Journal of Plant Process and Function, Vol. 13, No. 61, Year 2024 **page: 95-106**
https://doi.org/10.22034/13.61.95

Research Article

Enhancing the safflower performance and quality by brassinosteroids and salicylic acid foliar application under water stress conditions

Mehdi Naghizadeh[1*] and Kobra Maghsoudi[2]

[1] Department of Plant Productions, Agricultural Faculty of Bardsir, Shahid Bahonar University of Kerman, Kerman, Iran
[2] Graduate University of Advanced Technology, Kerman, Iran
(Received: 2023/06/14-Accepted: 2023/11/07)

Abstract

World-over, under biotic stress conditions, plant growth regulators are used to increase the growth and production of crops. To evaluate the effect of salicylic acid (SA; 0 and 1 mM) and Brassinosteroids (BRs; 0, 0.75 and 1 µM) foliar application on seed and oil yield and physiological and biochemical responses of safflower under water deficit (100 and 50% F.C., I100, and I50), an experiment was carried out in a factorial on a randomized complete block design with four replicates in the research greenhouse of Agriculture Faculty, Shahid Bahonar University of Kerman during 2023. Grain yield per plant and its components, as well as seed oil content and oil yield of safflower, were reduced under I50 treatment. Moreover, I50 stress, increased the concentrations of malondialdehyde (MDA), hydrogen peroxide (H_2O_2), and electrolyte leakage (EL) as well as osmolyte accumulation (soluble sugars and proline) and anti-oxidant enzymes activity of safflower leaves. In addition, SA and BRs application significantly increased the anti-oxidant enzyme activity and the osmolyte contents and, in contrast, decreased the concentrations of MDA and H_2O_2 as well as EL, however, the positive effect of SA on these parameters was highest when applied simultaneously with BRs. Also, SA, and BRs applied increased the seed oil content and oil yield of safflower, but the effect of BRs and SA together was greater than that of SA or BRs applied separately. Overall, water-stress alleviation and yield improvement in safflower by BRs and SA application was attributable to partly improved osmotic adjustment (accumulation of osmolytes), cell membrane stability and antioxidant activity under stress conditions. Foliar applications of SA and BRs had great potential in improving growth and seed and oil yield of safflower under water stress conditions.

Keywords: Cell membrane stability, Plant growth regulators, Oil yield

Introduction

Safflower (*Carthamus tinctorius* L.), even though it is known as one of the eldest and most multipurpose crops that have been traditionally grown aimed at coloration, stuffing foods and making red and yellow dyes, but since a century ago, safflower has been increasingly grown mainly due to the oil content of its seeds (Bella *et al.*, 2019; Ebrahimian *et al.*, 2019). In terms of seed oil quality, safflower as an oilseed crop contains 30–40% oil as well as 15–20% protein (Beyyavas *et al.*, 2011; Bella *et al.*, 2019). The oil of safflower includes linoleic and oleic acids (90% of the total fatty acid content) as unsaturated fatty acids, and the remaining 10% corresponds to saturated fatty acids (Zandalinas *et al.*, 2016). The seeds are also a rich source of minerals (Zn, Cu, Mn and Fe), vitamins (thiamine and β-carotene) and tocopherols α, β and γ (Ozturk *et al.*, 2008). Safflower is considered a drought-tolerant crop that is grown in arid and semi-arid areas of the world (Majidi *et al.*, 2011; Bella *et al.*, 2019). Due to their high tolerance to water shortages and droughts, safflower could be taken into consideration as an alternative crop in semi-arid ecosystems (Kar *et al.*, 2007; Ebrahimian *et al.*, 2019).

Beyyavas *et al.* (2011), Ozturk *et al.* (2008), and Bella *et al.* (2019) reported that drought stress is reported to cause reduced growth and seed yield in safflower. According to the research findings, vegetative, flowering, and seed filling stages are affected by drought, so that safflower yield and oil content were substantially decreased (Eslam *et al.*, 2010; Zandalinas *et al.*, 2016; Oguz *et al.*, 2022). Drought stress resulted in damaged machinery of the photosynthetic system, devaluation of the photosynthetic rate, impairment in the partitioning of assimilating, and ultimately reduction of yield (Ullah *et al.*, 2018; Saikia *et al.*, 2018; Ebrahimian *et al.*, 2019; Billah *et al.*, 2021).

World-over, under biotic or abiotic stress conditions, plant growth regulators are used to increase the growth and production of crops (Diaz-Vivancos *et al.*, 2017;

*Corresponding Author, Email: naghizadeh@uk.ac.ir

Faize and Faize, 2018). Of several PGRs, salicylic acid (SA) 1 mM has a very important effect in defense mechanisms against water stress in safflower (Shaki *et al.,* 2018) and wheat (Maghsoudi *et al.,* 2019b). Application of SA improves uptake of nutrients, anti-oxidant enzyme activities, regulation of stomatal, transpiration and photosynthetic rate as well as accumulation of osmolytes (such as proline, proteins, and carbohydrates), and as a consequence, the tolerance of crop increases to water stress (Sorahinobar *et al.,* 2016; Diaz-Vivancos *et al.,* 2017; Faize and Faize, 2018; Shaki *et al.,* 2018; Bano-Otalora *et al*., 2020). Brassinosteroids (BRs) a new type of polyhydroxy steroidal phytohormones have a considerable impact on the crops growth and production (Hasan *et al.,* 2011; Vardhini and Anjum, 2015). Furthermore, multiple reports show that in crops, an association exists among the BRs application and increased tolerance to abiotic stresses (Janeczko *et al.,* 2011; Zhiponova *et al.,* 2013; Wang *et al.,* 2014; Vardhini and Anjum, 2015; Bano-Otalora *et al*., 2020).

Although, several researchers (Wang *et al*., 2014; Vardhini and Anjum, 2015; Diaz-Vivancos *et al*., 2017; Faize and Faize, 2018; Hossain *et al*., 2021; Haddad *et al*., 2022; Pamungkas *et al*., 2022) have reported that application of SA and BRs can elevate plant tolerance to biotic and abiotic stresses, there is not enough information on the roles of SA and BRs applied in combination in alleviating drought stress in plants. Therefore, in this investigation, the effects of BRs and SA applied on the quantity and quality of safflower yield under drought stress conditions were studied. The premier objective of this investigation was to examine how far individual or combined application of BRs and SA could alleviate the adverse effects of drought stress on safflower.

Materials and methods

Plant materials, growth conditions, design and treatments: This study was conducted in the research greenhouse of the Agriculture Faculty, Shahid Bahonar University of Kerman, in 2023. This investigation was carried out as a factorial on randomized complete block design with four replicates. Experimental treatments were water stress (100 % (control treatment) and 50% field capacity, as I_{100} and I_{50}), BRs (0, 0.75 and 1 µM) and SA (0 and 1 mM). These concentrations were selected based on previous researches (Maghsoudi *et al.,* 2019a and Maghsoudi *et al.,* 2019b). Minimum and maximum temperatures in the greenhouse were 14 and 28˚C, respectively, where relative humidity varied between 55-60%. The safflower plants (cv. Isfahan) were exposed to a 14 h photoperiod.

All seeds were surface-sterilized in a 1% sodium hypochlorite solution for 10 minutes and rinsed thoroughly with distilled water. The seeds were germinated on moist filter paper placed in Petri dishes for 48 hours. The 10 days old seedlings were transplanted into 5-liter plastic pots.

The pots soil was fertilized with 150 kg ha^{-1} of urea before sowing and at the start of the stem elongation stage. All phosphorus (150 kg ha^{-1}) and potash (100 kg ha^{-1}) fertilizers were applied before sowing. Until the stem elongation stage, all plants were irrigated properly to maintain 100% F.C. However, from stem elongation to ripening, water stress treatments were initiated to maintain 50% F.C., while the control plants were regularly maintained at 100% F.C.

