Research Article

Abscisic Acid (ABA) mitigates drought stress in sunflower by enhancing water relations and osmotic adjustments

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Abstract

Drought stress significantly alters plant growth by disturbing plant water relations. Production and accumulation of compatible solutes improve plant water relations and help the plants to survive under water-limited conditions. Absciscic acid (ABA) could ameliorate the negative consequences of drought stress. Therefore, this two-year field study was conducted to infer the role ABA application (0, 5 and 10 μM) at bud initiation (BBCH-51) and flower initiation (BBCH-55) in improving plant growth under water deficiency imposed at various growth stages. Drought stress was imposed at BBCH-51 and BBCH-55 by skipping irrigation, whereas a well-watered treatment was also included for comparison. The water relations, accumulation of compatible solutes and achene yield were significantly reduced by drought stress imposed at BBCH-51 and BBCH-55. The negative impacts of drought stress were more prominent at BBCH-55 than BBCH-51. Hence, BBCH-55 proved more sensitive growth stage to drought stress than BBCH-51. The ABA application under stress-free conditions significantly reduced achene yield, with more reduction with higher ABA level. The ABA application of 5 μM either at BBCH-51 or BBCH-55 improved plant water relations, accumulation of compatible solutes and achene yield under drought stress whereas 10 μM ABA application was less effective. Therefore, it is recommended that 5 μM ABA application could ameliorate the negative impact of water deficit stress at BBCH-51 and BBCH-55 in sunflower.

Keywords: ABA; Compatible solutes; Irrigation schedule; Water shortage

Introduction

Sunflower is an edible oilseed crop having short growing season, successfully grown under a range of water-limited environments globally [1-3]. It is a high

yielding crop among edible oilseed crops; thus, could tighten up the production and consumption gap of edible oil in countries having huge edible oil import bill. Sunflower was introduced in Pakistan in

1960s as an oilseed crop and now recognized as top source of edible oil after cotton seed [4].

The supply of irrigation water is decreasing globally due to decreasing capacity and the annual inflow of water reservoirs [5]. Water availability usually remains insufficient and inconsistent in arid and semiarid climates. Therefore, low availability of irrigation water is a major limiting factor to attain the maximum yield potential of the field crops under such climates. The most of the cultivated areas in Pakistan are categorized as arid or semiarid, which face increasing shortage of freshwater availability that would decrease by 32% in 2050 [6].

Water shortage during growing season exerts severe negative impacts effects on crop production. The severity of these negative impacts varies greatly depending on the susceptibility of different growth and developmental stages. Soil water deficit restricts plant establishment, growth and development, which consequently ends with decreased crop productivity [7].

Water use efficiency is low in arid and semiarid areas due to high application and conveyance losses. Therefore, water use efficiency should be improved in these regions by appropriate agronomic measures. The timing of irrigation can significantly improve the water use efficiency in these regions [8]. Water use efficiency could be increased by using deficit or regulated deficit irrigation to achieve higher crop yield [9]. The accumulation of compatible solutes, e.g., proline, glycinebetaine and soluble sugars can enhance the capacity of field crops to acclimatize to drought stress. These solutes are highly soluble and low in molecular weight. Even higher cytosolic concentrations of these compatible solutes are not toxic for plants [10]. Compatible solutes detoxify reactive oxygen species produced under water deficiency, enhance membrane stability and protect enzymatic and protein structure, which collectively help plants to avoid cellular dehydration.

Under water deficient conditions, lower osmotic potential results from accumulation of compatible solutes which help plants in attracting water molecules into the cells and eventually cell turgor is maintained to relatively normal level [11].

The ABA is a vital phytohormone in crop plants which is involved in abiotic stress tolerance. Similarly, reduced water availability due to drought stress triggers ABA biosynthesis in plant tissue [12]. The ABA is subsequently translocate to the guard cells, where it stimulates stomatal closure and ultimately improves the water relations of the plant through adaptive physiological expressions, including lowering transpiration and increasing influx of water into the roots. The ABA plays vital role during dormancy functions like germination, root architecture modulation, vegetative growth and seed development. Thus, ABA could ameliorate the negative consequences of water deficiency [13].

