food supply. The problem of soil alkalinization due to $NaHCO_3$ and Na_2CO_3 may be more severe than that of soil salinization caused by neutral salts, like NaCl and Na_2SO_4. For example, in the northeast of China, alkalinized grassland has reached more than 70% (Kawanabe and Zhu, 1991); because soil salinization and alkalinization frequently co-occur, the conditions in naturally salinized and alkalinized soils are very complex; the total salt contents, their composition and the proportion of neutral to alkaline salts may vary in different soils. Grain productivity through green revolution has reached a ceiling, whereas the world population continues to grow (Akhtar and Saqib, 2008). Therefore, improving crop yields in normal and less productive soils, including saline and alkaline soils by combating those stresses is highly desirable to feed the ever-increasing population. Plants under saline conditions encounter three inevitable factors (Islam, 2001). First, salt decreases the osmotic potential of the soil solution effectively creating water stress for plants. It can result in specific ion toxicity due to excess accumulation of Na^+ or Cl^- in plant cells, which is the second effect on plants. Lastly, the interaction of salts with mineral nutrients may result in nutrient imbalances and deficiencies (Munns, 2002; Munns and Tester, 2008). Halophytes cope with this situation by actively taking up Na^+, which acts as an osmoticum to maintain the water potential gradients necessary for continuous water uptake (Ehret and Plant, 1999). These plants also generate a higher level of osmotically active compounds (proline, glycine betaine, etc.) in the cells in order to sustain adequate gradients for water uptake (Hasegawa et al., 2000). To induce tolerance against toxic

Na^+ sensed by plants, the regulation of K^+ uptake and/or prevention of Na^+ entry, efflux of Na^+ from the cell, and utilization of Na^+ for osmotic adjustment are the strategies commonly used by plants to maintain desirable Na^+/ K^+ ratios in the cytosol (Glenn and Brown, 1999). The Na^+, K^+, Ca^{2+}, and Mg^{2+} are the main cations of dissoluble mineral salts, and Cl^-, SO_4^{2-}, HCO_3^-, CO_3^{2-}, and NO_3^- are the corresponding main anions in saline and alkaline soils, which come from neutral salts or alkaline salts (Läuchli and Lüttge, 2002). Alkaline salts ($NaHCO_3$ and Na_2CO_3) induce much stronger destructive effects on plants than neutral salts (NaCl and Na_2SO_4) (Shi and Yin, 1993). When salinized soil contains HCO_3^- and/or CO_3^{2-}, which raise the soil pH, plants suffer damaging effects of both saline and alkaline stresses (Yang et al., 2008a). The contributory role of proline to osmotic adjustment has been reported by many researchers (Kavi Kishor et al., 2005; Ashraf and Foolad, 2007). Proline has also been considered as a carbon and nitrogen source for growth, a stabilizer for the membrane and some macromolecules and also a free radical scavenger under stress conditions. However, to date, researches into salt stress have emphasized NaCl as the main contributing factor to proline accumulation, but there is very little published information available regarding this issue under alkaline stress condition. Proso millet (*Panicum miliaceum* L.) is an important forage species of the largest genus *Panicum*, which includes more than 400 species (Roshevits, 1980). This plant naturally grows in hot and dry areas where a high salt content is the characteristic of most soils and it has been cultivated for both its high food and feed value. Foxtail millet (*Setaria italica* L.) is also widely cultivated in arid and semi-arid regions as a food and fodder crop. The morpho-physiological, cellular and molecular responses of many crop species to salinity/alkalinity stresses have been extensively investigated but, unfortunately, millets like Foxtail and Proso millets have not been explored in this way to date. Therefore, the present study was aimed to assess inter-species variation on growth and physiological responses to saline and alkaline stresses of Foxtail millet and Proso millet in their vegetative stage.

