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Research article

Phytochemical responses of camelina to brassinolide and boron foliar spray under irrigation regimes

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ABSTRACT

The high level of boron in dryland and semi-arid soils is an important issue that strongly affects growing and developing crops, especially under drought stress. Meanwhile, brassinosteroid (BR) as a noval stress hormone can improve resistance to abiotic stress in plants. To explore the appropriate foliar application of boron (0.5 and 1 %) and 24-Epi-brassinolide (0.5 and 1 μM) and their combinations on camelina (*Camelina sativa* L.) under irrigation regimes a field experiment was conducted during 2018–2020 years. Irrigation regime consisted of well irrigation from emergence until the end of the growing season (WI_0), withholding irrigation from flowering to silique formation (WI_1), and withholding irrigation from silique formation to harvest (WI_2)]. Our finding revealed that boron had a destructive effect on phytochemical parameters of camelina while application of brassinolide mitigated the boron impacts and improved the parameters. Results showed the highest increase in chlorophyll *a* (26.8 and 23.8 %) at $B_{0.5} + BR_{0.5}$ treatments under WI_1 and WI_2 conditions, respectively. Application of BR (0.5 and 1 μM) and low level of B ($B_{0.5\ \%}$) combination alleviated drought stress by improving osmolyte accumulation (proline) (5–9% increase), antioxidant enzymes, superoxide dismutase (SOD) (5.35–5.72 % increase), catalase (CAT) capacity (4.10 % increase) and secondary metabolites (total phenol and flavonoid) (6.78–10.26 % and 4.60–5.27 % increase). Further, malondealdehide (MDA) decreased (8.73 %) at BR and B combination and increased with a high level of B ($B_{1\%}$) application under-withholding irrigation. All of these results confirmed that BR and B synergistically (mainly $B_{0.5} + BR_{0.5}$ and $B_{0.5} + BR_1$) regulate the phytochemical properties response in the camelina plant to drought stress.

1. Introduction

Camelina (*Camelina sativa* L.), a Brassicaceae member, as an adaptable plant, can replace other oilseed crops in water shortage conditions [1,2]. Among oilseed crops, camelina has been reported as a high tolerant crop to drought conditions [3–5]. So It can growth to different stressful conditions [6–8] especially in water limited areas [9,10]. Camelina performs well on poor soils [8,11–13] and drier climates [6,14–17].

Although many studies reported that camelina has high efficiency in using of ground water [2,12,18], water restriction is a major

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environmental factor that limited crop productivity in arid and semi-arid regions [19–22]. Water deficit stress can directly affect oxidative defense system, physiological and biochemical activities including photosynthetic efficiency and secondary (bioactive) compounds [23–26]. Also, under stressful environments, Reactive oxidative species (ROS), increased and damage cell membranes and vital components of the cell, such as proteins, lipids, carbohydrates and nucleic acids, [27,28]. However, In plants, the antioxidant defense system can be reduced and remove ROS with enzymatic (including superoxide dismutase (SOD), catalase (CAT), peroxidase (POD)) and non-enzymatic (contains certain secondary metabolites such as total phenolic (TPC) and flavonoids (TFC) components) [29,30]. Additionally, to improve the water absorption from the environment, plants produce osmolytes substances such as proline, soluble protein and carbohydrate [31,32]. Based on previous research The growth, yield, oil content and physicochemical properties of camelina are affected by drought stress [33–36].