Several studies have reported that ABA improves plant water relations under drought stress and increases crop yield. The improvement in yield and plant water relations of sunflower by exogenously applied ABA has also been reported. However, there are limited reports on the role of different doses of ABA applied at BBCH-51 and BBCH-55 in improving plant water relations and yield. Therefore, this study was conducted to investigate the role of different doses of ABA in improving plant water relations and accumulation of compatible solutes under drought stress. Further, determining the sunflower growth stage which better responds to exogenous application of ABA was the other aim of the study.

Materials and Methods

Description of experimental site and seed collection

This two-year field study was conducted at Agronomic Research Farm, University of Agriculture, Faisalabad, Pakistan. The study area is characterized as semiarid [14]. The soil was sandy clay loam with

64.3, 17.8 and 17.9% sand silt and clay, respectively. The pH and EC of the soil were 8.14 and 1.39 dS m⁻¹, respectively. Similarly, the total organic matter, total nitrogen, available phosphorus and available potassium were, 0.58%, 0.59%, 7.31 ppm and 191 ppm, respectively. The

meteorological data were obtained from the near weather station for the entire experimental period and presented in (Figure 1). The seeds of sunflower hybrid (Hysun-33) were obtained from Pakistan Oil Seed Development Board, Regional Office, Faisalabad.

Figure 1. Meteorological data of the experimental site during experiment period

Experimental treatments

Drought stress was imposed at BBCH-51 (bud initiation) and BBCH-55 (flower initiation) growth stages of sunflower by withholding irrigation (Table 1). A well-

watered treatment where no irrigation was skipped was also included in the study. Similarly, ABA was applied at two different doses, (i.e., 5 and 10 µM) at the same growth stages where irrigation was

skipped and no application of ABA was regarded as control. The experiment was laid out according to randomized complete block design with the factorial arrangement. Drought stress was the main factor, whereas ABA application was regarded as sub-factor. All treatments of the study had three replications and the experiment was repeated for two years.

Crop husbandry

Two cultivations, with tractor-mounted cultivator, followed by planking were applied to prepare seedbed suitable for plant growth. Seeds were planted on 3rd week, i.e., 17th and 12th February during 1st and 2nd year of the study, respectively.

Seeds were sown on ridges with the help of dibbler by keeping seeds rate was kept 8 kg ha⁻¹. Ridges-to-ridge and plant-to-plant distances were kept 75 and 25 cm, respectively, which were maintained after thinning. Nitrogenous fertilizer as urea was applied at the rate of 150 kg N ha⁻¹ in two equal splits, i.e., just before sowing and with first irrigation. Phosphatic fertilizer was added in the soil just before the sowing at the rate of 100 kg P₂O₅ ha⁻¹ as diammonium phosphate. Standard plant protection measures were applied to avoid any stress except described in the treatments.

Table 1. Details of the drought stress and exogenous ABA treatments

Treatment	Code	Description
Irrigation Schedules (IS)	IS ₁₁₁₁ (Control)	4 irrigations [1 st irrigation at BBCH-16 (5 DAS), 2 nd irrigation at BBCH-51 (45 DAS), 3 rd irrigation at BBCH-55 (67 DAS), 4 th irrigation at BBCH-71 (90 DAS)]
	IS ₁₀₁₁	2 nd irrigation skipped at BBCH-51
	IS ₁₁₀₁	3 rd irrigation skipped at BBCH-55
Absciscic acid (ABA)	ABA ₀	No application (0 µM) of ABA
	ABA ₅ BBCH-51	5 µM exogenous application of ABA at BBCH-51
	ABA ₅ BBCH-55	5 µM exogenous application of ABA at BBCH-55
	ABA ₁₀ BBCH-51	10 µM exogenous application of ABA at BBCH-51
	ABA ₁₀ BBCH-55	10 µM exogenous application of ABA at BBCH-51

DAS= days after sowing

Determination of plant water relations

Leaf water potential (Ψ_w) was determined between 8.00 to 10.00 am from two randomly selected plants in each treatment. The fully expanded youngest leaf (3rd leaf, from the top) was used for Ψ_w measurements. The Ψ_w was measured with Scholander pressure chamber as described earlier [15]. The leaves used for Ψ_w measurements were immediately transported to lab and frozen (below -20 °C) for seven days, which were latter thawed to extract cell sap using disposable syringe. Osmotic potential (Ψ_s) was directly determined from the extracted sap using an osmometer (Wescor-5500).

