

APPRAISAL OF GUAR [*CYAMOPSIS TETRAGONOLOBA* (L.) TAUB.] ACCESSIONS FOR FORAGE PURPOSE UNDER THE TYPICAL SAUDI ARABIAN ENVIRONMENTAL CONDITIONS ENCOMPASSING HIGH TEMPERATURE, SALINITY AND DROUGHT

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Abstract

Guar (*Cyamopsis tetragonoloba* L. Taub.) being a summer annual legume is widely grown in some Asian countries primarily for three major purposes, i.e., cattle forage, as a green manure and for industry. Guar is relatively drought-tolerant compared to many other summer forages and it is well adapted to arid and semi-arid climates with high temperatures. We identified high-biomass-producing guar accessions in the hot, dry, and saline growth conditions of Saudi Arabia. We compared the performance of 24 introduced guar accessions as potential forage crop in Saudi Arabia, examining some key morphological traits and biomass production under water-limited and saline conditions in a greenhouse receiving natural sunlight and ambient temperature. The irrigation treatments were: 100% (Control), 80%, 60%, and 40% field capacity (FC), while salinity treatments were: Control (0 mM NaCl), 75 mM, 150 mM, and 200 mM NaCl. Accessions BWP 5595, 24320, Chiniot 1, Chiniot 2, Kaloorkot 2, and BWP 5599 had higher fresh and dry biomass than that of BR-99, BR-90, 027340, 24333, 24332, Khanewal Local 2, and Bhawana 2; the former accessions were therefore considered drought-tolerant, while the latter drought-susceptible. Accessions 24320, BWP 5595, Chiniot 1, Chiniot 2, and 22159 were found to be more salt-tolerant than BWP 5589, 24333, Bhowana 2, 24287, 027340, and BWP5596. Overall, BWP 5595 and 24320 are drought- and salt-tolerant, respectively, while Khanewal Local 2 and BWP 5589 are drought-sensitive and salt-sensitive, respectively. Future guar breeding programs could use these germplasm resources to improve crops against conditions of drought, high salinity, and heat in Saudi Arabia.

Key words: Guar water-deficit salinity forage crop.

Introduction

Guar or cluster bean (*Cyamopsis tetragonoloba* L. Taub.) is an important summer leguminous crop of some Asian countries particularly inflicted with arid and semi-conditions. It is primarily cultivated in Asia, and its uses are of multifarious nature, e.g., it is used for human consumption and cattle forage as well as green manure (Rao & Shahid, 2011; Satyavathi *et al.*, 2014 and Choy *et al.*, 2015). Its seed has 78–82% endosperm, which contains high levels of galactomannan gum, an increasingly important ingredient used in various products. The guar gum is of widespread use in food, cosmetics and pharmaceutical industries (Undersander *et al.*, 1991). Guar gum's richness and wide applicability for both food and non-food products has made it one of the most important industrial crops world-over (Pathak *et al.*, 2010; Sultan *et al.*, 2013).

In terms of drought tolerance potential of guar, it is contemplated as a non-thirsty crop (Sultan *et al.*, 2013). Therefore, it grows well in arid and semi-arid climates with high temperatures (Undersander *et al.*, 1991). Numerous studies on guar response to water deficit have been performed, especially in Pakistan and India, where guar is a common crop. For example, Ali *et al.* (2015) examined 36 different guar accessions from Pakistan to quantify their ability for high biomass production under hot and dry conditions. Upon ceasing irrigation for 20 and 40 days, all accessions under study showed a marked reduction in fresh and dry biomass. Moreover, accessions BR99, 5597, BWP 5595, BWP 5596, BWP 5609, BWP 5611, 24323, Chiniot Black, and Khushab Black produced greater biomass than that did by accessions 24321, Khushab White, Silanwali White, Sialkot Black, and

Khanewal Local 2; thus, the first group was considered drought-tolerant, while the latter group as drought-sensitive. Khanzada *et al.* (2003) experimented with four guar accessions under four water regimes (control, pre-flowering drought, post-flowering drought, and terminal drought). Water deficit conditions decreased leaf area, and seed yield, in all four accessions. However, S-807 and Esser performed better than accessions, Brooks and S-1183. In a separate study on 10 guar accessions, PI 323083 produced the highest yield (12.8 t ha⁻¹), with the remaining accessions averaging 9.5 t ha⁻¹ in dry matter yield, suggesting that guar could be an excellent alternative to water-thirsty forage species (e.g., alfalfa) in the UAE (Rao & Shahid, 2011).

Soils of arid and semi-arid zones are inflicted with multiple edaphic factors and thus few crops thrive on such soils (Tuna *et al.* 2007). Like some other crops known to grow well on soils hit with multiple stresses (Ashraf & McNeilly, 2004), guar grows well under a wide range of soil conditions including salinity, alkalinity and low soil fertility (Ali *et al.*, 2015). However, guar performs optimally on fertile, medium-textured, and sandy-loam alluvial soils, while heavy black soils are not well tolerated (Tran, 2015). Guar's response to soil salinity stress has also been explored in multiple studies. For example, Ashraf *et al.* (2005) examined the relationship between the root system and salt tolerance in 15 guar accessions. Salinity stress resulted in decreased root length, root dry weight, whole plant dry weight, and root nodule number across all accessions. These traits also exhibited inter-accession variation under both normal and stress conditions. Overall, accessions with better rooting systems correlated positively with seed yield, and were considered more salt-tolerant. The effect of irrigation with

diluted seawater on guar was investigated by Khan *et al.* (1989). The threshold EC_{iw} for a 50% reduction in seed production was estimated as $5.76 \text{ dS}\cdot\text{m}^{-1}$. Moreover, the net reproductive effort of plants treated with 30% seawater was 10.85%, versus 27.32% in control. Salt stress significantly decreased plant height, dry matter weight, and pod number, but not seed number per pod. Guar seeds germinated at high levels of salinity up to $16.57 \text{ dS}\cdot\text{m}^{-1}$. However, a separate study found that seed germination and plant growth significantly decreased with increasing seawater levels during irrigation; these results suggested that guar could be produced in the regions like the Red Sea shore basin if seawater is diluted with fresh water (Abusuwar & Abbaker, 2009). In another study, salt stress (3, 9, and $15 \text{ dS}\cdot\text{m}^{-1}$) was found to considerably suppress root length, root fresh and dry weights, plant height, shoot fresh and dry weights, as well as seed yield per plant in guar accessions. Among the tested accessions, 281/3 and 239/2 performed surpassed all at higher salinity levels (Ashraf, 2002). Similarly, while screening for salt tolerance a set of 29 diverse accessions, Rasheed *et al.* (2015) found that accessions 41671, Khaushab White, 5597, 24320, 24288, Sillanwali White, 24321, and Mardan White were the most salt-tolerant, while Chiniot White, BWP-5589, Kaloorkot White, Khanewal Local 2, and 24323 were the most salt-sensitive.

