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MORPHO-PHYSIOLOGICAL RESPONSES OF GUAR [*CYAMOPSIS TETRAGONOLOBA* (L.) TAUB.] TO MULTIPLE STRESSES OF DROUGHT, HEAT AND SALINITY

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Abstract

Plant responses to abiotic stresses such as drought, salt, and heat, have been studied separately, but in fact, plants are exposed simultaneously to a combination of stresses. Therefore, the objective of this study was to explore the morpho-physiological responses of the guar plant to the combination of heat, drought, and salinity with respect to that to an individual stress. Five treatments including heat, drought, salinity, a combination of multiple stresses and control were applied to two guar accessions namely "BWP-5595" and "24320". The results showed that heat stress enhanced biomass, plant height, leaf number, leaf elongation, and prolonged time to flowering. However, it reduced root length and water use efficiency, but it had moderately negative effect on leaf area, stomatal conductance and number of pods. Contrastingly, drought stress improved root length, water use efficiency, and leaf elongation. It affected negatively leaf area, plant height, and prolonged days to flowering. Drought stress moderately reduced leaf number, biomass, stomatal conductance, and number of pods. Comparatively, salinity stress had a moderately negative impact on all studied traits except leaf number, which was reduced significantly. However, the combination of multiple stresses severely affected all studied traits except water use efficiency. These results show that the adverse effects of the combination of all three stresses were more pronounced than those of either of the individual stresses. Of the two guar accessions used, BWP 5595 showed better performance than accession 24320 in most of the traits measured. This indicates that the effect of multiple stresses differs among the accessions of a species.

Key words: Abiotic stress, Biomass, Forage crop, Multiple stress.

Introduction

Guar (*Cyamopsis tetragonoloba* L. Taub.) is an important leguminous forage crop of some Asian countries particularly located in a range of arid and semiarid-conditions. It has multiple uses for humans, animals, and soil as a green manure (Rao & Shahid, 2011; Satyavathi *et al.*, 2014 and Choy *et al.*, 2015). The endosperm of the seed is highly rich in its content of galactomannan gum, which is used in food, pharmaceutical industries, and cosmetics (Undersander *et al.*, 1991). Consequently, guar becomes one of the most significant industrial crops globally (Pathak *et al.*, 2010; Sultan *et al.*, 2013). Guar is considered as a non-thirsty crop (Sultan *et al.*, 2013). It tolerates arid and semi-arid climates as well as high temperature conditions (Undersander *et al.*, 1991). Furthermore, it grows well under salinity, alkalinity, and low soil fertility conditions (Ali *et al.*, 2015).

Plants are prone to be exposed to multiple abiotic stresses within their natural habitat. Multiple stresses cause noticeable threats to plants (Rizhsky *et al.*, 2004; Mittler, 2006; Kissoudis *et al.*, 2014; Suzuki *et al.*, 2014; Pandey *et al.*, 2015; Jangale *et al.*, 2018). Therefore, there is a dire need to examine responses of different plants to the combination of multiple stresses. It is naïve to expect that combined application of multiple stresses make the plant to adopt unique stress-adaptation strategies compared with those when the plant is subjected to an individual stress.

Plants generally use specific strategies to counteract an individual stress. For example, under drought stress, plants undergo acclimatization by reducing water deprivation and increasing water uptake mediated by reduced vegetative growth and enhanced root system (Prasad *et al.*, 2008; Pandey *et al.*, 2015). In contrast, under heat stress, transpiration increases to reduce the canopy temperature, which is ascribed to increased leaf surface and number (Pandey *et al.*, 2015). However, under saline stress, plants have to counteract osmotic and ionic stress by either accumulating/synthesizing compatible organic osmolytes or by compartmentalizing toxic ions such as sodium and chloride in vacuole (Kumar *et al.*, 2017). It is naive to expect that plants under the combination of different stresses employ different adaptive mechanisms.