The difference between the values Ψ_s and Ψ_w was described at Turgor pressure (Ψ_p).

$$\Psi_p = \Psi_w - \Psi_s$$

Fresh weight (FW), turgid weight (TW) and dry weight (DW) of the collected leaf samples were measured as described earlier [16]. Relative water contents (RWC) of the leaves were calculated as below;

$$\text{RWC (\%)} = \frac{(\text{FW} - \text{DW})}{(\text{TW} - \text{DW})} \times 100$$

Compatible solutes content

Leaf samples were collected 10 days after ABA application to determine the leaf proline (Pro) contents and total soluble sugars (TSS). Similarly, Pro was calculated following [17].

Absciscic acid determination

High-performance liquid chromatography [HPLC, Model 1C, A-Shimadzu Ltd. Japan, Detector SPD-6AV (Shimadzu)] was used to determine ABA concentration following [18].

Statistical analysis

Normality and homogeneity of variance in the collected data was tested first. Shapiro-wilk normality test was used to assess the normality in the data and parameters with non-normal distribution were normalized by arcsine transformation technique. Homogeneity of variance was visually inspected by plotting the residuals before statistical analysis. The differences among the experimental years were tested by paired t test, which indicated significant differences. Therefore, the data of both years were analyzed, presented and interpreted separately. One-way analysis of variance (ANOVA) was then used to test the significance in the data for control, drought stress at BBCH-51 and drought stress at BBCH-55, separately. Least significance difference test at 5% probability was used to separate the treatment means where ANOVA indicated significant differences [19].

Results and Discussion

Leaf water and osmotic potential (-MPa)

Drought stress at BBCH-51 and BBCH-55 significantly influenced leaf water potential (Ψ_w) and osmotic potential (Ψ_s) of sunflower (Table 2). The ABA application under drought stress at BBCH-51 and BBCH-55 variably affected Ψ_w and Ψ_s . Significant reduction in Ψ_w and Ψ_s was noted with ABA application at BBCH-51 and BBCH-55 under well-watered treatment of the study. ABA application to the plants exposed to water stress at BBCH-51 and BBCH-55 significantly increased Ψ_w and Ψ_s compared to the control. The lowest Ψ_w and Ψ_s were recorded under well-watered treatment, whereas the highest Ψ_w and Ψ_s were recorded for 5 and 10 μ M ABA application at BBCH-51 and BBCH-55 (Table 2). Similar trend of Ψ_w and Ψ_s was recorded at BBCH-51 and BBCH-55 in both years of the study.

Drought stress at BBCH-51 and BBCH-55 made the Ψ_w more negative, whereas ABA application ameliorated the negative

impacts to significant extent. The Ψ_w was less negative with ABA application compared with no ABA application. The improvement in Ψ_w through ABA application served as a proxy of improved tolerance ending with moisture conservation. The ABA accumulation under water-limited environments enables the plants to partially close stomata [20, 21] which result in reduced transpiration; hence, moisture is conserved.

Drought stress either at BBCH-51 or BBCH-55 reduced Ψ_w , which resulted in a parallel decrease in Ψ_s . The accumulation of solutes in plant cells linked to osmotic adjustments was responsible for decreased Ψ_s [22, 23]. The Ψ_s became less negative with ABA application in the current study. A higher reduction in Ψ_s was noted with ABA application at BBCH-51 compared with BBCH-55. The accumulation of compatible solutes e.g., GB, Pro, and TTS helped the plants to improve Ψ_s under drought stress. The accumulation of these solutes detoxify reactive oxygen species, stabilize membranes and enzymatic structures ultimately hinder dehydration [24-26].