Saudi Arabia's arid climate and saline soil present particular challenges for the year-long sustainable production of forage crops. The average annual rainfall in Saudi Arabia is far lower than that in most other countries with a similar climate. Thus, arable land must be split between various types of crop production strategies. Despite this, forage crop productivity can be increased through cultivating better-adapted, heat- and drought-tolerant species, which may result in more sustainable yields. At present, the major forage crops in Saudi Arabia are sorghum and alfalfa, both of which are poorly adapted to the country's environment. Even with continuous irrigation (a requirement for both crops), they are relatively low yielding. Moreover, suitable irrigation water is in short supply, which results in the frequent use of seawater or brackish water, increasing soil salinity. Adding to the difficult conditions, daily temperatures frequently exceed 40°C . Therefore, the primary focus of this study was to identify guar accessions/lines that are high-biomass-producing in the hot, dry, and saline growth conditions prevalent in Saudi Arabia. The results would ultimately benefit agriculture and reinforce food security for Saudi Arabia.

Materials and Methods

The experiment was performed in a greenhouse having natural sunlight at the College of Science, King Saud University, Saudi Arabia. The morphological traits and biomass of 24 guar accessions obtained from Pakistan and already used by Ali *et al.* (2015) and Rasheed *et al.* (2015) were compared under drought- and salt-stressed conditions, respectively.

Seeds of each accession were sterilized separately with sodium hypochlorite (5%) for 10 min, rinsed twice with distilled water, and then soaked in distilled water for 30 min. One seed was sown in each saturated peat disc,

and then put in trays until germination. The trays were irrigated regularly until emergence of the third true leaf occurred. Polyvinyl chloride (PVC) pots (diameter, 12 cm; depth, 20 cm) were filled with three equal parts of peat moss, perlite, and pot soil. One healthy germinated seedling was transferred into each pot and irrigated regularly. One gram per liter of macronutrients (NPK 20:20:20) was added through the irrigation water three times before applying stress treatments to enhance the soil mix condition and improve the growth of the plants.

The experimental units were laid down following a Randomized Complete Block Design (RCBD) with three replicates. In the drought experiment, the irrigation settings were: 100%, 80%, 60%, and 40% field capacity (FC), whereas in the salinity experiment four levels of salinity were: Control (0 mM NaCl), 75 mM, 150 mM, and 200 mM of NaCl.

Drought and salinity treatments were applied 30 days after the sowing date. Morphological and biomass evaluations were conducted 40 days later. The traits evaluated were: plant height (cm), leaf number, leaf area (cm^2), leaf fresh weight, stem fresh weight, root fresh weight, leaf dry weight, stem dry weight, and root dry weight. All plant samples were subjected to an oven at 65°C for 48 h to determine dry weight.

Statistical analysis of data: Analysis of variance was performed on the collected data using SAS 9.1, SAS Inc., North Carolina, USA (Anon., 2002), and the least significant difference (LSD) test was used to discern differences among the mean values of the treatments and accessions.

Results

Water-deficit effect: The interaction between irrigation regimes and guar accessions for leaf fresh weight was highly significant ($p < 0.0001$) reflecting that the accessions responded differently to the water deficit conditions. Significant differences in leaf fresh weight also existed among the four irrigation regimes ($p < 0.0004$). At full FC and 80% FC, leaf fresh weight was the highest, with no significant difference between the two treatments. The 40% FC resulted in the lowest leaf fresh weight. Accessions also differed significantly ($p < 0.0001$), with BWP 5595, 24320, Chiniot 1, Kaloorkot 2, and BWP 5599 exhibiting highest leaf fresh weight, whereas the lowest leaf fresh weight was recorded in BR-99, BR-90, 027340, 24332, and Khanewal Local 2 (Fig. 1).

Water deficit markedly reduced stem fresh weight ($p < 0.0001$). Accessions BWP 5595, 24320, Kaloorkot, BWP 5599, Chiniot 1, and Chiniot 2 had higher stem fresh weights than those of BR-99, BR-90, 24333, Khanewal Local 2, and Bhowana 2. The interaction between the irrigation and accessions was highly significant ($p < 0.0002$) (Fig. 2).

Water limited regimes resulted in a marked suppression of plant height ($p < 0.0001$). The tallest accessions were found as: BWP 5595, 24288, BWP 5599, 24320, BWP 5589, BWP 5597, and 24332, while the shortest accessions were: BR-99, BR-90, 24287, Bhowana, and 22159. The difference between the accessions for plant height was highly significant ($p < 0.0001$) (Fig. 3).

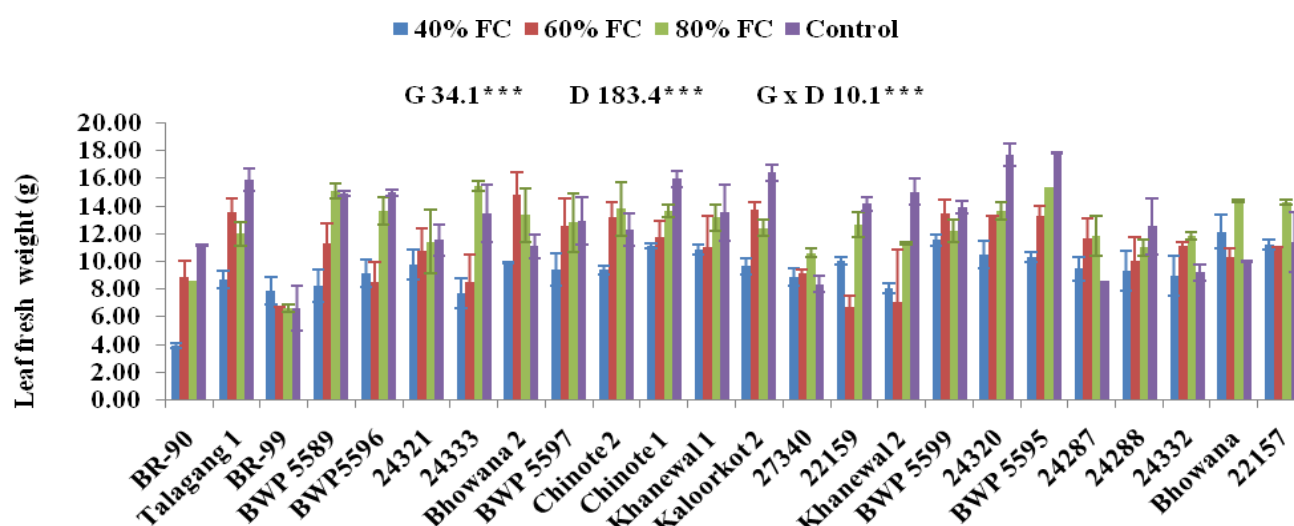


Fig. 1. Variation in leaf fresh weight (g, mean \pm S.E.) across 24 guar accessions grown under four water regimes (40% field capacity [FC], 60% FC, 80% FC, and Control at 100% FC). G, Accession; D, Drought; ***, significant at $p < 0.001$.