Measurement method: the amount of irrigation water in this research was determined based on the weight method and the determination of the percentage of moisture by weight. Therefore, the amount of water in the dry soil was determined in relation to the capacity of the field. In this way, in order to calculate the agricultural capacity of the soil, a certain amount of soil was poured into a pot with holes at the end for the exit of excess water, and it was saturated by adding water every 24 hours. The weight of this soil was recorded once. Until there was no change in the weight of soil saturated with water in two periods of time, this weight was recorded as the weight of the soil in the state of crop capacity. Then the soil under consideration was placed in an oven at a temperature of 110 degrees Celsius and after 48 hours, its weight was measured, recorded and calculated as the weight of dry soil. After that, the percentage of agricultural capacity of the soil was calculated (Romano and Santini, 2002). In order to create different percentages of the agricultural capacity of the field and apply drought stress, continuous weighing of the pots and calculation of the amount of water needed up to the corresponding treatment level were used. Furthermore, both SA and then BRs were sprayed on the leaves of all treatment plants in two steps: at the early growth stage (3–4 leaves) as well as the stem elongation stage. The SA and BRs were sprayed for three successive days to avouch that the uptake by the safflower plants has taken place. The pots not receiving BRs or SA were treated similarly with an equivalent amount of water. The fully expanded leaves were harvested at the stage of grain filling and simultaneously transferred and frozen to liquid N_2 until the determination of biochemical parameters.

Measurement of osmolytes (soluble sugars, proline, and protein): To measure soluble sugars, samples of leaves were placed in boiling distilled water contained in a water bath. The mixture was subjected for 10 minutes to centrifugation. To an aliquot of 0.5 ml of the supernatant, 1.5 ml of distilled water, 0.5 ml of 5% phenol, and 5 ml of H_2SO_4 was added. Then, vigorously shaking the mixture, it was placed for one minute in a boiling water bath. Then, after cooling the mixture to room temperature, the color change was measured at 485 nm using a spectrophotometer (Zhang *et al.,* 2006). The protocol described by Bates *et al.* (1973) was employed for determining proline and that of Bradford (1976) for soluble proteins.

Determination of antioxidant enzymes activity: The leaf samples were homogenized in 1 ml ice-cold of

[DOI: DOI: 10.22034/13.61.95]

0.1 M potassium phosphate buffer containing 1 mM ethylene di-amine-tetra-acetic acid and 2% (w/v) PVP. The mixture was centrifuged at 12000 *g* for 20 minutes, and the supernatant was used for the determination of enzyme activities. The activity of superoxide dismutase assay was determined using the modified protocol of Dhindsa and Matowe (1981). Moreover, the activity of catalase and ascorbate peroxidase were was assayedfollowing the protocol described by Nakano and Asada (1981). Also, the activity of peroxidase was appraised based on the rate of oxidation of guaiacol (Cakmak *et al.*, 1993).

Measurement of electrolyte leakage (EL), malondialdehyde (MDA) and hydrogen peroxide (H_2O_2): Leaves were washed with distilled water to remove solutes from the leaf surface. The samples were placed in tubes and incubated with 15 mL of distilled water. Tubes were kept at 25ºC for 24 h, and then, using a conductivity meter, the electrical conductivity (EC) of the electrolytes was measured. In the following, all samples were autoclaved at 60ºC for 15 minutes, and EC was measured again (Sullivan and Ross, 1979). EL was calculated by using equation 1:

$$EL = \frac{C_1}{C_2}$$

Where, C_1 and C_2 refer to the initial and final EC, respectively.

Furthermore, the content of malondialdehyde (MDA) was measured based on the method of Hodges *et al.* (1999). Leaf samples were crushed into a fine powder using a mortar in an ice bath and 5.0 mL of phosphate buffer (0.05 mol L^{-1}) with 1% polyvinylpyrrolidone (PVP) was used as the extraction buffer. The homogenate was centrifuged (15000 g, 15 min), and the supernatant was used to measure MDA. Also, the levels of hydrogen peroxide (H_2O_2) were measured (Veljovic-Jovanovic *et al.*, 2002). The leaves (100 mg) were extracted with 1.0 mL of TCA (0.1%, w/v) and centrifuged at 12 000 × g for 15 min. The supernatant (0.5 mL) was carefully collected, and 0.5 mL of phosphate buffer (pH 7.0) along with 1.0 mL of potassium iodide (1 M) was added. The absorbance of the mixture was read at 390 nm. H_2O_2 concentration was expressed as µmol g^{-1} FW.

Measurement of yield and its components: At maturity, the number of capitula, seed number per capitulum, and 1000-seed weight were recorded on 10 plants randomly selected from the two middle rows. Also, plants in two middle rows were harvested, and grain yield and oil yield were determined. The seed yield of each plot was determined, and seed and oil yields per hectare were calculated. The oil content of seeds in percent was calculated by the nuclear magnetic resonance spectrometer (NMR) at 25°C, according to Colnago *et al.* (2011). Oil content was determined based on dry weight (DB, %), and oil yield was determined as kg/ha.

The collected data were subjected to analysis of variance using SAS v.9.1 software. Duncan's Multiple Range test ($P \leq 0.05$) was used to determine a significant difference among treatment means.

Results

The results of this research showed that foliar application of brassinosteroids and salicylic acid were significant effect on total physiological and biochemical parameters as well as grain yield and its components of safflower under water stress conditions (Table 1)

Osmolaytes (soluble sugars, proline, and proteins): According to the results of the interaction effects of salicylic acid, brassinosteroids and drought stress, it was determined that the concentration of soluble sugars of safflower leaves increased considerably (29.05%) under drought stress conditions (I_{50}). Furthermore, under water and non-water stress conditions, safflower plants treated by brassinosteroids (BRs) and salicylic acid (SA) had higher soluble sugar concentrations than that in the untreated plants. However, the effect of the applied of SA and BRs simultaneously on the content of soluble sugars was greater than that applied singly of SA or BRs (Figure 1A).

The treatment of I_{50} significantly increased the proline content by 59.78%. Also, SA and BRs applied in combination caused a remarkable enhance in the content of proline under normal conditions (I100). However, under I_{50}, foliar application of BRs 0.75 µM, BRs 1 µM, SA 1 mM, BRs 0.75 + SA 1 mM, and BRs 1 + SA 1 mM caused an increase of 35.90%, 33.93%, 33.07%, 48.93%, and 50.50% in concentration of proline, respectively (Figure 1B). Water stress caused a significant reduction (13.76%) in soluble proteins. Indeed, water-stressed plants fed with BRs and SA accumulated a higher protein content than that in the control plants. The negative impact of I_{50} on the concentration of proteins was alleviated by foliar application of BRs and SA; however, the influence of BRs and SA applied in combination on the content of proteins was greater compared to that when BRs or SA were applied singly (Figure 1C).

The activity of antioxidant enzymes: The activity of catalase (CAT) in safflower leaves remarkably increased (39.08%) due to a water deficit. In the I_{50} treatment, it was found that BRs and SA applied separately or in combination considerably increased the CAT activity of water-starved plants. However, the influence of BRs and SA applied in combination on CAT was greater compared to that when BRs or SA were applied separately. Under water stress conditions, the effect of foliar application of SA on the CAT activity was more significant than that of BRs applied. Furthermore, there was no significant difference between BRs 0.75 µM and 1 µM (Figure 2A).

BRs and SA, applied separately or in combination, considerably promoted the activity of peroxidase (POX) in I_{100} treatment. Indeed, the POX activity rose significantly in the I_{50} treatment by 21.45% in safflower leaves compared to the control treatment (I_{100}

[DOI: DOI: 10.22034/13.61.95]

Table 1. The variance analysis (Mean Squared) of the effects of brassinosteroids and salicylic acid on some physiological and biochemical parameters and grain yield and yield components of safflower under water stress conditions

Sources of variation	df	Soluble sugars	Proline	Protein	POX	CAT	APX	SOD
Replication	3	58.33^{ns}	9.31^{ns}	11.32^{ns}	16.13^{ns}	15.73^{ns}	9.70^{ns}	25.13^{ns}
Salicylic acid (A)	1	117.18*	17.45*	2523*	26.13**	21.73*	10.65^{ns}	40.18*
Brassinosteroids (B)	2	113.63*	25.73**	36.73**	20.12*	24.13*	11.98^{ns}	42.48*
Drought stress (C)	1	113.10*	28.83**	32.00**	23.41*	31.51**	27.85**	56.43**
(A) × (B)	2	131.41**	21.03*	25.85*	21.13**	24.26*	13.21^{ns}	44.21*
(A) × (C)	1	122.55*	17.45*	35.00**	28.12**	22.24*	11.32^{ns}	39.19*
(B)× (C)	2	145.32**	19.87*	27.89*	20.16*	24.03*	13.45^{ns}	42.52*
(A) × (B) × (C)	2	156.41**	25.89**	29.72*	22.02**	23.76*	10.31^{ns}	41.70*
Erorr	33	96.31	12.13	18.2	12.33	18.46	12.48	34.11

**, * and ^{ns}: Significant different in levels of 1%, 5% and non-significant respectively.

Continue of table 1.

