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ORIGINAL RESEARCH ARTICLE

Drought stress tolerance based on selection indices of resistant crops variety

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ABSTRACT

BACKGROUND AND OBJECTIVES: The stress caused by dryness can affect plant growth and physiology. Several coping mechanisms (recovery, avoidance, tolerance and drought escape) have been developed to mitigate the impact of drought stress, and most strategies involve survival during stress condition. The aim of this study was to compare the morphological and physiological characteristics of two varieties of sorghum forage (Pegah and Speedfeed) under drought stress conditions in order to provide beneficial and functional recommendations to farmers in the study area.

METHODS: This study was performed as a spit-plot plot in a complete randomised design with 3 replications for two years in Esfahan, Iran. Experimental treatments included drought stress at three levels for two varieties of sorghum. Mechanisms of sorghum response to drought stress, including physiological and morphological alterations, were also proposed. Treatment means were compared by the Duncan test at 5% and 1% levels of probability. The statistical analysis was applied to the data using the R software.

FINDINGS: Lower irrigation showed a gradual decrease in plants height, number of leaves per plant, stem diameter, nitrogen and crude protein, with an increase in the length and weight of their panicle. Compared to Pegah variety, Speedfeed cultivar with 12% increase enhanced the contents of chlorophyll (1.7 times) in the two years of experiment. It could be concluded that Speedfeed variety exhibited better yield and quality characteristics against drought stress compared to Pegah variety. Considering the tolerance index and the harmonic mean index, Pegah showed the highest sensitivity to drought stress.

CONCLUSION: This study indicated that sorghum had several adaptive mechanisms for dealing with drought stress, so that it could be applied as a suitable alternative for other crops with higher water needs such as Zea.

NUMBER OF REFERENCES	NUMBER OF FIGURES	NUMBER OF TABLES
41	4	2

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INTRODUCTION

Over 80% of Iran is located in arid and semiarid regions and has extremely dry periods with no precipitation and strong evapotranspiration (Nouri *et al.,* 2020). Moreover, in this country, precipitation does not have appropriate spatial and temporal distributions (Amiri and Eslamian, 2010). Agriculture accounts for 72 billion cubic meters (94%) of the country's total water output. Models Crops under drought stress have predicted severe droughts by the end of the 21st century, causing severe water crises and double yields (Kang *et al.,* 2019; Yu *et al.,* 2018). As a result, finding a solution to reduce water consumption and increase its productivity seems to be necessary more than ever. Drought stress is one of the most significant factors in reducing the growth, development and production of cultivated plants. This stress represents a loss of 55% of crop varieties worldwide (Bray *et al.,* 2000). Water stress is more or less affected by all aspects of the plant, including morphology, physiology and metabolism (Zhao *et al.,* 2020; Deepak *et al.,* 2019; Jabereldar *et al.,* 2017; Tariq *et al.,* 1325). Therefore, drought has become the world's most destructive strain (Zhao *et al.,* 2020; Mohammadi and Shams, 2012). Decline in irrigation, as a beneficial economic strategy mostly targeting the use of water volume unit, is known as a step towards the stability of agricultural commodities. This means that the trained plants receive less water than they need. Today, global deficiency is considered as a method for increasing the water consumption efficiency by eliminating the irrigation systems that have the least contribution to productivity or use more water (Karam *et al.,* 2007). Although, with less aggregation, the plant is slightly exposed to water stress, by adjusting the irrigation and implementing optimization steps, the unit of water volume can be used (Kirnak *et al.,* 2002). The problem is that farmers are looking for an alternative product rather than the crops that require more water. In search of the best measure of genotype selection under water stress, various selection criteria have been recommended to choose the genotypes with optimal performance under stress and non-stress conditions among other groups (Fernandez, 1992; Naghavi *et al.,* 2013; Afolabi *et al.,* 2020). Estimating the drought tolerance indices is fundamental in assessing the drought-tolerant hybrids (Hussain *et al.,* 2019). In recent years, sorghum drought resistance has been considered as an appropriate alternative for maize with higher water requirements (Smith and Friedriksen, 2008). Sorghum is one of the top five cereal types after wheat, rice, maize and Hordeum which is ranked as the fourth largest grain in the world. Sorghum provides food for millions of animals. For example, over 55% and approximately 33% of sorghum seeds are used for human consumption and livestock feed in the world, respectively (Aghaalikhani *et al.,* 2012). The quality and value of sorghum feed are similar to that of maize, but its use is significantly different. Sorghum can be used for dry matter or direct grazing, whereas maize silage is the most expendable feed type (Sarshad *et al.,* 2021). In addition, a good growth capacity of sorghum after harvest has made it economically more valuable and suitable than corn (De Oliveria Santos *et al.,* 2020). In Iran, the lack of fodder is one of the main livestock issues (Karimi *et al.,* 2018). Considering the lack of rich pastures and the pressure posed by cattle on them, evaluation of the cultivation of these plants is especially important. Regarding the compromise of sorghum, drought conditions on the one hand and dryness of large areas in Iran on the other hand, investigation of plant yield in drought stress in the country seems essential. Considering the growth of population, their need to provide the required food and poultry and the need for research on plants and drought-resistant forage, such as sorghum, have received more attention (Iqbal *et al.,* 2015). Optimizing plant water use efficiency in arid and semiarid areas is one of the most important factors in sustainable agriculture. An appropriate method for dealing with drought in the agricultural sector should be based on cultivation of drought-resistant plants instead of highly consumed plants, leading to an increase in the yield and quality of the product. Consequently, the aim of the current study is to evaluate the impacts of drought stress on different varieties of sorghum in terms of morpho-physiological traits and compare morphological and physiological characteristics of two varieties of sorghum forage (Pegah and Speedfeed) under drought stress conditions in order to provide beneficial and functional recommendations to farmers. This study has been carried out in Isfahan, Iran, within 2017 and 2018 crop seasons.

