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SCREENING OF DIVERSE LOCAL GERMPLASM OF GUAR [*CYAMOPSIS TETRAGONOLOBA* (L.) TAUB.] FOR SALT TOLERANCE: A POSSIBLE APPROACH TO UTILIZE SALT-AFFECTED SOILS

MIAN JAHAN ZAIB RASHEED[1], KAFEEL AHMAD[1], MUHAMMAD ASHRAF[2,3*], FAHAD AL-QURAINY[3], SALIM KHAN[3] AND HABIB-UR-REHMAN ATHAR[4]

[1]*Department of Botany, University of Sargodha, Sargodha, Pakistan*
[2]*Pakistan Science Foundation, Islamabad, Pakistan*
[3]*Department of Botany and Microbiology, King Saud University, Riyadh, Saudi Arabia*
[4]*Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan 60800, Pakistan*
**Corresponding author's e-mail: ashrafbot@yahoo.com*

Abstract

Lack of good quality water and soil salinity reduces crop productivity world-over. The development of salt stress tolerant cultivars/lines by screening and selection is of considerable value to enhance crop growth and yield. Though a number of breeding programs are underway to develop salt tolerant cultivars in wheat, barley, maize, and even grasses, a low amount of work done for improving salt tolerance in a potential leguminous forage crop guar –widely grown in subcontinent due to rapid increase in its demand for its commercial use. Thus, the present study was focused on efforts to develop salt tolerant cultivars of guar. The growth responses of 31 accessions/lines/cultivars of a potential leguminous crop (*Cyamopsis tetragonoloba*) to salt stress were assessed at the vegetative growth stage. A considerable variation in salinity tolerance was found in a set of lines/cultivars of guar using agronomic traits. Under saline conditions, Khanewal Local2, Chiniot White, 27340, 24323, BWP-5589 produced the lowest shoot fresh and dry biomass in relative terms, while genotypes/lines 5597, 24288, Br 99, Khushab white, Sillanwali white and Mardan white had greater fresh and dry biomass. Klorkot white and 24323 had maximum plant height under non-saline conditions, whereas genotypes/line 5597 and 24288 was maximal in plant height under salt stress conditions. Moreover, genotypes/lines Khanewal Local2 followed by Chiniot White and 27340 were the lowest in plant height. Growth attributes and relative salt tolerance of guar genotypes were used to group genotypes/lines as salt tolerant, moderately tolerant and salt sensitive using Hierarchical Cluster method following squared Euclidean distance. It was found that genotypes/lines 41671, Khaushab White, 5597, 24320, 24288, Sillanwali White, 24321, Mardan White were the most salt tolerant, while Chiniot White, BWP-5589, Kalorkot White, Khanewal Local 2, 24323were the most salt sensitive. The availability of considerable amount of genotypic variability in the germplasm suggested that further crop improvement for salt tolerance is possible in this potential leguminous forage crop.

Key words: Guar [*Cyamopsis tetragonoloba* (L.) Taub.], Salt stress, Plant height, Hierarchical cluster method, Squared euclidean distance method.

Introduction

Salt stress is one of the most serious threats to plant growth and crop productivity in arid and semi arid regions of the world. Various scientists have already proposed various strategies to overcome this problem. However, development of salt tolerant crop cultivars is the only economically viable way to overcome this problem (Ashraf *et al.*, 2008). Moreover, selection of available germplasm of a crop species for salinity tolerance with subsequent breeding is one of the most potential procedures to enhance crop salt tolerance (Munns & Tester, 2008). This is evidenced from the available published scientific reports, for example, Dewey (1960) selected some strains of *Agropyron desertorum* out of 60 strains. Similarly, Ulfat *et al.* (2007) selected five salt tolerant canola cultivars out of 32 cultivars. Long ago, Kingsbury *et al.* (1984) selected some salt tolerant wheat lines at the early growth stages. Keeping this information in mind, scientists suggested exploring intra-specific variation for salt tolerance in available germplasm. Moreover, success of this approach depends on availability of genetic variation, appropriate selection pressure and plant growth stage at which it is being selected (Athar & Ashraf, 2009). For example, Ashraf *et al.* (1986;1987) selected highly salt tolerant individuals of seven grass species using a high selection pressure at the seedling stage.