Boron is an necessary micronutrient for crop production that plants require in extremely small concentrations [37,38]. However, the *Brassicaceae* family have a high consumption of boron [39,40]. For example *Brassica napus*, a member of the Camelina family, is highly sensitive to B deficiency conditions [41]. It also is essential to plant development in which it participates in carbohydrate and phenol metabolism [42], water relations [43], cell wall integrity [44–46]. In addition, The problem of boron deficiency is more critical than other micronutrients [47]. Nevertheless, the excessive accumulation of B limits plant growth and productivity in areas with excessive dryness [48,49]. It is proven that excess of B is frequent in the drylands and semi-arids zones [50,51]. The main reason is high evaporation and transpiration and very limited washing in these regeons [52,53]. Recent reports have indicated that during co-combination of water deficiency and boron excess in the soil, devastating effects of drought stress can have an noticeable influence on the effects of B toxicity on plants [52–54]. However, different evidences show that climate change will intensify the stress of drought and the expansion of deserts in arid and semi-arid regions, and this will lead to the aggravation of soils with boron toxicity, which will ultimately lead to the vulnerability of plants and reduce their production [49,53].

Brassinosteroids (BRs) are a new class of phytohormones whose importance in many plant processes and reducing the effects of various abiotic stresses has been well proven [55–59]. BR influences photosynthesis [60], photomorphogenesis, cell elongation, seed germination, and xylem differentiation [58]. Numerous studies have proven the role of BR in drought resistance in higher plants [58, 61,62]. BR participated in many metabolic processes of plants including chlorophyll function [63] and antioxidant metabolism [64]. Therefore it is concluded that BR could increase the plant tolerance to drought, salt and nutrient toxicity, via the enhancement of antioxidant defense enzymes and accumulation of important osmolytes [65–68]. Recent reports documented that BR affects nutrient availability by regulating root growth and development during periods of low nutrient availability [69,70]. Also, phytohormones are effective in plant reaction to B availability (deficiency and excess) [71]. In this regard, interactions between BR and B deficiency in Arabidopsis root growth have been recorded. It may be due to the effect of B deficiency on the reduction of bioactive BRs and then the suppression of BR effect on cell division and development and subsequent root growth [70].

Numerous studies on plants of the Camelina family well reflect the effect of brassinosteroids in reducing drought stress through biochemical and enzymatic changes. Brassinosteroid and jasmonic acid, individually and in combination, had a significant effect on the photosynthetic system and antioxidant defense system, increasing resistance and improving the growth of *Brassica rapa* under drought stress conditions [72]. In another report, foliar application of epibrassinolide (EBL) reduces the destructive effects of drought stress in *Brassica napus* by reducing membrane damage, improving yield, and increasing antioxidant capacity [73].

The existing reports indicating the effective role of the plant growth-promoting (brassinosteroids) in modulating the damage caused by non-biotic stresses. Currently, no comprehensive and targeted study has been reported on the combined use of brassinosteroid*boron on physiological behavior of the relatively new crop, Camelina, under drought stress conditions. The main hypothesis of our study was that the combined application of brassinosteroid and boron would be an agronomic tool to reduce the negative effects of drought by increasing physiological and biochemical traits in camalina. Therefore, what motivated the authors to conduct this study was to determine which of the brassinosteroid and boron treatments could reduce the negative effects of water deficit on biochemical parameters, photosynthetic pigments, and membrane lipid peroxidation.

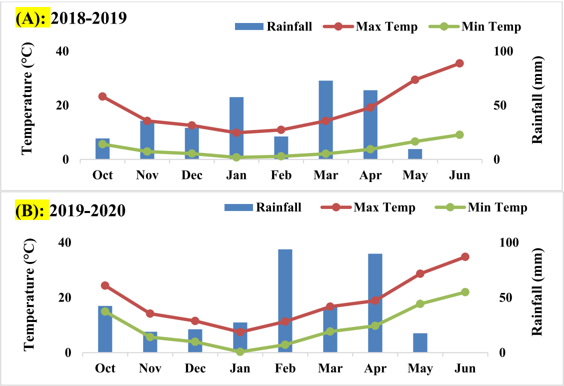

Fig. 1. Maximum, minimum temperatures (°C) and rainfall (mm) during the 2018–2019 (A) and 2019–2020 (B) years at the experimental site.