MATERIALS AND METHODS

Site description and planting

A spit-plot experiment was performed in a randomized block design with 3 replications within 2017 and 2018 crop seasons. The treatments comprised irrigation managements with 3 levels: High irrigation (HI) (100% irrigation), Medium irrigation (MI) (80% full irrigation), and Low irrigation (LI) (60% full irrigation), with sorghum varieties: Pegah (late mature) and Speedfeed (early mature). Varieties belonging to Iranian fodder sorghum cultivars, were gained from the Seed Improvement Research Centre of Isfahan, Iran. The harrowing and ploughing were performed prior to sowing in order to prepare the soil. Seeds were sown on the mound at a density of 250,000 plants/ha in early June (calculated according to prevailing plantan dates in the area) for two years. The length of each edge was set to 12 m and the separate between the edges was 60 cm in each plot. Therefore, spacing among the seedlings in the row was 60 cm. Prior to planting, the physical and chemical properties of the soil were Clay: 18%, Silt: 12%, Sand: 70%, EC: 1.23 dS/m, pH: 7.2, Organic Carbon: 0.97%, Absorbable potassium: 700 ppm, Absorbable phosphorus: 40.50 and Total nitrogen: 0.1%. Potassium sulfate and ammonium phosphate fertilizers were connected with the doses of 150 and 250 kg/ha individually. When the plants reached 40-cm tall with a water framework system, urea fertilizer was utilized as dressing. The water system was established by drip-strip and water system cycle was based on a steady cycle and water net prerequisite of plant (evaporation pan class A). Water requirement was estimated according to daily evapotranspiration values of reference tree (ETO) and crop specific coefficient (KC) of the combined Penman-Montes-FAO model (Allen *et al.,* 1998). The irrigation water quality included EC: 1.9 dS/m, pH: 7.2, HCO^3: 4.6 meq/L, Cl: 9.2 meq/L, SO_4^{-2}: 6.5 meq/L, Ca^{2+}: 6.6 meq/L, Mg^{2+}: 3 meq/L, and Na^+:10.3 meq/L. Water consumption was also measured by a calibrated meter. Water consumptions during the growing season, during 18 to 20 irrigations in 100%, 80% and 60% full irrigation treatments, were 5,038, 42,250 and 3,350 m^3/ha in 2017 and 4,400, 5,445 and 3,225, in 2018 respectively. The total rainfalls in the whole area were 14 mm and 25.2 mm for 2017 and 2018, respectively.

Laboratory analyses

Important crop traits, including plant height, stem diameter, length, leaf number, and weight of the panicle, were measured. In order to determine the dry weight, one kilogram sample was placed in a temperature of 70-75 °C for 24 hours to dry. Ash content was determined using the dry ash method when weighing an empty crucible and placing 2 g of the sample in a crucible at 550 °C in a furnace until the sample turned gray after complete heating. The crucible was placed in desiccator and allowed to cool, and then the sample was reweighed and calculated. The kjeldahl method was applied for determination of total nitrogen and crude protein (Bremner, 1982). Total chlorophyll content was estimated as sum of chlorophyll a and b and expressed as mg/g fresh weight. Drought tolerance indices were calculated using Eqs. 1 to 4 (Bonea and Urechean, 2011).

$$\text{Tolerance index (TOL)}=(Yp-Ys) \quad (1)$$

$$\text{Mean productivity (MP)}=(Ys + Yp)/2 \quad (2)$$

$$\text{Geometric mean productivity (GMP)}=(Yp*Ys)^{½} \quad (3)$$

$$\text{Harmonic mean index (HAR)}=2(Ys.Yp)/Ys+Yp \quad (4)$$

Where, $\hat{Y}s$ and $\hat{Y}p$ are the means of all genotypes under stress and well water conditions, respectively.