Guar (*Cyamopsis tetragonoloba* L.) is one of prospective spring-summer legume forage crop. Guar is mainly grown in arid and semi-arid regions of Pakistan, India, United States and South Africa (References cited in (Ashraf *et al.*, 2005) . Guar plants have a degree of water stress tolerance due to its ability to extract water from deep soil layers by its deep tap root system (Francois *et al.*, 1990). It is important leguminous forage crop for livestock. Seeds of guar contain galactomannans which is being used in wide range industries such as pharmaceutics, textile, paint, cosmetics, detergents and food industry (Francois *et al.*, 1990; Jukanti *et al.*, 2015). In view of these agro-botanical characters of this crop, this is gaining considerable attention to grow on abiotic stress hit areas (Ali *et al.*, 2015). It has also been found that local germplasm of guar is highly diverse in nature and possibly has adaptations to different climatic conditions such as cool environment of Khyber Pakhtoon Kha (KPK) or dry environment of Bahawalpur. A few reports are available on salt tolerance potential of this important forage crop (Deepika & Dhingra, 2014). For example, Ashraf *et al.* (2005) found limited growth of guar accessions under salt stress conditions. Such limited growth of guar plants might have been due to various environmental and genetic factors. Keeping these facts in mind, the present study was aimed to assess genotypic differences in 31 genotypes of guar to salt stress. This will greatly help in identifying salt tolerant genotypes which can be grown on salt affected land.

Materials and Methods

Seeds of 31 genotypes of guar [*Cyamopsis tetragonoloba* (L.) Taub.] were collected from various locations of District Jhang, Faisalabad, Bhakkar, Khaushab, Bahawalpur, Sialkot and Sargodha from Pakistan. The experiment was conducted in a randomized complete block design with four replicates in wire-net house of the University College of Agriculture, Sargodha, Pakistan during summer, 2014. The aluminium alloy trays of 7 feet long, 4 feet wide and 10 inch in depth (7′ x 4′ x 10″) were lined with polythene sheets having central drainage hole sealed with glass wool. The aluminium alloy trays were then filled with 240 kg normal garden soil. For appraising physico-chemical characteristics of the soil used, 12 soil samples were collected randomly from the containers and analysed following standard protocols. The texture of the soil was loam, saturation percentage 43, TSS 0.53%, organic matter 1.21%, pH 7.5, K 132 mg/kg and P 8.1 mg/kg. The seeds were sown with a drill in line with a distance of 25 cm (line to line). For uniform plant growth, thinning was done after 7 days of seed germination. An amount of 2.714 kg NaCl was dissolved in 480 litre water and irrigated each tray with 480 litre of that saline water to raise ECe 10 mS/cm. Electrical conductivity of non-saline control soil was 0.354 mS/cm. Plants were allowed to grow in non-saline and saline growth medium till maturity. Data for plant height was recorded. Plants of each genotype of guar were harvested and fresh biomass of shoots was recorded. Fresh shoots were oven-dried at 70°C for a period of one week, and dry weights were recorded. Plant height and dry weight stress tolerance indices were also calculated using following formulae:

$$PHSTI = \frac{\text{Plant height of stressed plants}}{\text{Plant height of control plants}} \times 100$$

$$FMSTI = \frac{\text{Fresh weight of stressed plants}}{\text{Fresh weight of control plants}} \times 100$$

$$DMSTI = \frac{\text{Dry weight of stressed plant}}{\text{Dry weight of control plant}} \times 100$$

Statistical analysis of the data: The data for all variables obtained from the experiment were subjected to a two-way analysis of variance (ANOVA) using the COSTAT computer package (CoHort 6.3 Windows, Berkeley, USA). Genotypes of guar were subjected to Hierarchical Cluster Methods by assessing between-groups linkage. Number of groups and group distance were assessed following squared Euclidean distance method. Genotypes of guar were then ranked as salt tolerant, moderately salt tolerant and salt sensitive based on stress tolerance indices using the statistical package SPSS 19 (SPSS Inc., USA).