Sources of variation	df	Electerolyt leakage	Malondialdehyde	H_2O_2	1000-grain weight	number of capitula	seed number per capitulum	Grin yield
Replication	3	95.83^{ns}	5.16^{ns}	32.12^{ns}	60.12^{ns}	21.32^{ns}	42.31^{ns}	10.21^{ns}
Salicylic acid (A)	1	178.36**	16.43*	78.21**	110.23**	45.32*	87.23**	25.32**
Brassinosteroids (B)	2	123.52*	19.38*	65.32*	98.54*	51.00*	94.32**	18.32**
Drought stress (C)	1	163.32**	35.92**	94.10**	124.01**	65.12**	61.02*	17.32*
(A) × (B)	2	132.80*	33.82**	64.32*	110.02**	52.32*	95.15**	29.32**
(A) × (C)	1	167.27**	18.56*	91.51**	89.23*	44.08*	89.63**	28.01**
(B)× (C)	2	154.46**	19.85*	87.23**	108.45**	50.32*	58.08*	17.63*
(A) × (B) × (C)	2	125.23*	18.20*	88.02**	100.45**	50.41*	74.32*	26.35**
Erorr	33	110.31	10.46	45.12	65.23	34.12	47.23	12.32

**, * and ^{ns}: Significant different in levels of 1%, 5% and non-significant respectively.

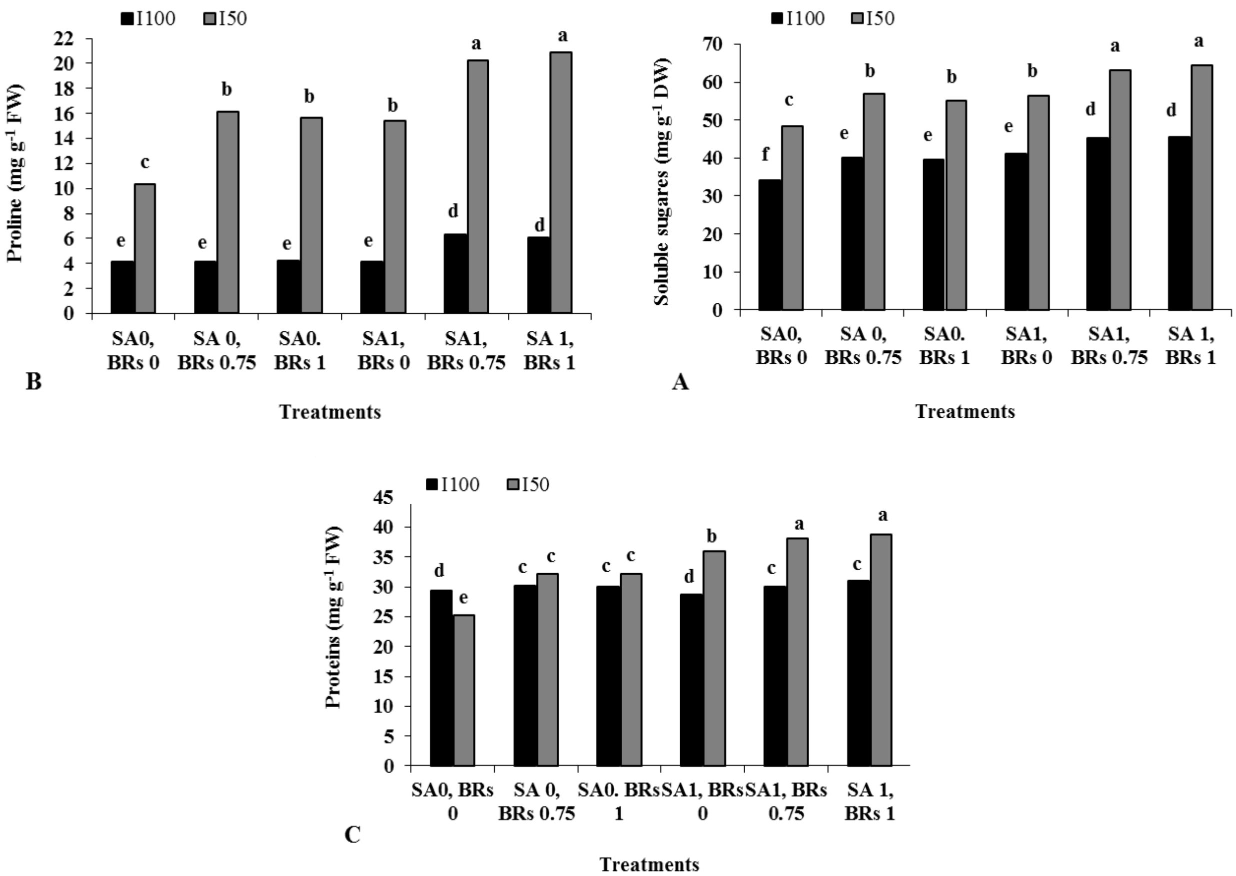

Figure 1. Effect of brassinosteroids (BRs µM) and salicylic acid (SA mM) application on the concentrations of soluble sugars (A), proline (B), and proteins (C) of safflower leaves under water stress (I_{50}) and non-stress (I_{100}) conditions. Means within each figure bearing the same letters do not differ significantly at P ≤ 0.05.

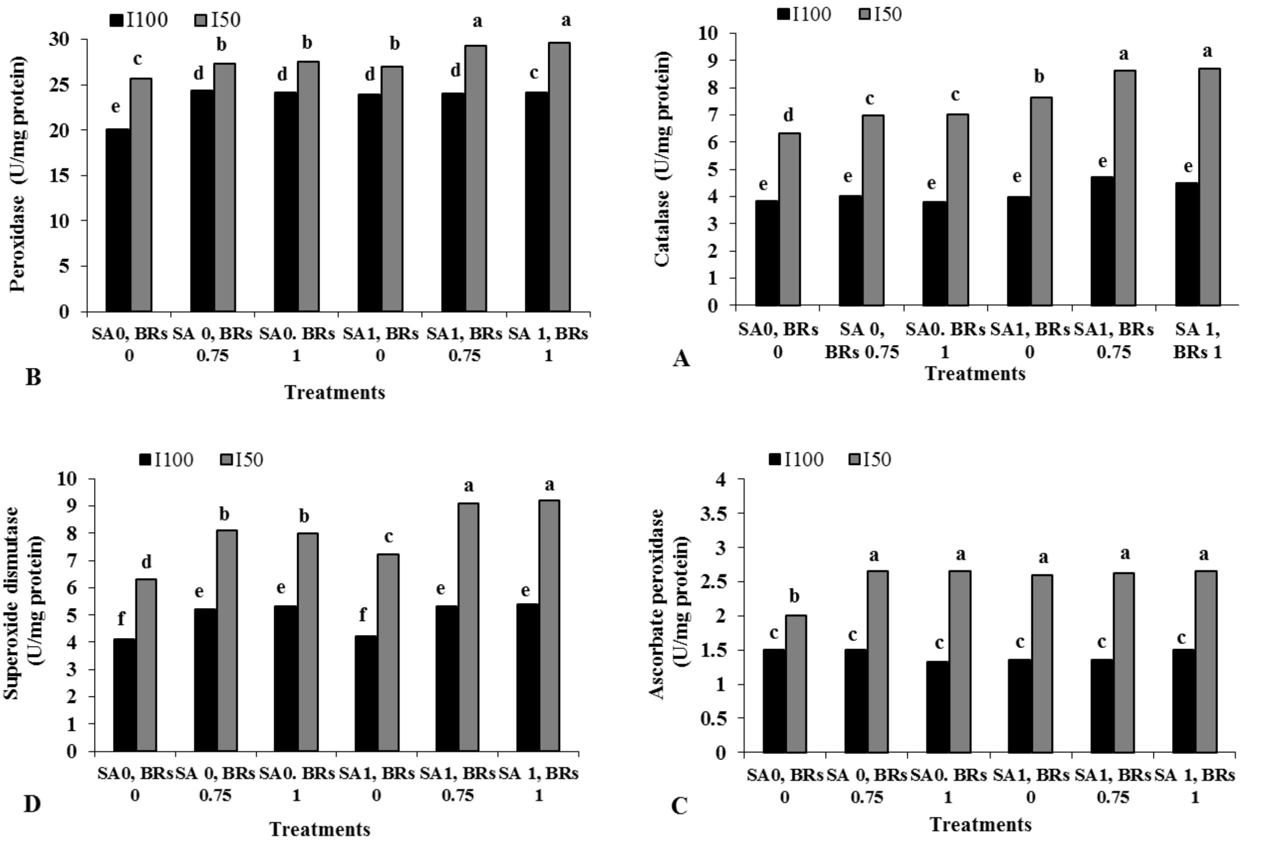

Figure 2. Effect of brassinosteroids (BRs µM) and salicylic acid (SA mM) application on the activities of catalase (A), peroxidase (B), ascorbate peroxidase (C), and superoxide dismutase (D) of safflower leaves under water stress (I_{50}) and non-stress (I_{100}) conditions. Means within each figure bearing the same letters do not differ significantly at P ≤ 0.05.

treatment). Furthermore, SA-treated and BRs-treated safflower plants had greater activity of POX than that in the plants grown solely in water deficit conditions; however, the effect of the combination of BRs and SA on the activity of POX was greater compared to that when BRs or SA were applied separately (Figure 2B).

