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RELATIONSHIP OF PHOTOSYNTHETIC PIGMENTS AND WATER RELATIONS WITH SALT TOLERANCE OF PROSO MILLET (*PANICUM MILIACEUM* L.) ACCESSIONS

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Abstract

An experiment was conducted to assess whether accumulation of photosynthetic pigments and maintenance of water relation attributes relate to the growth of proso millet accessions differing in salt tolerance. Salt stress significantly decreased leaf chlorophyll *a* and *b* contents, RWC, water potential (Ψ_w), and osmotic potential (Ψs), whereas it did not alter leaf chlorophyll *a/b* ratio except in accessions 008214 (salt tolerant), 008217, 008221 and 008223 (all three salt sensitive) under saline regimes where it enhanced chlorophyll *a/b* ratio. Leaf turgor potential also increased in all accessions under saline conditions and accessions 008213 (moderately tolerant), 008214 (tolerant) and 008236 (sensitive) were relatively higher than the others in turgor potential. The salt tolerant accessions (008211, 008214, 008220, and 008226) were higher, while the salt sensitive (008236, 008242 and 008208) were lower in leaf chlorophyll *a* content under saline conditions. Chlorophyll *b* was higher in 008213 and lower in 00823 (both moderately tolerant). Accessions 008211 and 008220 (both tolerant) and 008216 (moderately tolerant) were higher in RWC and 008215 (moderately tolerant) and 008242 (sensitive) in leaf osmotic potential than those of the other accessions under salt stress. Of the *P. miliaceum* tolerant accessions, 008214 was the lowest in leaf water and osmotic potentials. The relatively tolerant accessions 008211, 008214, 008220 and 008226 were higher only in chlorophyll *a* content under saline conditions. Overall, due to differential response of all the accessions to salt stress in terms of their performance in photosynthetic pigments and water relations none of these attributes except chlorophyll *a* can be related to the tolerance of *P. miliaceum* accessions differing in salt tolerance.

Introduction

Inside the plant, soluble salts at higher concentrations cause hyperosmolality, ion toxicity and disequilibrium of nutrients that adversely affect plant growth and development (Naheed *et al.,* 2007; Noreen *et al.,* 2009; Nawaz & Ashraf, 2009). High concentrations of particularly Na^+ and Cl^- in the cytosol are inhibitory to a variety of metabolic and cellular processes (Zhu, 2001; Munns & Tester, 2008). It is now well accepted that high salt-induced negative water potential in soil results in water loss from plant cells and thus plants experience water stress (Zhu, 2002; Ashraf, 2004). In addition, high concentration of salts causes membrane dysfunction, triggers the production of reactive oxygen species, impairs photosynthesis, and causes death of plant tissues (Mittler, 2002; Munns & Tester, 2008; Ashraf, 2009). It has been demonstrated that in most plants exposed to salt stress, destruction of chloroplast structure (Santos, 1998) is influenced by Na^+ toxicity and/or salt-induced oxidative stress (Mittler, 2002; Ashraf, 2009). However, the inhibitory role of NaCl stress on overall photosynthetic machinary of plant is found to be dependent on various factors such as gas exchange characteristics, photochemical quenching capacity, photosynthetic pigments, type of species and cultivar (Dubey, 2005).

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Considerable genetic variation for tolerance to salinity has already been observed in some crops at inter- and intra-cultivar level e.g., wheat, grasses, rice (Ashraf, 1994), pea (Noreen *et al.,* 2007; Ashraf, 2007; 2009a), radish (Noreen & Ashraf, 2009b), safflower (Siddiqi *et al.,* 2007; 2009), maize (Ali *et al.,* 2008) and *Panicum miliaceum* (Sabir & Ashraf, 2007; 2008). Thus, a significant difference for salinity tolerance occurs in major and minor crops, but it is not yet fully known how far such inter- or intra-cultivar variation is due to physiological attributes.

In a previous study (Sabir & Ashraf, 2007), using some growth parameters as selection criteria, considerable inter-accession variation for salt tolerance was observed in 18 accessions of Proso millet (*Panicum miliaceum* L.). However, the present study was carried out with the premier objective whether the accessions categorized as salt tolerant due to better growth performance are also superior in maintaining water relations and photosynthetic pigments under salt stress.