Earlier, the response of guar has been examined to individual stresses, e.g., drought (Khanzada *et al.*, 2003; Rao & Shahid, 2011; Ali *et al.*, 2015; Alshameri *et al.*, 2017), and salinity (Khan *et al.*, 1989; Ashraf, 2002; Ashraf *et al.*, 2005; Abusuwar & Abbaker, 2009; Rasheed *et al.*, 2015; Alshameri *et al.*, 2017)., whereas little information exists in the literature on guar response to heat stress. Moreover, the effects of combined application of heat, drought, and salinity have not been previously studied. Therefore, the aim of the current research was to uncover the responses of guar to multiple stresses of heat, drought, and salinity and compare them with those to either of the individual stresses. The guar accession PWP 5595 was reported to be highly drought tolerant but moderately salt tolerant (Ali *et al*., 2015; Alshameri *et al*., 2017), whereas, the accession 24320 showed high tolerance to salinity and moderate tolerance to drought (Rasheed *et al*., 2015; Alshameri *et al*., 2017). Thus, the two accessions with varying degree of tolerance to drought and salinity were exposed simultaneously to the combination of salinity, drought and heat to uncover their responses to the three stresses applied jointly or singly.

Materials and Methods

A field trial was carried out at the College of Sciences, King Saud University, Saudi Arabia. A guar accession BWP-5595 (highly tolerant to heat and drought, but moderately tolerant to salinity), and another accession 24320 (highly tolerant to heat and salinity, and moderately tolerant to drought) were used in this study. Seed sterilization, sowing and transplanting were performed as mentioned in our previous study (Alshameri *et al*., 2017). The design of the experiment was a Randomized Complete Block Design (RCBD) with three replicates.

After thirty days of sowing, five treatments were applied to the plants for a period of three weeks. The treatments comprised heat (H; 42°C), drought (D; 40% field capacity), salinity (S; 200 mM NaCl), multiple stress (M; "heat @ 42°C + drought @ 40% field capacity + S @ 200 mM NaCl") and control (C; no stress). To simulate the environmental heat stress, a growth chamber established *in situ*. The temperature inside the chamber was recorded +10°C more than that outside the chamber, which ranged between (32-36°C) during the experimentation.

The morpho-physiological measurements made were: biomass, plant height, leaf number and area, leaf elongation, root length, stomatal conductance, water use efficiency (WUE), days to flowering and number of buds. Leaf area meter (DT Area Meter, Model MK2; Delta T Devices, Cambridge, UK) and a portable infrared gas analyzer-based photosynthesis system (LI-6400; LiCor, Inc., Lincoln, NE, USA) were used to determine leaf area and stomatal conductance, respectively.

Statistical analysis: Analysis of variance of data for each attribute was performed using a computer software, SAS 9.1, SAS Inc., North Carolina, USA (Anonymous, 2002). The significant differences among the mean values were determined using the LSD test at 5% probability.

Results

Our results show a highly significant interaction between stress treatments and accessions for all traits studied, indicating that the accession difference was significantly affected by a specific stress. Fig. 1 illustrates the influence of stress treatments on some morpho- physiological characteristics of guar accession "BWP 5595". The five stress treatments showed significant differences in biomass (p<0.0001). In the plants under heat stress and control conditions, biomass was the highest, with no significant difference between them. Multiple stresses produced the lowest biomass. Accessions also varied significantly (p<0.0001), where BWP 5595 produced the highest biomass (Fig. 2A).

