

SOME PHYSIOLOGICAL AND GENETIC DETERMINANTS OF SALT TOLERANCE IN SORGHUM (*SORGHUM BICOLOR* (L.) MOENCH): BIOMASS PRODUCTION AND NITROGEN METABOLISM

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Abstract

Soil salinization is the most important limiting factor in plant productivity all over the world. To fulfill the food, feed, fodder and industrial raw material demands of growing population, development of salt tolerant and high yielding crop genotypes are necessary. The genotypes having efficient N metabolism produce high biomass/plant productivity under saline conditions are tolerant one. This study was conducted to explore the salinity induced changes in nitrogen metabolism of sorghum. A sand culture experiment with four sorghum genotypes was conducted in NIAB wire-house under natural conditions in plastic pots containing 0, 10 dS m⁻¹ NaCl salinity solutions along with 1/5 Hoaglands nutrient solution. Physiological parameters like dry biomass of shoots and roots, leaf proteins, total nitrogen, total free amino acid, nitrate reductase activity, and NO₃ were reduced due to salinity in all sorghum genotypes. Salinity influence was more pronounced in Noor medium sensitive and FJ-115 sensitive. On the basis of results obtained for nitrogen metabolism, sorghum lines JS-2002 and Sandalbar can be categorized as tolerant, Noor medium sensitive and FJ-115 as sensitive one.

Introduction

Crop production has been decreased by both biotic and abiotic stresses. Among abiotic stresses, soil salinity and drought are two major problems to agricultural land (Ashraf *et al.*, 2008). Salinity diminishes seedling growth and development (Wang *et al.*, 2013) by causing ion toxicity, physiological drought, mineral imbalance and osmotic stress in the growth medium (Culha & Cakirlar, 2011) which negatively affect plant productivity and nitrogen metabolism (Ashraf *et al.*, 2005; Ashraf & Ashraf, 2012). Reports indicate a severe reduction in proteins (Yildiz, 2007) and the synthesis of new proteins in wheat (Hameed *et al.*, 2010). Salinity interferes with nitrogen metabolism by disturbing the uptake of nitrate (Ullrich, 2002) because NO₃ is replaced by chloride resulting reduction and transportation of NO₃ (Engels & Kirkby, 2001) which causes disturbance in long range signaling mechanisms (Forde, 2002). Salinity also reduces the activity of nitrate reductase activity (NRA) and adversely affects plant nitrogen metabolism (Memon, 1999).

Sorghum (*Sorghum bicolor* L.) is the crop of arid and semi arid areas of the world and is moderately tolerant to salinity (Almodares *et al.*, 2007; Gates, 2009). Sorghum is a cereal crop which has capability to produce even under water deficit conditions which makes it an important grain crop that can be used as a source of food, fiber, fuel, feed, and chemical/biofuels in the global agro-ecosystem. Sorghum is a leading tropical crop having larger genomes and more genes like sugarcane which is one of the world's most efficient biomass-producing crop and the leading biofuel source in the world (Paterson *et al.*, 2009).

Salt tolerance is the relative growth rate of the crops plants in response to varying salinity levels and the plants may be classified in to tolerant and sensitive genotypes/varieties depending on their response to salts in the soil (Iqbal *et al.*, 2006). The differential tolerance of susceptible and tolerant genotypes of crops indicates that

they have salinity tolerant mechanism involving changes in their physiological and biochemical metabolism. This mechanism can be inducted to other crops by genes manipulation. Currently there is a need to elucidate the physiological marker/traits and to identify genes associated with trait of interest and to transfer them to plant species for acquired characters. It is also very important to search out the transcriptional factors of gene regulation for salt tolerance (Ashraf *et al.*, 2003; 2005). The tolerant varieties can be selected by screening using various morphological, physiological and biochemical parameters.

The main hypothesis of present study was to determine the effects of NaCl on growth and nitrogen metabolism in sorghum genotypes differing in their salt tolerance potential. Thus the objective of present study is to evaluate genotypic variations in N metabolism in different sorghum genotypes under salinity.