Materials and Methods

Description of 18 accessions of proso millet (*Panicum miliaceum* L.) and growth conditions are presented in Sabir & Ashraf (2007). Data for the following physiological attributes were measured and presented in this manuscript.

Chlorophyll contents: Following Arnon (1949), chlorophylls *a* and *b* pigments and chlorophyll *a/b* ratio were determined.

Leaf water potential (Ψ_w): At 6:00 a.m. 3rd leaf from top was used for the measurement of leaf water potential using a pressure chamber (Scholander type, Arimad-2-Japan).

Osmotic potential (Ψ_s): The same leaf as used for Ψ_w measurement was also used for Ψ_s determination. The leaf material was frozen for two weeks, after this it was thawed, and the sap was used for Ψ_s determination in an osmometer (VAPRO, Model 5520, USA).

Turgor potential: Turgor potential was estimated following Nobel (1991).

$$\Psi p = \Psi w - \Psi s$$

Relative water content (RWC): The 3rd leaf from top was excised, weighed fresh (FW) and placed in deionized H_2O for 24 h to re-hydrate. After this time, turgid weight (TW) was recorded. Then the leaves were dried at 65 °C for 48 h and recorded dry weight (DW) RWC was calculated as:

$$RWC = [(FW - TW)/(FW - DW)] \times 100$$

Statistical analysis of data: Data for all variables were subjected to analysis of variance using the COSTAT computer package. The mean data were compared according to Snedecor & Cochran (1980).

Results and Discussion

In the present study, photosynthetic pigments like chlorophyll *a*, *b* and chlorophyll *a*/*b* ratio of different accessions of *Panicum miliaceum* presented in Fig. 1, show that NaCl stress considerably declined the leaf chlorophyll *a* and *b* contents, however leaf chlorophyll *a*/*b* ratio remained almost unaffected except in accessions 008214, 008217, 008221 and 008223 where it increased under saline regimes. Some earlier reports showed reduction in chlorophyll *a* and *b* content in pea (Noreen & Ashraf, 2009a), sunflower (Akram *et al.,* 2007; 2009), wheat (El-Hendawy *et al.,* 2005; Shahbaz & Ashraf, 2007; Shahbaz *et al*., 2008) and radish (Noreen & Ashraf, 2009b). Furthermore, the accessions differed significantly in chlorophyll a and *b* contents. Accessions 008211, 008214, 008220, and 008226 had greater leaf chlorophyll *a* than that of others under saline conditions, whereas accession 008236 followed by 008242, 008208 and 008223 was the lowest in leaf chlorophyll *a* under salt stress. Leaf chlorophyll *b* was considerably higher in accession 008213 under normal and salt stress conditions than that in the other accessions, whereas accession 008223 was the lowest of all accessions in leaf chlorophyll *b* under salt stress (Fig. 2). Although *P. miliaceum* accessions differed significantly in leaf chlorophyll '*a/b*' ratio, salinity x accessions interaction was non-significant. However, accessions 008214, 008217, 008221 and 008223 were higher in chlorophyll *a*/*b* ratio as compared to the other accessions under saline conditions (Fig. 2). The decrease in chlorophyll '*a*' and '*b*' in the *Panicum* accessions might have been due to salt-induced acceleration of chlorophyll enzymes degradation (Hernandez *et al*., 1993; 1995; Hernandez & Almansa, 2002), and/or disorder of chloroplast structure and related proteins (Singh & Dubey, 1995). In a study on sunflower, Conceicao (2004) suggested that salt-induced reduction in chlorophyll content is more related to reduced chlorophyll synthesis than chlorophyllase-mediated degradation. Although salinity may reduce the chlorophyll content, the degree of reduction in total chlorophyll depends on salt tolerance of plant species (Hamada & El-Enany, 1994). For example, in salt-tolerant *Avicennia marina*, no reduction in chlorophyll contents took place, but in the salt-sensitive pea (*Pisum sativum*) chlorophyll was decreased (Ball & Anderson, 1986).