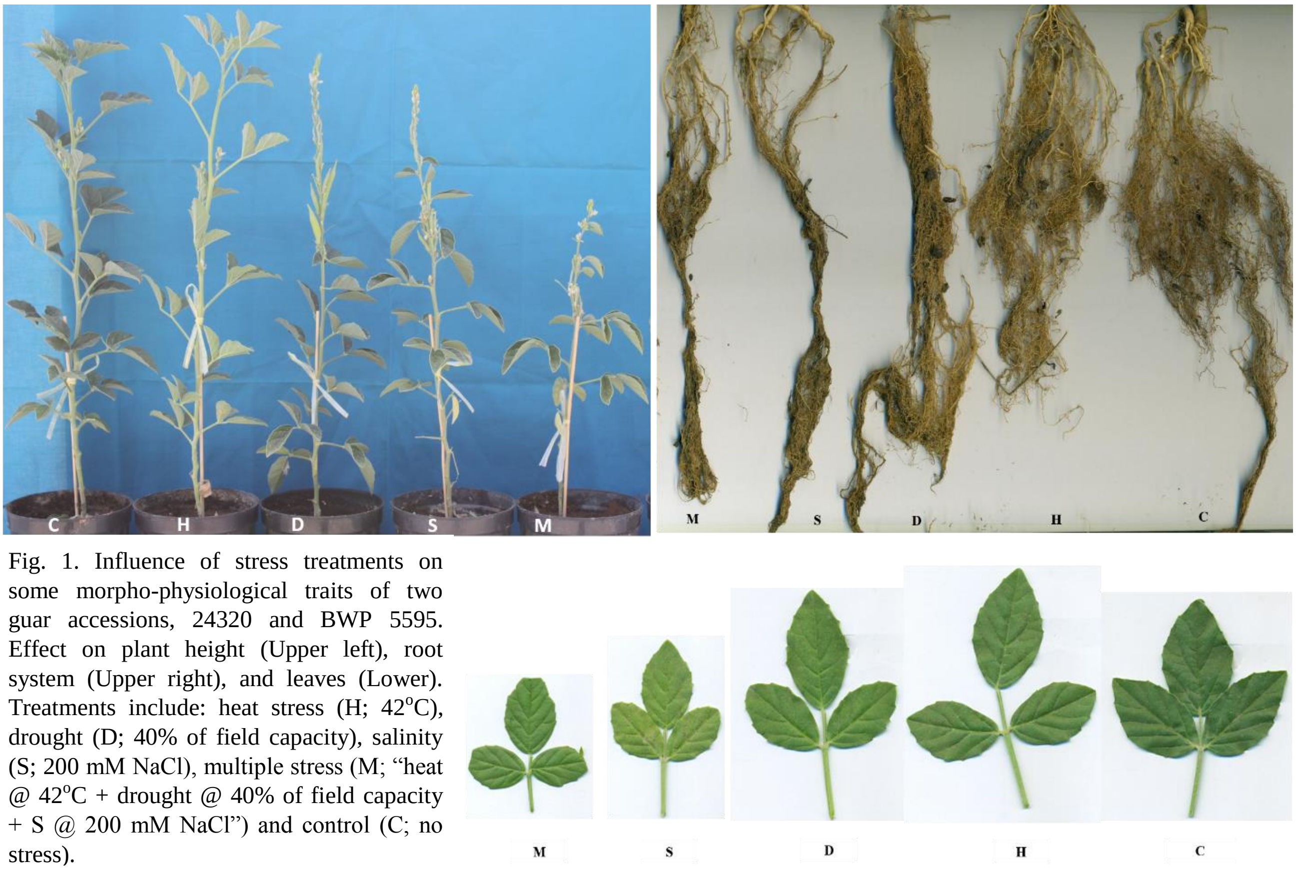

Fig. 1. Influence of stress treatments on some morpho-physiological traits of two guar accessions, 24320 and BWP 5595. Effect on plant height (Upper left), root system (Upper right), and leaves (Lower). Treatments include: heat stress (H; 42°C), drought (D; 40% of field capacity), salinity (S; 200 mM NaCl), multiple stress (M; "heat @ 42°C + drought @ 40% of field capacity + S @ 200 mM NaCl") and control (C; no stress).