Results

Plant growth

The plant dry matter yield of both Foxtail millet and Proso millet declined with SS and AS and the decline was mostly caused by a reduction in leaf and stem biomass. However, Proso millet produced a significantly greater amount of dry matter than Foxtail millet (Fig 1). A marked relative reduction (37 and 62% under SS and AS, respectively) in the shoot dry mass was observed in Foxtail millet, as compared to Proso millet (22 and 45%, respectively). Moreover, decreases of 40 and 17% more than the control were also recorded for root dry mass under the AS condition in Foxtail millet and Proso millet, respectively. The values for the root/shoot ratio increased with the stress treatments and reached a maximum in Foxtail millet under the AS condition (data not shown). The RGR and NAR of both species decreased significantly under AS condition. The reduction percentages of RGR and NAR of alkaline treated Foxtail millet were 44 and 33%, whereby 31 and 27% in the case of Proso millet, respectively (Table 1). It is noteworthy that a noticeable reduction of the LAR was observed only in Foxtail millet under AS but no statistical differences were observed among the treatments in Proso millet.

Relative water content

Stress treatments caused a significant decrease in the RWC and rate of reduction was greater in AS than in SS in both tested species (Fig 2). The relative reduction was more marked in Foxtail millet than in Proso millet. The RWC was almost the same between the two species under control treatment (87 and 88% in Foxtail millet and Proso millet, respectively), however, under stress conditions, it tended to be lower in Foxtail millet (70 and 61% under SS and AS, respectively) than in Proso millet (74 and 68% under SS and AS, respectively).

Ionic concentrations

Sodium

The Na concentrations in the leaves, stems and roots increased under both stresses, and the increases under the AS condition were significantly greater than those under SS in all plant parts of both species with the exception of the roots of Foxtail millet, which accumulated a significantly higher Na concentration under the SS condition (Table 2). Compared to Proso millet, Foxtail millet accumulated almost three times more Na under the SS condition and two times more Na under the AS condition in the leaves and stems. Interestingly, the roots of Proso millet attained a higher concentration of Na (2 times higher) than the roots of Foxtail millet under AS.

Potassium

The AS caused a significant decrease in the K concentration of the studied plant segments in both species except for the leaves of Proso millet. The leaves of both species achieved the highest concentration of K under the SS condition compared to the other treatments (Table 3). Significantly lower concentrations of K were observed in all of the plant parts in Foxtail millet under AS compared to under SS; however, the similar tendency was observed only in the roots but not in the leaves and stems of Proso millet.

Na/K ratio

The ratio of increased under both stresses and it was higher under AS compared to SS in all plant parts (Table 4). Foxtail millet showed greater values than Proso millet except in the AS treated Proso millet roots.

Calcium

The Ca concentration was noticeably reduced by SS and AS in the leaves and by AS in the stems of Foxtail millet. The leaves accumulated a higher concentration than the stems and roots of both species. Saline stress caused a significant decrease in the root Ca concentration of both species, whereas AS increased the roots Ca concentration more markedly in Foxtail millet. The relative reduction due to stresses was greater in Foxtail millet than in Proso millet.

Magnesium

The Mg concentration was inhibited significantly by the stresses in the leaves and roots of both species. The relative inhibition was greater (39 and 52% under SS and AS) in the

Table 1. Effects of SS and AS on the RGR, NAR and LAR of Foxtail millet and Proso millet. The values are the means (± S.E) of three replicates. Means followed by the same letter within each line are not significantly different (p<0.05).

Growth parameters	Foxtail millet			Proso millet		
	Control	SS	AS	Control	SS	AS
RGR (mg g^{-1} day^{-1})	59.3±4.04^{a}	46.7±5.67^{ab}	33.2±4.58^{b}	54.2±0.58^{a}	46.9±1.53^{ab}	37.5±4.63^{b}
NAR (mg cm^{-2} day^{-1})	0.110±0.01^{a}	0.091±0.01^{ab}	0.074±0.00^{b}	0.140±0.01^{a}	0.122±0.00^{ab}	0.102±0.02^{b}
LAR (cm^2 g^{-1})	537.57±11.5^{a}	513.29±6.57^{ab}	448.21±12.72^{b}	389.43±12.46^{a}	385.43±7.60^{a}	367.95±6.94^{a}