Leaf relative water content (RWC) of all *P. miliaceum* accessions significantly declined with increase in NaCl concentration (Fig. 2). Accessions also differed significantly in this water relation parameter. Leaf RWC was found to be the highest in accession 008216 followed by 008220 and 008211 under saline conditions, whereas accession 008210 was the lowest in RWC under saline conditions. Extent of salt-induced effects on relative water content has been used as one of the vital water relation parameters for assessing degree of salt tolerance in maize (Premachandra *et al.,* 1990), *Vigna radiata* (Nandwal *et al.,* 1998), sorghum (Jones *et al.,* 1980), safflower (Siddiqi & Ashraf, 2008), and pea (Noreen & Ashraf, 2009a).

Application of NaCl to the growth medium resulted a significant reduction (more negative values) in Ψ_w of all *P. miliaceum* accessions except in accessions 008215 and 008242, where it remained almost unchanged (Fig. 2). Of the *P. miliaceum* accessions, accession 008208 followed by 008213, 008214 and 008242 was the lowest in Ψ_w under salt stress (Fig. 2). Leaf water potential is used as one of the promising parameters of salt tolerance in a number of crops such as *Vigna mungo* (Ashraf, 1989), wheat (Kingsbury & Epstein, 1984), safflower (Siddiqi & Ashraf, 2008), *Pisum sativum* (Noreen & Ashraf, 2009a) and sunflower (Akram *et al.,* 2009). However, in the present study leaf water potential was not associated with salt tolerance of *P. miliaceum* accessions.

Leaf osmotic potential in all accessions of *P. miliaceum* significantly decreased ($p<0.001$) under saline conditions. Accession 008208 followed by 008213, 008214 and 00836 was the lowest and 008215 and 008242 the highest in leaf osmotic potential of all *P. miliaceum* accessions under saline conditions. Decreased osmotic potential adversely affects the capability of plants to take up water from the surroundings (Munns, 2002). The adverse effects of osmotic stress also depend upon degree of salt imposition (Munns, 2002). The reduction in osmotic potential in salt stressed plants mainly occurs due to high accumulation of Na^+, Cl^- and K^+ (Hasegewa *et al*., 2000).