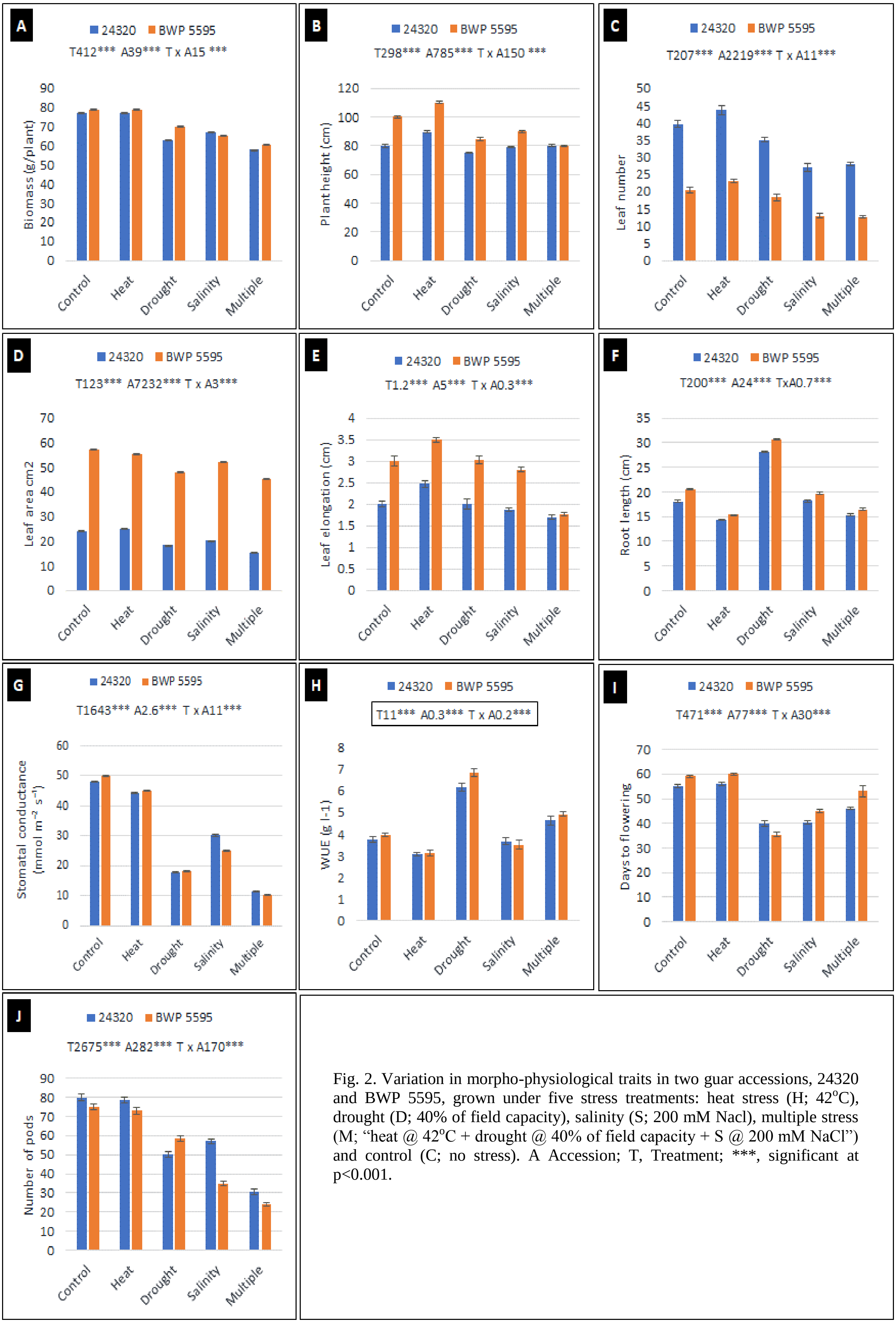

Fig. 2. Variation in morpho-physiological traits in two guar accessions, 24320 and BWP 5595, grown under five stress treatments: heat stress (H; 42°C), drought (D; 40% of field capacity), salinity (S; 200 mM Nacl), multiple stress (M; "heat @ 42°C + drought @ 40% of field capacity + S @ 200 mM NaCl") and control (C; no stress). A Accession; T, Treatment; ***, significant at $p<0.001$.

Stress treatments led to a marked reduction in plant height (*p*<0.0001). Heat stress increased plant height significantly compared with that in control, whereas drought and multiple stresses had no significant differences between them while decreasing plant height even more than that under salinity stress. Plant height differed between the two accessions significantly (*p*<0.0001); the tallest accession being BWP 5595 (Fig. 1 and Fig. 2B).

Leaf number per plant differed significantly among stress treatments. Salinity and multiple stresses decreased leaf number significantly with no significant differences between them, whereas heat stress increased leaf number compared with that in the control (Fig. 2C). Accession 24320 recorded the highest number of leaves with highly significant differences (*p*<0.0001) among the stress treatments. Stress treatments affected leaf area significantly (*p*<0.0001). Multiple stresses recorded the lowest leaf area followed by that under drought, salinity, or heat stress imposed singly (Fig. 2D). The two studied accessions also varied significantly, leaf area being highest for accession BWP 5595. Leaf elongation was markedly affected by stress treatments. Heat stress increased leaf length compared with that due to drought, which did not differ significantly from control. Multiple stresses reduced leaf length followed by salinity stress (Fig. 1 and Fig. 2E). Accession BWP 5595 had significantly higher leaf length than that of the other accession.