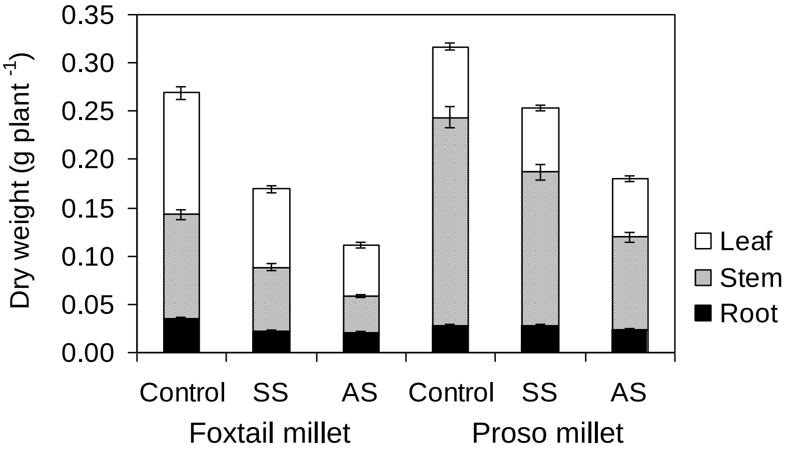

Fig 1. Effects of SS and AS on the dry weight of leaves, stems and roots of Foxtail millet and Proso millet. The values are the means (± S.E) of three replicates.

roots of Foxtail millet than Proso millet (23 and 40% under SS and AS, respectively) but the rates of reduction were greater in the leaves and stems of Proso millet than Foxtail millet. The significant inhibition was mainly observed in the stems of AS treated plants of both species (Table 6).

Nitrogen and proline

The total N content decreased in all plant parts under both stresses and the reductions were more severe in AS than in SS (Fig 3). Significant reductions were observed in Foxtail millet under both SS and AS, showing values (relative reduction $plant^{-1}$) of 25 and 63%, respectively. However, a significant reduction (54%) was observed only under AS but not under SS (14%) in Proso millet. The proline concentration increased under SS and AS conditions and the increase was greater under SS than under AS for both species (Fig 4). Furthermore, these results demonstrated that Foxtail millet produced 14.7 and 12.6 times more proline than the control under SS and AS conditions, respectively; while those values in Proso millet were only 5.2 and 2.3.

Discussion

Plant growth

The decreased biomass weights of plants under saline and alkaline conditions are correlated with the reduced leaf area, which results in decreases of photosynthetic area and Pn (Yang et al., 2008a). It is thought that a decreased Pn under stress could have reduced the shoot growth and development, thus finally leading to lower biomass production compared to the control (Campbell and Nishio, 2000). In the present study, the lower stress-induced reduction of growth in Proso millet compared with Foxtail millet (Fig 1) might be attributed to the lower reduction of the RGR (SS:21/13% and AS:44/31% for Foxtail millet/Proso millet, respectively) and also NAR in the salt-stressed plants (Table 1). These results indicate that Proso millet is a comparatively saline and alkaline tolerant species with the inhibitory effect of alkalinity being stronger than that of salinity. We suppose that a high pH appearing in the rhizosphere might be a primary factor for a more pronounced inhibition of plant growth by disturbing some mineral nutrition and other physiological functions. This finding is also in agreement with the previous studies (Sharma et al., 2001; Nuttall et al., 2003). The reduction of plant growth at a higher saline concentration was mainly due to the reduction of the photosynthetic area as reported by Marcelis and Van-Hooijdonk (1999) and James et al. (2002). The other factors mainly depend on the cumulative effects of leaf water and osmotic potential, biochemical constituents (Sultana et al., 1999; Dixit and Chen, 2010), contents of photosynthetic pigments (Koyro, 2006) and ion toxicities in the cytosol (James et al., 2006). The RGR value reflects the life-sustaining activities of plants, and is considered an optimum index for degrees of stress and plant responses to stresses. Severe salt stress generally leads to growth arrest and even to death of plants (Parida and Das, 2005). In our research, the decreases of RGR under AS (44 and 31% in Foxtail millet and Proso millet, respectively) were greater

Table 2. Effects of SS and AS on Na concentration (mg g^{-1} DW) in the leaves, stems and roots of Foxtail millet and Proso millet. The values are the means (± S.E) of three replicates. Means followed by the same letter within each line are not significantly different ($p<0.05$).

