Research Article

Morphological and biochemical responses of *Sorghum bicolor* (L.) Moench under drought stress

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Abstract
Sorghum is an important forage crop, and both quantity and quality of this crop are affected by drought stress. Accordingly, in order to investigate the effect of drought stress on quantity and quality of morpho-physiological traits, a split-plot experiment was conducted based on randomized complete block design with four replicates in Isfahan, Iran, during 2017 and 2018 crop seasons. Treatments were irrigation regimes with four levels (control, preventing irrigation at pollination, seed milky, and seed doughy stages) and three varieties of sorghum (Sepideh, Kimia, and Payam). The results showed that drought stress negatively influenced morphological and yield-related traits of sorghum, while its effect was positive on some quality-related traits such as total soluble carbohydrate, crude protein, and proline contents. According to the results, drought stress based on prevention of irrigation at doughy stage (representing moderate drought stress) caused inconsiderable reduction in sorghum yield. In addition, drought stress has effect on relationships between morpho-physiological traits in sorghum. Considering morphological and yield-related traits together with susceptibility (stress susceptibility index) and tolerance (geometric mean product) indices indicated that Payam variety is more proper to be used in both drought stress and non-stress conditions. Furthermore, both Kimia and Payam varieties were shown to be suitable varieties based on quality-related traits, but because of having low NDF Payam variety might be more suitable.

Keywords Crude fiber · Crude protein · Proline · Cell wall—hemicellulose free · Correlation plot

Abbreviations

ADF	Cell wall—hemicellulose Free
DM	Dry matter
NDF	Neutral detergent fiber
SSI	Stress susceptibility index
TSC	Total soluble carbohydrate
TS	Total starch
GMP	Geometric mean productivity

1 Introduction

Environmental stresses have been negatively affected on production of different crops (particularly cereals) under worldwide. Drought is the most important environmental stress which has limited the production of crops in arid and semiarid regions [1, 2]. Sorghum (*Sorghum bicolor* L. Moench), as a forage crop, is a serious crop of cereal family highly tolerant to drought in comparison with other crops of this family [3, 4]. It is the fifth most cultivated cereal in the world after wheat, rice, maize, and barley [4], but in some countries, e.g., Sudan it could be categorized as the most economical crop [5]. Sorghum is on par with maize, in terms of feed quality and its commercial value, though

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there is a significant difference associated with their types of use. Sorghum is able to be consumed in different forms, i.e., silage, wet forage (high moisture), dried fodder, and direct grazing as pasture or after harvesting by livestock but maize is normally consumed as silage form [6]. Also, capability of being harvested as a multiple cuttings crop makes sorghum a more suitable crop in terms of economic benefit compared to maize [7].

The altered responses of different plant classes and order to environmental condition are related to their genetics backgrounds. Additionally, plants belonging to the same family such as maize and sorghum, and in a closer range, different genotypes of the same species, can have perceptible different responses to drought stress. Researchers have been examining different plants' varieties to categorize them into tolerant, semi-tolerant, and sensitive varieties in response to drought stress in order for making suggestions to farmers [8–12]. Ogbaga, Stepien (5) categorized two different varieties of sorghum as tolerant and sensitive to water shortage according to biochemical and physiological characteristics, since the study on response of different genetic backgrounds of different crops' genotypes or varieties (especially sorghum) to stressful condition is required and it needs higher attention [13, 14].

According to Ogbaga, Stepien (5), sorghum, as a highly drought-tolerant crop, has remarkably obtained slight consideration in terms of its mechanisms to cope with water shortage and its change feed quality under drought stress condition. Therefore, the aim of this study was to assess the effects of drought stress at different life stages of sorghum includes pollination, seed milky, and seed doughy stages on its morpho-physiological traits and also its forage qualities regarding different genetic backgrounds.

2 Materials and methods

2.1 Field experiment

A split-plot experiment was conducted based on randomized complete block design with four replicates in Isfahan, Iran, during 2017 and 2018 crop seasons. Treatments were irrigation regimes with four levels (control, preventing irrigation at pollination, seed milky, and seed doughy stages) and sorghum varieties (Sepideh (semi-sensitive to stress), Kimia (sensitive to stress), and Payam (resistant to stress)). The varieties are among the Iranian cultivars of grain sorghum, which were provided from the seed and plant improvement institute, Karaj, Iran. Drought stress was applied by preventing irrigation at four different stages consisting of a) control (no irrigation prevention), b) prevention irrigation in pollination stage (PIP), c) prevention irrigation in milky grain stage (PIM), and d) prevention irrigation in doughy grain stage (PID) on three common cultivated sorghum varieties in Iran, where drought stress arranged in main plot and variety in sub-plot.

After a deep tillage and disk lever, seeds were planted on the ridge with density of 167 thousand seeds per hectare in early May (based on common planting date of the region) for both years. Length of each ridge in every plot (four plots or replicates per treatment) was 7 m and distance between ridges was 60 cm; therefore, the distance of planted seeds was 10 cm. Based on soil test results (Table 1) and suggestion of agronomists in the station, ammonium phosphate fertilizer was applied at the rate of 250 kg.ha^{-1} at planting time and 100 kg. ha^{-1} urea fertilizer was applied as dressing at just before flowering stage. Based on normal irrigation in the region, every seven-day irrigation was done till the stage of irrigation treatments. One week after applying the last treatment of stop irrigation (doughy grain), soil water availability was measured. For calculating the soil water content, each plot in depth 0–30, sampling was done and inside the container (avoid evaporation) dried in an oven at 80 °C for 72 h. Soil water availability was 0.13, 0.10, 0.06, and 0.03 cm^{-3} cm^{-3} for control, PIP, PIM, and PID, respectively. The average precipitation in Field Station was 98.5 mm and 112.3 mm for 2017 and 2018, respectively.

2.2 Measurement of traits

Morphological and grains-related traits such as plant height, tiller number per plant, panicle length, panicle weight, number of grains per panicle, 1000-grain weight, grains tannin [15] and grains yield per unit area were studied after harvesting.

Plant's samples were cut in the field and instantly transfers to laboratory were forced dried in an oven at 64 °C for 74 h. The dried samples were then grounded and passed through a 2-mm sieve and biochemical traits were then assessed. Biochemical traits as compositional characteristics were contained total protein by Talei et al. method [16], dry matter, raw fiber, Neutral Detergent Fiber (NDF), Cell wall—hemicellulose Free (ADF) [17], crude ash, crude fat [18], total soluble carbohydrate (TSC) [19], total starch (TS) [20], and proline [21].