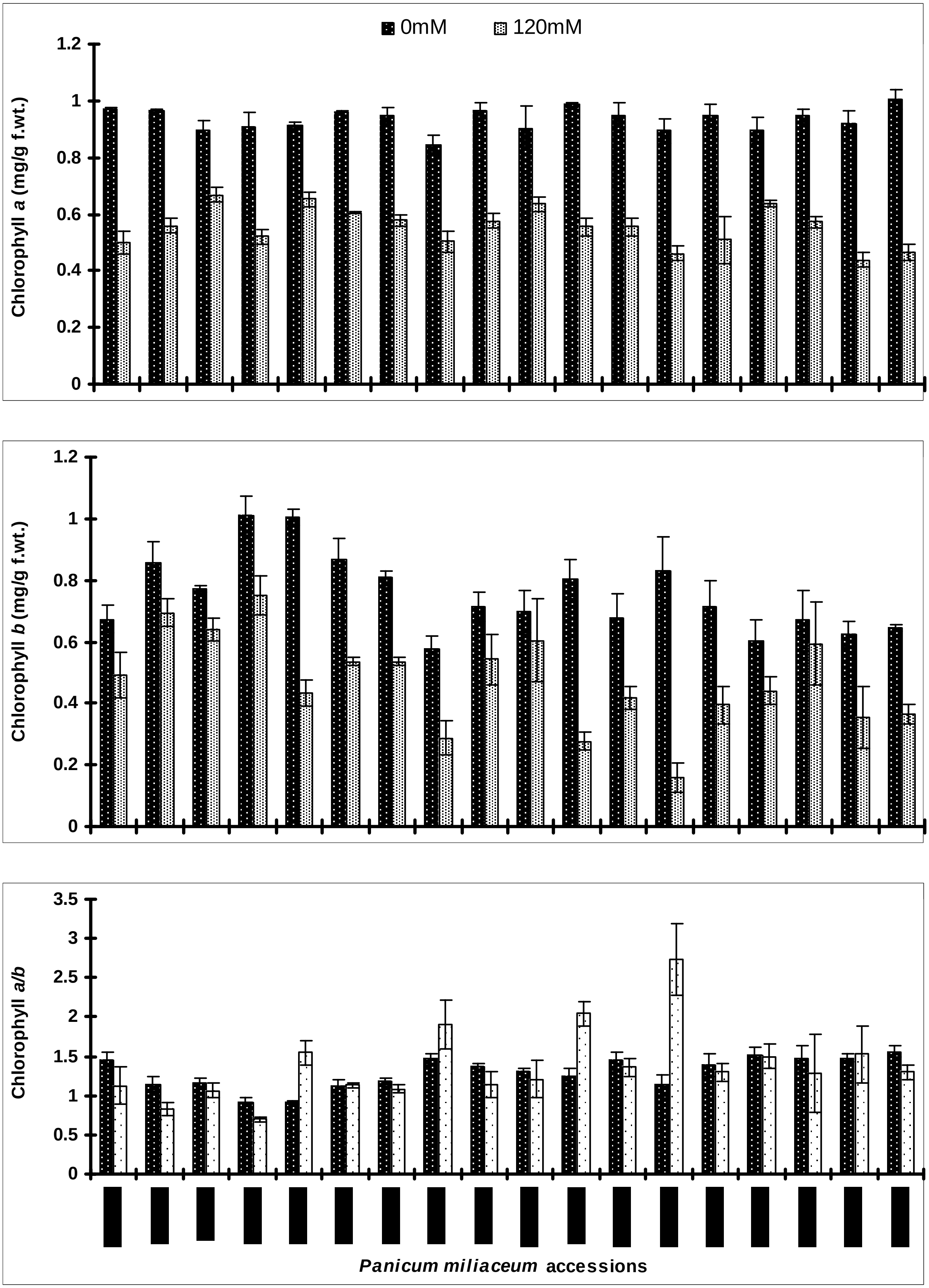

Fig. 1. Chlorophyll *a*, *b* and *a/b* of 18 accessions of *Panicum miliaceum* L. when 21 days old plants were subjected to salt stress (120 m*M* NaCl) for 3 weeks (Mean + S.E; *n* = 4).