Leaf turgor pressure (MPa)

Turgor pressure (Ψ_p) was significantly reduced by drought stress imposed at BBCH-51 and BBCH-55, and the reduction was higher under drought stress at BBCH-55 during both years of the study. Exogenous ABA application decreased Ψ_p under well-watered conditions, whereas improved Ψ_p under drought stress imposed at BBCH-51 and BBCH-55 in each experimental year. The highest and the lowest Ψ_p under well-watered conditions was recorded with no ABA application and 10 μ M ABA application at BBCH-55, respectively (Table 2). Similarly, the highest and the lowest Ψ_p under drought stress at BBCH-51 was noted with 5 μ M ABA application at BBCH-51 and no ABA application, respectively. Likewise, the highest and the lowest Ψ_p under drought stress at BBCH-55 was noted with 5 μ M ABA application

at BBCH-55 and no ABA application, respectively (Table 2).

Decreased Ψ_s under droughty stress is regarded as major physiological adaptation for maintaining Ψ_p . However, the activities of certain enzymes which cause starch breakdown and other substances resulting in negative Ψ_s are increased by ABA application. The Ψ_p was significantly decreased by drought stress at BBCH-51

and BBCH-55. The reduced Ψ_p is directly related to decreased Ψ_w in the current study [26, 27]. The application of ABA significantly improved Ψ_p , and the improvement was higher with ABA application at BBCH-51 compared with BBCH-55. Conserved plant moisture due to stomatal closure was responsible for the improved Ψ_p [28, 29].

Table 2. Interactive effect of irrigation schedules and exogenous application of ABA on water relations of sunflower

Treatments	WP (-MPa)		OP (-MPa)		TP (MPa)		RWC (%)	
	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2
No water stress (IS_{1111})								
ABA ₀	0.586a	0.592a	1.480a	1.496a	0.894a	0.903a	86.08a	86.92a
ABA ₅ BBCH-51	0.625b	0.632 b	1.507b	1.523b	0.882ab	0.892ab	85.68a	85.95ab
ABA ₅ BBCH-55	0.655b	0.662b	1.532c	1.548c	0.877ab	0.886ab	84.93b	85.44b
ABA ₁₀ BBCH-51	0.717c	0.724 c	1.579d	1.596d	0.862bc	0.871bc	83.27c	84.32c
ABA ₁₀ BBCH-55	0.781d	0.789d	1.620e	1.637e	0.839c	0.848c	82.82c	84.14c
Water stress at BBCH-51 (IS_{1011})								
ABA ₀	1.117d	1.128d	1.784f	1.803f	0.667d	0.674d	76.90d	77.39c
ABA ₅ BBCH-51	0.815a	0.824a	1.597a	1.614a	0.781a	0.790a	79.84a	80.92a
ABA ₅ BBCH-55	0.917b	0.927b	1.668cd	1.686cd	0.751bc	0.759bc	78.11bc	77.91c
ABA ₁₀ BBCH-51	0.848 a	0.857a	1.619b	1.636b	0.771ab	0.779ab	78.69b	79.53b
ABA ₁₀ BBCH-55	0.992 c	1.002c	1.719e	1.737e	0.727c	0.735c	77.59c	77.99c
Water stress at BBCH-55 (IS_{1101})								
ABA ₀	1.327e	1.341e	1.881d	1.901d	0.554e	0.560e	73.85c	74.92d
ABA ₅ BBCH-51	1.050d	1.061b	1.682b	1.700b	0.632d	0.639d	74.20c	75.15cd
ABA ₅ BBCH-55	0.892a	0.901a	1.626a	1.643a	0.734a	0.742a	75.85a	77.02ab
ABA ₁₀ BBCH-51	0.982bc	0.993bc	1.673bc	1.691bc	0.691bc	0.699bc	74.98b	75.85cd
ABA ₁₀ BBCH-55	0.953 b	0.964b	1.655b	1.673b	0.702b	0.709b	75.15b	76.05bc
LSD 0.05	0.0337	0.0341	0.0218	0.0220	0.0241	0.0253	0.634	0.975

ABA = abscisic acid; ABA₀, control (no ABA application); AB₅BBCH-51 = 5 μ M exogenous application of ABA at BBCH-51; AB₅BBCH-55 = 5 μ M exogenous application of ABA at BBCH-55; AB₁₀BBCH-51 = 10 μ M exogenous application of ABA at BBCH-51; AB₁₀BBCH-55 = 10 μ M exogenous application of ABA at BBCH-55; IS = irrigation schedule; LSD = least significant difference; OP = osmotic potential; RWC = relative water contents; TP = turgor pressure; WP = water potential. Means with different letters in a column significantly differ at $P < 0.05$

Leaf relative water contents (%)

Leaf relative water contents (RWC) were significantly influenced by drought stress imposed at BBCH-51 and BBCH-55 during both study years. Similarly, ABA application decreased the RWC under well-

watered conditions, while improved RWC under drought stress in each year of the study. The highest and the lowest RWC under well-watered conditions were observed for no ABA application and 10 μ M

ABA application at BBCH-51 and BBCH-55, respectively (Table 2).