Statistical analysis

The analysis of variance (ANOVA) was conducted for each year to verify the statistical differences among the two sorghum varieties, various irrigation levels (3 levels) and their interaction. Moreover, correlation analyses of the studied parameters were performed by a linear regression model. Several data sets were altered for logarithm to provide the prerequisites of ANOVA for homogeneity of variance and normality. Treatment means were compared using the Duncan test at 5% and 1% levels of probability. The statistical analysis was applied to the data using the R software (version 4.3.19).

RESULTS AND DISCUSSION

According to analysis of variance, the effect of experimental years on all morphological characteristics, except for the weight of the plate at the level of 1%, was significant (Table 1). The irrigation effect on all morphological traits, except for number of leaves and plant height, was also significant. The effect of varieties on all traits, except for the length of the plate and plant height, was significant. The interaction of factors was also different in other traits.

The results of variance analysis of physiological traits indicated that the effect of experimental years on all studied characteristics was not significant at the level of 5% (Table 2). The effect of irrigation on all traits, except for chlorophyll and ash content, was important, and the effect of varieties on all traits, except for ash content, was important. The interaction of factors in most traits, except for chlorophyll content and dry weight plant, was not significant.

Results of mean comparison showed that the difference in mean plant height in the first year between the two varieties was insignificant and, in the second year, it was higher in HI than in MI and LI and only in LI it was significantly higher (Fig. 1). In general, stem diameter had the highest value in Speedfeed variety in MI compared to other treatments, while both varieties in LI showed the lowest value in both years (Fig. 1). Due to sorghum's resistance to environmental stresses, most of its vegetative and functional characteristics remained unaffected by the increase of temperature in the second year. Jabereldar *et al.* (2017) reported similar results. The dry matter, protein and minerals (ash) of Speedfeed are higher than Pegah variety. While, its soluble fibers and lignin or, in other words, digestibility is less than that of Pegah (Mokhtarpour *et al.,* 2000). Due to the lack of significance of dry matter in water stress levels with control treatment, it has been reported that sorghum can drain more moisture from the soil in low water conditions (Pardales Jr and Kono 1990). As a result, the average number of leaves of the two varieties was meaningfully higher in the first year than in the second year. No marked difference was observed in the mean length of panicle in the two varieties in different irrigation years. The weight of the panicle showed different values, with values for Pegah cultivar being less than the values observed in HI levels in both years. Finally, the mean DM in Speedfeed variety at HI level was significantly higher than Pegah variety and it was vice versa at MI (Fig. 1). Naseri *et al.* (2011) showed that shortening of the length of the panicle due to the impact of drought

Table 1: Two-way ANOVA results of morphological variables of the two varieties of sorghum at different years under different irrigation levels

Variables	df	Plant height (cm)	Leaf number	Panicle length (cm)	Panicle weight (g)	Stem diameter (mm)
Time (A)	1	4436.89 **	86.64**	29.5**	11.82ns	15**
Irrigation (B)	2	1111.84 ns	0.18ns	19.62**	192.99**	21.5**
A×B	2	145/74 ns	1.69ns	7.7*	3ns	0.1ns
Variety (C)	1	76 ns	32.2**	7.62ns	132.44*	455.5**
B×C	2	568/92*	1.4ns	0.91ns	142.87*	2.4**
A×C	1	756.43*	1.22ns	2ns	0.45ns	0.1ns
A×B×C	2	645/13*	0.09*	11 ns	117.21*	0.08ns

ns: not significant; * and **: significant at $p < 0.05$ and $p < 0.01$, respectively

Table 2: Two-way ANOVA results of variables of the two varieties of sorghum at different years under different irrigation levels

Variables	df	Chlorophyll content (mg/g)	Nitrogen (%)	Dry matter weight (g)	Crude protein (%)	Ash (%)
Time (A)	1	0.07ns	0.0003ns	0.008ns	0.01ns	1.88ns
Irrigation (B)	2	0.2ns	0.1**	25.14**	4.69**	3.73ns
A×B	2	3.7**	0.005ns	2.27 ns	0.2 ns	1.97ns
Variet y(C)	1	13.9**	0.1**	404.27**	4.89**	3.75ns
B×C	2	33.6**	0.007ns	60*	0.3ns	3ns
A×C	1	31.7**	0.003ns	1.36ns	0.13ns	1.69ns
A×B×C	2	3.6**	0.007ns	2.6ns	0.28ns	2.59ns

ns: not significant; * and **: significant at $p < 0.05$ and $p < 0.01$, respectively