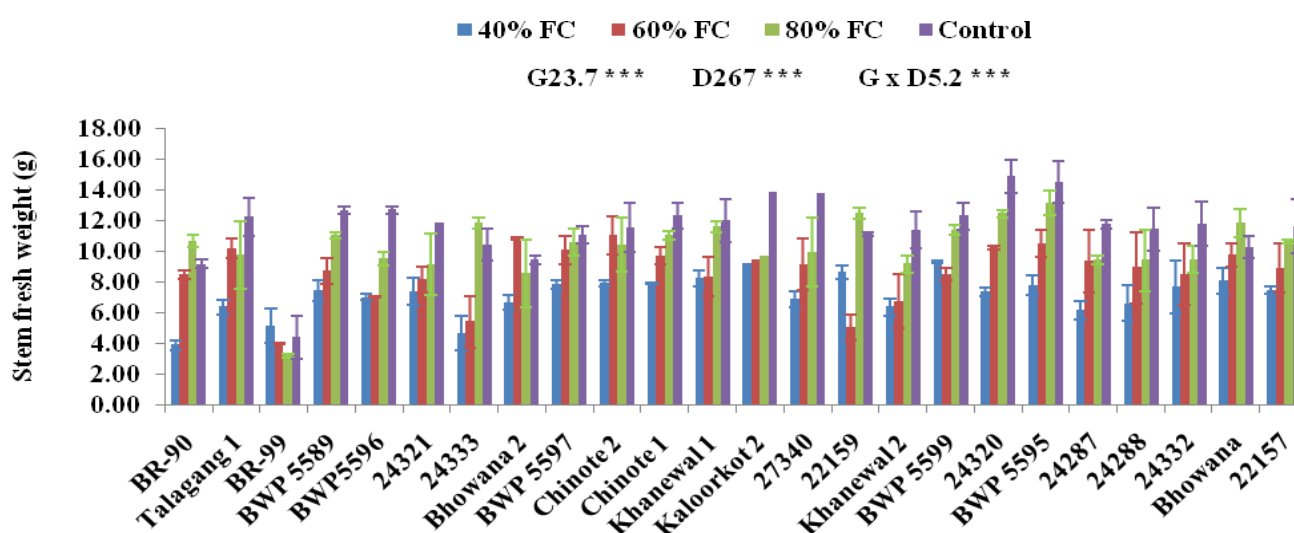


Fig. 2. Variation in stem fresh weight (g, mean \pm S.E.) across 24 guar accessions grown under four water regimes (40% field capacity [FC], 60% FC, 80% FC, and Control at 100% FC). G, Accession; D, Drought; ***, significant at $p < 0.001$.

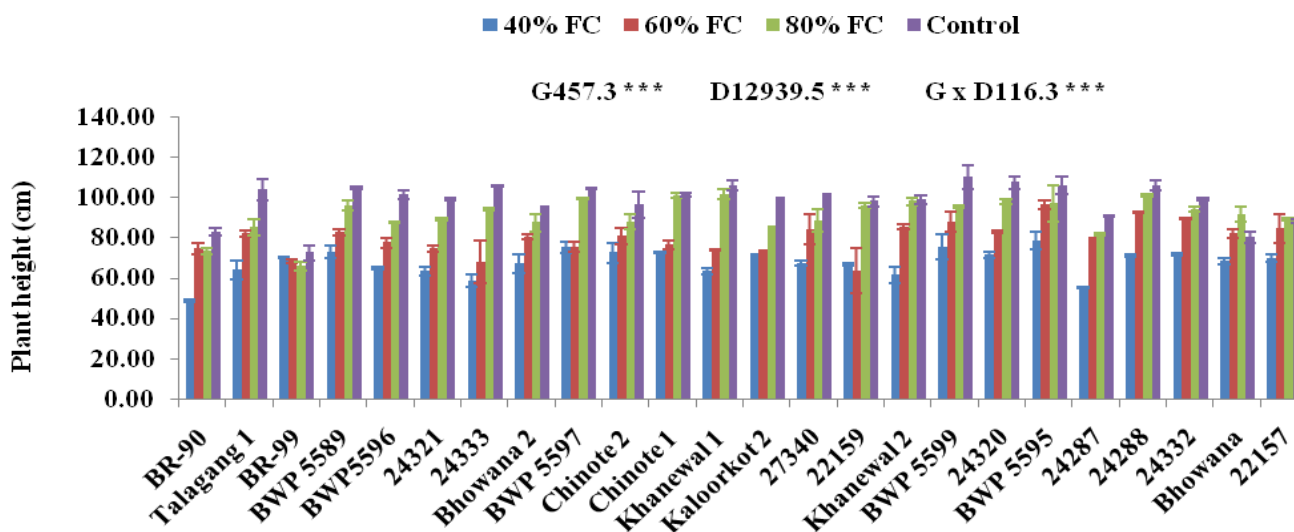


Fig. 3. Variation in plant height (cm, mean \pm S.E.) among 24 Guar accessions grown under four water regimes (40% field capacity [FC], 60% FC, 80% FC, and Control at 100% FC). G, Accession; D, Drought; ***, significant at $p < 0.001$.

Leaf number per plant was reduced significantly by low supply of water ($p < 0.0001$) (Fig. 4). Leaf number was highest in accessions BWP 5595, 22157, Chiniot 1, Kaloorkot, BWP 5599, 24320, Khanewal Local 1, and BWP 5589, but lowest in BR-90, 027340, 24288, 24332, 24287, BR-99, and Bhowana.

Leaf area was decreased significantly under water deficit stress ($p < 0.0001$). Accessions Chiniot 2, Khanewal Local 1, Chiniot 1, BWP 5589, 22159, BWP5596, 24288, and Kaloorkot 2 exhibited the largest leaf area, while accessions BR-99, 22157, Bhowana, 24320, Khanewal Local 2, and BWP 5595 exhibited the lowest (Fig. 5).

Leaf dry weight was significantly different across irrigation treatments ($p < 0.0001$). The highest leaf dry weight was recorded for accessions 24320, BWP 5595, BWP 5599, Chiniot 1, 24288, and Kaloorkot 2, while the lowest was observed in accessions BR-99, BR-90, BWP 5596, 22159, and 027340 (Fig. 6).

Water deficit negatively but significantly affected stem dry weight ($p < 0.0001$), which was highest in accessions BWP 5595, 24332, 24288, 2430, and Bhowana, but lowest in BR-99, BR-90, BWP 5596, 24333, BWP 5597 and Khanewal Local 2 (Fig. 7).