Similarly, the highest and the lowest RWC under drought stress imposed at BBCH-51 were recorded with 5 μ M ABA application at BBCH-51 and no application of ABA, respectively. In the same way, the highest and the lowest RWC under drought stress imposed at BBCH-55 were recorded with 5 μ M ABA application at BBCH-55 and no application of ABA, respectively (Table 2). Similar trend for all treatments was recorded during both experimental years.

The RWC were severely reduced under drought stress at BBCH-51 and BBCH-55. Earlier study has also reported that drought stress significantly reduced RWC of sunflower. The decrease in RWC was higher under drought stress at BBCH-55 compared to BBCH-51. ABA application significantly improved RWC. The increases RWC are directly linked with moisture conservation by partial and gradual stomatal closure, deeper root penetration and accumulation of dehydrin protein. Similar findings have been reported in various earlier studies conducted on different plant species [29, 30]. Some contrasting reports also exist where ABA had no effect on RWC of maize plants subjected to drought stress [31].

Compatible solutes

Drought stress at BBCH-51 and BBCH-55 significantly altered the accumulation of compatible solutes, i.e., glycinebetaine (GB), leaf proline (Pro) and total soluble sugars (TSS) during both years of the study. Similarly, the accumulation of compatibles solutes was also influenced by different doses of ABA application at BBCH-51 and BBCH-55 (Table 3) during each study year. Overall, drought stress increased the accumulation of GB. The accumulation of GB was increased with ABA application under stress-free conditions, whereas decreased under drought stress. The highest and the lowest GB accumulation under stress-free conditions was recorded for 10 μ M ABA application at BBCH-51 and no ABA application, respectively. When drought stress was imposed at BBCH-51 growth stage, the highest accumulation of GB was noted with no ABA application,

whereas the lowest was recorded with 5 μ M ABA application at BBCH-51. Likewise, the highest and the lowest GB accumulation under drought stress at BBCH-55 growth stage was recorded for no ABA application and 5 μ M ABA application at BBCH-55 (Table 3).

Like GB, the accumulation of Pro and TSS was increased under drought stress imposed at BBCH-51 and BBCH-55. The ABA application under stress-free conditions increased Pro and TSS accumulation, whereas decreased under drought stress. The highest and the lowest Pro and TSS accumulation under stress-free conditions was recorded for 10 μ M ABA application at BBCH-51 and no ABA application, respectively. Similarly, the highest Pro and TSS accumulation under drought at BBCH-51 growth stage was noted with no ABA application, whereas the lowest was recorded with 5 μ M ABA application at BBCH-51. Likewise, the highest and the lowest Pro and TSS accumulation under drought stress at BBCH-55 was recorded for no ABA application and 5 μ M ABA application at BBCH-55 (Table 3). Similar trend was recorded for both study years.

The endogenous ABA accumulation was increased under drought stress. Exogenous ABA application increased endogenous ABA accumulation under stress-free conditions as well as under drought stress. The highest and the lowest accumulation of endogenous ABA under drought stress imposed at BBCH-51 was noted with 10 μ M ABA application at BBCH-51 and no ABA application, respectively. Likewise, the highest and the lowest endogenous ABA accumulation under drought stress at BBCH-55 was recorded for 10 μ M ABA application at BBCH-55 and no ABA application, respectively (Table 3). Similar trend was recorded for both study years.