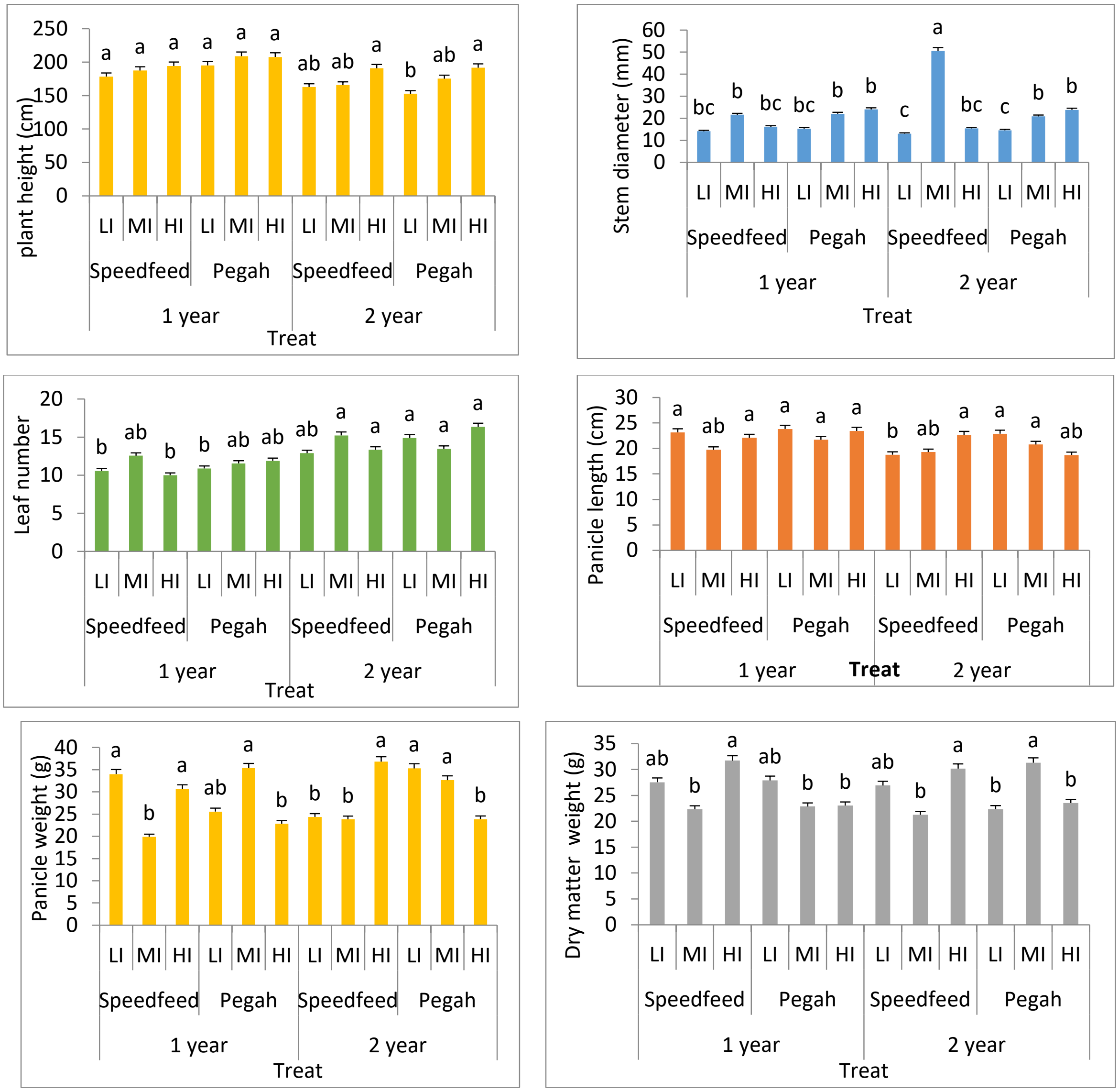

Fig. 1: Variations in morphological properties of the two varieties of sorghum at different years under different irrigation levels. Different lower-cases for interaction on the bars show significant differences (Duncan, P < 0.05). (HI): High irrigation, (MI): Medium irrigation and (LI): Low irrigation

stress on the development of the panicle was reported as contradictory.

Results showed that the amount of nitrogen was not significantly different in any treatment, but it decreased with the decrease of irrigation (Fig. 2). The amount of forage nitrogen in HI treatment had the highest amount (averagely 1.4%) and decreased with the decrease of access to water. Therefore, the lowest amount upto an average of 1.2% was related to the treatment of 60% of complete irrigation. The study of drought stress evaluation on forage quality of millet forage implied that the highest amount was related to complete irrigation of plants (Keshavarz *et al.,* 2013). Considering the reduction of nitrogen due to water stress, it can be said that one of the most detrimental effects of water stress is the disruption of nutrient uptake and accumulation, leading to reduced crop yields and animal feed, in addition to loss of