Genotypes	Treatments	Na		
		Leaf	Stem	Root
Foxtail millet	Control	0.86±0.04^{c}	1.72±0.09^{c}	1.85±0.29^{c}
	SS	26.30±1.81^{b}	28.60±1.53^{b}	20.44±1.79^{a}
	AS	41.02±3.93^{a}	37.67±4.15^{a}	13.60±0.93^{b}
Proso millet	Control	0.84±0.02^{c}	1.56±0.05c	2.45±0.11^{c}
	SS	8.53±0.38^{b}	10.92±0.79^{b}	21.38±1.01^{b}
	AS	19.28±1.76^{a}	22.14±1.00^{a}	28.73±2.43^{a}

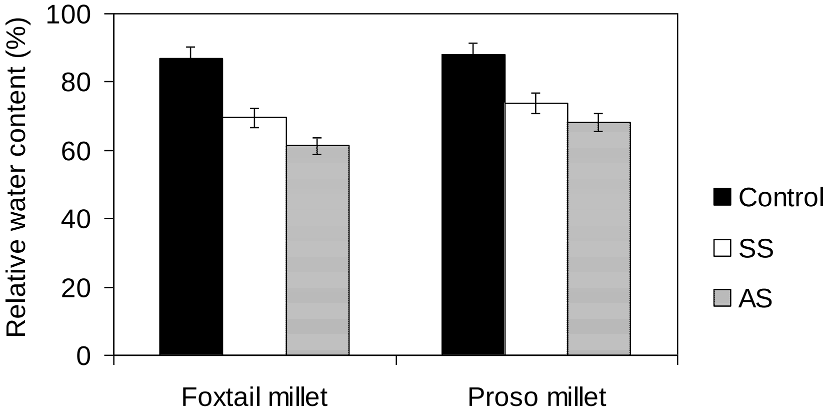

Fig 2. Effects of SS and AS on the RWC in the leaves of Foxtail millet and Proso millet. The values are the means (± S.E) of three replicates.

than that under SS (21 and 13% in Foxtail millet and Proso millet, respectively)(Table 1). This more injurious effect by AS compared with SS is consistent with the previous study reported by Yang et al. (2007). The RGR is the product of NAR and LAR, where NAR is largely the net result of carbon gain (Pn) and carbon losses (respiration) expressed per unit leaf area. The AS exerts the same stress factors as SS but under AS plants have to deal with the stress of an elevated pH. The AS induced severe reductions in water content in plants (Fig 2). These results indicate that high pH due to AS in the soil surrounding the roots might cause damage to root structures and functions such as reduced water uptake (Fig 2), and inability to prevent accumulation of Na^{+} (Table 2) and to uptake the essential elements like K, Ca, Mg (Table 3, 5 and 6) following reduced LAR and NAR (Table 1). These may be the main reasons explaining the lower RGR value under AS than under SS of Foxtail millet and Proso millet. The injurious effects of salinity are commonly thought to be a result of low water potentials and ion toxicities (Munns, 2002).

Relative water content

Under saline conditions, plants suffer from osmotic shock due to lower osmotic potential and synthesize different metabolites to maintain turgor (Orcutt and Nilsen, 2000). However, in our study, the RWC decreased under SS and AS, and a more marked reduction was also observed under AS in Foxtail millet compared to Proso millet (Fig 2), which may represent the cumulative effects of a greater reduction in the leaf area and LAR, as well as severe damage to root structures by a higher concentration of Na. Nonetheless, Foxtail millet plants have to face a more pronounced water deficit under AS, imposed by a low external water potential due to a higher concentration of Na accumulation in extracellular regions reaching a toxic threshold, causing severe damage to plant tissues. Our results suggest that the better water relation in plant under stress conditions obviously contributed to the maintaining of higher plant growth in Proso millet than in Foxtail millet.