Materials and Methods

Present study was conducted with two tolerant (JS-2002 and Sandalbar) and two sensitive (Noor and FJ-115) sorghum genotypes (Kausar *et al.*, 2012). Four uniform healthy seeds of above selected sorghum genotypes were grown in plastic pots (diameter, 12"; depth, 15") containing washed fine river sand saturated with 0 and 10 dS m⁻¹ NaCl solutions. The experiment was repeated three times in a completely randomized design with three replications. After one month, two plants from each replicate of each treatment were harvested and kept in oven at 70±2°C for two days and dry biomass of roots and shoots was recorded. Fresh material was taken from the third plant and total soluble proteins were determined through Lowry *et al.*, (1951) method. Similarly fresh leaves were used for the analysis of total free amino acids (Hamilton & Van Slyke, 1943). Nitrate reductase activity (NRA) was determined using the method of Sym (1984) and Nitrate

(NO₃) as described by Cataldo *et al.*, (1975). Total nitrogen was determined by digesting dry leaf material through H₂SO₄ and H₂O₂ method (Wolf, 1982) using micro-Kjeldhal method (Bremner, 1965).

Results

Dry biomass production of shoots and roots of all sorghum genotypes was negatively affected by salt stress (Fig. 1a & b). All sorghum genotypes showed significant differential response to saline environment. The maximum dry biomass of shoot and root was noted in Sandalbar followed by JS-2002, Noor and FJ-115.

Nitrogen (N) contents in shoots and roots significantly decreased in all sorghum genotypes due to salt stress. However, the variations among different

genotypes were non significant in case of shoot N contents (Table 1). The maximum amount of N was estimated in shoots of FJ-115 followed by Sandalbar, JS-2002 and Noor. In contrast, in the case of roots variations among all cultivars were found significant and the highest N contents were recorded in genotype JS-2002 followed by Sandalbar, FJ-115 and Noor (Fig. 2a & b).

Total soluble leaf proteins significantly decreased due to presence of salinity in the root zone in all sorghum genotypes. The variations among different sorghum genotypes were significant (Table 1). The maximum leaf proteins were estimated in Sandalbar followed by JS-2002, Noor and FJ-115. Interaction between salinity and genotype was also significant.

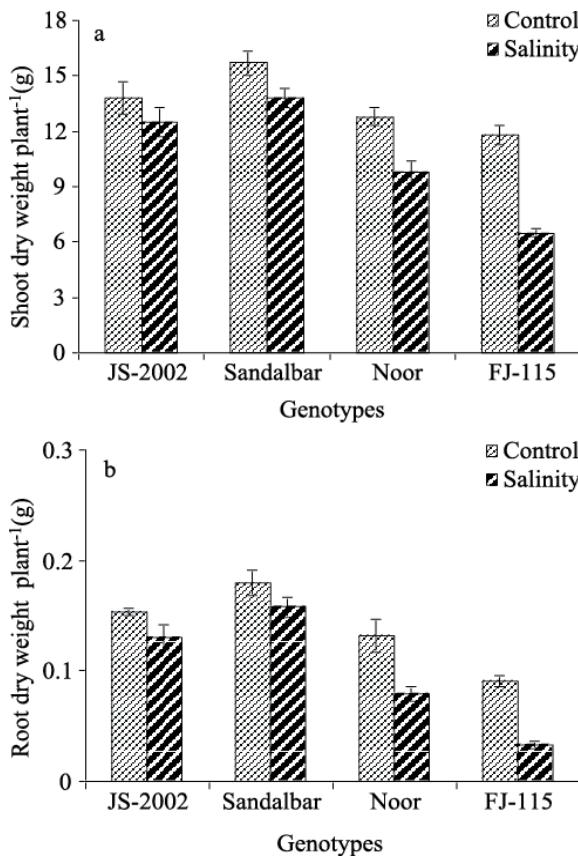


Fig. 1. Effect of salinity on shoot and root dry weights of four sorghum genotypes.