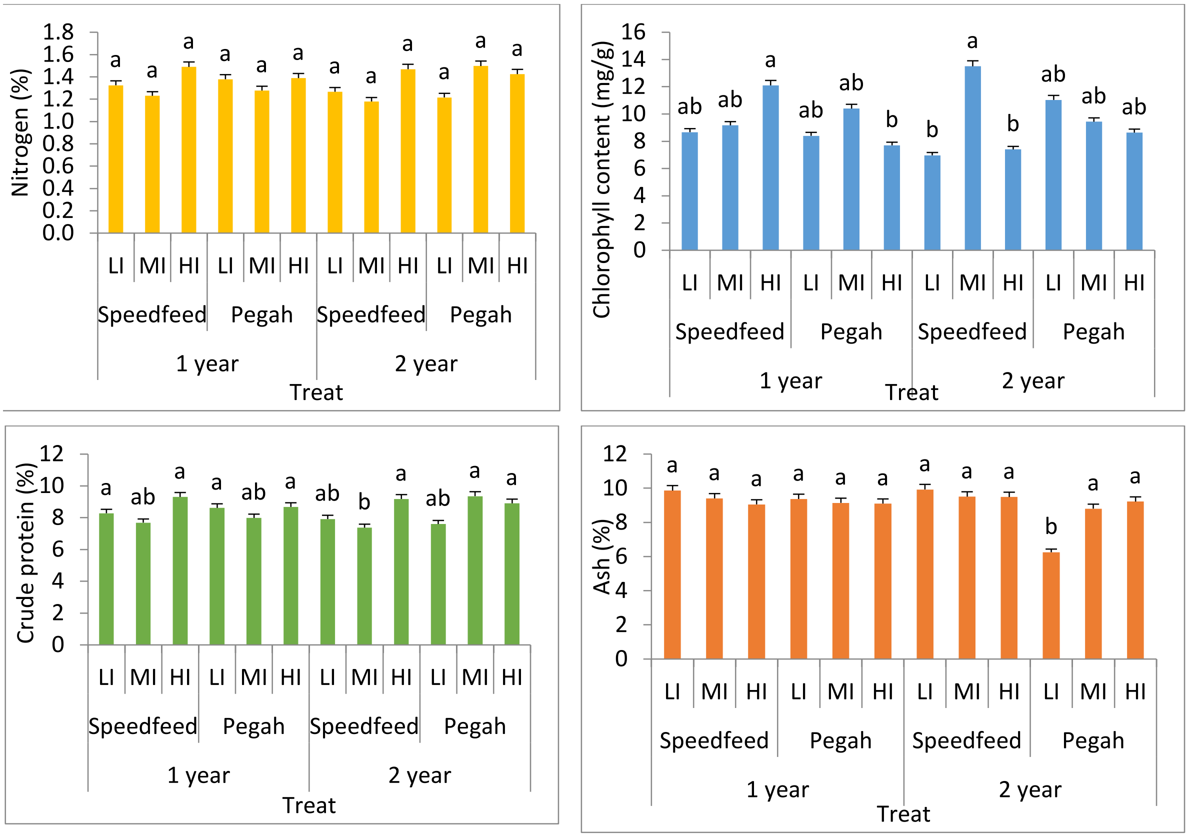

Fig. 2: Variations in chemical properties of the two varieties of sorghum under different irrigation levels in different years. Different lower-cases for interaction on the bars show significant differences (Duncan, $P < 0.05$). (HI): High irrigation, (MI): Medium irrigation and (LI): Low irrigation

fertilizer (Irannejad, 1991). Bock (1984) stated that in order to absorb nitrogen, it must move in aqueous solution to reach the roots. Therefore, providing the right amount of water is one of the most efficient ways to move nitrate to the roots through the mass flow. The amount of crude protein differences was small, so that the values were higher in HI than in LI, but the values were not significant. Compared to Pegah variety, Speedfeed cultivar with a 12% increase had a higher chlorophyll content in both years of experiment (Fig. 2). According to results, during the two years of experiment and due to the temperature difference, the amount of crude protein of sorghum in both years was nearly 8% higher in natural irrigation than in the two levels of low irrigation stress applied in the study. Barati *et al.* (2015) explored the effect of low irrigation regimes on barley and showed that stress reduced the protein concentration. The ash percentage of Pegah variety had the lowest amount in the second year compared to other treatments. Accordingly, Speedfeed variety in full irrigation produced about 19% more crude protein than Pegah variety at 60% full irrigation. The interaction between irrigation and variety demonstrated a slight difference among the irrigation regimes in terms of forage ash percentage of the two sorghum varieties. Therefore, it could be stated that the ash contents of the two varieties in the present study were not affected by irrigation treatments. In a study comparing the physiological traits of the two sorghum cultivars in response to water restriction, it was observed that there was a difference in the leaf chlorophyll content of the cultivars (Goche *et al.*, 2020). Drought stress can lead to changes in chlorophyll content and thus changes in photosynthetic efficiency (Xu *et al.*, 2020). These findings showed that the effect of water