2.3 Statistical analysis

Data were collected and analysis of variance (ANOVA), mean comparison, correlation coefficient, and principal component analysis (PCA) for determination of relationship between measured traits were carried out by Agricola library in R statistical software (3.5.2). In order to measure

Table 1 Physical and chemical properties of the soil

Total Nitrogen%	Absorbable phosphorus (ppm)	Absorbable potassium (ppm)	pH	Organic carbon%	EC(dS/m)	Absorbable elements (mEq/lit)				Soil Texture		
						Copper	Zinc	Manganese	Iron	Sand (%)	Silt (%)	Clay (%)
0.1	40.5	700	7.2	0.97	1.23	0.3	1.04	1.1	0.6	70	12	18

susceptibility and tolerance of sorghum varieties, stress susceptibility index (SSI) and geometric mean productivity (GMP) were assessed according to Fischer and Maurer [22] and Fernandez [23], respectively, using the following formula:

$$\mathrm{SSI} = \frac{1 - \frac{Y_s}{Y_p}}{1 - \frac{Y_s}{\overline{Y}p}}$$

$$\mathrm{GMP} = \sqrt{\left(Y_s \times Y_p\right)},$$

where Y_s, Y_p, and $\overline{Y}p$ are grain yield under stress condition, under normal condition, and average yield across all three varieties under normal condition, respectively.

3 Results

The analysis of variance of agronomical traits (Table 2) indicated that the stages of water stress have significant effect on agronomical traits includes plant height, tiller number, panicle length, thousand-seed weight, panicle weight, grains weight per panicle, and grain yield. The analysis of variance (Table 2) indicated that, there were significant differences between cultivars in terms of plant height, tiller number, and panicle length; however, there was no significant difference in terms of traits for thousand-grain weight, panicle weight, grains weight per panicle and grain yield among cultivars. There was a significant interaction between the stress and the variety for 1000-grain weight.

Table 3 represents mean comparison for morphological and yield-related traits regarding stress levels and varieties of sorghum. Plant height, tiller number, panicle length, 1000-grain weight, panicle weight, and total grain weight were highest in control condition (no water stress). Different levels of irrigation showed no difference one another for tiller number. No difference between control treatment and PID stage was observed for plant height and panicle weight. The PID stage showed higher mean values related to morphological and yield-related traits than the two other levels of PIM and PIP stages. Lowest mean values for morphological and yield-related traits were recorded for PIP stage, though the difference between PIP and PIM stages was not significant for plant height. The PIM and PID stages also showed no significant differences related to plant height, panicle length, 1000-grain weight, and total grain weight. Among compared varieties, Kimia variety obtained highest mean values for plant height, tiller number, panicle weight, and grain weight, but lowest mean value for panicle length. Payam variety had the

Table 2 Variance analysis of some agronomical traits in sorghum varieties under irrigation regimes

S.O.V	Mean Squares							
	df	Plant height	Tiller number	Panicle length	1000-grain weight	Panicle weight	Grains weight per panicle	Grain yield
Replication	2	11.19	0.01	0.58	4.48	489.9	407.27	824037
Stress (stage)	3	30.5**	0.02*	7.65**	339.4**	7530.2**	5468.5**	71978384**
Error (a)	6	2.38	0.003	0.62	4.42	150.2	142.1	773269
Variety	2	108.3**	0.27**	132.3**	10.8ns	416.4ns	391.6ns	189605ns
S×V	6	1.77ns	0.002ns	0.70ns	15.8*	648.5ns	566.9ns	1171188ns
Error (b)	16	2.79	0.006	0.99	5.11	565.1	340.1	1449261
CV (%)	–	1.51	5.63	3.36	9.83	26.24	27.63	17.6

**, *, ns: respectively, significant at level of 1 and 5 % and no-significant

SOV; source of variance, df: degree of freedom, and CV; Coefficient of variation

Table 3 Mean comparison of drought stress and variety of sorghum for morphological grain-related traits

Factors	Treatment	Plant height (cm)	Tiller number	Panicle length (cm)	1000-grain weight (g)	Panicle weight (g)	Grain weight (g)	Grain yield (kg. ha^{-1})
Drought tress	PIP	108.56c	1.31a	28.44c	50.19c	14.38c	31.52c	3240.07c
	PIM	110.11bc	1.39a	29.44b	90.22b	22.96b	68.00b	5893.64b
	PID	111.67ab	1.39a	29.89b	106.30b	26.41a	82.59b	9188.41a
	Control	112.78a	1.41a	30.56a	115.70a	28.21a	84.89a	8990.18a
Variety	Sepideh	110.92b	1.38b	31.58a	88.08a	22.35b	64.11b	6711.26b
	Kimia	114.58a	1.53a	25.75b	97.33a	24.08a	73.31a	6961.09a
	Payam	106.83c	1.23c	31.42a	86.39a	22.53b	62.83b	6811.88ab

Mean with the same letter(s) is not significantly different using Duncan's multiple range tests ($P \leq 0.05$)

PID; Prevented Irrigation at Doughy, PIM; Prevented Irrigation in Milky, PIP; Prevented Irrigation in Pollination

lowest mean values for plant height and tiller number but Sepideh and Payam varieties had no significant difference of panicle length, panicle weight, and total grain weight. All three varieties showed statistically equal mean values for 1000-grain weight.

The highest grain yield was shared between control and PID stage, but its lowest value observed in PIP stage. Although Kimia variety obtained highest grain yield mean, its difference with Payam variety was not significant. The lowest grain yield of sorghum was recorded for Sepideh variety. However, under control condition all three varieties shared statistically equal grain yield (Fig. 1). Payam variety obtained the highest grain yield under PIP stage, but the lowest under PIM stage. The highest grain yield under PID stage was obtained by Kimia variety. Since the difference between control irrigation and PIP stage related to grain yield was the highly significant, stress susceptibility index (SSI) and geometric mean product (GMP) index were calculated based on grain yield of these two treatments (Table 4). Consequently, the highest GMP and SSI were estimated for

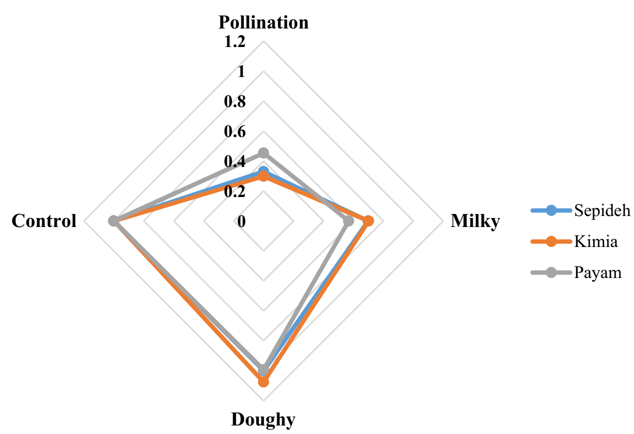


Fig. 1 Spider plot comparing yield of three sorghum varieties under different stress levels by preventing irrigation compared to control (treatment/control)

Payam variety. Although Kimia variety showed the lowest GMP and SSI, its difference with Sepideh was not significant.