The ascorbate peroxidase (APX) activity also increased by 25% under the I_{50} regime. Also, foliar application of BRs or SA had no considerable effect on the APX activity under I_{100} treatment, whereas, in I_{50} treatment, activity of APX was enhanced by the BRs application, SA, and combination of BRs and SA by about 24.50% (Figure 2C). The activity of superoxide dismutase (SOD) also increased by 35.12% under I_{50} treatment. Furthermore, SA foliar application did not exhibit any marked effect on its activity under control treatment, whereas, in these conditions, SOD activity increased with the application of BRs and a combination of SA and BRs. In addition, under I_{50} treatment, BRs 0.75 µM, BRs 1 µM, SA 1mM, BRs 0.75+SA 1 mM, and BRs 1+SA 1 mM supplementation caused an increase of 21.97%, 21.00%, 12.58%, 30.54%, and 31.31% in SOD activity, respectively (Figure 2D).

Electrolyte leakage (EL), malondialdehyde (MDA) and hydrogen peroxide (H_2O_2): I_{50} treatment caused a marked increase in electrolyte leakage (EL). It was found that BRs and SA applied separately or in combination considerably improved the EL of safflower plants under water stress conditions. However, the effect of the combination of BRs and SA on the EL was higher compared to that when BRs or SA were applied singly (Figure 3A). Besides, drought stress caused a considerable enhance of 45.53% in the levels of malondialdehyde (MDA). In contrast, under normal conditions, foliar application of BRs 0.75+SA 1 mM and BRs 1+SA 1 mM reduced the concentration of MDA in safflower leaves by about 34.40%. Also, in I_{50} treatment, the levels of MDA were reduced with BRs and SA applied separately or in combination; however, the effect of BRs and SA applied in combination on the content of MDA was higher compared to that when BRs or SA applied singly (Figure 3B).

Water stress applied as I_{50} increased the hydrogen peroxide (H_2O_2) contents by 66.47% in leaves of safflower compared to control conditions (I_{100}). In contrast, SA and BRs application decreased the content of H_2O_2 in water-stressed safflower plants. In addition, the effect of SA and BRs combination, on the content of H_2O_2 was higher than that by SA or BRs applied separately (Figure 3C).

Yield and yield components: Drought stress significantly reduced 1000-grain weight, a number of capitula, and seed number per capitulum of safflower by 23.55%, 47.13%, and 18.11%, respectively (Figure 4A,

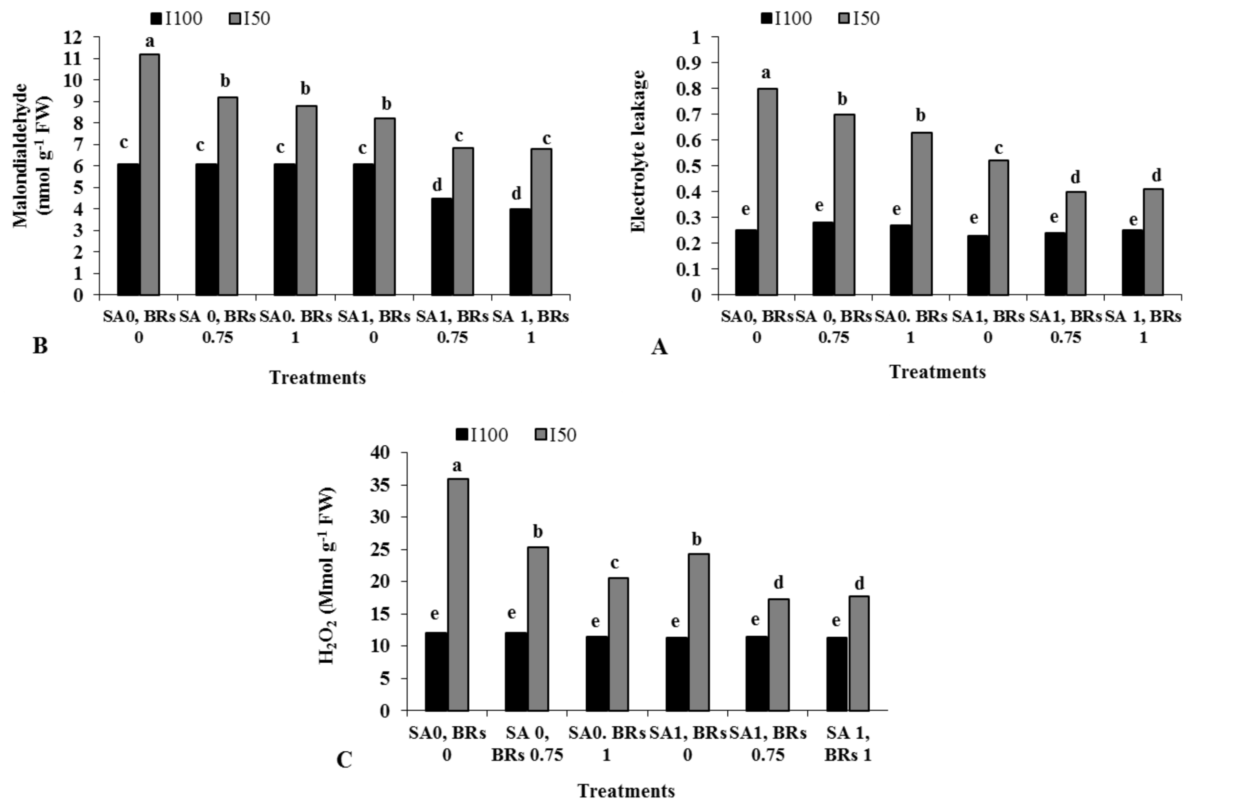

Figure 3. Effect of brassinosteroids (BRs μM) and salicylic acid (SA mM) application on the electrolyte leakage (A), malondialdehyde (B), and H_2O_2 (C) of safflower leaves under water stress (I_{50}) and non-stress (I_{100}) conditions. Means within each figure bearing the same letters do not differ significantly at $P \leq 0.05$.