Ionic concentrations

Under saline conditions, halophytes usually accumulate inorganic ions in vacuoles to decrease the water potential in the plant because energy consumption to absorb inorganic ions is far less than that needed to synthesize organic compounds (Khan et al., 2000; Munns, 2002; Moghaieb et al., 2004; Shi and Sheng, 2005), and they generally compartmentalize Na^{+} in vacuoles to avoid Na^{+} toxicity in the cytosol (Serrano and Rodriguez-Navarro, 2001; Zhu, 2003). Additionally, halophytes usually absorb Na^{+} and inhibit K^{+} uptake under saline and alkaline stresses (Hasegawa et al., 2000; Islam, 2001; Tammam et al., 2008). In our study, Na concentration was induced under both stresses in all the plant segments and K concentration was reduced in the stems and roots of both species, indicating that there is a competitive inhibition between the absorption of Na^{+} and K^{+}. However, in leaves,

Table 3. Effects of SS and AS on K concentration (mg g^{-1} DW) in the leaves, stems and roots of Foxtail millet and Proso millet. The values are the means (± S.E) of three replicates. Means followed by the same letter within each line are not significantly different ($p<0.05$).

Genotypes	Treatments	K		
		Leaf	Stem	Root
Foxtail millet	Control	38.84±1.55[a]	42.88±1.80[a]	3.31±0.41[a]
	SS	41.15±2.01[a]	36.46±2.82[a]	2.84±0.427[a]
	AS	32.95±0.34[b]	23.50±2.48[b]	0.88±0.07[b]
Proso millet	Control	16.27±0.62[b]	27.61±0.65[a]	5.89±0.09[a]
	SS	20.20±0.87[a]	20.10±1.11[b]	3.50±0.31[b]
	AS	18.19±0.37[ab]	19.67±0.28[b]	1.87±0.12[c]

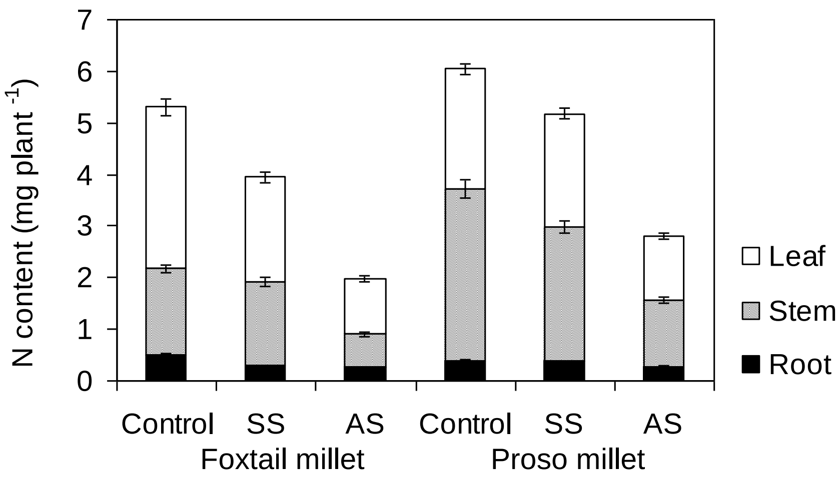

Fig 3. Effects of SS and AS on total N content in the leaves, stems and roots of Foxtail millet and Proso millet. The values are the means (± S.E) of three replicates.