Table 4 Stress susceptibility and tolerance indices of sorghum varieties

Variety	Yield (drought)	Yield (normal)	SSI	GMP
Sepideh	2922.50	8871.41	−3.74	4997.60
Kimia	2701.69	9057.92	−3.82	4926.42
Payam	4096.02	9041.20	−2.00	6068.36

Biochemical traits were statistically analyzed (Table 5), and the results of statistical variance analysis showed that the stages of water stress have significant effect on all studied biochemical traits. The results also indicated that, there were significant differences between cultivars in terms of crude protein, crude ash, NDF, and phenol contents, while other biochemical traits including proline, total starch, water-soluble carbohydrate, crude fat, ADF, crude fiber, and dry matter were not significant (Table 5). There was a significant interaction between the stress and the variety for the proline, NDF, and phenol contents.

Mean comparison of some biochemical traits related to the quality of sorghum is presented in Table 6. Highest phenol content was obtained in control and PID stage treatments, while its lowest content was recorded for PIP stage. Control treatment also showed highest mean values for crude fat and total starch contents, while the lowest for crude fiber, neutral detergent fiber, and proline content. Longest stress period (PIP stage) showed maximum content of crude fiber, neutral detergent fiber, crude ash, total soluble carbohydrate, crude protein, and proline content, but minimum for phenol content, crude fat, and total starch content. Lowest content of total soluble carbohydrate, crude protein, and proline was shared between control and PID stage treatments. Dry matter presented no statistical difference among stress levels. Related to acid detergent fiber, PIP and PIM stages shared highest, while control and PIP stage shared lowest mean values. Although PIP stage led to maximum content of crud ash, no statistical difference was found among the other three stress levels. Comparing used varieties showed that no significant difference was detectable among these three varieties regarding dry matter, crude fiber, acid detergent fiber, crude fat, total soluble carbohydrate, total starch, and proline (Table 6). However, Payam var. showed highest content of phenol, and crude ash. Crude protein mean was the highest in Kimia var. Sepideh and Kimia var. showed low but no statistically different mean for phenol and crude ash. Similarly, no difference was detectable between Sepideh and Payam var. regarding neutral detergent fiber (sharing high content) and crude protein (sharing low content).

Due to the high significant difference between PIP stage and control treatments among stress levels, evaluation of relationships related to measured traits was separately carried out for these two treatments. Figures 2 and 3 represent the correlation plots among measured traits in this study under control and PIP stage treatments, respectively. There were differences in values of the correlations between measured traits, but in some cases the sign of the correlation was also changed. Correlation of plant height with 1000-grain weight, panicle weight, grain weight, grain yield crude fiber, and total soluble carbohydrate was negative and its correlation with phenol, ADF, crude protein, and proline was positive under control irrigation, while these correlations changed their signs under water stress condition. Tiller number showed negative correlations with 1000-grain weight, panicle weight, seed weight, grain yield crude fiber, crude fat, total soluble carbohydrate and positive with phenol, ADF, crude protein, crude protein under no water stress condition but these correlation's signs were changed under stress condition. Except for the correlation of panicle length with tannin,

Table 5 Variance analysis of some biochemical traits in sorghum varieties under irrigation regimes

SOV	dF	Mean Squares Proline	Crude protein	Total starch	Total soluble carbohydrate	Crude fat	Crude Ash	ADF	NDF	Crude fiber	DM	Phenol
Replication	2	0.01	0.46	2.78	0.004	0.46	0.10	1.18	0.02	0.05	0.46	0.01
Stress	3	0.21**	40.4**	588.6**	1.21**	4.26**	1.01**	47.2**	16.3**	2.63**	0.03ns	2.14**
Error (a)	6	0.0031	0.41	46.68	0.04	0.13	0.03	1.47	0.52	0.07	0.33	0.45
Variety	2	0.006ns	1.35*	2.89ns	0.09ns	0.08ns	0.14*	0.09ns	1.37*	0.09	0.22ns	0.31**
S× V	6	0.019**	2.00ns	4.97ns	0.06ns	0.13ns	0.03ns	0.60ns	1.66**	0.06ns	0.16ns	0.17**
Error (b)	16	0.003	0.28	13.28	0.05	0.06	0.03	0.32	0.22	0.04	0.10	0.02
CV (%)	–	5.13	4.58	4.96	10.57	8.02	8.68	16.39	8	11.00	0.34	9.75

**, *, ns: respectively, significant at level of 1 and 5 % and no-significant

SOV; source of variance, df: degree of freedom, and CV; Coefficient of variation

Table 6 Mean comparison of drought stress and variety levels in sorghum for biochemical traits

Factors	Treatment	Phenol (%)	Dry matter (%)	Crude fiber (%)	NDF (%)	ADF (%)	Crude ash (%)	Crude fat (%)	Total soluble carbohydrate (%)	Total starch (%)	Crude protein (%)	Proline (%)
Drought tress	PIP	0.89c	94.43a	2.54a	7.56a	5.82a	2.43a	2.16c	2.54a	63.35c	14.86a	1.21a
	PIM	1.23b	94.36a	1.84b	6.07b	5.03a	1.71b	3.47ab	2.11b	72.05b	12b	1.02b
	PID	1.86a	94.43a	1.51c	5.26c	1.64b	1.79b	3.27b	1.78c	75.84b	10.3c	0.9bc
	Control	1.88a	94.5a	1.3d	4.4d	1.37b	1.78b	3.72a	1.76c	82.75a	10.42c	0.87c
Variety	Sepideh	1.32b	94.41a	1.75a	6.12a	3.44a	1.82b	3.24a	2.04a	73.64a	11.65b	0.98a
	Kimia	1.44b	94.31a	1.75a	5.45b	3.39a	1.94b	3.08a	1.97a	73.91a	12.28a	1.03a
	Payam	1.64a	94.57a	1.9a	5.9a	3.56a	2.03a	3.14a	2.14a	72.96a	11.76b	0.99a