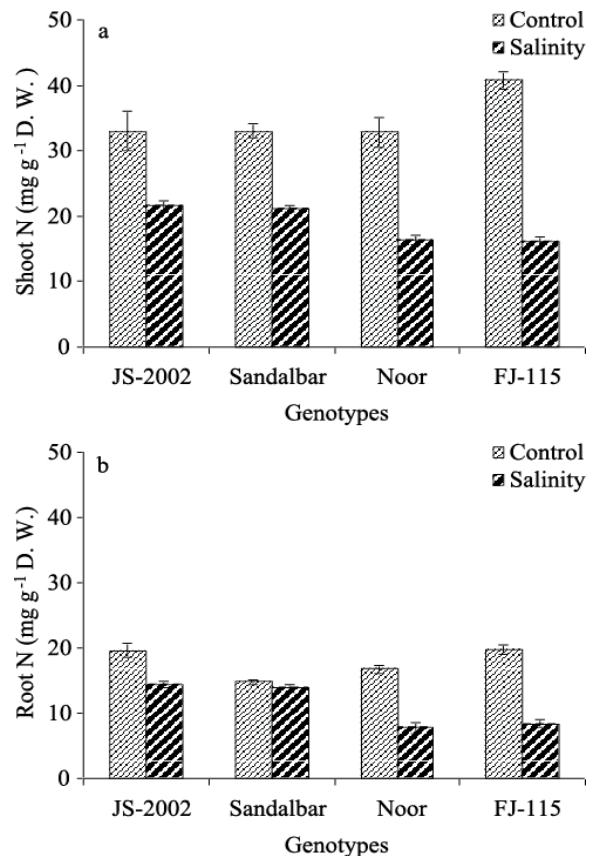


Fig. 2. Effect of salinity on shoot and root N of four genotypes of sorghum.

Table 1. Mean squares values from analyses of variance of data for nitrogen metabolism of four genotypes of sorghum subjected to NaCl (100 mM) stress.

| Source of variance | df | Total nitrogen in shoots (mg g ⁻¹ D.W.) | Total nitrogen in roots (mg g ⁻¹ D.W.) | Total proteins (mg g ⁻¹ F.W.) | Leaf NRA (μ mol NO ₂ g ⁻¹ F.W. h ⁻¹) | Total free amino acids (mg g ⁻¹ F.W.) | Leaf nitrate (m mol NO ₃ D.W.) |
|---------------------|-----------|--|---|--|--|--|---|
| Genotypes (G) | 1 | 1955.176** | 465.344** | 5.415** | 427.562** | 1.649** | 14.259** |
| NaCl treatments (S) | 3 | 17.738 ^{NS} | 26.930** | 1.276** | 6472.848** | 0.910** | 12.030** |
| G x S | 3 | 29.501* | 69.735** | 0.214** | 28.171** | 0.204** | 2.280** |
| Error | 16 | 7.417 | 4.182 | 0.004 | 1.014 | 0.002 | 0.144 |
| Total | 23 | | | | | | |

*, **, *** = Significant at p<0.05, 0.01, and 0.001, respectively. NS = Non-significant

Total free amino acids were significantly increased by the application of salinity in all sorghum genotypes (Table 1). The variations among the genotypes were significant. The maximum total free amino acids were accumulated in Sandalbar followed by FJ-115 and minimum was in Noor followed by JS-2002 (Fig. 4). The interaction between the salinity and genotypes was significant. In the saline medium the Sandalbar proved the best and successful in accumulating the highest amount of total free amino acids which was the lowest in Noor.

Nitrate reductase activity (NRA) was significantly reduced in plants grown in NaCl stress environment in all sorghum genotypes (Fig. 5). The variations among different sorghum genotypes were significant (Table 1). The maximum NRA was recorded in JS-2002 followed by Sandalbar, while, it was minimum in Noor and FJ-115. The interaction between salt stress and sorghum genotypes was significant. The response of all cultivars of sorghum was similar under saline and non-saline medium.