Salinity effect: Leaf fresh weight varied significantly at the four salinity regimes ($p < 0.0001$) and among the accessions ($p < 0.0001$). Accessions BWP 5595, 24320, Chiniot 2, 22159, and Chiniot 1 exhibited the highest leaf fresh weights, whereas the lowest in BR-99, 027340, 24333, 24287, and Bhowana 2 (Fig. 8).

Salinity stress considerably reduced stem fresh weights of all accessions ($p < 0.0001$). Accessions 24320, Chiniot 1, BWP 5595, Kaloorkot 2, Chiniot 2, 22159, and BWP 5599 had higher stem fresh weights than those of BR-99, 24333, BWP 5589, Talagang 1, BR-90, and Bhowana 2. The effect of the salinity and accession interaction was highly significant ($p < 0.0001$; Fig. 9).

Increasing salt level of the growth medium caused a significant reduction in plant height of all guar accessions ($p < 0.0001$). The tallest accessions were 24320, Kaloorkot 2, Chiniot 1, BWP 5599, BWP 5597, and 027340, whereas the shortest BR-99, 24333, Bhowana, BWP 5596, 22157, Bhowana 2, and 24287. The differences among the accessions in plant height were highly significant ($p < 0.0001$) (Fig. 10).

Salt stress markedly reduced leaf number per plant ($p < 0.0001$) (Fig. 11). The highest leaf number was in accessions 24320, Kaloorkot 2, BWP 5595, Chiniot 1, and 22159, whereas the lowest in accessions BWP 5597, 24321, BWP 5596, BR-99, Bhowana 2, 24288, and 24333.

Salinity stress significantly reduced the leaf area of all accessions ($p < 0.0001$). Accessions Khanewal Local 1, Chiniot 1, BWP 5596, 22159, Chiniot 2, and BWP 5597 exhibited the largest leaf area, while the lowest was found in BWP 5595, 24287, BR-99, 22157, 24332 and 24320 (Fig. 12).

Differences in leaf dry weight were highly significant among the salinity treatments ($p < 0.0001$). Accessions with highest leaf dry weights were 2430, Chiniot 1, BWP 5595, Kaloorkot 2, and BWP 5599, whereas those with the lowest were 24333, BR-99, 24321, BWP 5597, BWP 5596, 24332, and Bhowana 2 (Fig. 13).

Stem dry weight was negatively but significantly affected by the salinity treatments ($p < 0.0001$). Accessions 24320, Kaloorkot 2, 24332, Chiniot 1, Chiniot 2, BWP 5595, and BWP 5599 had the highest, whereas accessions BR-99, 24333, BR-90, Talagang 1, and 24287 had the lowest stem dry weight (Fig. 14).

Discussion

Water starvation is among the most important challenges of the modern world for agriculture. Addressing this challenge is cumbersome particularly in areas with poor and saline agricultural soils receiving low-quality irrigation water (Athar & Ashraf, 2009). High temperatures lead to soil water deficit, which then automatically increases soil salinity levels. In Kingdom of Saudi Arabia all three environmental cues are prevalent in conjunction. Therefore, in countries like Saudi Arabia, recent breeding programs have focused on producing crops that are simultaneously drought, salinity, and heat-tolerant. Several forage crop species are reported to be highly salt-tolerant, but most of them when tested for drought tolerance they proved to be drought-sensitive, or vice versa (Ashraf *et al.*, 2002; Ashraf *et al.*, 2005; Deepika & Dhingra, 2014; Ali *et al.*, 2015; Cheeseman, 2015). Under such circumstances, a crop cultivar/line resilient to high temperature, drought and salinity seems to be plausible for maximizing forage production on lands inflicted with all three cues.

Numerous studies have demonstrated that guar accessions have a high potential for salt, drought, and heat tolerance. Thus, guar is thought to be a promising leguminous forage crop in countries with unfavorable agricultural conditions, such as India, Pakistan, and other such countries (Francois *et al.*, 1990; Ashraf *et al.*, 2002; Teolis *et al.*, 2009; Deepika & Dhingra, 2014; Ali *et al.*, 2015). Since these countries are highly similar to Saudi Arabia in terms of harsh environmental factors, so testing guar accessions under Saudi conditions could allow the plant's introduction to the country, which will certainly meet the recent dramatic increase in animal feeding needs, thereby contributing to the reduction of hunger.

We found that the interaction between irrigation regimes and accessions was highly significant for all measured variables, indicating a strong significant effect of both water deficit and accessions on guar growth and development. Moreover, all studied traits differed significantly among the four irrigation regimes indicating that water deficits had a considerable influence on guar. Accessions were diverse in their response to irrigation regimes, affirming their value for use in breeding programs.

Accessions BWP 5595, 24320, Chiniot 1, Kaloorkot 2, and BWP 5599 Chiniot 1, and Chiniot 2 were found to have the highest fresh and dry biomass, indicating their drought tolerance compared with accessions BR-99, BR-90, 027340, 24333, 24332, Khanewal Local 2, and Bhowana 2; the latter accessions exhibited the lowest biomass and are likely to be considered as drought-susceptible. These results are in agreement with those of Ali *et al.* (2015) who also reported a marked variation among different accessions of guar including all being studied here.

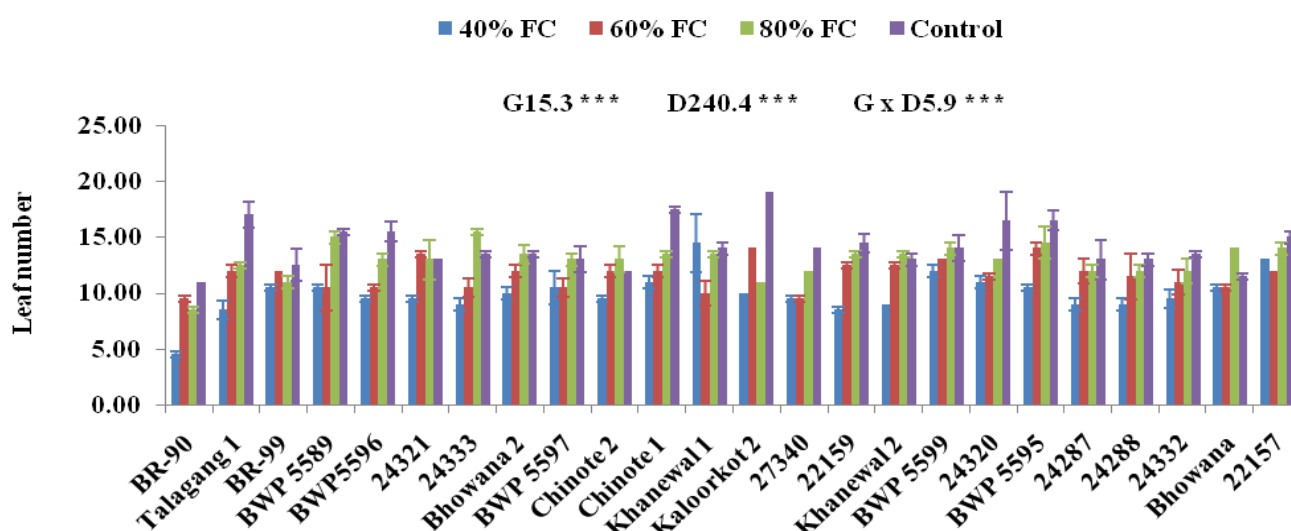


Fig.4: Variation in leaf number (mean ± S.E.) across 24 guar accessions grown under four water regimes (40% field capacity [FC], 60% FC, 80% FC, and Control at 100% FC). G, Accession; D, Drought; ***, significant at $p < 0.001$.