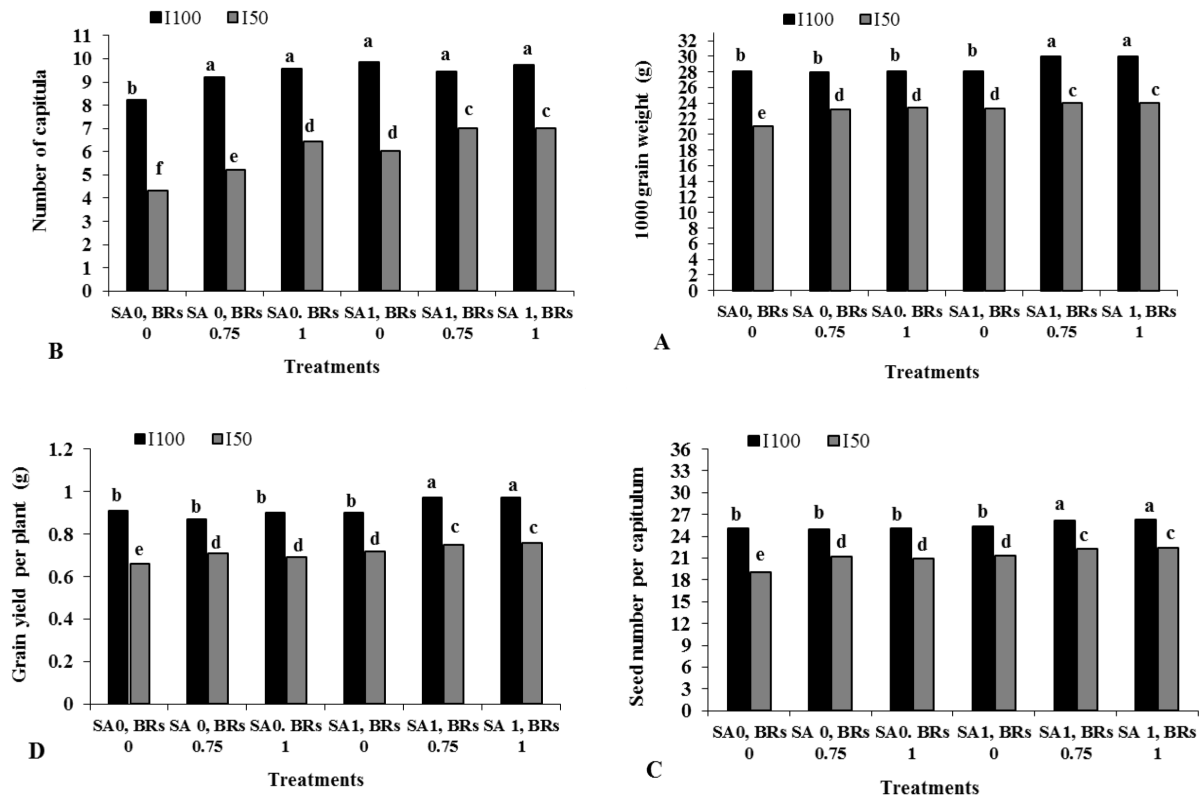

Figure 4. Effect of brassinosteroids (BRs μM) and salicylic acid (SA mM) application on the 1000-grain weight (A), number of capitula (B), seed number per capitulum (C), and grain yield per plant (D) of safflower under water stress (I_{50}) and non-stress (I_{100}) conditions. Means within each figure bearing the same letters do not differ significantly at $P \leq 0.05$.

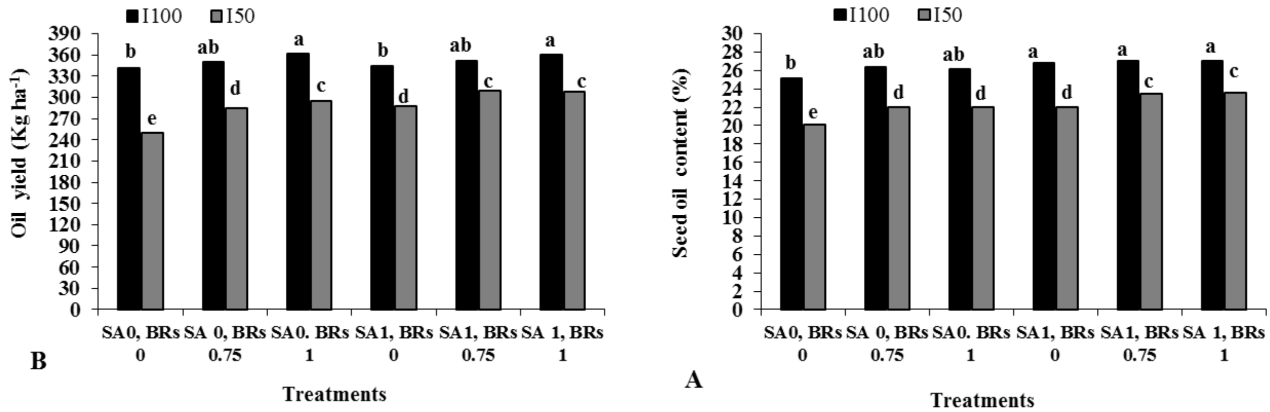

Figure 5. Effect brassinosteroids (BRs µM) and salicylic acid (SA mM) application on the seed oil content (A) and oil yield (B) of safflower under water stress (I_{50}) and non-stress (I_{100}) conditions. Means within each figure bearing the same letters do not differ significantly at P ≤ 0.05.

4B, and 4C). Indeed, foliar application of BRs and SA were induced to improve the negative effects of water deficit on these parameters. However, the effect of BRs and SA applied in combination with 1000-grain weight, the number of capitula and, seed number per capitulum spike was greater compared to that when BRs or SA applied separately (Figure 4A, 4B and 4C). Furthermore, under I_{100} treatment, combined and separately application of BRs and SA caused an increase in these parameters (Figures 4A, 4B and 4C). Grain yield per plant of safflower reduced by 27.47% under I_{50} treatment. Also, combination of BRs and SA promoted grain yield in I_{100} treatment. Furthermore, SA-treated and BRs-treated safflower plants had greater grain yield per plant than that in the plants grown solely in water stress conditions; however, the effect of the combination of BRs and SA on grain yield per plant was higher compared to that when BRs or SA applied separately (Figure 4D).

Seed oil content and oil yield: Drought stress treatment (I_{50}) caused a marked decrease of 19.94% in the seed oil content of safflower. It was found that PGRs applied separately or in combination significantly improved the seed oil content of water-starved plants. However, the effect of a combination of BRs 0.75µM + SA 1mM and BRs 1µM + SA 1mM on the seed oil content was greater compared to that when PGRs were applied separately (Figure 5A). Also, under normal conditions, foliar application of SA 1mM, SA 1mM+ BRs 0.75µM, and SA 1mM+ BRs 1µM caused a considerable enhance in the seed oil content of safflower (Figure 5A). Water stress applied as I_{50} reduced the oil yield of safflower by 26.68% compared to control conditions (non-water stress). In contrast, the application of PGRs increased the oil yield of safflower under stress and non-stress conditions. So that, under non-water stress conditions, foliar application of BRs 0.75µM, BRs 1µM, SA 1mM+ BRs 0.75µM, and SA 1mM+ BRs 1µM increased the oil yield by 2.57%, 5.54%, 2.84%, and 5.28%, respectively (Figure 5B). Furthermore, BRs 0.75µM, BRs 1µM, SA 1mM, SA 1mM+ BRs 0.75µM, and SA 1mM+ BRs 1µM application increased the oil yield by 12.31%, 15.25%, 12.89%, 19.00%, and 18.83%, respectively, under drought stress conditions (Figure 5B).

Discussion

All plant growth stages, from germination to maturity, are affected by abiotic and environmental stresses (Wang *et al.*, 2018; Bangar *et al.*, 2019; Haddad *et al.*, 2022; Pamungkas *et al.*, 2022). Among the abiotic stresses, drought is a major menace, with adverse effects on the physiological and biochemical responses and via disordering activities of plants, including the rate of carbon assimilation, reduced turgor, enhanced oxidative damage, and variation in leaf gas exchange, thereby leading to a remarkable decline in plant production (Nadeem *et al.*, 2018; Kamanga *et al.*, 2018; Hossain *et al.*, 2021). In plants, a main component of drought tolerance is the manufacture and accumulation of osmolytes, a process known as osmoregulation or osmotic adjustment (Nadeem *et al.*, 2019). Osmoregulation, as a considerable adaptation mechanism under water deficit status in plants, helps maintain cell turgor via the solutes accumulation (Choudhury *et al.*, 2017; Bechtold, 2018; Li *et al.*, 2018). Different plants improve their metabolism under water deficiency via the accumulation of proline, carbohydrates, and amino acids (Nadeem *et al.*, 2019). Similarly, the results of this research showed that the concentration of osmolytes, including soluble sugars and proline, in safflower leaves increased considerably under drought stress conditions (Figure 1). Prior research has suggested that the accumulation of proline contributes to an increase in osmotic stress endurance (Choudhury *et al.*, 2017; Ullah *et al.*, 2018; Saikia *et al.*, 2018; Nadeem *et al.*, 2019). During drought stress, proline plays a main role and acts as a signaling compound to adjust mitochondria function and affect cell proliferation utilizing activating particular genes, which are essential for stress recovery (Mohamed *et al.*, 2017; Li *et al.*, 2018). Accumulation of proline helps in cell membrane stability by decreasing lipid oxidation via protection of cellular redox potential and scavenging free radicals (Nadeem *et al.,* 2019; Oguz *et al.,* 2022).