2. Materials and methods

2.1. Site information

This study was conducted during the 2018 to 2020 growing seasons at research field of Tarbiat Modares University, Iran, NV (35° 44' N, 51° 09' E and 1265 masl). The climate type of the site according to the Köppen is arid to semi-arid with low rainfall distribution during late autumn and early spring [Fig. 1(A and B)]. Textural class of the soil (0–30 cm depth) was silty loam (pH = 7.5) and soil available B concentration was 0.50 ppm.

2.2. Experimental arrangement and treatments

The experiment was laid out as a randomized complete block design (RCBD) with split-plot arrangement of treatments in each year (three replications). Irrigation regimes (IR) were assigned to the main plots, including: (WI_0) [well irrigation (control)], (WI_1) withholding irrigation from full flowering (growth stage BBCH: 65) to silique formation (growth stage BBCH: 73), and (WI_2) withholding irrigation from silique formation to one week prior to harvesting (growth stage BBCH: 89). Also the foliar spray were divided into ten treatment groups: (1) non sprayed (control); (2) distilled water; (3) boron 5 %; (4) boron 1 %; (5) BR 0.5 μM; (6) BR 1 μM; (7) B 0.5 % + BL 0.5 μM; (8) B 0.5 % + BR 1 μM; (9) B 1 % + BR 0.5 μM; and (10) B 1 % + BR 1 μM were the sub-plot factor.

2.3. Cultural practices and treatments application

Winter camelina (*Camelina sativa* L.) seeds (Soheil cultivar) were obtained from Biseton Shafa Co, Kermanshah Province, Iran. Nitrogen (urea) and Phosphorus (triple superphosphate) were applied based on soil tests at a rate of 50 kg ha^{-1} and 30 kg ha^{-1}, respectively before sowing. The seeds were sown on November 1, 2018 and November 3, 2019 at a rate of 6.5 kg ha^{-1}, with spacing of 20 cm and depth of 1.0 cm. Each plot consisted of six planting lines with a length of 4.2 m and 20 cm row space. Also 0.5 m and 1.5 m distance was kept between plots and blocks, respectively, for lateral water movement and other interferences.

Drought stress was applied by withholding water and irrigation intervals were adjusted based on 50 and 70 % of available soil water for control and stressed plots. This means that for stress plants, all plots were watered completely before the beginning drought stress, then water was withheld from the two different growth stages (flowering and seed filling stage) until soil water potential became 70 % of available soil water (ASW) and continued throughout the ending stages. The control plots were irrigated at the same time to 100 % field capacity (FC) after depleting 50 % of available soil water. TDR (Time Domain Reflectometry) was used to monitor soil moisture. According to Mokhtassi-Bidgoli et al. [74], irrigation scheduling was based on maximum allowable depletion (MAD) over 30 cm soil depth Eq. (1) and the required volume of water was calculated according to Eqs. (2) and (3)

$$MAD = (FC - \theta / FC - PWP) \qquad \text{Eq. (1)}$$

$$ASW = FC - PWP \qquad \text{Eq. (2)}$$

$$Vd = MAD \times ASW \times Rs \times 10 \qquad \text{Eq. (3)}$$

The solutions of BR were firstly dissolved with ethanol, then the volume was made by using deionized water in a volumetric flask. Boron as H_3BO_3 (Merck, Germany) at 0.5 and 1 %, was used and mixed in distilled water. Solutions were applied as a foliar application to the camelina plants at different concentrations of each compound separately and mixed with surfactant (0.1 % Tween-20). Foliar applications were conducted two times with an interval of 24 h, since the last sufficient irrigation day at the two growth stages (flowering and seed filling). Each plot was uniformly sprayed with a respective solution (Cone type nozzle under operating pressure of 0.2–0.3 MPa).

2.4. Pigments assay

For the determination of Chl a, b and carotenoids concentrations, Leaves sample (0.5 g) were extracted in 5 ml acetone (80 %) solution, and after centrifugation, pigments were determined by absorption at 663, 645 and 470 nm by spectrophotometer following the method of Arnon [75].