Root length was influenced significantly by the stress treatments. Drought stress increased root length compared with in the control, whereas heat stress decreased to a maximum extent followed by that in multiple stresses and salinity stress (Fig. 1 and Fig. 2F). Accession BWP 5595 had significantly longer root system than the other accession.

Stress treatments affected stomatal conductance significantly. Multiple stresses resulted in the lowest values of stomatal conductance, followed by those by drought, salinity, or heat (Fig. 2G). Accession 24320 had higher stomatal conductance than that of the other accession.

Water use efficiency was affected significantly by stress treatments. Drought and multiple stresses improved water use efficiency, whereas heat and salinity stresses reduced water use efficiency compared with that in the control (Fig. 2H). Accession BWP 5595 was more efficient in water use compared with the other accession.

Stress treatments resulted in significant differences in days to flowering. Heat stressed as well as control plants, resulted in prolonged days to flowering with no significant differences between them. Drought led to fewer number of days to flowering, followed by that by salinity and multiple stresses (Fig. 1 and Fig. 2I). Accession 24320 reached flowering earlier than BWP 5595.

Number of buds was affected significantly (*p*<0.0001) due to the stress effects. Multiple stresses reduced number of pods more than that by salinity, drought or heat (Fig. 2J). The two accessions also varied in number of buds; accession 24320 being higher in number of buds.

As illustrated in Fig. 3, multiple stresses of heat, drought, and salinity on guar plant showed specific responses compared those to an individual stress. For example, heat stress enhanced biomass, plant height, leaf number, leaf elongation, and prolonged days to flowering. It reduced root length and water use efficiency. However, it had a moderately negative effect on leaf area, stomatal conductance and number of pods. Contrastingly, drought stress improved root length, water use efficiency, and leaf elongation. It adversely influenced plant height, leaf area, and prolonged days to flowering. Whereas, it had moderately reduced biomass, leaf number, stomatal conductance, and number of pods. Comparatively, salinity stress had moderately negative impact on all traits studies except leaf number, which was reduced significantly. However, multiple stresses negatively affected all studied traits except water use efficiency, which was improved, and root length, which was moderately reduced.

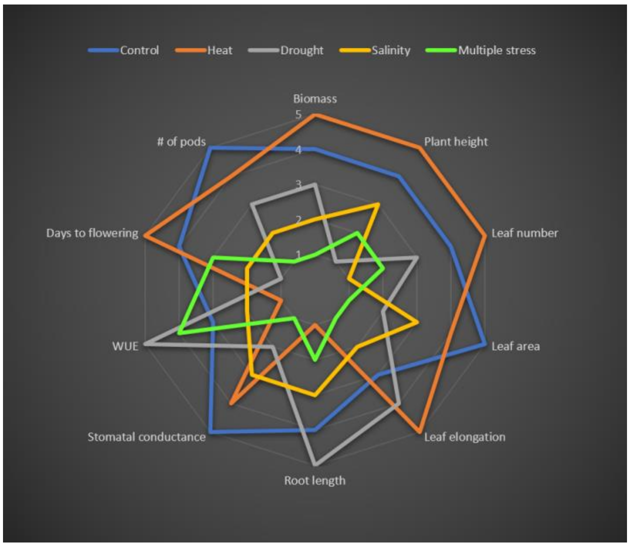

Fig. 3. Radar chart illustrates the comparative sharing, unique or cumulative responses of guar to the combined application of heat, drought, and salinity compared with either of the individual stresses. Numbers from 1-5 represent the severity of stress, where 1 is the most severe

Discussion

Plants become acclimatized to drought stress through increasing the consumption and minimizing the loss of water. On the other side, adaptive strategy of heat tolerance involves rising transpiration to reduce canopy temperature, which is achieved through increased leaf number and area (Pandey *et al*., 2015).