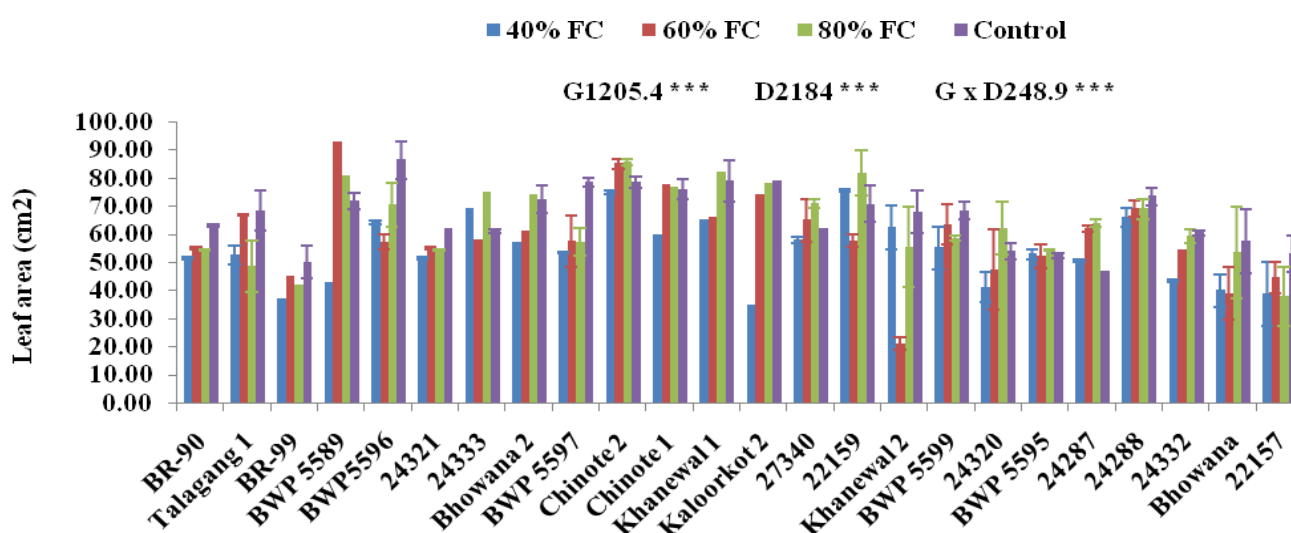


Fig. 5. Variation in leaf area (cm²; mean ± S.E.) among 24 guar accessions grown under four water regimes (40% field capacity [FC], 60% FC, 80% FC, and Control at 100% FC). G, Accession; D, Drought; ***, significant at $p < 0.001$.

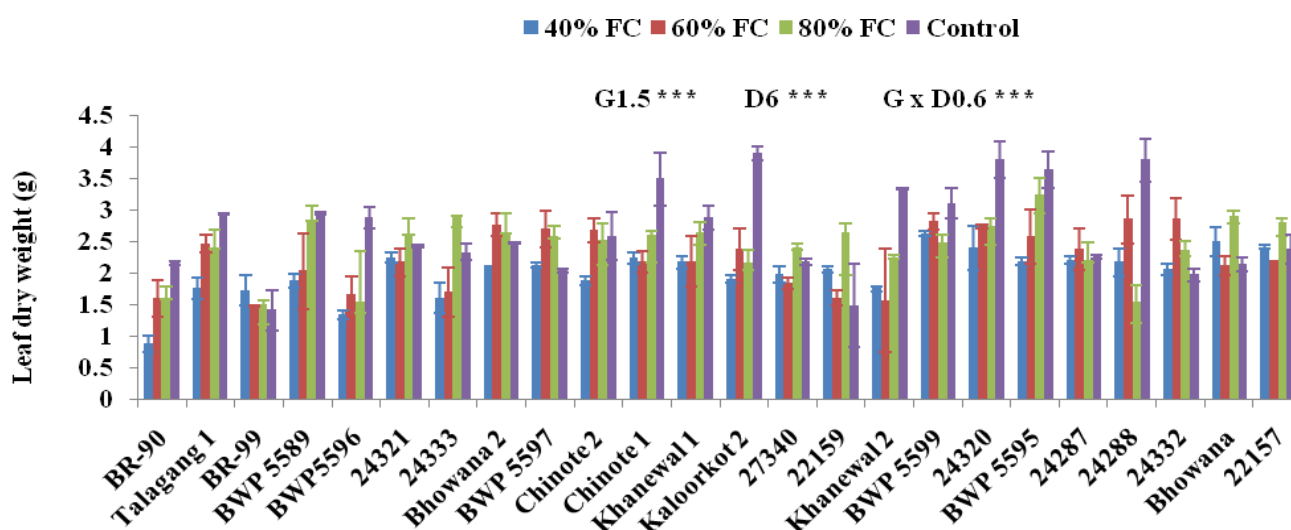


Fig. 6. Variation in leaf dry weight (g; mean ± S.E.) across 24 guar accessions grown under four water regimes (40% field capacity [FC], 60% FC, 80% FC, and Control at 100% FC). G, Accession; D, Drought; ***, significant at $p < 0.001$.