the concentration of Na and K increased under SS, which implies that there was no competitive inhibition for absorption Na^+ and K^+ in the leaves. No competitive inhibition between Na^+ and K^+ uptake was observed by Saneoka et al. (1995, 1999) in maize and wheat under SS. The acquisition of K^+ was inhibited more by AS than by SS of both species, possibly due to the high pH under AS which increased the interference with the selective absorption of K^+ to Na^+ in the roots and elevated intracellular Na concentration to a toxic level. A more markedly decreased acquisition of K in *Chloris virgata* under AS than under SS was noticed by Yang et al. (2008a). Recently some investigations also reported that both Na and K concentrations increased with elevating salinity in the shoots of *Suaeda glauca* and *Kochia sieversiana* (Yang et al., 2007, 2008c), in the leaf blade of bread wheat (Hidhab) (Benderradji et al., 2011). Thus, the pattern of Na and K accumulation to SS and AS in halophytes may be varied by their genotypic nature. Those antagonistic-synergistic effects for uptaking Na and K may need to investigate further. The Na/K ratios have been shown to increase with rising salinity in many halophytes (Yang et al., 2007; 2008b) and a high Na/K ratio implies metabolic disorders (Brady et al., 1984). In the present study, AS sharply increased the Na/K ratio and Foxtail millet showed higher ratios than Proso millet except in the roots (Table 4). It is thought that the severe depressive effect of alkalinity over salinity on plant growth could be related to a greater increase of Na and decline of K content in aerial plant parts. Proso millet inhibited the transportation of Na from roots to shoots which resulted in a higher ratio of Na/K in Proso millet roots. Yang et al. (2008a) reported the similar results whereby a high pH caused by alkaline stress may enhance interference with the selective absorption of Na/K in roots and may increase intracellular Na to a toxic level. The Ca^{2+} and Mg^{2+} accumulation is inhibited by salt stress in many plants (Khan et al., 1999; Islam, 2001; Yousif, 2010). In our observation, the Ca accumulation was inhibited significantly in the Foxtail millet leaves under SS and AS, and stems under AS. In case of Proso millet, the inhibition was insignificant in the leaves under SS and stems under both stresses (Table 5), indicating that Proso millet is more tolerant than Foxtail millet. The Mg concentration also decreased in the leaves and roots of both species under SS and AS and the extent of decreases under AS were higher than under SS. It may be due to the high pH under AS reducing the availability of Ca and Mg in the root zones by precipitating them into $CaCO_3$ and $MgCO_3$.

Nitrogen and proline

Decreased nitrogen uptake under SS and AS conditions may be due to the interaction between Na^+ and NH_4^+ and/or between Cl^- and NO_3^- that ultimately reduces the growth of crops. Moreover, the lower accumulation of Na^+ in Proso millet as compared to Foxtail millet is thought to be the result of a higher N uptake due to the reduced antagonistic effects of Na^+-NH_4^+ in roots and the lower influence of Na^+ on NH_4^+ loading into the xylem. Na^+-NH_4^+/Cl^--NO_3^- interactions under stresses from a biochemical perspective indicate a decreased N accumulation that ultimately reduces growth and yield of crops as described by Bar et al. (1997). N deprivation adversely affects plant growth and development by reducing the photosynthetic area (Marcelis and Van-Hooijdonk, 1999; James et al., 2002), having cumulative effects on the leaf water and osmotic potential (Mori et al., 2000; Munnns,

Table 4. Effects of SS and AS on Na/K ratio in the leaves, stems and roots of Foxtail millet and Proso millet. The values are the means of three replicates.

Genotypes	Treatments	Na/K		
		Leaf	Stem	Root
Foxtail millet	Control	0.03	0.04	0.56
	SS	0.76	0.79	7.20
	AS	1.49	1.60	15.45
Proso millet	Control	0.02	0.06	0.42
	SS	0.72	0.54	6.04
	AS	1.15	1.13	23.63