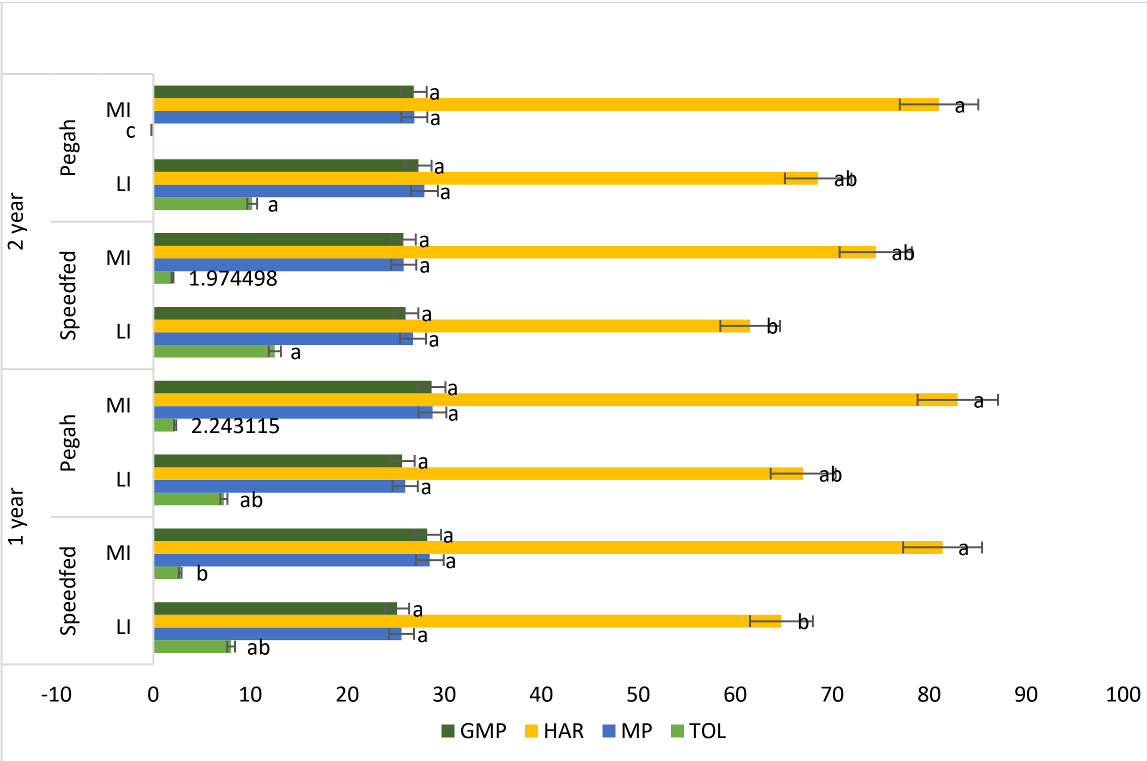

Fig. 3: Values of drought tolerance indices for maize hybrids under different irrigation levels. Different lower-cases for interaction on the bars show significant differences (Duncan, P < 0.05). Medium irrigation (MI) and Low irrigation (LI)

stress on plant chlorophyll could be very different depending on the environmental conditions and genotype of the plants (Jnandabhiram and Sailen Prasad, 2012). The superiority of the chlorophyll content of sorghum over other plants can be related to its genetic characteristics. There are many differences between plant species and even different cultivars of a species in terms of changes in a trait. Some plants or cultivars of a plant, under drought stress conditions, can prevent the reduction of leaf chlorophyll by maintaining the relative content of leaf water and photosynthetic power (Najafinejad et al., 2019). Drought stress is a key environmental factor that inhibits photosynthesis. Drought stress typically affects the stomatal conductance and photosynthetic activity in leaves, and since photosynthesis is necessary for plant materialization, drought stress reduces photosynthetic efficiency, growth retardation, and relative growth rate (Maddah and Farhangian Kashani, 2011). Sorghum can grow and produce crops in a hot, dry environment that is unappropriate for most crops. As a result, among the two varieties, Speedfeed seemed to have a higher content of cholorophyll under water deficit. The increase in chlorophyll content at 60% stress in the second year observed in the present study was in agreement with the results presented by Goche *et al.* (2020).