The accumulation of compatible solutes such as GB, Pro and TSS are considered as the most important mechanism of drought tolerance [30]. Drought stress resulted in significant production of GB in current study and higher GB was accumulated under drought stress at BBCH-55. The GB helps the plants to maintain Ψ_p under adverse

environmental conditions, particularly drought stress [32, 33]. Moreover, GB protects enzymes, functional proteins, lipids and sustained electron flow through thylakoid membrane [34, 35] all of which collectively result in improved drought tolerance. The higher accumulation of GB in plants is considered as a sign of high drought tolerance ability. The ABA application improved GB accumulation which helped the plants to cope drought stress through improved moisture availability. Partial stomatal closure and deeper root proliferation are responsible for improved moisture [36, 37].

The TSS play a significant role in osmoregulation under drought stress. The accumulation of TSS was increased under drought stress and higher amounts of TSS were accumulated under drought stress at BBCH-55 compared to BBCH-51. The breakdown of polysaccharides was responsible for higher TSS accumulation, which ultimately helped in osmotic adjustment [38, 39].

Drought stress significantly increased Pro accumulation with higher amount accumulated under drought stress at BBCH-51. The Pro biosynthesis increases when Ψ_w is low. As a result of which reduced Pro oxidation occurs in mitochondria which increases leaf Pro concentration [40-42]. The ABA application lowered Pro accumulation compared with no ABA application which can be linked with the exogenous ABA application [41]. Stomatal closure and conserved moisture due to ABA application might also be responsible for reduced Pro accumulation [43].

The endogenous ABA contents were increased under drought stress and higher increase was noted under drought stress at BBCH-55. The ABA-mediated stomatal regulation was responsible for improved endogenous ABA contents [44-47]. The ABA application did not affect endogenous ABA contents under stress-free conditions [48] has reported similar findings earlier, and highlighted that leaf ABA content may remained half of the applied ABA, however, leaf size and volume of sap extracted for ABA concentration analysis potentially have

significant influence. The application of ABA at higher doses results in higher endogenous ABA contents which improved metabolism [49] and decreased endogenous ABA concentration [50].

Achene yield (kg ha⁻¹)

Achene yield was significantly reduced by drought stress, and reduction was severe when drought was imposed at BBCH-55. The exogenous application of ABA decreased the achene yield under well-watered conditions compared to no application of ABA. However, achene yield was improved under drought stress at BBCH-51 and BBCH-55 with ABA application compared with no ABA application (Table 3). The highest and the lowest achene yield under stress-free conditions was recorded for no ABA application and 5 μ M ABA application at BBCH-55, respectively. When drought stress was imposed at BBCH-51, the highest achene yield was noted with 5 μ M ABA application at BBCH-51, whereas the lowest was recorded with 10 μ M ABA application at BBCH-55. Likewise, the highest and the lowest achene yield was recorded with 5 μ M ABA application at BBCH-55, whereas the lowest was recorded with 10 μ M ABA application at BBCH-51 (Table 3).

Drought stress significantly hampered the achene yield; however, ABA application helped to improve achene yield to significant extent. The reduction in achene yield was higher under drought stress at BBCH-55 compared to BBCH-51. The reduction in achene yield can be directly linked with disturbed plant water relations and growth. The ABA-mediated yield improvement can be explained with improved plant water relations and osmotic adjustments in the current study. The higher reduction in achene yield at BBCH-55 might be linked with pollen abortion as flowers were initiating at this stage. The application of 5 μ M ABA either at BBCH-51 or BBCH-55 improved the achene yield compared with no application. However, higher dose, i.e., 10 μ M lowered achene yield compared to control indicating that it might be toxic for plants.

Table 3. Interactive effects of irrigation schedules and exogenous application of ABA on compatible solutes, ABA contents in leaf and achene yield of sunflower