Nitrate (NO_3) contents were significantly affected by NaCl stress in all sorghum genotypes (Table 1). The genotypic differences were noted in different sorghum

genotypes. The highest NO_3 was measured in JS-2002 followed by FJ-115 and it was the lowest in Sandalbar followed by Noor (Fig. 6). The interaction between salt stress and genotypes was significant. The lowest amount of NO_3 was estimated in Sandalbar followed by Noor under salinity stress.

Discussion

Salinity has pronounced effect on growth and disturbed the nitrogen metabolism of all tested sorghum genotypes (Figs. 1-6). On the basis of biomass, sorghum genotypes Sandalbar and JS-2002 showed higher performance than others and can be categorized as salt tolerant. There are many reports which indicate that the tolerant genotypes under saline conditions produced higher biomass and yield (Ashraf *et al.*, 2006; Krishnamurthy *et al.*, 2007). The variation in biomass among the sorghum genotypes might be due to their genetic potential (Ashraf *et al.*, 2006). Reduction in plant growth due to the adverse effect of salinity has been also reported in many other crops (Ashraf *et al.*, 2006; Krishnamurthy *et al.*, 2007).

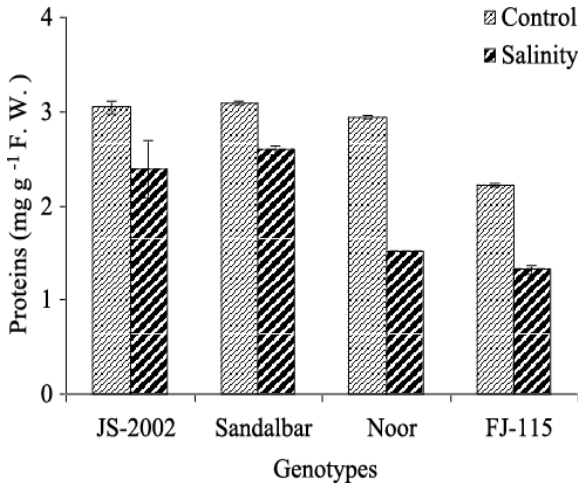


Fig. 3. Effect of salinity on protein of four genotypes of sorghum.

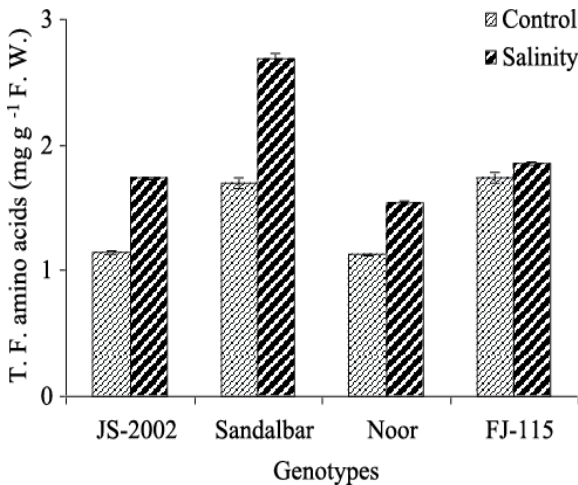


Fig. 4. Effect of salinity on total free amino acids of four genotypes of sorghum.

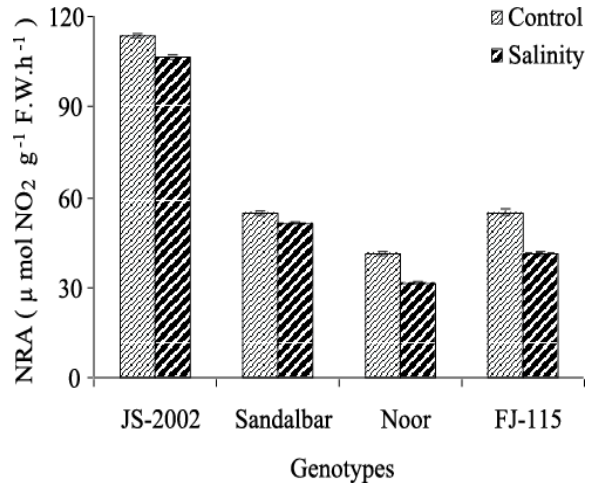


Fig. 5. Effect of salinity on NRA of four genotypes of sorghum.