Reactive oxygen species (ROS) manufacture is a primary response of abiotic-stressed plants and acts as a messenger to activate defense mechanisms in plants (Bechtold, 2018; Li *et al.,* 2018). Under water deficit, ROS such as hydrogen peroxide, hydroxyl radical, superoxide radical and singlet oxygen are produced and accumulate, which damage macromolecules and cell structure (Choudhury *et al.*, 2017; Bano-Otalora *et al*., 2020). ROS are signaling compounds that act at low concentrations and trigger different responses under abiotic stresses, such as drought stress. When the level exceeds the defense mechanism, ROS causes oxidative stress to lipids and proteins as well as nucleic acids, leading to changes in the intrinsic attributes of biomolecules and cell death (Choudhury *et al.,* 2017; Khater *et al.,* 2018). In this research, drought stress caused an increase in the hydrogen peroxide content (as a ROS) and malondialdehyde (MDA) levels in the leaves of safflower (Figure 3C), showing a considerable association of the ROS with MDA as an index of lipid peroxidation of the membrane. Numerous researchers have reported that ROS mediated lipid peroxidation leads to impairment of membrane functions, thereby causing remarkable membrane leakage and raising electrolyte leakage (EL) from cells (Khater *et al.,* 2018; Nadeem *et al.,* 2019). As observed in the drought-treated safflower plants in this research, can be related to increased ROS production as presented in other investigations (Choudhury *et al.,* 2017; Khater *et al.,* 2018; Nadeem *et al.,* 2019). The association of ROS with MDA and EL is a common phenomenon that occurs in many plants under abiotic stress conditions (Kamanga *et al.,* 2018; Billah *et al.,* 2021).

In the plant cells, enzymatic and non-enzymatic antioxidants adjust the defensive mechanism of ROS, and maintaining a higher concentration of antioxidants or antioxidant enzymes has proven to be an adaptive response under stress conditions (Li *et al.,* 2018; Nadeem *et al.,* 2019; Hossain *et al.,* 2021). Enzymatic antioxidants comprise peroxidase (POX), superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT) and non-enzymatic antioxidants include glutathione, ascorbate, tocopherols, carotenoids, phenolics and ascorbic acid (Sahitya *et al.,* 2018). Between enzymatic antioxidants, the activity of SOD leads to the detoxification of ROS such as superoxide radicals and hydrogen peroxide. APX helps to generate $NADP^+$ and changes superoxide radicals to water. APX also helps to remove superoxide radicals, whereas dehydroascorbate reductase (DHAR), glutathione reductase (GR) assist by providing a substrate for reactions. Based on the results of this study, under drought stress, it has been recorded that POX, SOD, APX, and CAT activities increased in the leaves of safflower (Figure 2). In this regard, Nadeem *et al.* (2019) reported that enhanced antioxidant activities would help to ameliorate drought tolerance by protecting from oxidative stress. Based on the results of this study, in summary, water stress enhanced the accumulation of key organic osmolytes (Figure 1) and the activities of some critical antioxidant enzymes (Figure 2), as well as raised EL and the concentrations of MDA and H_2O_2 in the leaves of safflower (Figure 3).

The useful effects of brassinosteroids (BRs) and salicylic acid (SA) application have been previously reported on various plants under abiotic stress conditions (Wang *et al.,* 2014; Vardhini and Anjum, 2015; Diaz-Vivancos *et al*., 2017; Shaki *et al.,* 2018; Faize and Faize, 2018). In other words, multiple reports display that a potent association exists among the application of BRs and SA and increased tolerance to different abiotic stresses in crops (Zhiponova *et al.,* 2013; Wang *et al.,* 2014; Sorahinobar *et al.,* 2016). The application of BRs and SA is believed to affect the growth and physiological processes of almost all crops (Vardhini and Anjum, 2015; Bano-Otalora *et al*., 2020). It is important, foliar application of SA and BRs significantly increased the antioxidant enzymes activities (Figure 2) and the concentrations of osmolytes (Figure 1), and in contrast, reduced the levels of H_2O_2 and MDA as well as EL in water-stressed safflower (Figure 3). Similar to the results of this study, the beneficial effects of the application of BRs and SA have been earlier reported on different plants under abiotic and biotic stressful cues (Wang *et al.,* 2014; Dong *et al.,* 2017; Pamungkas *et al.,* 2022) however, not many data exist on the useful effects of simultaneous application of BRs and SA. The results of this study showed that the positive effects of SA on the accumulation of osmolytes, antioxidant enzymes activities and cell membrane stability was high when applied with BRs (Figures 1, 2 and 3). Dong *et al*. (2017), Faize and Faize, (2018) and Shaki *et al.* (2018) reported that BRs and SA directly or indirectly effect on different physio-biochemical processes in plants exposed to various stresses. However, its effectiveness to mitigate stress depends on type of plant species and concentration of PGRs. Some researchers reported that PGRs can enhance the activity of antioxidants, resulting in ameliorate the stress-induced ROS damage (Hasan *et al.,* 2011; Dong *et al.,* 2017; Haddad *et al.,* 2022).

Zaharah *et al.* (2012) reported that the effect of BRs on the increase in carbohydrate content is due to enhanced capacity of photosynthetic and the efficient transfer of these compounds from the production center to the consumption center. BRs show by their influence on the expression of genes encoding the enzymes involved in the carbohydrates metabolism, and the control of the transfer of these compounds to their consumption centers in the carbohydrates accumulation (Yu *et al.,* 2004). In addition, BRs enhance ethylene production, resulting in the hydrolysis of polysaccharides and starch, as well as the soluble sugars production (Zaharah *et al.,* 2012). The results of this experiment are consistent with the results of some researchers regarding the effect of BRs on increasing the amount of soluble sugar (Figure 1). Furthermore,

[DOI: DOI: 10.22034/13.61.95]

similarity results of this study, some scholar presented that treating the plant via BRs caused an enhance in the amount of necessary amino acids and proteins, particularly proline, occurred to protect the plant from abiotic stresses (Zaharah *et al.,* 2012; Dong *et al.,* 2017). Increased levels of soluble proteins have been reported in the treatment of BRs in mung bean (Bajguz, 2011; Hasan *et al.*, 2011; Dong *et al.*, 2017; Haddad *et al.*, 2022). Similar to the results of this study, several reports suggest that BRs and SA regulate the expression of different genes in plants, so the application of these PGRs can increase the expression of antioxidant activity regulating genes. These PGRs have a significant potential for antioxidant activity in stress conditions (Vardhini and Anjum, 2015; Sorahinobar *et al.,* 2016; Shaki *et al.,* 2018; Pamungkas *et al.,* 2022).

Water deficits at each stage can affect plant growth as a result of decreased crop production, especially in grain filling stage (Wang *et al.,* 2018; Bangar *et al.,* 2019; Billah *et al.,* 2021). The results of this research confirmed that in safflower, yield and its components depend strongly on water availability, and when drought stress was applied to the stem elongation stage, the number of capitula, seed number per capitulum and 1000-seed weight of safflower were greatly reduced compared with control conditions, thereby reducing the seed yield (Figure 4). Similarly, Beyyavas *et al.* (2011) and Camas *et al.* (2007) reported a considerable correlation between seed yield and the number of capitula per plant, the number of seeds per capitulum and 1000-seed weight. In this research, seed yield was reduced significantly under water stress conditions, as formerly reported by Nabipour *et al.* (2007), Istanbulluoglu (2009), and Ghamarnia and Sepehri (2010). Furthermore, Sharrifmoghaddasi and Omidi (2010). Also, Nabipour *et al.* (2007), Movahhedy-Dehnavy *et al.* (2009), and Ebrahimian *et al.* (2019) reported water deficit effects considerably on yield and all components of yield. The achievement of safflower development in an area greatly depends on the content of seed oil and oil yield (Beyyavas *et al.,* 2011; Bella *et al.,* 2019). The results of this research showed that seed oil content was reduced under drought stress conditions (Figure 5), confirming previous observations from Beyyavas *et al.* (2011), Ghamarnia and Sepehri (2010), Jabbari *et al.* (2010), and Ebrahimian *et al.* (2019). Oil yield variation was mainly driven by the variation of seed yield and seed oil content (Beyyavas *et al.,* 2011; Ebrahimian *et al.,* 2019).

In this research, foliar application of BRs and SA was induced to improve the negative effects of water deficit on seed yield and its components, as well as the seed oil content of safflower (Figures 4 and 5). However, the influence of BRs and SA applied in combination on these characteristics was greater compared to that when SA or BRs applied singly (Figures 4 and 5). Recently, it has been reported that BRs stimulate cell division independently of other plant growth regulators (PGRs). However, BRs respond to the anoxic levels of auxin and enhance the effectiveness of each other, thereby increasing the height and fresh weight of the plant (Vardhini and Anjum, 2015; Bano-Otalora *et al*., 2020). Bajguz (2011) introduced the idea that BRs have a significant influence on the development and growth of the Chlorella vulgaris plant. Similar to the present experiment in peanut butter (*Arachis hypogaea*) treated with BRs, an increase was observed in growth parameters and grain yield per plant. Improving the growth of plants treated by BRs is dependent on enhanced content of soluble proteins and carbohydrates as well as increased levels of DNA and RNA (Vardhini and Anjum, 2015; Oguz *et al.,* 2022).