Results

Imposition of salinity stress (10 mS/cm) significantly ($p \leq 0.001$) reduced the plant fresh and dry weights of all 31 genotypes of guar (Table 1; Fig. 1). Genotypes were significantly varied under both normal and salt stress conditions. Plant fresh biomass was maximal in genotypes Kalorkot white, 24323 and 5597under non-saline conditions, whereas the same was true for genotypes Br99, 24288 and 5597 under saline conditions (Fig 1). Moreover, the minimum shoot fresh biomass was found in lines Khanewal Local2, Chiniot White, 27340 and BWP-5595. However, relative reduction in fresh weight of shoot was maximal in Khanewal Local 2 followed by 24323 and 27340.

A significant reduction in dry weights of shoots of all genotypes of guar (*Cyamopsis tetragonolaba*) was recorded due to growth medium salinity stress (Table 1; Fig. 1). However, the effect of salt stress in reducing shoot dry weight was small in most of the cultivars. Moreover, maximum reduction in shoot dry weight was found in 24323 followed by Kalorkot white, BWP-5589 and Khanewal Local 2. Moreover, minimum reduction in shoot dry weight due to salinity stress was observed in genotypes 5597, 24288, Khushab white and Mardan white.

Plant height was also significantly reduced in all genotypes of guar due to imposition of salt stress (Table 1; Fig. 1). Of all lines, Khanewal Local2, Chiniot White, and 27340 had minimum plant height under saline conditions, but highest plant height was recorded in genotypes 24288 and 5597 followed by BWP-5609 and Khushab White.

To avoid confounding effect of growth potential of a genotype/line of guar, decrease in growth attribute over control and stress tolerance indices were calculated to assess relative stress tolerance in each genotype. Based on relative stress tolerance, genotypes were grouped as salt tolerant, moderately tolerant and salt sensitive (Table 2). Cumulative results slightly changed the ranking of cultivars. For example, genotypes 41671, Br 99, Khushab white, and 5597 were highly salt tolerant, whereas BWP-5589, Kalorkot white, Khanewal Local 2 and 24323 were the most salt sensitive genotypes/lines. Moreover, hierarchical cluster method was employed to group the cultivars by calculating squared Euclidean distance also supported similar results (Fig. 2). Hierarchical Cluster analysis showed that Khanewal Local2, Chiniot White, 27340, 24323, BWP-5589 are the salt sensitive genotypes, while genotypes 41671, Br 99, Khushab white, Sillanwali white, Mardan white, and 5597 are salt tolerant.

Table 1. Mean squares from the analysis of variance of the data for shoot fresh and dry weights, and shoot lengths of guar accessions/lines/cultivars when four week old plants were subjected to salt stress at the vegetative growth stage.

Source of variation	df	MS shoot fresh weight	MS shoot dry weight	MS shoot length	MS moisture contents
Salinity stress	1	1063***	97.12***	7546***	517.94***
Varieties	30	35.02***	22.52***	76.73***	5.89***
Salinity x Varieties	30	25.71***	16.95***	43.51***	5.86***
Error	186	0.0289	0.102	6.54	0.038
Total	247				

*** Significantly different at 0.001 P level

Table 2. Ranking of 31 guar (*Cayamopsis tetragonoloba* L.) lines on the basis of shoot fresh weight, shoot dry weight and plant height at 10 mS/cm salinity stress.

Classes	Genotypes
Tolerant	41671, Khaushab White, 5597, 24320, 24288, Sillanwali White, 24321, Mardan White, Br 99, Karor White, Hafizabad White, BWP-5611, Klorkot Black
Moderately tolerant	Br90, 22190, 24332, 24287, 22157, Sialkot White, BWP-5595, 24333, BWP-5609,
Sensitive	BWP-5596, Bhowana White, BWP-5599, 27340, Chiniot White, BWP-5589, Kalorkot White, Khanewal Local 2, 24323