In our study, individual stresses such as drought and salinity, as well as multiple stresses, affected the vegetative biomass negatively, whereas heat stress improved it with no significant differences with that of the control. This might have been due to high thermal tolerance of guar because guar has been reported to germinate optimally at 30°C with optimum rooting temperature of 30–35°C (Stafford & McMichael, 1990). Our results agree with those of Ali *et al*., (2015), Rasheed *et al*., (2015), and Alshameri *et al*., (2017) in which a negative influence of drought and salinity applied individually has been reported on the biomass production of guar. Similarly, Rollins *et al*., (2013) reported a negative effect of drought stress, but no effect of heat stress on the biomass of barley (*Hordeum vulgare*).

Drought as well as multiple stresses did not show significant differences between them, and they both resulted in decreased plant height even more than that by salinity stress. In contrast, heat stress increased plant height significantly compared with the control. The results of individual stresses of drought and salinity match with the previous results of Ali *et al*., (2015), Rasheed *et al*., (2015), and Alshameri *et al*., (2017).

Leaf number, area, and length were affected variably by abiotic stresses. Salinity and multiple stresses decreased leaf number significantly with no significant differences between them, whereas heat stress increased leaf number compared with that of the control. Multiple stresses resulted in the lowest leaf area followed by drought, salinity, and heat stresses compared with the control. Heat stress increased leaf length compared with the drought, which did not differ significantly with the control. Multiple stresses reduced leaf length followed by salinity stress. Our results are analogous to those of other researchers (Ali *et al*., 2015; Rasheed *et al*., 2015 and Alshameri *et al*., (2017) who found a negative influence of drought and salinity applied singly on guar leaf number and area. Other studies on other plants also presented similar results under different stresses imposed individually. For instance, leaf number, size, and expansion in cassava (*Manihot esculenta*) were declined due to drought stress (Alves & Setter, 2004), whereas heat stress increased leaf number and elongation in maize (*Zea mays* L.) and grain-sorghum [*Sorghum bicolor* (L.) Moench.] (Bos *et al*., 2000; Prasad *et al*., 2006). In contrast to our results for guar under heat, drought and salinity as well as multiple stresses, in *Arabidopsis thaliana* only heat and drought increased leaf size and decreased leaf number (Vile *et al*., 2012).

Drought stress increased root length compared with that of the control, whereas heat and salinity stresses decreased it. Similar to our findings, Prasad *et al*., (2008) reported that heat stress reduced number, length, and diameter of roots in grain-sorghum [*Sorghum bicolor* (L.) Moench], but drought stress enhanced root growth which is necessary for water absorption from deeep layers of soil. Under multiple stresses of heat, drought and salinity, root length decreased which does not corroborate with the findings of Vile *et al*., (2012) who found that biomass allocation occurred preferably in roots under only heat and drought imposed in combination.

Multiple stresses resulted in the lowest stomatal conductance, followed by drought, salinity, and heat, respectively. These findings are partially in agreement with those of Rizhsky *et al*., (2002) who found that stomata of *Nicotiana tabacum* plant remained closed under either individual or multiple stresses of heat and drought. We observed that heat stress gave rise higher value of stomatal conductance compared with those in all other stresses. Rizhsky *et al*., (2002) also found that heat shock increased stomatal conductance even higher than that of the control in the tobacco plant.

Compared with the control, drought and multiple stresses improved water use efficiency, whereas heat and salinity stresses reduced it. Our findings are parallel to those of Alghamdi *et al*., (2015) who found that severe drought stress improved water use efficiency of *Vicia faba* genotypes.

In view of our results, drought, salinity and multiple stresses led to reduce days to flowering, whereas heat treatment resulted in prolonged days to flowering with no significant differences between them. Multiple stresses reduced the number of formed pods, followed by salinity, drought and heat. These findings partially contrast to those of Savin and Nicolas (1996), Prasad *et al*., (2006) and Pradhan *et al*., (2012) who observed that heat, drought and their combination delayed flowering of *Triticum aestivum*. Moreover, they found that grain weight and yield were reduced which is in alignment with our results.

The response of the two studied guar accessions differed significantly under different stress treatments of individual and multiple stresses (heat, drought and salinity stresses). Accession BWP 5595 showed better performance than accession 24320 in most of the traits studied under different stresses applied individually or in combination. This shows that the influence of multiple stresses significantly differs among the accessions of a particular species.

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