TOL and HAR indices showed an intensive tolerance and better performance than MP and GMP (Fig. 3). Based on TOL and HAR indices, Pegah had the highest sensitivity to drought stress. The large amount of TOL index can be a good criterion for selecting the variety which is tolerant to drought stress. Speedfeed cultivar appeared to be more responsive to water shortage in chlorophyll content and photosynthetic efficiency than Pegah cultivar. Pegah is a relatively late Iranian cultivar suitable for forage silage production because of its high soluble sugar content and is considered genetically pure. Speedfeed is one of

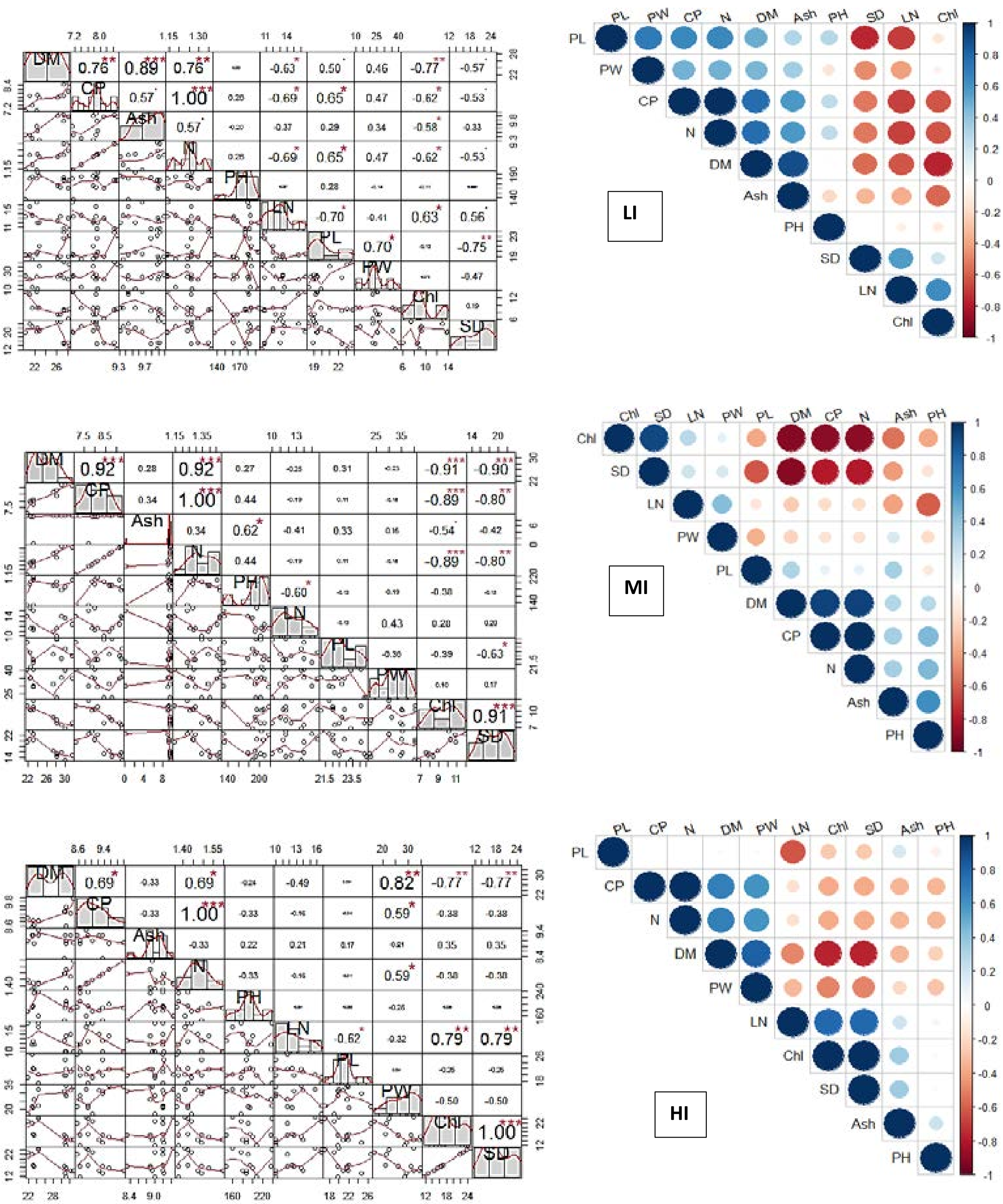

Fig. 4: Correlation plot of sorghum traits under irrigation levels; (HI): High irrigation, (MI): Medium irrigation and (LI): Low irrigation. DM: dry matter, CP: crude protein, PH: plant height, LN: leaf number, PW: panicle weight, PL: Panicle lengh, Ash: Ash content, and Chl: Total Chlorophyll.

the varieties of Australian hybrid cultivar modified by Pacific Seyed Australia. This variety is multi-grained and is suitable for direct grazing and, in terms of low sugar content, it is not suitable for producing forage silage. Compared to Pegah variety, Speedfeed hybrid variety has a shorter growth period due to its multiplicity and, except for amount of soluble sugar. It has also preference over Pegah variety in terms of some qualitative parameters.