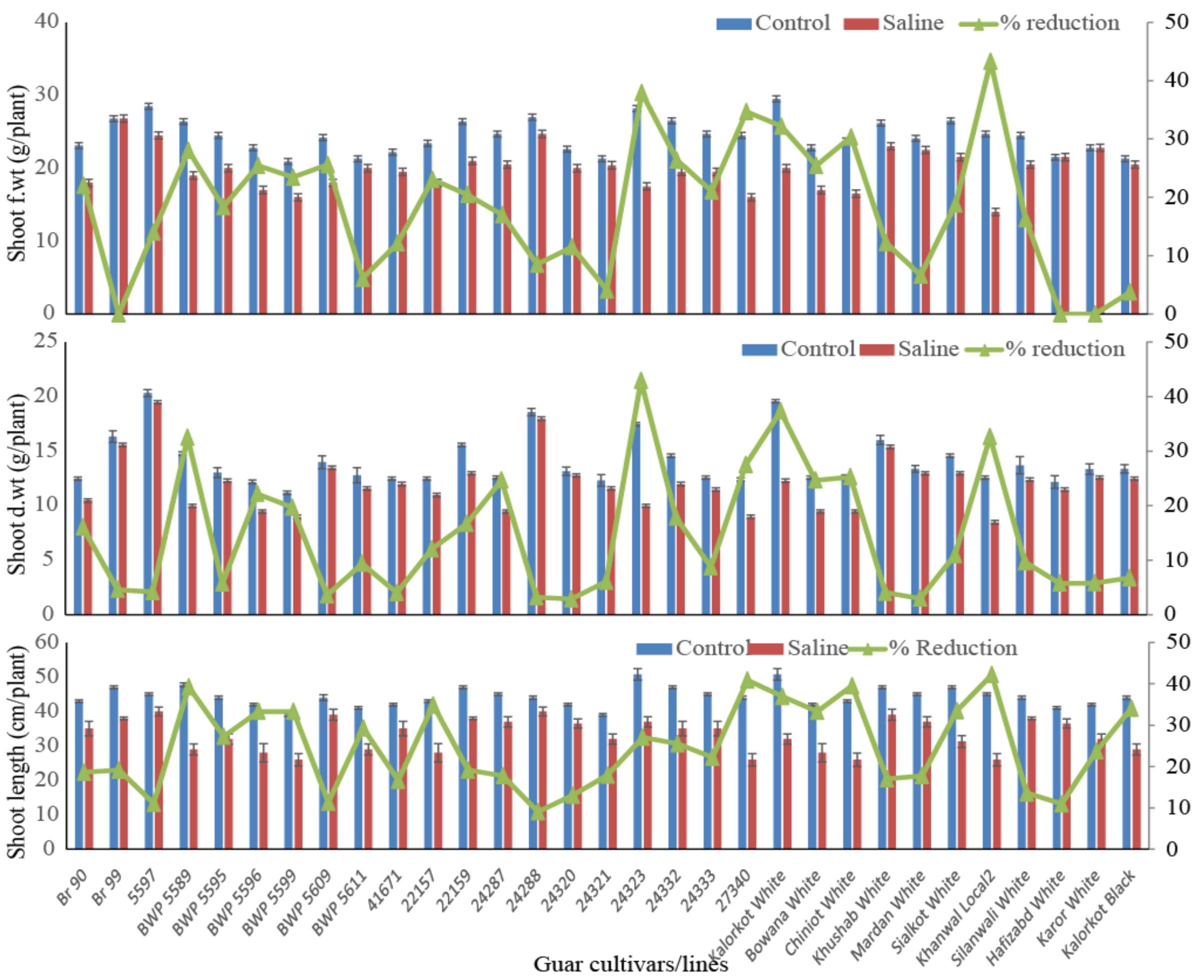

Fig. 1. Fresh and dry weights of shoots, and shoot lengths of guar accessions/lines/cultivars when four week old plants were subjected to salt stress at the vegetative growth.