Treatments	GB ($\mu\text{mol g}^{-1}$ d. wt.)		Pro ($\mu\text{mol g}^{-1}$ f. wt.)		TSS (mg g^{-1} d. wt.)		ABA ($\mu\text{mol g}^{-1}$ f. wt.)		Achene yield (kg ha^{-1})	
	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2
No water stress (IS_{1111})										
ABA ₀	12.03c	9.23d	4.14d	4.08d	72.33e	70.22e	0.13a	0.12b	2809.40a	3015.18a
ABA ₅ BBCH-51	12.56b	10.29b	4.72b	4.66b	79.39b	78.26b	0.14a	0.13ab	2717.45b	2888.53b
ABA ₅ BBCH-55	11.69c	9.45d	4.47c	4.40c	75.14d	73.64d	0.14a	0.13ab	2645.85bc	2724.59cd
ABA ₁₀ BBCH-51	12.95a	10.7a	4.92a	4.83a	82.49a	80.52a	0.14a	0.14a	2589.15c	2777.74c
ABA ₁₀ BBCH-55	11.88c	9.8c	4.64bc	4.58bc	77.29c	75.55c	0.15a	0.14a	2479.64d	2640.76d
Water stress at BBCH-51 (IS_{1011})										
ABA ₀	17.81a	15.36a	7.08a	7.00a	90.11ab	89.22a	0.73e	0.67e	1942.23c	2094.71c
ABA ₅ BBCH-51	14.74f	12.68e	5.46e	5.39e	84.16e	83.75d	1.54b	1.41b	2451.94a	2630.92a
ABA ₅ BBCH-55	15.26e	13.16d	5.98d	5.90d	86.82d	85.56c	0.87d	0.79d	1837.91d	1983.36d
ABA ₁₀ BBCH-51	15.88d	13.95c	6.45c	6.35c	87.91c	86.13c	1.82a	1.67a	2205.19b	2357.42b
ABA ₁₀ BBCH-55	16.75bc	14.66b	6.73b	6.63b	89.12b	88.31b	0.94c	0.86c	1536.79e	1665.28e
Water stress at BBCH-55 (IS_{1101})										
ABA ₀	18.24a	16.64a	6.93a	6.85a	93.21a	92.21a	0.78e	0.72e	1860.72c	1906.39c
ABA ₅ BBCH-51	17.50b	15.95b	6.01c	5.89cd	91.88bc	90.19b	1.35d	1.24d	1763.59d	1785.51d
ABA ₅ BBCH-55	16.59d	14.76d	5.25e	5.16f	87.92e	87.17d	1.98b	1.82b	2448.79a	2518.82a
ABA ₁₀ BBCH-51	17.85b	16.07b	6.59b	6.49b	92.66ab	91.78a	1.77c	1.62c	1394.14e	1487.21e
ABA ₁₀ BBCH-55	16.98c	15.16c	5.80d	5.72e	90.95cd	89.15c	2.48a	2.28a	2044.45b	2068.63b
LSD 0.05	0.392	0.347	0.174	0.177	1.072	0.825	0.023	0.016	91.856	95.572

ABA = abscisic acid; ABA₀, control (no ABA application); AB₅BBCH-51 = 5 μM exogenous application of ABA at BBCH-51; AB₅BBCH-55 = 5 μM exogenous application of ABA at BBCH-55; AB₁₀BBCH-51 = 10 μM exogenous application of ABA at BBCH-51; AB₁₀BBCH-55 = 10 μM exogenous application of ABA at BBCH-55; IS = irrigation schedule; LSD = least significant difference; GB = glycinebetaine; ns = non-significant; Pro = proline; TSS = total soluble sugars. Means with different letters in a column significantly differ at $P < 0.05$

Conclusion

Sunflower water relations, accumulation of compatible solutes and achene yield were significantly reduced by drought stress imposed at BBCH-51 and BBCH-55. The negative impacts of drought stress were more prominent at BBCH-55 than BBCH-51. Hence, BBCH-55 proved more sensitive growth stage to drought stress than BBCH-51. The ABA application under stress-free conditions significantly reduced achene yield, with more reduction with higher ABA level. The ABA application of 5 μ M either at BBCH-51 or BBCH-55 improved plant water relations, accumulation of compatible solutes and achene yield under drought stress whereas 10 μ M ABA application was less effective. Therefore, it is recommended that 5 μ M ABA application could ameliorate the adverse impacts of drought stress at BBCH-51 and BBCH-55.

Authors' contributions

Conceived and designed the experiments: S Hussain, S Farooq & MA Bashir, Performed the experiments: S Hussain & M Jan, Analyzed the data: S Atta & MM Maqbool, Contributed reagents/ materials/ analysis tools: A Husain & MN Mushtaq, Wrote the paper: S Hussain & M Jan.

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