Mean with the same letter(s) is not significantly different using Duncan's multiple range tests ($P \leq 0.05$)

PID; Prevented Irrigation at Doughy, PIM; Prevented Irrigation in Milky, PIP; Prevented Irrigation in Pollination, NDF; Neutral Detergent Fiber , ADF; Cell wall—hemicellulose Free

dry matter, and total soluble carbohydrate, all other correlations of panicle length were different under altered stress conditions. Correlations of 1000-grain weight with ADF, crude fat, total soluble carbohydrate, total starch, crude protein, and crude protein were also different under water stress and no stress treatments. Similarly, panicle weight changed its correlation signs with tannin, phenol, crude fiber, ADF, crude ash, total starch, and crude protein in response to water stress. Total grain weight showed correlations with different signs under control and stress condition related to tannin, crude fiber, ADF, crude ash, total starch, and crude protein traits.

Under control condition, the correlations of grain yield with dry matter, crude fiber, and crude ash were negative and its correlations with tannin, phenol, and total starch were positive, but these signs reverted under drought stress condition in sorghum varieties. Tannin showed reversed sign related to its correlations with phenol, dry matter, total starch, crude protein, and crude protein under the two different irrigation systems. Also, dry matter, NDF, total soluble carbohydrate, total starch, and crude protein showed different correlation trends with phenol under water stress and no stress conditions. Dry matter showed different signs of correlation with all remained biochemical traits except for crude fiber and NDF. Similarly, crude fiber correlations with other biochemical traits showed changed signs under different water stress conditions, but for NDF and ADF. ADF, total soluble carbohydrate, crude protein, and crude protein were reversely correlated with NDF under different water regimes. Under water stress condition, ADF showed different signs of correlations with crude ash, crude fat, and TS other than no stress condition. All remained correlation of crude ash showed a sign change in response to water stress condition. Total soluble carbohydrate with crude fiber, total soluble carbohydrate with total starch, and total soluble carbohydrate with crude protein are other correlations that reversed their signs under different water stress conditions according to the results presented in Figs. 2 and 3.

In order to more precisely consider the relationship changing between control condition (without stress) and prevention irrigation in pollination stage, principal component analysis was conducted separately for these two conditions, and the results are summarized in Fig. 4. First and second components accounted for about 64% and 66% accumulatively, under normal irrigation and water stress condition, respectively. Approximately all associations between measured traits and the angles between these traits were affected by change in irrigation regime; however, some of these associations were completely altered according to the change in their angles from acute to obtuse and vice versa. These results were somewhat in accordance with the results of correlation plots in which

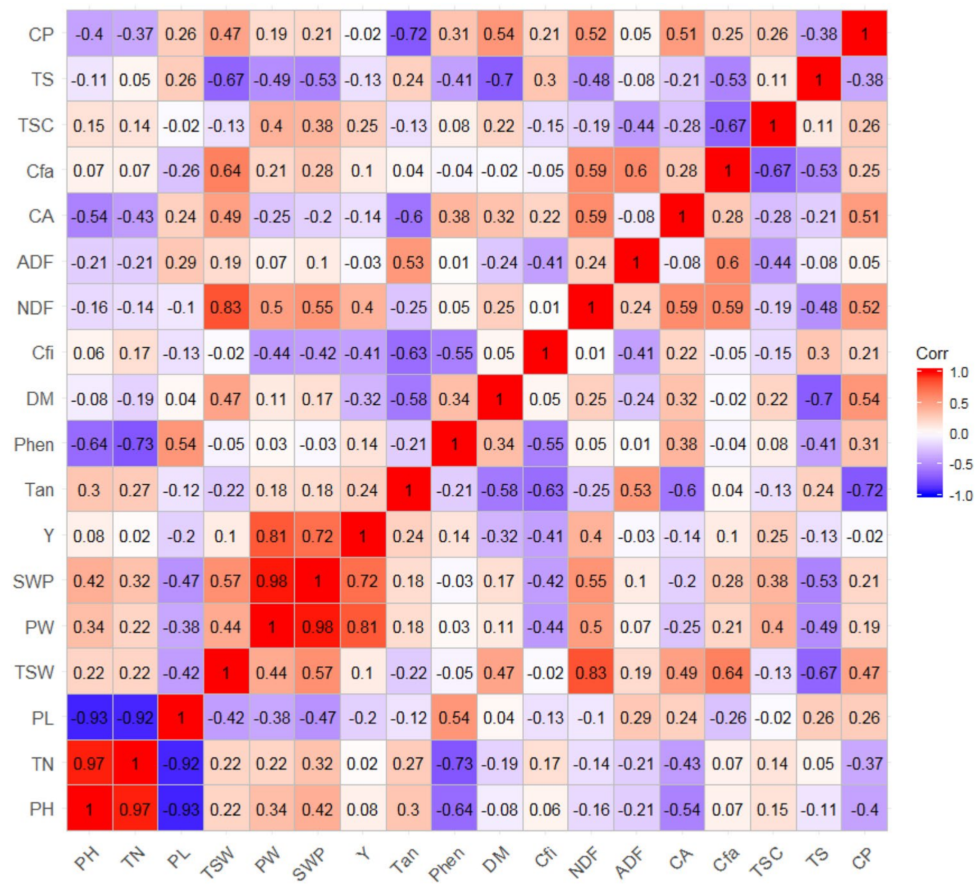


Fig. 2 Correlation plot for measuring association between measured traits of sorghum under normal condition. (PH; Plant height, TN; Tiller number, PL; Panicle length, TSW; 1000-seed weight, PW; Panicle weight, SWP; Seed weight per panicle, Y; Grain yield, Tan; Proline, Phen; Phenol, DM; dry mater, Cfi; Crude fiber NDF; Neutral Detergent Fiber, ADF; Cell wall—hemicellulose Free , CA; Crude Ash, Cfa; Crude fat, TSC; Total soluble carbohydrate, TS; Total starch, CP; Crude protein)

water stress caused changes in the relationships between measured traits of sorghum varieties.