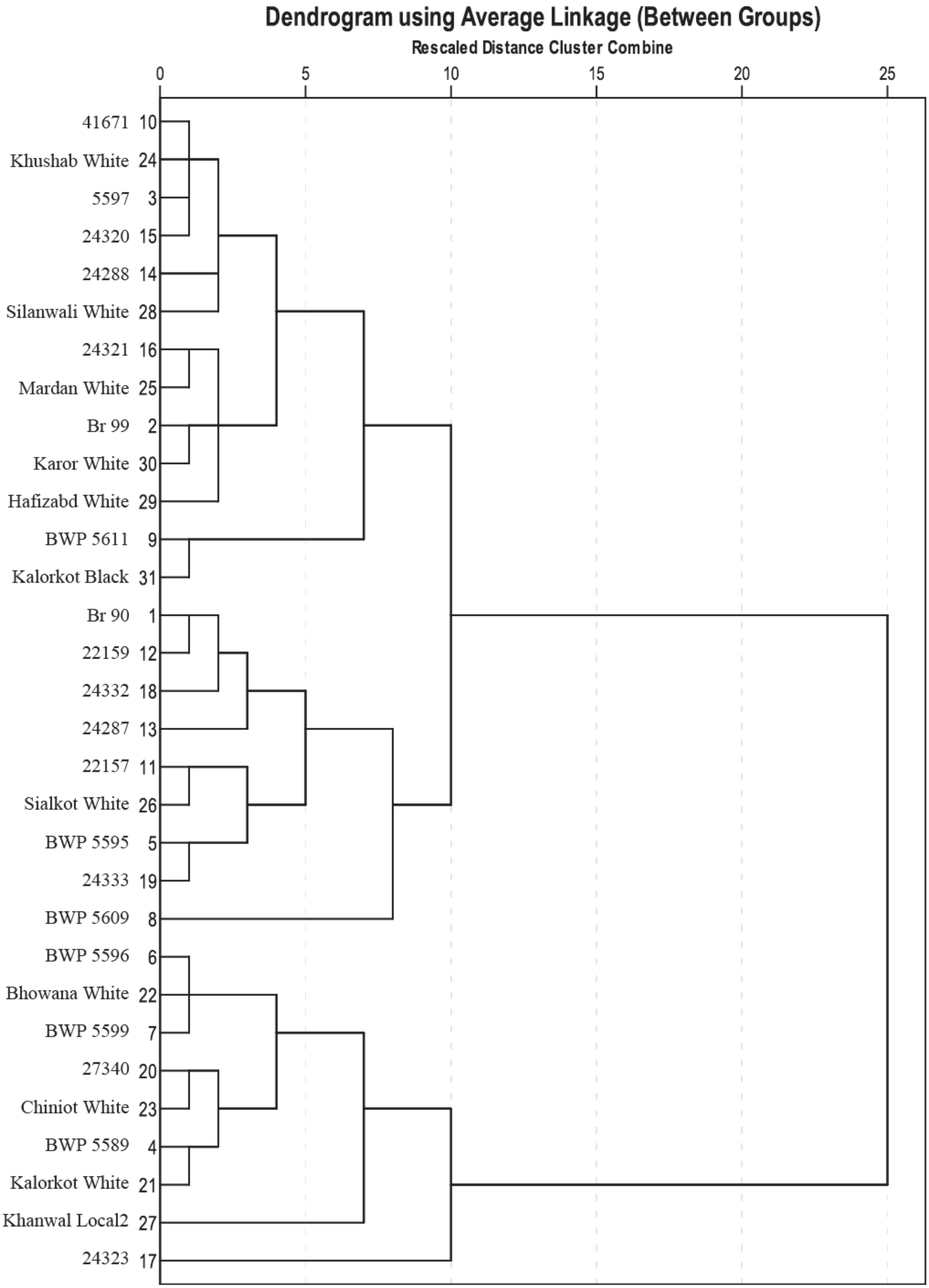

Fig. 2. Hierarchical Cluster Method analysis of 31 guar genotypes to group as salt tolerant, moderately tolerant and salt sensitive genotypes. Number of groups and differentiation was measured by squared Euclidean distance method using SPSS 19 statistical package.