The correlation between the studied traits at LI level showed a strong negative correlation among the amount of panicle length, stem diameter and number of leaves, between the dry weight and chlorophyll content, and between nitrogen and the number of leaves (Fig. 4). However, the amount of raw protein had a strong positive relationship with nitrogen and the amount of ash in dry weight. According to Abdi and Habibi (2017), fiber up to a low amount is needed for forage crops to have a high quality of, but its higher content can negatively affect the quality of forage. At MI level, chlorophyll content and stem diameter were strongly correlated with dry weight, crude protein, and nitrogen traits, while the dry matter content was strongly correlated with crude protein and nitrogen, and nitrogen content was strongly correlated with ash. However, at HI level, the dry weight had a strong negative correlation with chlorophyll and stem diameter, while a strong positive correlation was observed between crude protein and nitrogen and between chlorophyll and ash (Fig. 4)**.** Evaluating the impact of drought stress through the association among the traits under different stress treatments displayed that the drought stress induction had no significant effect on yield-related and morphological traits in sorghum compared to control. However, it was capable of changing the relationship among the traits, and this was verified by the correlation plot results. The results obtaioned by Cho *et al.* (2006) revealed a significant reduction in yield and biomass yield-related characteristics of sorghum under severe water shortage conditions. If the moisture content decreases, the intensity and amount of nutrient uptake would change. Since some element transfer systems, such as diffusion, require less moisture to absorb nutrients, reducing the moisture content to the critical threshold can trigger the process of absorption and transfer of some nutrients by the roots. However, other traits, including mass flow, are highly dependent on the amount of moisture, and by reducing the moisture, the elements would be transferred by the flow and the process would have a negative absorption (Taize and Zeiger, 1998; Aravind *et al.,* 2016).

CONCLUSION

The development of drought tolerant cultivars suitable for a wide range of agro-climatic conditions, particularly in arid and semiarid regions, is important to avoid the adverse effects of water stress. Forages have a key role in producing protein and energy required by livestock. In forage selection, the improvement of forage yield and quality is of particular importance and one of the main factors is the introduction of improved cultivars. The results demonstrated that the quality-related traits were implicated in stress tolerance mechanisms. These factors could enhance the nutritional quality of cereals. The difference between nutrient contents was non-significant for different irrigation levels. Besides, the relationships among the studied traits were affected by the drought stress. The lower irrigation significantly affected all the tested parameters. The decrease in fodder yield with drought application could be attributed to lower plant height, the number of leaves per plant, stem diameter, and panicle weight. The yield-related and morphological traits along with tolerance and susceptibility indices revealed that the Speedfeed variety exhibited better yield and quality characteristics against drought stress than Pegah variety. It could be concluded that Sorghum variety was compatible against drought stress. Hence, a mild water stress along with saving water resources could increase the sorghum quality. This study, among others, focused merely on the effects of drought stress occurring at particular plant growth stages. For a more comprehensive understanding of the characteristics, it is essential to perform an in-depth study covering the growth and developmental stages of the whole plant. Despite the closeness of the ecological characteristics and nutritional composition of sorghum to those of corn, the relative advantages of sorghum against drought stress have led to its significant superiority over corn. Encouraging farmers and ranchers to grow sorghum and use this fodder in production units is one of the important results of this study.

AUTHOR CONTRIBUTIONS

H. Heidari Sharifabad, the corresponding author, contributed in supervising the first author in the data analysis and interpreting the results. R. Daneshvar rad prepared the manuscript, performed the data analysis, and plotted the figures. M. Torabi and R. Azizinejad designed the field experiments. H. Salemi and M. Heidarisoltanabadi prepared the text and figures.

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CONFLICT OF INTEREST

The authors declare no potential conflict of interest regarding the publication of this work. In addition, the ethical issues including plagiarism, informed consent, misconduct, data fabrication and, or falsification, double publication and, or submission, and redundancy have been completely witnessed by the authors.

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ABBREVIATIONS

%	Percent
^{o}C	degrees centigrade
ANOVA	Analysis of variance
Ca^{2+}	Calcium
Chl	Total Chlorophyll
cm	Centimeter
CP	Crude protein
df	Degrees of freedom
dS/m	DeciSiemens/meter
DM	Dry matter
EC	Electrical conductivity
Eq.	Equatioon
ETO	Evapotranspiration
FAO	Food and Agriculture Organization
Fig.	Figure
g	Gram
GMP	Geometric mean productivity
ha	hectares
HAR	Harmonic mean
HCO^{3}	Bicarbonate
HI	High irrigation
KC	Crop specific coefficient
kg	Kilogram
LI	Low irrigation
LN	Leave number
meq/L	*Milliequivalents per liter*
Mg^{2+}	*Magnesium*
mg/g	*Milligrams* per gram
MI	Medium irrigation
mm	Millimeter
m^3	Cubic meter
MP	Mean productivity
Na^+	Sodium
ns	not significant
SO_4^{-2}	Sulphate
pH	potential of hydrogen
PL	Panicle lengh
ppm	Part per million
PW	Panicle weight
TOL	Tolerance

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