4 Discussion

Among the phenological stages of sorghum reproductive development, the pollination stage is more sensitive to drought stress, as the transfer of pollen grains from male to female organs and contact with the eggs in the ovary requires sufficient moisture [24, 25]. So that, preventing irrigation at pollination stage of sorghum plants caused highest decrease in yield due to the lack of insemination of eggs inside the ovary. Due to the previous formation of seeds in milky stage, the reduction rate of the yield was less than the pollination stage. Decreased yield in milky level is due to a decrease in the rate of transfer of starch from the leaves and stems to the grain; however, prevention of irrigation in milky stage led to a reduction in these traits compared to control with no drought stress. The existence of a significant correlation between starch content and yield under stress conditions confirms this relationship (Fig. 4). Because a large proportion of starch is transferred from the leaf and stem to the seed before the dough stage, in most of the morphological and yield-related traits, especially grain yield [26], no statistical significant differences between stress at doughy stage and control treatment were detectable, and the damage caused by the cessation of irrigation in the dough stage was minimal.

These results showed that water deficit stress after or at ending stages of sorghum grain filling might have no significant negative impact on morphological and also yield components; therefore, farmers should be able to manage their water sources to provide more water in period of before grain filling to reach maximum productivity.

Moreover, assessing the influence of drought stress by evaluation of association between traits under different stress treatments showed that induction of drought stress, in addition to direct decrease in morphological and yield-related traits in sorghum, is able to change the relationship between these traits as well, and this issue was verified by the results of correlation plot and principal component analysis. The change in the relationship between biochemical traits and yield under stress conditions is due to plant response mechanisms. For example, shift of starch from grain to leaf for osmotic regulation through the production of proline and other amino acids can be one of these