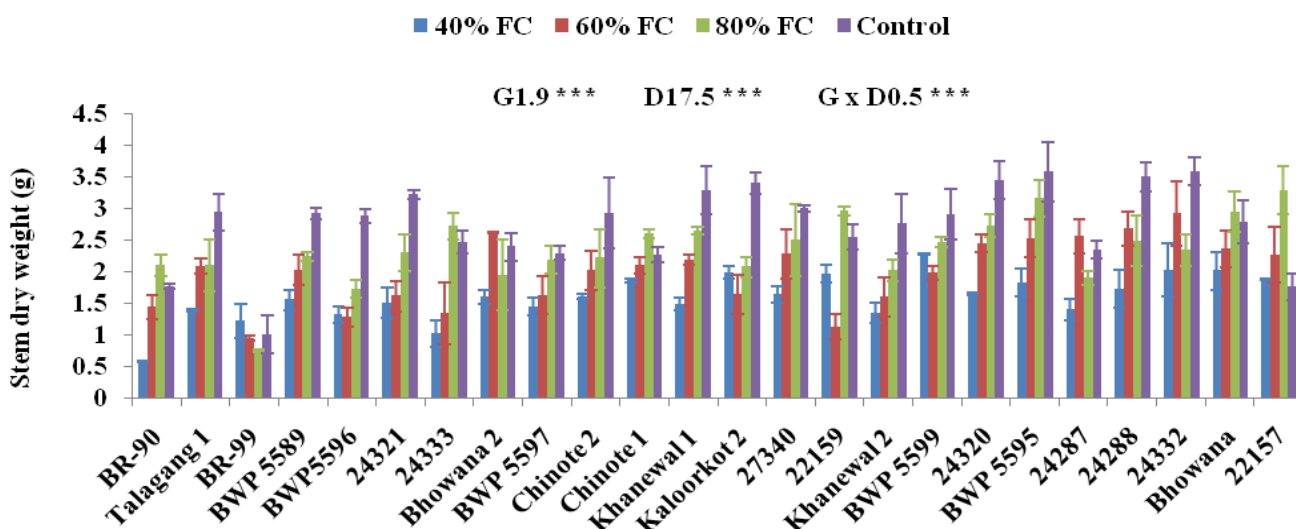


Fig. 7. Variation in stem dry weight (g; mean ± S.E.) across 24 guar accessions grown under four water regimes (40% field capacity [FC], 60% FC, 80% FC, and Control at 100% FC). G, Accession; D, Drought; ***, significant at $p < 0.001$.

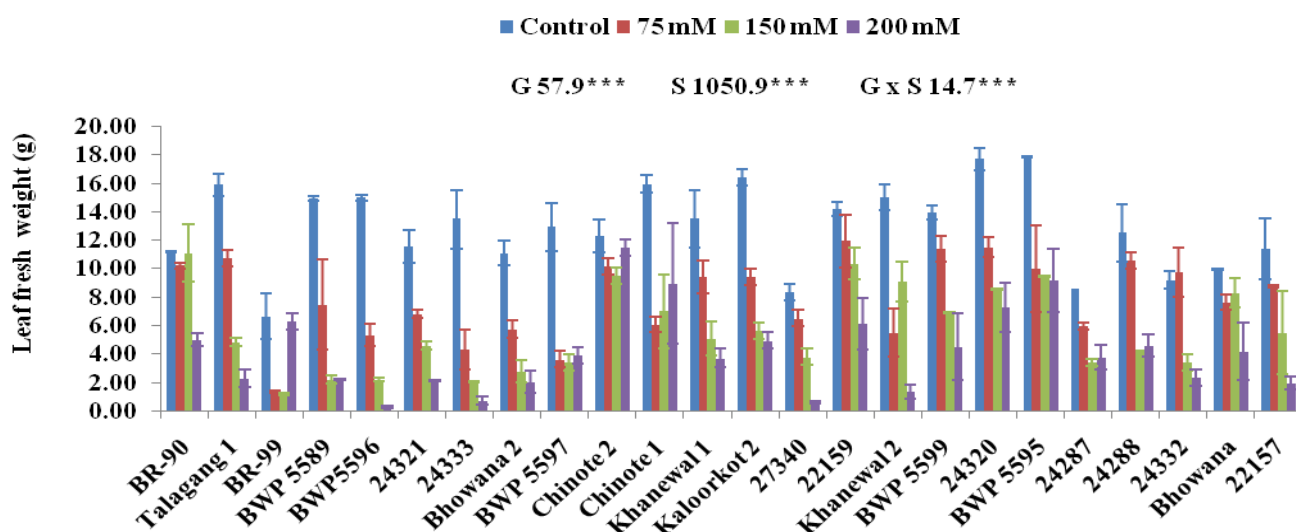


Fig. 8. Variation in leaf fresh weight (g; mean ± S.E.) of 24 guar accessions grown under four salinity regimes (NaCl: 75 mM, 150 mM, 200 mM, and a no-salt Control). G, Accession; S, Salinity; ***, significant at $p < 0.001$.

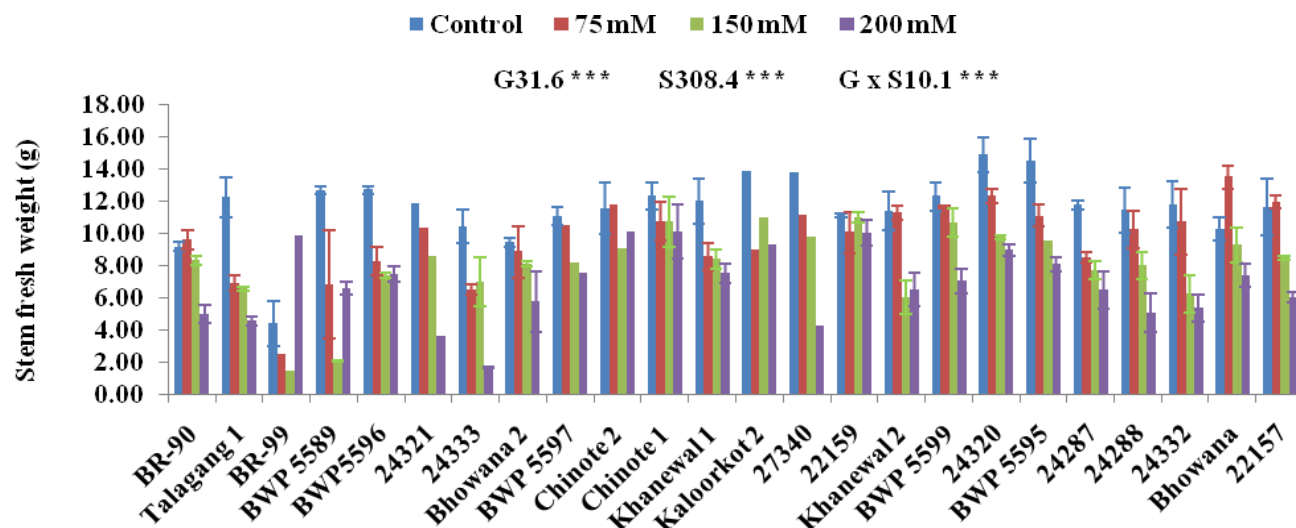


Fig. 9. Variation in stem fresh weight (g; mean ± S.E.) of 24 guar accessions grown under four salinity regimes (NaCl: 75 mM, 150 mM, 200 mM, and a no-salt Control). G, Accession; S, Salinity; ***, significant at $p < 0.001$.