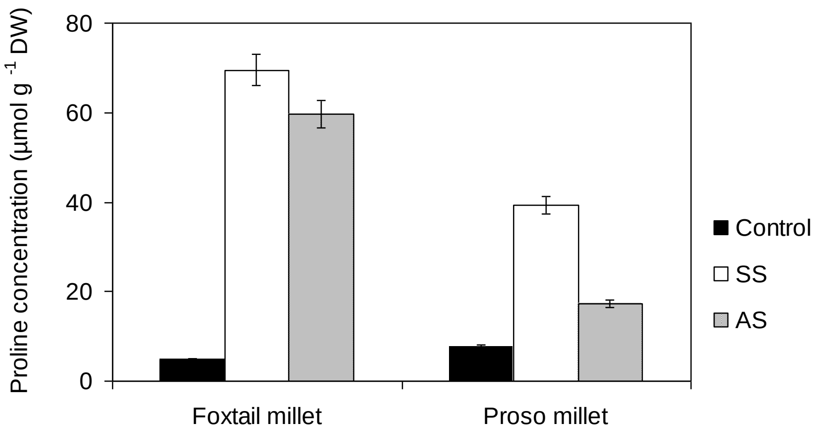

Fig 4. Effects of SS and AS on the proline concentration in the leaves of Foxtail millet and Proso millet. The values are the means (± S.E) of three replicates.

2002), and increasing ion toxicities in the cytosol (James et al., 2006). In this case, we predict that a more markedly decreased leaf area, RWC and increased Na accumulation under AS in Foxtail millet induced higher-level inhibition of the NAR, ultimately mediated by a reduced nitrogen content (Fig 3). The roles of proline have been widely reported as cell osmotic adjustment, membrane stabilization and the detoxification of injurious ions and correlation with stress tolerance in plants exposed to salt stress (Kavi Kishor et al., 2005; Ashraf and Foolad, 2007; Tammam et al., 2008). It is evident from our study that the proline concentartion of both species increased under SS and AS (Fig 4). These results suggest that the induction of proline concentration is related to the changes in not only salinity, but also alkalinity. It is common for proline to be correlated with stress tolerance (Kavi et al., 2005; Ashraf and Foolad, 2007; Younis et al., 2009) but the significance of proline accumulation in osmotic adjustment is still being debated and varies according to the species (Lutts et al., 1996; Rodriguez et al., 1997). Our results indicate that the role of proline accumulation is not only being osmolyte and protectant, but it may also have other roles related to alkaline stress, which should be further investigated.

Materials and methods

Plant material and culture conditions

The experiment was conducted at the Graduate School of Biosphere Science, Hiroshima University, Higashi-Hiroshima, Japan. The seeds of Foxtail millet (*Setaria italica* L. cv: *BARI kaun-3*) and Proso millet (*Panicum miliaceum* L., cv: *BARI china-1*) were collected from Bangladesh Agricultural Research Institute (BARI), Gazipur, Bangladesh. The seeds of both species were surface-sterilized with 5% thiophanate-methyl for 5 min and air-dried. The seeds were sown into 5 L plastic pots containing a soil mixture of granite regosol soil and perlite (2:1 v/v). After germination, 20 uniform seedlings were kept at an identical distance in each pot. Pots were maintained under greenhouse conditions. Plants were irrigated with nutrient solution at each watering using an irrigation system. The basal nutrient solution contained 8.3 mM NO_3-N, 0.8 mM NH_4-N, 0.5 mM P_2O_5, 2.2 mM K_2O, 0.7 mM MgO, 2.1 mM CaO, 11 µM MnO, 5 µM B_2O_3 and 13 µM Fe. To simulate saline stress (SS) and alkaline stress (AS) conditions in nature (Kawanabe and Zhu 1991; Chen et al., 2009; Liu et al., 2010), two stress treatments were applied: neutral salts of NaCl and Na_2SO_4 (9:1 molar ratio) and alkaline salts of $NaHCO_3$ and Na_2CO_3 (9:1 molar ratio). At four weeks after sowing, plants were subjected to stress treatments every day until water was drained-out from the bottom of the pot. Before applying 100 mM SS and AS treatments for 7 days, plants were subjected to SS and AS of 25, 50 and 75 mM concentrations every 3 days alternatively for the hardening of plants. The pH/EC (S/m) of saline and alkaline solutions was 6.9/1.217 and 9.2/0.930, respectively. Each treatment was applied to three replicates located randomly in the greenhouse in order to avoid positional effects.

Table 5. Effects of SS and AS on Ca concentration (mg g^{-1} DW) in the leaves, stems and roots of Foxtail millet and Proso millet. The values are the means (± S.E) of three replicates. Means followed by the same letter within each line are not significantly different ($p<0.05$).