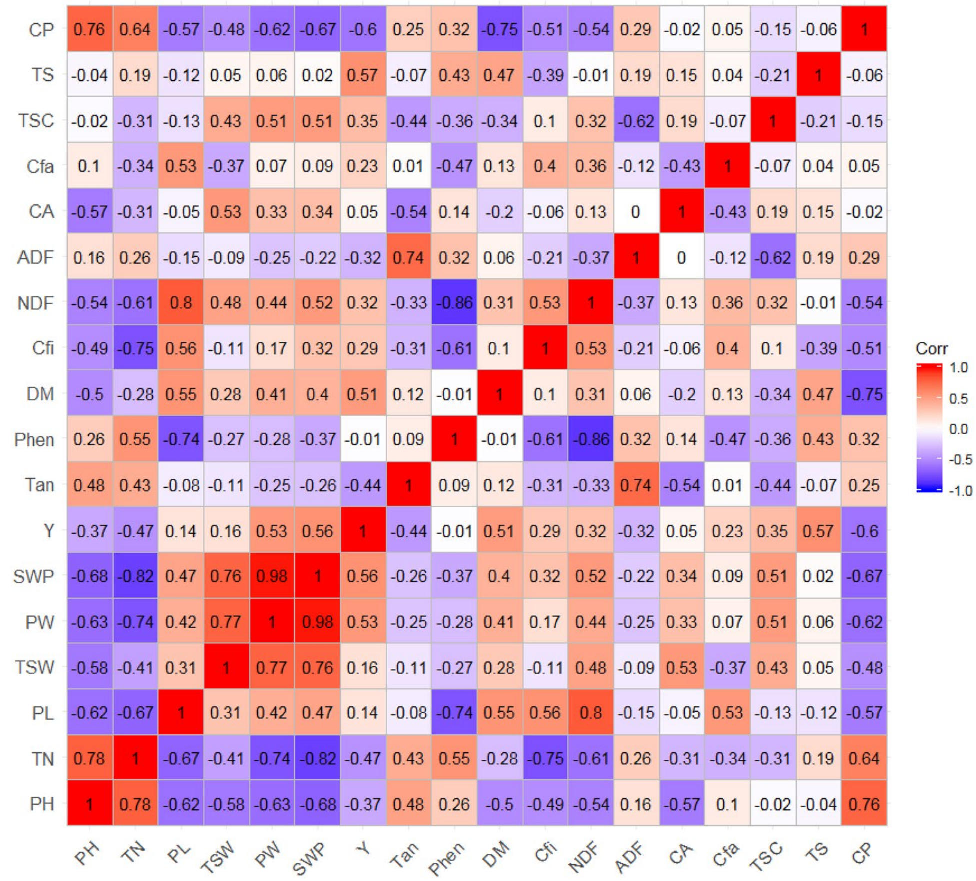


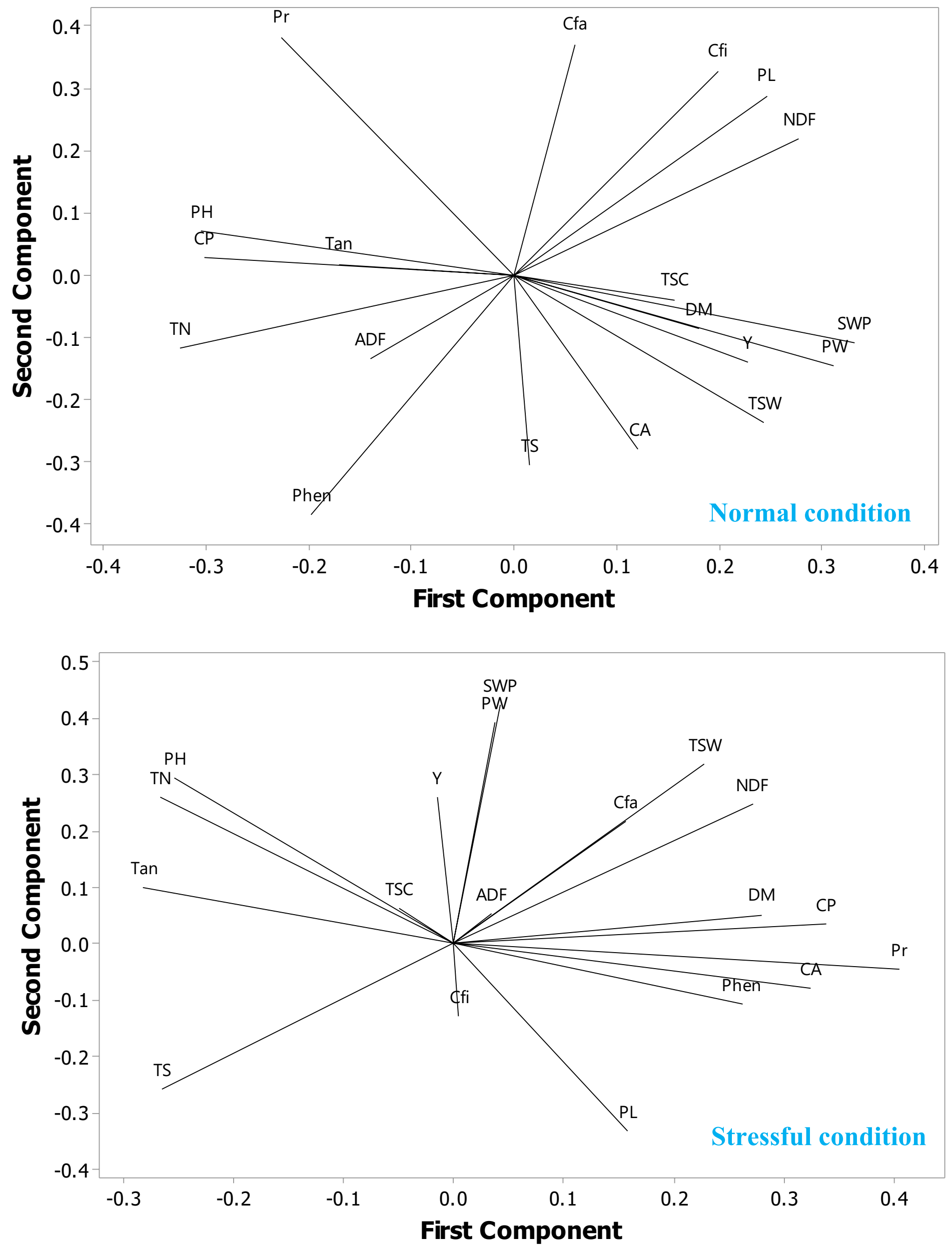
Fig. 3 Correlation plot for measuring association between measured traits of sorghum under stress condition (prevented irrigation at pollination stage). (PH; Plant height, TN; Tiller number, PL; Panicle length, TSW; 1000-seed weight, PW; Panicle weight, SWP; Seed weight per panicle, Y; Grain yield, Tan; Proline, Phen; Phenol, DM; dry mater, Cfi; Crude fiber NDF; Neutral Detergent Fiber, ADF; Cell wall—hemicellulose Free , CA; Crude Ash, Cfa; Crude fat, TSC; Total soluble carbohydrate, TS; Total starch, CP; Crude protein).

mechanisms [27]. The study by Craufurd and Peacock [28] showed that grain yield of sorghum lines (both early and late flowering) was affected by both the timing and the severity of the drought stress. Also, in consistent with our results, this study indicated that moderate drought showed no significant effect on most of growth-related traits, but they were negatively impacted by drought with longer duration (initiating at early stages of flowering). However, Cho, Toler [29] study resulted in significant decrease in yield-related traits and biomass production of sorghum under severe water deficit, but they showed that symbiosis of *Glomus intraradices* with sorghum plants was able to suppress the negative influences of drought stress up to a point. In line with our results, Symanczik, Lehmann [30] showed that drought stress in addition to decrease water uptake capability, it influences the uptake of nutrients in sorghum indicating that drought stress might cause deficiency in some nutrients as well.

Kimia variety showed highest mean values for most of the measured morphological and yield-related traits; however, low rate differences with other two varieties were observed. On the other hand, the interaction effect of drought stress and varieties showed that best variety, according to higher grain yield, under stress at pollination stage was Payam var., but Kimia and Sepideh at milky, and Kimia at doughy stage. Although a different response was obtained by inducing stress at different growth stages, the highest difference among varieties was observed at pollination stage where the highest grain yield was achieved by Payam var. Based on abovementioned results, the highest negative effect of stress on morphological and yield component traits was observed in stress at pollination stage; therefore, SSI and GMP indices were evaluated to assess the response of the varieties to drought stress. Subsequently, highest tolerance (GMP) and lowest susceptibly (highest SSI) were achieved by Payam var. According to the morphological traits and yield assessment, Payam var. could be introduced as a more suitable variety under drought stress in general. Two different hybrids, E-57 and TX-671, were compared in response to drought stress in a study carried out by Wright, Smith [31]. Their results, as in line with our results, showed that E-57 was able to preserve stomatal opening enabling it to maintain proper rate of photosynthesis leading to a significant difference between the two hybrids related to growth of both root and shoot and also plant production.

Some measured traits related to sorghum quality as a forage crop consisting of crude protein, proline, crude

Fig. 4 Loading plot extracted from principal component analysis for showing association among measured traits of sorghum. A; under normal condition, B; stressful condition by preventing irrigation at pollination stage. PH; Plant height, TN; Tiller number, PL; Panicle length, TSW; 1000-seed weight, PW; Panicle weight, SWP; Seed weight per panicle, Y; Grain yield, Tan; Proline, Phen; Phenol, DM; dry mater, Cfi; Crude fiber NDF; Neutral Detergent Fiber, ADF; Cell wall—hemicellulose Free , CA; Crude Ash, Cfa; Crude fat, TSC; Total soluble carbohydrate, TS; Total starch, CP; Crude protein).



fiber, NDF, ADF, crude ash, and total soluble carbohydrate showed their highest mean values in drought stress started at pollination stage. These traits were decreased as stress duration reduced from pollination stage to milky and doughy stages, and were the lowest in control with no drought, indicating that higher drought led to heighten the production of these biochemical traits. The accumulation of soluble sugars, protein and proline in plants under drought stress is a result of a series of metabolism interaction which has impact on formation or their transfer in the leaf [32, 33]. High thickness of these, beside a role which it has in reducing water potential, prevents from the oxidative destruction and helps in keeping the structure of proteins and membrane under average dehydration during drought period [34]. Conversely, phenol content, dry matter, crude fat, and total starch were maximum in control treatment and were decreased as the severity of drought (drought duration) increased. The results also showed that in most of the cases no significant difference was observed between control and stress initiation at doughy stage. According to Abdi and Habibi [35], fiber is required up to a low point for high quality of forage crops, while its higher content negatively influences the forage quality. Hence, PIP stage, as the most severe drought stress, is not suitable for the quality of the sorghum. However, some other traits such as crude protein and proline contents that increase forage quality were higher under drought stress conditions. Since both quality and quantity of forage are important, sorghum plants that tolerate some not-severe levels of drought stress would show both characteristics. Therefore, based on the results of the current study, inducing drought stress at doughy stage could slightly decrease yield of sorghum, which indicates the resistance of it to stress. This case could use for managing water consumption in this plant under limited water conditions. Besides, assessment of influences of severe drought stress (longer duration of stress) showed that quality-related traits influenced the relationship among these traits and also their relationships with morphological and yield component traits. Zhang and Kirkham [36] evaluated antioxidant capability of sorghum in comparison with *Helianthus annuus* and reported that sorghum has a higher antioxidant capability under drought stress condition which indicates high suitability of sorghum as forage crop according to its high antioxidant activities. Antioxidant activities resulted in elimination of reactive oxygen species and change it to low-risk material, that this the process is a kind of mechanism of stress tolerance in plants. Antioxidant activity includes enzyme activity such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POX) and ascorbate peroxidase (APX) and other enzymes, including glutathione cycle and ascorbate [37]. As well as secondary compounds and metabolites similar phenolic compounds and flavonoids also have antioxidant properties and reduce the negative effects of stress [38].