The present investigation showed that foliar application of BRs and SA considerably played an efficient role in improving and increasing the production and quality (seed oil content) of safflower under drought stress conditions.

Conclusion

The results of this research suggest that water stress affects the seed and oil yield and some of physiological and biochemical responses of safflower. Besides, SA and BRs applied separately or in combination effectively improved antioxidant activity, accumulation of osmolytes, cell membrane stability as well as seed yield in safflower plants under drought stress; however, the positive effects of SA were considerably when it was applied with BRs. Furthermore, SA and BRs caused a significant increase in seed oil content and oil yield of safflower, but the effect of SA + BRs was higher than that of BRs or SA applied singly. Water-stress alleviation and yield improvement in safflower by BRs and SA application was attributable to partly improved osmotic adjustment (accumulation of osmolytes), cell membrane stability and antioxidant activity under stress conditions. Altogether, SA and BRs foliar application demonstrated the potential to improve growth and increase seed and oil yield of safflower under drought stress conditions.

The present research was conducted in greenhouse conditions. Therefore, it is suggested to evaluate the effect of SA and BRs application on the yield and quality of safflower on a larger scale and in a field experiment.

Acknowledgement

We hereby acknowledge Shahid Bahonar University of Kerman for the financial support provided for the implementation of the project with the number 1401/36578.

References

[DOI: DOI: 10.22034/13.61.95]

Bajguz, A. (2011). Suppression of *Chlorella vulgaris* growth by cadmium, lead and copper stress and its restoration by endogenous brassinolide. *Archives of Environmental Contamination and Toxicology*, *60*, 406-416.

Bangar, P., Chaudhury, A., Tiwari, B., Kumar, S., Kumari, R., & Bhat, K. V. (2019). Morphophysiological and biochemical response of mungbean (*Vigna radiata* L.) varieties at deferent developmental stages under drought stress. *Turkish Journal of Biology, 43*, 58-69.

Bano-Otalora, B., Madrid, J. A., & Rol, M. A. (2020). Melatonin alleviates circadian system disruption induced by chronic shifts of the light-dark cycle in *Octodon degus*. *Journal of Pineal Research*, *68*, 1-13.

Bates, L. S., Waldran, R. P., & Teare, I. D. (1973). Rapid determination of free proline for water studies. *Plant and Soil, 39*, 205-208.

Bechtold, U. (2018). Plant life in extreme environments: How do you improve drought tolerance? *Frontiers in Plant Science*, *9*, 543-551.

Bella, S. L., Tuttolomondo, T., Lazzeri, L., Matteo, R., Leto, C., & Licata, M. (2019). An agronomic evaluation of new safflower (*Carthamus tinctorius* L.) germplasm for seed and oil yields under Mediterranean climate conditions. *Agronomy*, *9*, 468-472.

Beyyavas, V., Haliloglu, H., Copur, O., & Yilmaz, A. (2011). Determination of seed yield and yield components of some safflower (*Carthamus tinctorius* L.) cultivars, lines and populations under the semi-arid conditions. *African Journal of Biotechnology, 10*, 527-534.

Billah, M., Aktar, S., Brestic, M., Zivcak, M., Khaldun, A. B. M., Uddin, M. S., Bagum, S. A., Yang, X., Skalicky, M., & Mehari, T. G. (2021). Progressive genomic approaches to explore drought- and salt-induced oxidative stress responses in plants under changing climate. *Plants, 10*, 142-156.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, *72*, 248-254.

Cakmak, I., Strbac, D., & Marschner, H. (1993). Activities of hydrogen peroxide-scavenging enzymes in germinating wheat seeds. *Journal of Experimental Botany*, *44*, 27-132.

Camas, N., Cirak, C., & Esendal, E. (2007). Seed yield, oil content and fatty acids composition of safflower (*Carthamus tinctorius* L.) grown in Northern Turkey conditions. *Journal of the Faculty of Agriculture*, *22*, 98-104.

Choudhury, F. K., Rivero, R. M., Blumwald, E., & Mittler, R. (2017). Reactive oxygen species, abiotic stress and stress combination. *Plant Journal*, *90*, 856-867.

Colnago, L. A., Azeredo, R. B. V., Marchi, N. A., Andrade, F. D., & Venan-cio, T. (2011). Rapid analyses of oil and fat content in agri-food products using continuous wave-free precession time domain NMR. *Magnetic Resonance in Chemistry*, *49,* S113-120.

Dhindsa, R. S. & Matow, W. (1981). Drought tolerance in two mosses: Correlated with enzymatic defense against lipid peroxidation. *Journal of Experimental Botany*, *32*, 79-91.

Diaz-Vivancos, P., Bernal-Vicente, A., Cantabella, D., Petri, C., & Hernandez, J. A. (2017). Metabolomics and biochemical approaches link salicylic acid biosynthesis to cyanogenesis in peach plants. *Plant and Cell Physiology, 58*, 2057-2066.

Dong, Y. J., Wang, W. W., Hu, G. Q., Chen, W. F., Zhuge, Y. P., Wang, Z. L., & He, M. R. (2017). Role of exogenous 24-epibrassinolide in enhancing the salt tolerance of wheat seedlings. *Journal of Soil Science and Plant Nutrition, 17*, 41-54.

Ebrahimian, E., Seyyedi, S. M., Bybordi, A., & Damalas, C. A. (2019). Seed yield and oil quality of sunflower, safflower, and sesame under different levels of irrigation water availability. *Agricultural Water Management*, *218*, 149-157.

Eslam, B. P., Monirifar, H., & Ghassemi, M. T. (2010). Evaluation of late season drought effects on seed and oil yields in spring safflower genotypes. *Turkish Journal of Agriculture and Forestry, 34,* 373-380.

Faize, L. & Faize, M. (2018). Functional analogues of salicylic acid and their use in crop protection. *Agronomy*, *8,* 5-12.

Ghamarnia, H. & Sepehri, S. (2010). Different irrigation regimes affected water use, yield and other yield components of safflower (*Carthamus tinctorius* L.) crop in a semi-arid region of Iran. *Journal of Food, Agriculture and Environment*, *8*, 590-593.

Haddad, N., Choukri, H., Ghanem, M. E., Smouni, A., Mentag, R., Rajendran, K., Hejjaoui, K., Maalouf, F., & Kumar, S. (2022). High temperature and drought stress effects on growth, yield and nutritional quality with transpiration response to vapor pressure deficit in lentil. *Plants*, *11*, 95-112.

Hasan, S. A., Hayat, S., & Ahmad, A. (2011). Brassinosteroids protect photosynthetic machinery against the cadmium induced oxidative stress in two tomato cultivars. *Chemosphere, 84*, 1446-1451.

Hodges, D. M., De Lond, J. M., Forney, C. F., & Prange, R. K. (1999). Improving the thiobarbituric acid-reactive substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. *Planta*, *207,* 604-611.

Hossain, A., Farooq, M., el Sabagh, A., Hasanuzzaman, M., Erman, M., & Islam, T. (2021). Morphological, Physiobiochemical and Molecular Adaptability of Legumes of Fabaceae to Drought Stress, with Special Reference to Medicago *sativa* L. In The Plant Family Fabaceae; Springer, Berlin/Heidelberg, Germany.

Istanbulluoglu, A. (2009). Effects of irrigation regimes on yield and water productivity of safflower (*Carthamus tinctorius* L.) under Mediterranean climate conditions. *Agricultural Water Management*, *96*, 1792-1798.

Jabbari, M., Ebadi, A., Tobeh, A., & Mostafaii, H. (2010). Effects of supplemental irrigation on yield and yield components of spring safflower genotypes. *Recent Research in Science and Technology*, *2*, 23-28.

Janeczko, A., Oklestkova, J., Pociecha, E., Koscielniak, J., & Mirek, M. (2011). Physiological effects and transport of 24-epibrassinolide in heat-stressed barley. *Acta Physiologiae Plantarum*, *33*, 1249-1259.