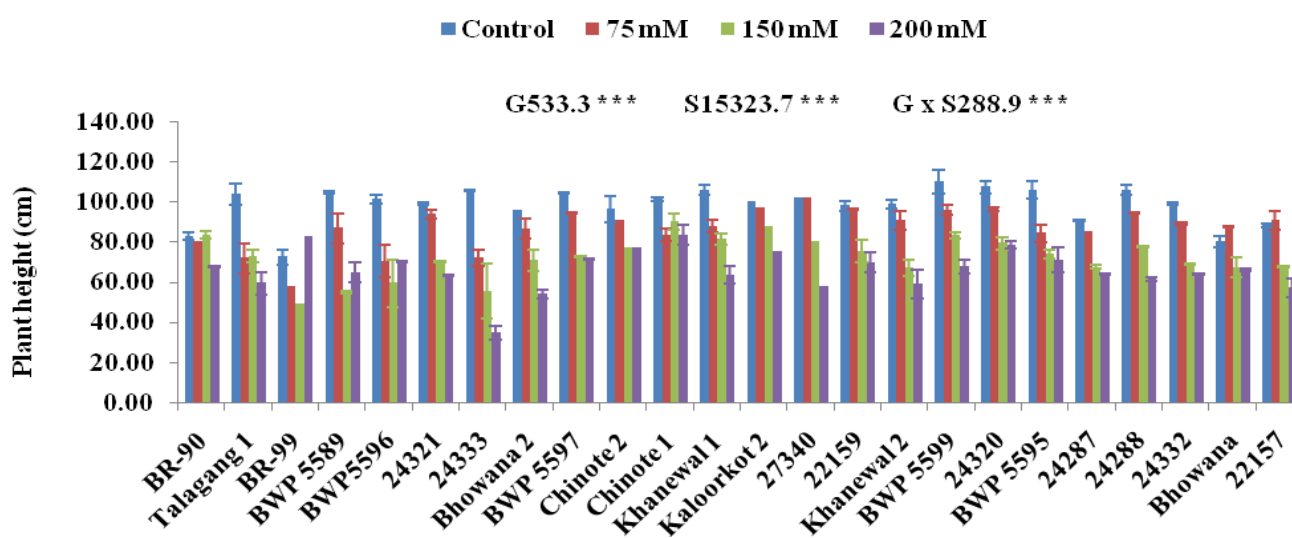


Fig. 10. Variation in plant height (cm; mean \pm S.E.) of 24 guar accessions grown under four salinity regimes (NaCl: 75 mM, 150 mM, 200 mM, and a no-salt Control). G, Accession; S, Salinity; ***, significant at $p < 0.001$.

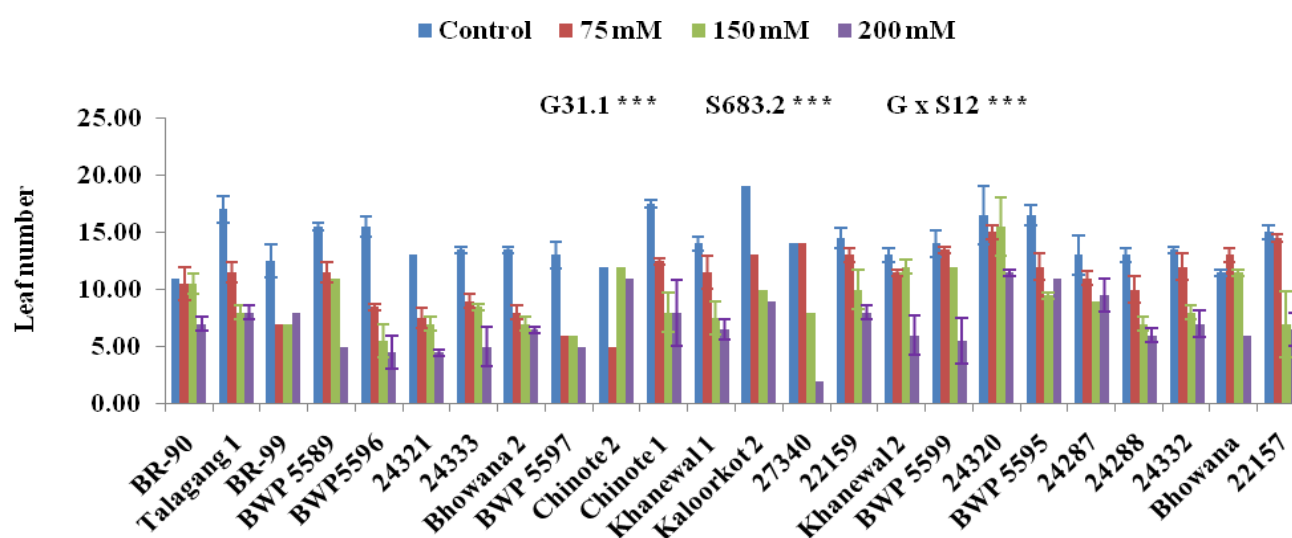


Fig. 11. Variation in leaf number (mean \pm S.E.) of 24 guar accessions grown under four salinity regimes (NaCl: 75 mM, 150 mM, 200 mM, and a no-salt Control). G, Accession; S, Salinity; **, significant at $p < 0.001$.

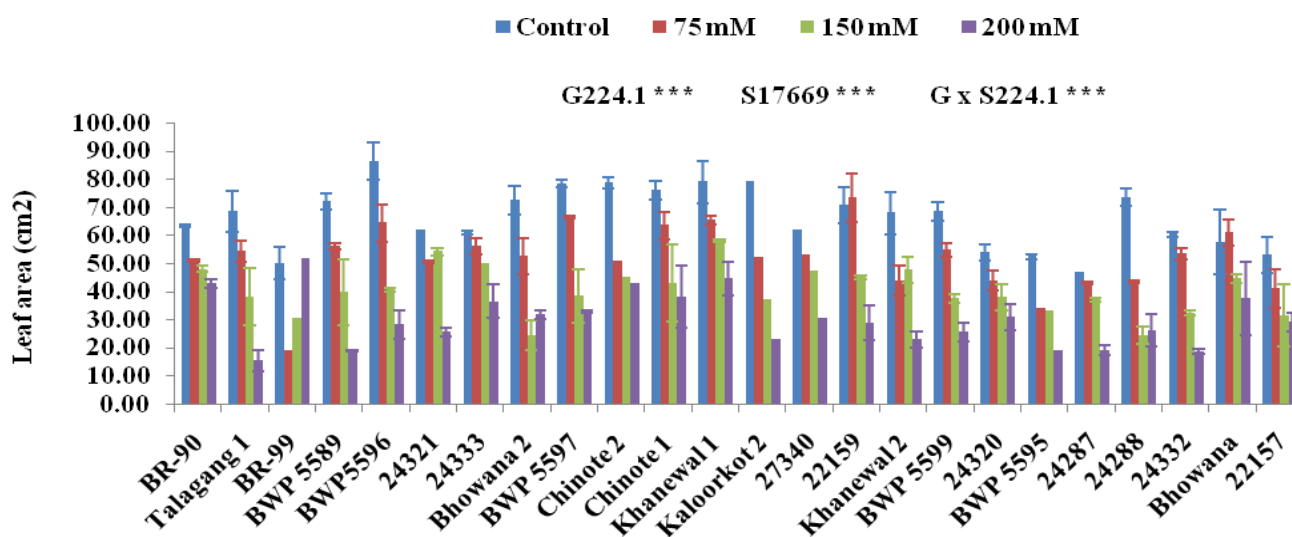


Fig. 12. Variation in leaf area (cm²; mean \pm S.E.) of 24 Guar accessions grown under four salinity regimes (NaCl: 75 mM, 150 mM, 200 mM, and a no-salt Control). G, Accession; S, Salinity; ***, significant at $p < 0.001$.