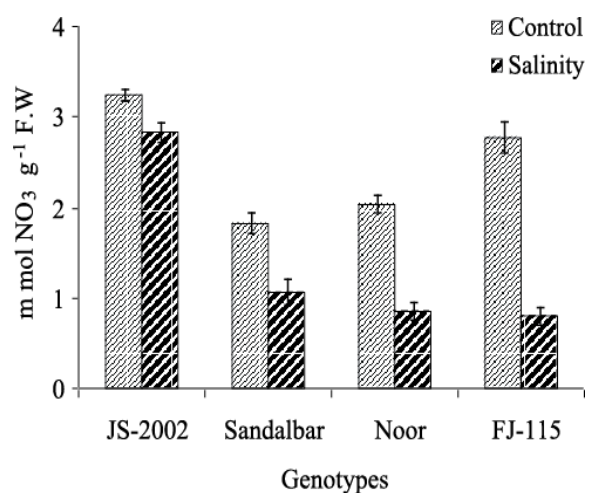


Fig. 6. Effect of salinity on NO_3 contents in four genotypes of sorghum.

Under salinity stress, N contents were higher in JS-2002 and Sandalbar than Noor and FJ-115. Literature also confirmed that salt tolerant genotypes maintained the higher N contents under saline condition than that of sensitive ones and were successful in producing high biomass (Culha & Cakırlar 2011). Same was the case with protein contents which were the highest in Sandalbar closely followed by JS-2002 under salinity stress environments (Fig. 3). Sorghum genotypes FJ-115 and Noor failed to produce high biomass due to the disturbance in their N metabolism under saline conditions. Nitrate reductase activity (NRA) was negatively affected by NaCl salinity in all sorghum genotypes (Fig. 5). The highest NRA was recorded for JS-2002 followed by Sandalbar, FJ-115 and Noor. Total free amino acids were significantly increased by the application of salinity in all sorghum genotypes. However, in the saline medium the Sandalbar performed the best and accumulated maximum amount of total free amino acids than others (Fig. 4). Significant reduction in NO_3^- was observed under NaCl stress in all genotypes. The minimum reduction in NO_3^- content was estimated in JS-2002 followed by Sandalbar, Noor and FJ-115 in salinity stress conditions (Fig. 6). Salt stress decreases the productivity and growth by adversely affecting the morphological, anatomical, biochemical and physiological characteristics and changing metabolic activities (Dhanapackiam & Ilyas, 2010).

Reports in literature also confirmed that salinity retards the activities of many enzymes especially NRA (Khan & Ashraf, 1990). Excessive salts in soils negatively affect the enzymes which control nitrogen metabolism, NRA acts as a key enzyme in nitrogen metabolism. So, reduction in NRA affects the N metabolism which disturbs the synthesis of proteins/ hormones/ enzymes and biomass production in plants (Hamid *et al.*, 2008). To adjust the environmental conditions plants under saline conditions decrease their cellular osmotic potential by increasing the concentrations of free amino acids, inorganic cations and insoluble particles (Loukehaich *et al.*, 2011).

The reduction in biomass in present study may be due to lower transport rate of essential ions like NO_3^- that reduced the N compounds and increased Na^+ in plant under high salinity (Ashraf & Sarwar, 2002). Several workers have proved that tolerance of plants in their rooting medium is under genetic control (Bavei *et al.*, 2011). Genetic variations are basis for improvement in plants (Akber *et al.*, 2009). These genetic differences provide information about lines/ varieties of sorghum that could be grown in salt-affected areas to enhance crop productivity, and also for determining degree of salt tolerance in different plant species to further utilize them in breeding programme.

Conclusion

Salt tolerant sorghum genotypes maintained high dry biomass, total N contents, proteins contents, total free amino acids, NRA and NO_3^- , thus can be used as physiological and biochemical markers to identify salt tolerant genotypes of sorghum and developing high yielding salt tolerant sorghum varieties.

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