Also, their study was consistent with our result related to increase some quality-related and biochemical traits of sorghum under drought stress condition. Additionally, in agreement with the result of the current study, Nxele, Klein [39] showed that antioxidant activities of sorghum plants, representing higher quality of the forage, were higher under drought stress condition. Arivalagan and Somasundaram [40] showed that drought stress caused inhibition in overall protein production, but it led to higher proline, amino acid, glycine betaine, and total carbohydrate content compared to control condition which is in agreement with our results. On the other hand, Qadir, Bibi [41] showed that the crude protein, ADF, and NDF in sorghum plants were increased in response to drought stress condition indicating that drought has altered effect on quality of this plant as forage crop.

Comparing used varieties in the current study regarding quality-related traits showed that no difference was observable for dry matter, crude fiber, ADF, crude fat, total soluble carbohydrate, and total starch. Payam var. showed highest mean values for phenol and crude ash, while Kimia var. obtained highest contents of NDF and crude protein. Both Kimia and Payam vars. were pointed out to be proper varieties based on quality-related traits, but because of having lowed NDF Payam might be more proper. Wright, Smith [31] showed that response of the two hybrids of sorghum (E-57 and TX-671) were different related to quality-related traits such as proline and protein content which is in line with the result of the current study. In another study by Masojídek, Trivedi [42], the combination of drought stress and light stress led to different responses in three used varieties of sorghum. Two different sweet sorghum inbred lines consisting of M-81E and Roma were responded alternatively to drought stress. In the study of Guo, Tian [43] quality-related traits containing of oxido-reductive activity and photochemical efficiency of PSII were lower in Roma than in M-81E under drought stress condition. In the study of Qadir, Bibi [41], 40 sorghum lines, which were cytoplasmic male sterile, showed different contents of crude protein, ADF, NDF, and total soluble carbohydrate under both drought stress and normal condition indicating high variability and selection ability in sorghum resources to reach higher-tolerant genotypes.