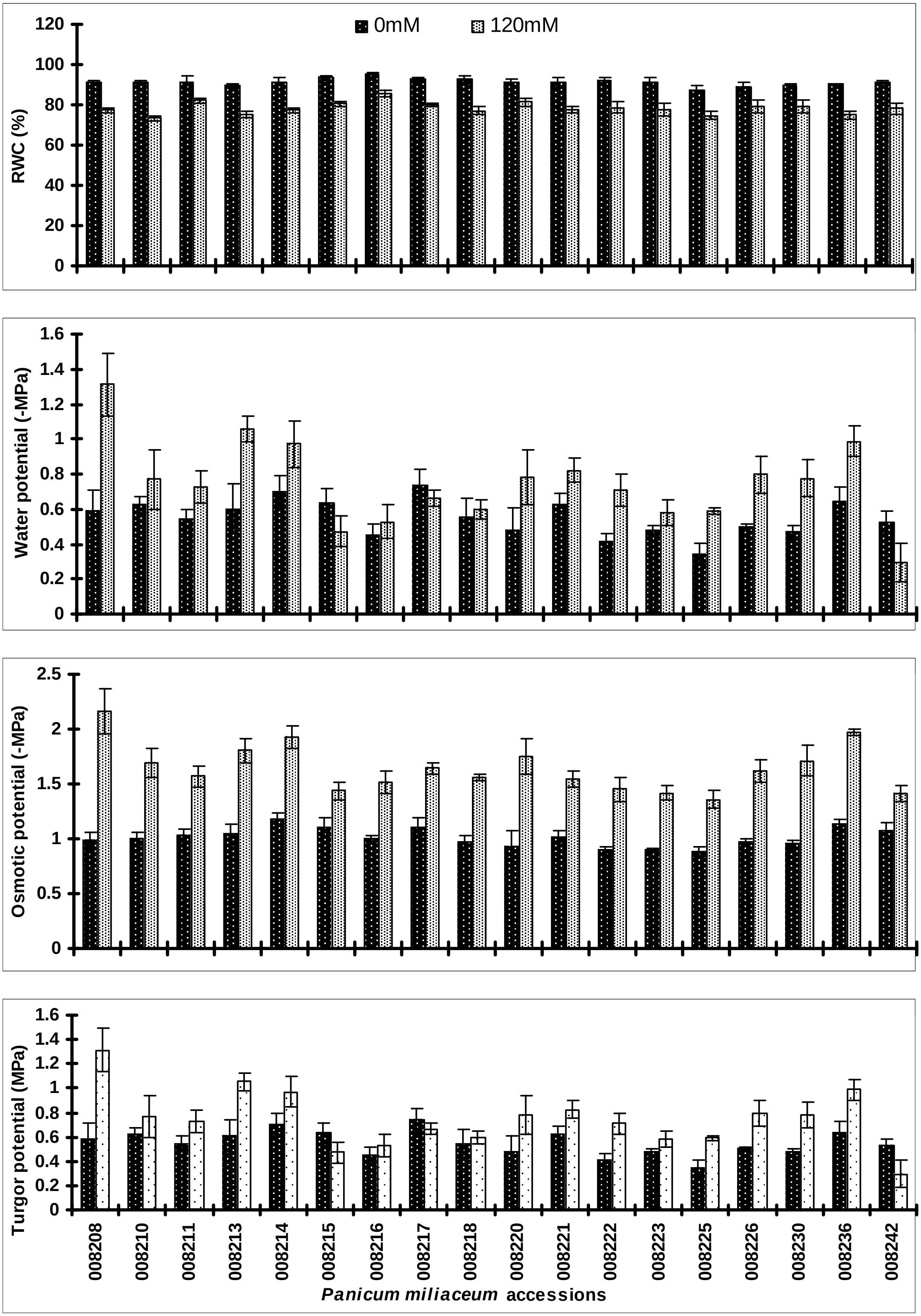

Fig. 2. Relative water content, water potential, osmotic potential and turgor potential of 18 accessions of *Panicum miliaceum* L. when 21 days old plants were subjected to salt stress (120 m*M* NaCl) for 3 weeks (Mean + S.E; *n* = 4).

Imposition of salt stress significantly increased the turgor potential of all accessions except of accessions 008215, 008217 and 008242 where it significantly decreased. Of all accessions, 008213, 008214 and 008236 were higher in turgor potential than the other accessions under salt stress (Fig. 2). Similarly, in another study a significant reduction in Ψ_p was found in different cultivars of safflower under salt stress and response of all lines varied significantly (Siddiqi & Ashraf, 2008). Maintenance of high turgor potential of plant cells under saline conditions was thought to be one of the vital water relation attributes for sustaining growth under salt stress (Greenway & Munns, 1980). However, the results for leaf water potential presented in the present study do not show a linear relationship with the extent of salt tolerance of the diverse *P. miliaceum* lines, because most of the low biomass producing lines (salt sensitive) had higher values of leaf turgor potential than those of the high biomass producing lines (salt tolerant). These results support some earlier studies in which salt sensitive lines of different species maintained higher leaf turgor than salt tolerant ones eg., Citrus (Walker *et al.,* 1983), sugarbeet (Heuer & Plaut, 1989), sorghum (Yang *et al.,* 1990), and wheat (Kingsbury & Epstein, 1984).

In conclusion, salt stress reduced the chlorophyll *a* and *b* pigments and water relation attributes adversely under salt stress but no linear response was determined in relatively tolerant, moderately tolerant and sensitive of all the accessions *P. miliaceum* to salt stress in terms of their performance in photosynthetic pigments and water relations except chlorophyll *a* content. Thus, none of these physiological attributes except chlorophyll *a* can be related to the tolerance of *P. miliaceum* accessions differing in salt tolerance.

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