Kamanga, R. M., Mbega, E., & Ndakidemi, P. (2018). Drought tolerance mechanisms in plants: Physiological responses associated with water deficit stress in *Solanum lycopersicum*. *Advances Crop Science Technology*, *6*, 1-8.

Kar, G., Kumar, A., & Martha, M. (2007). Water use efficiency and crop coefficients of dry season oilseed crops. *Agricultural Water Management*, *87*, 73-82.

Khater, M. A., Dawood, M. G., Sadak, M. S., Shalaby, M. A. F., El-Awadi, M. E., & El-Din, K. G. (2018). Enhancement the performance of cowpea plants grown under drought conditions via trehalose application. *Middle East Journal of Agriculture Research*, *7*, 782-800.

Li, P., Zhang, Y., Wu, X., & Liu, Y. (2018). Drought stress impact on leaf proteome variations of faba bean (*Vicia faba* L.) in the Qinghai–Tibet Plateau of China. *Biotechnology, 8*, 110-118.

Majidi, M. M., Tavakoli, V., Mirlohi, A., & Sabzalian, M. R. (2011). Wild safflower species (*Carthamus oxyacanthus* Bieb.): A possible source of drought tolerance for arid environments. *Australian Journal of Crop Science, 5*, 1055-1063.

Maghsoudi, K., Arvin, M. J., & Ashraf, M. (2019a). Mitigation of arsenic toxicity in wheat by the exogenously applied salicylic acid, 24-epi-Brassinolide and silicon. *Journal of Soil Science and Plant Nutrition*, *20*, DOI:10.1007/s42729-019-00147-3.

Maghsoudi, K., Emam, Y., Ashraf, M., & Arvin, M. J. (2019b). Alleviation of field water stress in wheat cultivars by using silicon and salicylic acid applied separately or in combination. *Crop and Pasture Science*, *70*, 36-43.

Mohamed, I. H. & Hanan, H. L. (2017). Improvement of drought tolerance of soybean plants by using methyl jasmonate. *Physiology and Molecular Biology of Plants*, *23*, 545-556.

Movahhedy-Dehnavy, M., Modares-Sanavy, S. A. M., & Mokhtassi-Bidgoli, A. M. (2009). Foliar application of zinc and manganese improves seed yield and quality of safflower (*Carthamus tinctorius* L.) grown under water deficit stress. *Industrial Crops and Products*, *30*, 82-92.

Nabipour, M., Meskarbashee, M., & Yousefpour, Y. (2007). The effect of water deficit on yield and yield components of safflower (*Carthamus tinctorius* L.). *Pakistan Journal of Biological Sciences*, *10*, 421-426.

Nadeem, M., Li, J., Wang, M., Shah, L., Lu, S., Wang, X., & Ma, C. (2018). Unraveling field crops sensitivity to heat stress: Mechanisms, approaches, and future prospects. *Agronomy, 8*, 128-135.

Nadeem, M., Li, J., Yahya, M., Wang, M., Ali, A., Cheng, A., Wang, X., & Ma, C. (2019). Grain legumes and fear of salt stress: Focus on mechanisms and management strategies. *International Journal of Molecular Sciences*, *20*, 799-809.

Nakano, Y. & Asada, K. (1981). Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant Cell Physiology*, *22*, 867-880.

Oguz, M. C., Aycan, M., Oguz, E., Poyraz, I., & Yildiz, M. (2022). Drought stress tolerance in plants: Interplay of molecular, biochemical and physiological responses in important development stages. *Physiologia*, *2*, 180-197.

Ozturk, E., Ozer, H., & Polat, T. (2008). Growth and yield of safflower genotypes grown under irrigated and non-irrigated conditions in a highland environment. *Plant Soil and Environment*, *54*, 453-460.

Pamungkas, S. S. T., Suwarto, S., & Farid, N. (2022). Drought stress: Responses and mechanism in plants. *Reviews in Agricultural Science*, *10*, 168-185.

Romano, N. & Santini, A. (2002). Methods of Soil Analysis, Physical Methods. SSSA Book Series N.5. Publisher, Soil Science.

Sahitya, U. L., Krishna, M. S. R., Prasad, G. S., Kasim, D. P., & Deepthi, R. S. (2018). Seed antioxidants interplay with drought stress tolerance indices in chilli (*Capsicum annuum* L.) seedlings. *BioMed Research International, 14*, 101-112.

Saikia, J., Sarma, R. K., Dhandia, R., Yadav, A., Bharali, R., Gupta, V. K., & Saikia, R. (2018). Alleviation of drought stress in pulse crops with ACC deaminase producing rhizobacteria isolated from acidic soil of Northeast India. *Scientific Reports*, *8*, 360-369.

Shaki, F., Ebrahimzadeh Maboud, H., & Niknam, V. (2018). Growth enhancement and salt tolerance of Safflower (*Carthamus tinctorius* L.), by salicylic acid. *Current Plant Biology*, *13*, 16-22.

Sharrifmoghaddasi, M. & Omidi, A. H. (2010). Study of interrupting irrigation effects at different growth stages on grain and oil yields of new safflower varieties. *Advances in Environmental Biology*, *4*, 387-391.

Sorahinobar, M., Niknam, V., Ebrahimzadeh, H., Soltanloo, H., Behmanesh, M., & Enferadi, S. T. (2016). Central role of salicylic acid in resistance of wheat against *Fusarium graminearum*. *Journal of Plant Growth Regulation, 35*, 477-491.

Sullivan, C. Y. & Ross, W. M. (1979). Selecting for drought and heat resistance in grain sorghum. In: Stress Physiology in Crop Plants. (eds. Mussel, H. and Staples, R. C.). John Wiley and Sons, New York.

Ullah, A., Manghwar, H., Shaban, M., Khan, A. H., Akbar, A., Ali, U., Ali, E., & Fahad, S. (2018). Phytohormones enhanced drought tolerance in plants: A coping strategy. *Environmental Science and Pollution Research*, *25*, 33103-33118.

Vardhini, B. V. & Anjum, N. A. (2015). Brassinosteroids make plant life easier under abiotic stresses mainly by modulating major components of antioxidant defense system. *Journal of Environmental Sciences*, *2,* 1-16.

Veljovic-Jovanovic, S., Noctor, G., & Foyer, C. H. (2002). Are leaf hydrogen peroxide concentrations commonly overestimated? The potential influence of artefactual interference by tissue phenolics and ascorbate. *Plant Physiology and Biochemistry*, *40*, 501-507.

Wang, L., Dong, S., Liu, L., Ma, Y., Li, S., & Zu, W. (2018). Transcriptome profiling reveals PEG-simulated drought, heat and combined stress response mechanisms in soybean. *Computational Biology Chemistry*, *77*, 413-429.

Wang, X. H., Shu, C., Li, H. Y., Hu, X. Q., & Wang, Y. X. (2014). Effects of 0.01% brassinolide solution application on yield of rice and its resistance to autumn low-temperature damage. *Acta Agriculturae Jiangxi*, *26*, 36-38.

Yu, J. Q., Huang, L. F., Hu, W. H., Zhou, Y. H., Mao, W. H., Ye, S. F., & Nogues, S. (2004). A role for brassinosteroids in the regulation of photosynthesis in *Cucumis sativus*. *Journal of Experimental Botany, 55*, 1135-1143.

Zaharah, S. S., Singh, Z., Symons, G. M., & Reid, J. B. (2012). Role of brassinosteroids, ethylene, abscisic acid, and indole-3-acetic acid in mango fruit ripening. *Journal of Plant Growth Regulator, 31*, 363-372.

Zandalinas, S. I., Rivero, R. M., Martinez, V., Gomez-Cadenas, A., & Arbona, V. (2016). Tolerance of citrus plants to the combination of high temperatures and drought is associated to the increase in transpiration modulated by a reduction in abscisic acid levels. *BMC Plant Biology*, *16*, 105-111.

Zhang, Z. J., Li, H. Z., Zhou, W. J., Takeuchi, Y., & Yoneyama, K. (2006). Effect of 5-aminolevulinic acid on development and salt tolerance of potato (*Solanum tuberosum* L.) microtubers in vitro. *Plant Growth Regulation*, *49*, 27-34.

Zhiponova, M. K., Vanhoutte, I., Boudolf, V., Betti, C., Dhondt, S., Coppens, F., & Mylle, E. (2013). Brassinosteroid production and signaling differentially control cell division and expansion in the leaf. *New Phytologist*, *197*, 490-502.

[DOI: DOI: 10.22034/13.61.95]