5 Conclusion

Overall results showed that drought stress negatively influenced morphological and yield-related traits of sorghum, but its effect on some quality-related traits such as total soluble carbohydrate, crude protein, and proline contents was positive, in addition to being involved in stress tolerance mechanisms, these factors can increase the quality of grain nutrition. Therefore, according to the results of the current study, occurring drought stress based on prevention of irrigation at doughy stage (representing moderate drought stress) could be suitable for production of sorghum. In addition, relationships among all measured traits were influenced by drought stress. Also, considering morphological and yield-related traits along with susceptibility (stress susceptibility index) and tolerance (geometric mean product) indices indicated that Payam is more proper to be used in both drought stress and non-stress conditions. Additionally, both Kimia and Payam varieties are proper varieties based on quality-related traits, but because of having lowed NDF Payam variety might be more proper.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Ijaz M, Qamar S, Bukhari SA, Malik K (2019) Abiotic stress signaling in rice crop. Adv Rice Res Abiotic Stress Toler 65:551–69
2. Zandalinas SI, Mittler R, Balfagón D, Arbona V, Gómez-Cadenas A (2018) Plant adaptations to the combination of drought and high temperatures. Physiologia plantarum. 162(1):2–12
3. Satyavathi CT, Solanki R, Kakani R, Bharadwaj C, Singhal T, Padaria J et al (2019) Genomics assisted breeding for abiotic stress tolerance in millets Genomics Assisted Breeding of Crops for Abiotic Stress Tolerance. Springer, New York, pp 241–55
4. Reddy PS (2019) Breeding for abiotic stress resistance in sorghum. Elsevier, Breeding Sorghum for Diverse End Uses, pp 325–40
5. Ogbaga CC, Stepien P, Johnson GN (2014) Sorghum (Sorghum bicolor) varieties adopt strongly contrasting strategies in response to drought. Physiologia plantarum. 152(2):389–401
6. Galyuon IK, Gay A, Hash CT, Bidinger FR, Howarth C (2019) A comparative assessment of the performance of a stay-green sorghum (Sorghum bicolor (L) Moench) introgression line developed by marker-assisted selection and its parental lines. Afr J Biotechnol 18(26):548–63
7. Carlson R, Tugizimana F, Steenkamp PA, Dubery IA, Hassen AI, Labuschagne N (2019) Rhizobacteria-induced systemic tolerance against drought stress in Sorghum bicolor (L) Moench. Microbiol Res 93:126–38
8. Lux A, Luxová M, Hattori T, Inanaga S, Sugimoto Y (2002) Silicification in sorghum (Sorghum bicolor) cultivars with different drought tolerance. Physiologia Plantarum. 115(1):87–92
9. Hattori T, Inanaga S, Araki H, An P, Morita S, Luxová M et al (2005) Application of silicon enhanced drought tolerance in Sorghum bicolor. Physiologia Plantarum. 123(4):459–66
10. Zahedi MB, Razi H, Saed-Moucheshi A (2016) Evaluation of antioxidant enzymes, lipid peroxidation and proline content as selection criteria for grain yield under water deficit stress in barley. J Appl Biol Sci 10(1):71–8
11. Saed-Moucheshi A, Razi H, Dadkhodaie A, Ghodsi M, Dastfal M (2019) Association of biochemical traits with grain yield in triticale genotypes under normal irrigation and drought stress conditions. Aust J Crop Sci 13(2):272–89
12. Saed-Moucheshi A, Hasheminasab H, Khaledian Z, Pessarakli M (2015) Exploring morpho-physiological relationships among drought resistance related traits in wheat genotypes using multivariate techniques. J Plant Nutr 38(13):2077–95
13. Saed-Moucheshi A, Pessarakli M, Mikhak A, Ostovar P, Ahamadi-Niaz A (2017) Investigative approaches associated with plausible chemical and biochemical markers for screening wheat genotypes under salinity stress. J Plant Nutr 40(19):2768–84
14. Riasat M, Kiani S, Saed-Mouchehsi A, Pessarakli M (2019) Oxidant related biochemical traits are significant indices in triticale grain yield under drought stress condition. J Plant Nutr 42(2):111–26
15. Waniska RD, Hugo LF, Rooney LW (1992) Practical methods to determine the presence of tannins in sorghum. J Appl Poult Res 1(1):122–8
16. Talei D, Valdiani A, Puad M (2013) An effective protein extraction method for two-dimensional electrophoresis in the anticancer herb (Andrographis paniculata Nees.) Biotechnol Appl Biochem 60:521–526
17. Asp N-G, Schweizer T, Southgate D, Theander O (1992) Dietary fibre analysis Dietary Fibre—A Component of Food. Springer, New York, pp 57–10
18. Hollmann J, Themeier H, Neese U, Lindhauer MG (2013) Dietary fibre fractions in cereal foods measured by a new integrated AOAC method. Food Chem 140(3):586–9
19. Sluiter A, Hames B, Ruiz R, Scarlata C, Sluiter J, Templeton D et al (2008) Determination of structural carbohydrates and lignin in biomass. Lab Anal Proc 67:1–16
20. McCleary B, Solah V, Gibson T (1994) Quantitative measurement of total starch in cereal flours and products. J Cereal Sci 20(1):51–8
21. Ábrahám E, Hourton-Cabassa C, Erdei L, Szabados L (2010) Methods for determination of proline in plants. Springer, New York, pp 317–318
22. Fischer R, Maurer R (1978) Drought resistance in spring wheat cultivars I Grain yield responses. Aust J Agri Res 29(5):897–912
23. Fernandez GC (1992) editor Effective selection criteria for assessing plant stress tolerance. Proceeding of the International Symposium on Adaptation of Vegetables and other Food Crops in Temperature and Water Stress, Aug 13-16, Shanhua, Taiwan
24. Barnabás B, Jäger K, Fehér A (2008) The effect of drought and heat stress on reproductive processes in cereals. Plant, Cell Environ 31(1):11–38
25. Liu JX, Liao DQ, Oane R, Estenor L, Yang XE, Li ZC, Bennett J (2006) Genetic variation in the sensitivity of anther dehiscence to drought stress in rice. Field Crops Res 97(1):87–100
26. Seiler C, Harshavardhan VT, Rajesh K, Reddy PS, Strickert M, Rolletschek H, Sreenivasulu N (2011) ABA biosynthesis and degradation contributing to ABA homeostasis during barley seed development under control and terminal drought-stress conditions. J Exp Bot 62(8):2615–2632
27. Zadehbagheri M, Azarpanah A, Javanmardi S (2014) Proline metabolite transport an efficient approach in corn yield improvement as response to drought conditions. Nature 566:76–485
28. Craufurd P, Peacock J (1993) Effect of heat and drought stress on sorghum (Sorghum bicolor). II. Grain yield. Exp Agri 29(1):77–86
29. Cho K, Toler H, Lee J, Ownley B, Stutz JC, Moore JL et al (2006) Mycorrhizal symbiosis and response of sorghum plants to combined drought and salinity stresses. J Plant Physiol 163(5):517–28
30. Symanczik S, Lehmann MF, Wiemken A, Boller T, Courty P-E (2018) Effects of two contrasted arbuscular mycorrhizal fungal isolates on nutrient uptake by Sorghum bicolor under drought. Mycorrhiza. 28(8):779–85
31. Wright G, Smith R, Morgan J (1983) Differences between two grain sorghum genotypes in adaptation to drought stress III Physiological responses. Aus J Agri Res 34(6):637–51
32. Xiang Y, Huang Y, Xiong L (2007) Characterization of stress-responsive CIPK genes in rice for stress tolerance improvement. Plant Physiol. 144:1416–1428
33. Mohammadkhani N, Heidari R (2008) Drought-induced accumulation of soluble sugars and proline in two maize varieties. World Appl. Sci. J 3(3):448–453

34. Erdei L, Tari I, Csiszar J, Pecsvaradi A, Horvath F et al. (2002) Osmotic stress responses of wheat species and cultivars differing in drought tolerance: some interesting gene. Proc. 7th Hungarian Congress Plant Physiol, 46: 63–65
35. Abdi M, Habibi M. (2017) Effect of drought stress on quantitative and qualitative traits of two forage sorghum cultivars in Jiroft region. Agroecology Journal.13(3)
36. Zhang J, Kirkham M (1996) Antioxidant responses to drought in sunflower and sorghum seedlings. New Phytologist. 132(3):361–7
37. Tanou G, Molassiotis A, Diamantidis G (2009) Induction of reactive oxygen species and necrotic death-like destruction in strawberry leaves by salinity. Environ Exp Bot 65:270–281
38. Facchini PJ, Park SU (2003) Developmental and inducible accumulation of gene transcripts involved in alkaloid biosynthesis in opium poppy. Photochemistry. 64(1):177–186
39. Nxele X, Klein A, Ndimba B (2017) Drought and salinity stress alters ROS accumulation, water retention, and osmolyte content in sorghum plants. South Afr J Bot 108:261–6
40. Arivalagan M, Somasundaram R (2016) Induction of drought stress tolerance by propiconazole and salicylic acid in Sorghum bicolor is mediated by enhanced osmoregulation, compatible solutes and biochemical accumulation. J Appl Adv Res 1(2):41–52
41. Qadir M, Bibi AB, Sadaqat HA, Awan FS (2019) Physio-biochemical responses and defining selection criteria for drought tolerance in Sorghum bicolor. Maydica. 64(2):8
42. Masojídek J, Trivedi S, Halshaw L, Alexiou A, Hall DO (1991) The synergistic effect of drought and light stresses in sorghum and pearl millet. Plant Physiol 96(1):198–207
43. Guo Y, Tian S, Liu S, Wang W, Sui N (2018) Energy dissipation and antioxidant enzyme system protect photosystem II of sweet sorghum under drought stress. Photosynthetica. 56(3):861–72