Genotypes	Treatments	Ca		
		Leaf	Stem	Root
Foxtail millet	Control	2.84±0.00[a]	1.94±0.06[a]	0.84±0.08[b]
	SS	2.44±0.09[b]	1.64±0.13[ab]	0.59±0.01[c]
	AS	2.12±0.07[c]	1.48±0.10[b]	1.12±0.05[a]
Proso millet	Control	2.19±0.02[a]	1.47±0.15[a]	1.12±0.03[a]
	SS	2.16±0.04[ab]	1.35±0.10[a]	0.86±0.03[b]
	AS	2.07±0.03[b]	1.18±0.04[a]	1.17±0.09[a]

Table 6. Effects of SS and AS on Mg concentration (mg g^{-1} DW) in the leaves, stems and roots of Foxtail millet and Proso millet. The values are the means (± S.E) of three replicates. Means followed by the same letter within each line are not significantly different ($p<0.05$).

Genotypes	Treatments	Mg		
		Leaf	Stem	Root
Foxtail millet	Control	1.92±0.10[a]	1.50±0.09[a]	0.33±0.02[a]
	SS	1.33±0.16[b]	1.41±0.33[ab]	0.20±0.02[b]
	AS	1.26±0.05[b]	0.71±0.09[b]	0.16±0.01[b]
Proso millet	Control	2.82±0.14[a]	0.99±0.03[a]	0.80±0.03[a]
	SS	1.79±0.17[b]	0.91±0.14[a]	0.62±0.01[b]
	AS	1.34±0.07[b]	0.37±0.03[b]	0.48±0.01[b]

Plant sampling and measurements

Plants in each pot were sampled and separated into the leaves, stems (culms) and roots before the application of treatments and at 16 d after treatment initiation. The separated segments were wiped with tissue towel paper to remove moisture and their fresh weights were measured. The fresh samples were kept frozen in liquid nitrogen, then freeze-dried and we measured the dry weight. Dry samples were ground into fine powder using a vibrating sample mill (Model TI-100, Heiko Seisakusho Ltd., Tokyo, Japan) for chemical analysis. Leaf samples were taken in triplicate from a composite pool of physiologically mature leaves of each genotype. The leaf area was measured using a leaf area meter (AMM-5 type leaf area meter, Hayashi-Denko, Tokyo, Japan) and the leaves were oven-dried at 80°C for 72 h and the dry weight was determined. The leaf area ratio was calculated as the total leaf area per unit leaf dry mass. The RGR was calculated using the method of Kingsbury et al. (1984). The RWC of the leaf was estimated according to the method of Saneoka et al. (1995). The Na and K concentrations were determined after digestion by nitric acid–hydrogen peroxide, using a flame photometer (ANA 135, Eiko Instruments Inc., Tokyo, Japan). The Ca and Mg concentrations were determined using an atomic absorption spectrophotometer (U-3310 Hitachi Co. Ltd., Tokyo, Japan). Proline was determined spectrophotometrically following the ninhydrin method described by Bates et al. (1973), using l-proline as a standard. The total N content was determined using a Kjeldahl nitrogen digester and distillator (Kjeldatherm Type TT100 & Vapodset Type 20, Gerhardt, Germany).

Statistical analysis.

Data were examined using one-way ANOVA and presented as the mean ± S.E. for each treatment and species (n=3). Multiple comparisons of means of data treatments within the plants were performed using Duncan's test at the 0.05 significance level (all tests were performed with SPSS Version 16.0 for Windows).

Conclusion

In summary, the responses of Foxtail millet and Proso millet under saline and alkaline conditions are complex and may involve various physiological and biochemical reactions depending upon their genetic ability. Proso millet showed a more favorable leaf area, LAR, NAR, RGR and Na-K levels under saline and alkaline conditions by reducing stress-induced changes in all physiological and biochemical functions. Meanwhile, the deleterious effects of alkaline stress on all plant traits were always higher than that of saline stress, and thus Proso millet may have evolved specific mechanisms to tolerate saline and alkaline stresses and these should be investigated further.

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