Discussion

Forecast for a rapid increase in human population of world will cause a large increase in demand for food and energy. To meet this challenge, it is imperative to double the crop production. This has become a more daunting task on poor agricultural soils receiving low quality irrigation water, particularly on salt affected lands (Athar & Ashraf, 2009). Breeding programs have been intensified to develop new cop cultivars with improved yield and salt tolerance and more attention has been given to main food crops such as wheat and rice. During the past decade, it has been suggested to exploit the potential of other alternative forage crops, halophytes on salt affected land (Koyro *et al.*, 2013; Cheeseman, 2015). Guar (*Cyamopsis teragonoloba*) has been paid much attention during past decade as one of promising leguminous forage crops (Francois *et al.*, 1990; Ashraf *et al.*, 2002; Teolis *et al.*, 2009; Deepika & Dhingra, 2014; Ali *et al.*, 2015), which is mainly grown for producing fluid additive for a range of industries (Alexander *et al.*, 1988; Teolis *et al.*, 2009). Though a number of other alternative species exist, which are sufficiently salt tolerant to thrive on salt affected lands, these species lack sufficient amount of drought tolerance to exist on saline dry-lands (Ashraf *et al.*, 2002; Ashraf *et al.*, 2005; Deepika & Dhingra, 2014; Ali *et al.*, 2015; Cheeseman, 2015). In the present study, screening was employed to select salt tolerant genotypes using some agronomic traits.

Salt tolerance of a crop species is mainly attributed to a wide range of morpho-physiological attributes. However, crop improvement for salt tolerance depends on existence of genetic variability in a crop species. It can be deciphered from a number of published research reports that screening and selection of germplasm of a crop species is economically viable strategy to identify salt tolerant genotypes or lines which can produce reasonable crop yield on lands plagued with salt (Ashraf *et al.*, 1986; Munns *et al.*, 2006; Munns *et al.*, 2010; Munns & Gilliham, 2015). In the present study, salt stress adversely affected the growth of all genotypes/accessions/lines of guar under study. The adverse effect of salt stress was more prominent on shoot fresh weight, shoot moisture contents and plant height. However, a prominent reducing effect of salt stress on growth was found in only 5 genotypes out of 31 genotypes/cultivars/lines. These results can be explained in view of the findings of Francois *et al.* (1990) who reported that threshold level of salt tolerance is 5 and 8.8 dS/m at the vegetative growth and reproductive growth stages. However, more drastic effect of salt stress on guar accessions was found by Ashraf *et al.* (2002) at 15 dS/m salinity stress. Similarly, Teolis *et al.* (2009) found that guar germplasm has ability to thrive on 200 mM NaCl but with a drastic reduction in growth. The adverse effects of salt stress on plant growth might have been attributed to reduced leaf area with low rate of photosynthesis as has earlier been observed in canola (Athar *et al.*, 2015; Khalid *et al.*, 2015). Plant height and branching pattern along with appropriate leaf orientation are important agronomic traits for light use efficiency in photosynthesis and growth. The salinity-induced lesser reduction in plant height in genotypes 5597, Khushab White, 41671, 24288 and Mardan White might have been advantageous for better growth under salt stress. These results are similar to those of Ashraf *et al.* (2002) in which it was suggested that plant height is good agronomic indicator for salt tolerance in guar. Since most of salt tolerant guar genotypes such as 5597, Br99, Hafizabad White etc. maintained plant moisture contents (Calculated as Fresh wt – Dry wt), it is assumed that these genotypes might have developed some osmo-regulatory mechanisms. This argument can further be supported by the fact that guar plant contains a dual purpose polysaccharide –galactomannan which plays an important role in maintaining plant water status (Alexander *et al.*, 1988). However, this cannot be explained in some other salt tolerant genotypes such as 41671 and Khushab White in which shoot moisture contents were almost equal to those in the salt sensitive genotypes. Overall, a significant amount of genetic variability exists in the available guar germplasm with respect to salt tolerance at adult vegetative growth stage.

Genotypes/lines/accessions/cultivars of guar were grouped using agronomic traits (shoot fresh biomass, shoot dry biomass and plant height) as well as stress tolerance indices of these traits (see Material and Methods). In view of grouping of genotypes of guar at adult vegetative growth stage it is evident that genotypes 41671, Khushab White, 5597, 24320, Sillanwali White, 24321, Mardan White, Br-99, Karor White, Hafizabad White, BWP-5611 and Klorkot Black are salt tolerant, while 24323, Khanewal Local2, Klorkot White, BWP-5589, Chiniot White, 27340, BWP-5599, Bhowana White and BWP-5596 are highly salt sensitive. Though it is clear from the results of the present study that a significant genotypic variation was found in a set of 31 available genotypes/lines, a further study is required to assess its physiological basis of salt tolerance.

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