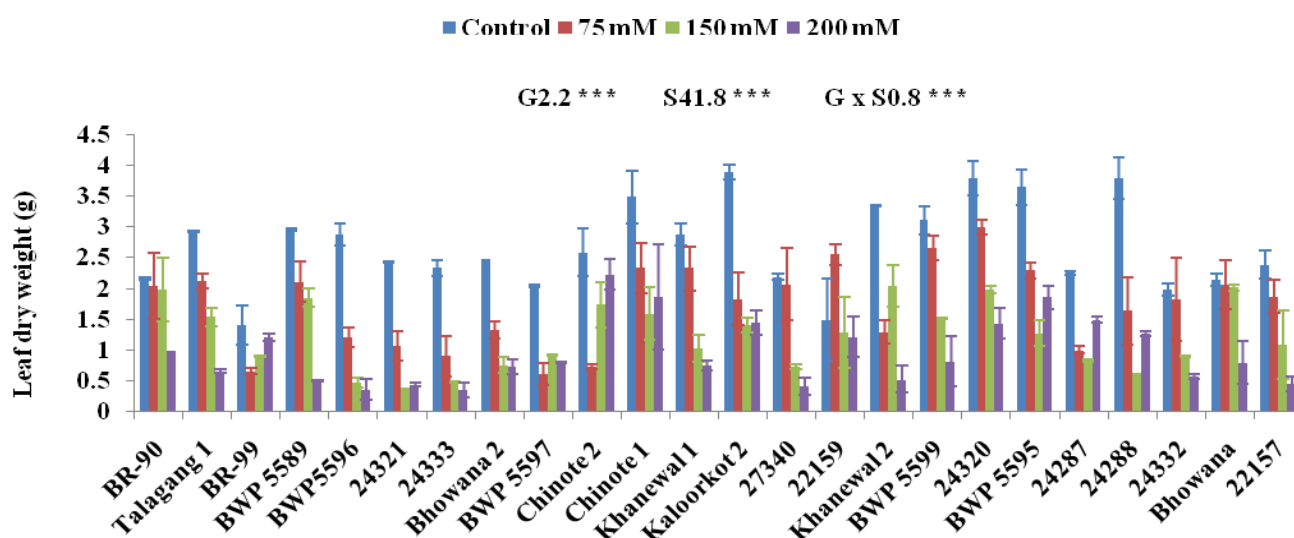


Fig. 13. Variation in leaf dry weight (g; mean \pm S.E.) of 24 guar accessions grown under four salinity regimes (NaCl: 75 mM, 150 mM, 200 mM, and no-salt Control). G, Accession; S, Salinity; ***, significant at $p < 0.001$.

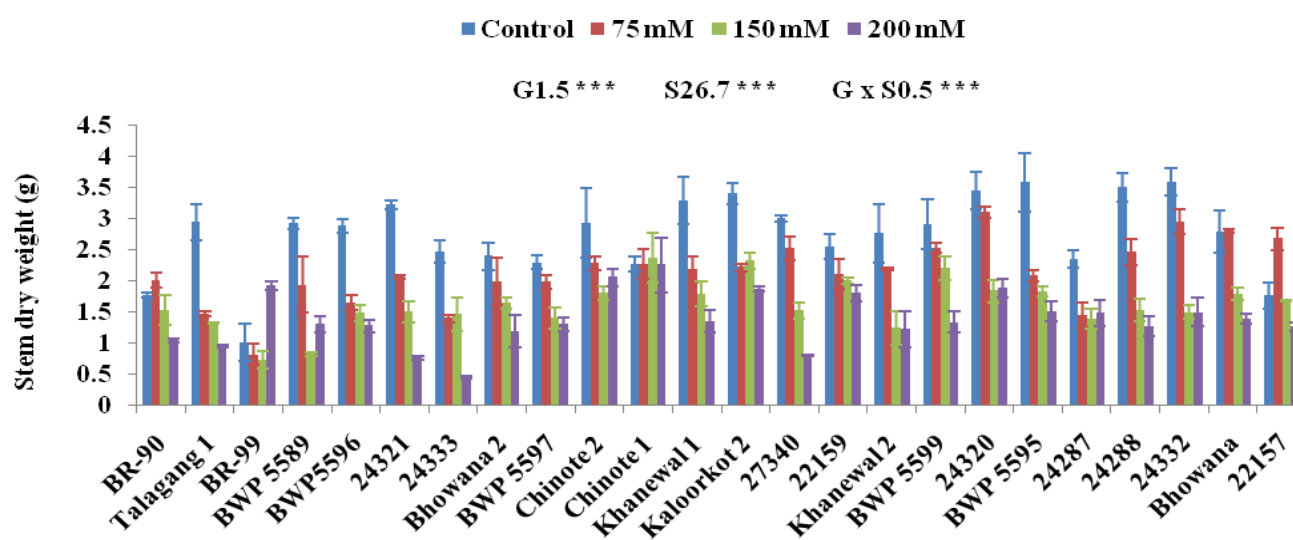


Fig. 14. Variation in stem dry weight (g; mean \pm S.E.) of 24 guar accessions grown under four salinity regimes (NaCl: 75 mM, 150 mM, 200 mM, and no-salt Control). G, Accession; S, Salinity; ***, significant at $p < 0.001$.

Guar morphology and biomass differed significantly among the four salinity regimes. Moreover, accessions varied significantly in their response to salinity stress. These differential responses indicate that the germplasm could be useful for future selection and breeding programs to improve guar salinity tolerance. Specifically, our data showed that accessions 24320, BWP 5595, Chinote 1, Chinto 2, and 22159 were found to be highly salt-tolerant as compared to the others. The accessions BWP 5589, 24333, Bhowana 2, 24287, 027340, and BWP5596 were ranked as salt-sensitive. These results are similar to those of Rasheed *et al.* (2015) who also reported a great magnitude of variation for salinity tolerance in a set of guar accessions including those studied here. The most tolerant accessions to drought and salinity were found to be BWP 5595 and 24320, whereas the most sensitive accessions were Khanawal2 and BWP 5589, respectively.

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