2.5. Antioxidant enzymes activity

The terminal leaves on several stems from each plant were harvested and frozen in liquid nitrogen for all analyses. Protein assay according to the Bradford [76] method and using bovine serum albumin (BSA) as a standard. The enzyme activities were measured using a Spectrophotometric (Specord 200, Analytical Jena, Germany) Method. For determination of SOD activity (unit mg^{-1} protein) after prepared of reaction, mixture contained extraction buffer, EDTA (0.1 mM), Na_2CO_3 (50 mM, pH 10.2), L-methionine (12 mM), nitroblue tetrazolium chloride (NBT) (75 μM), enzyme extract (300 μL), and riboflavin (1 μM) and extracted enzyme. The activity of SOD was assayed by monitoring the inhibition of photochemical reduction of NBT at 560 nm [77]. For catalase (CAT) activity, samples of camelina leave thawed in 3 ml of K-phosphate buffer (25 mM, pH 6.8). then, it was measured as a decrease of absorbance at 240 nm due to H_2O_2 consumption, against enzyme units mg^{-1} protein [78]. The peroxidase (POD) activity was assayed by using the method of

[78]. In brief, the assay reaction mixture (3 mL) was composed of sodium acetate buffer (50 mM with pH 5.0), H2O2 (40 mM), guaiacol (20 mM), and crude extract. The activity was expressed as changes in the absorbance at 470 nm against enzyme unit mg^{-1} protein [78]. Eventually, Nakano and Asada [79] method was used to measure Ascorbate peroxidase (APX) activity. In brief, the homogenate was containing leaves samples homogenized in Na-phosphate buffer (1 mL of 50 mM, pH 7.8) and then placed in a centrifuge. This reaction mixture contains crud extract, Na-phosphate buffer (50 mM) and H_2O_2 (44 μM). Finaly, the activity was measured by recording the decrease in absorbance at 290 nm for 1 min.

2.6. Proline measurement

Proline was estimated according to assay Bates et al. [80]. The leaves sample were dissolved with 3 ml 3 % aqueous sulfosalicylic acid. The mixture was centrifuged, then ninhydrin acid and glacial acetic acid (2:2) were added to 2 ml extraction and was placed for a 1-h at 100 °C. after cooled extraction, 4 ml toluene was added. A spectrophotometer recorded absorbance at 520 nm.

2.7. Total phenolic, flavonoid and anthocyanin content

Preparation of extracts for TPC assay leaves sample were homogenized in 10 mL acetone (80 %). The supernatant extracts were mixed with 100 μL of Folin-Ciocalteu reagent, then 300 μL sodium carbonate was added to the solution then ancobated for half-hour at 40 °C, the absorbance calculated with 765 nm against the blank. Total phenol was determined by a standard curve based on the solution of mg Gallic acid per gram of dry weight 82].

For TFC analysis leaves were homogenized in methanol (80 %). The supernatant extracts were added to sodium nitrite. Then, 0.5 mL extract was mixed with AlCl3 and followed by 2 % ethanol and centrifuged. the absorbance was measured at 420 nm. Total flavonoid content was calculated by a standard curve based on the solution of mg Quercetin per gram of dry weight [81].

For anthocyanin determination, 0.5 g of leaves sample were extracted in methanol: hydrochloric acid (99:1, v/v) and then centrifuged at 12000×*g* for 15 min. The supernatant was kept overnight in the dark condition, and finally anthocyanin content was calculated at 550 nm using an extinction coefficient of 33000 M^{-1} cm^{-1} [82].

2.8. Membrane lipid peroxidation assay

The measurement of malondialdehyde (MDA) was considered as a measure to determine of membrane lipid peroxidation. leaves sample (0.2 g) were homogenized in 10 % trichloroacetic acid (TCA) and centrifuged (at 10,000×*g* for 15 min) and filtration, the supernatant was added to 0.5 % thiobarbituric acid (TBA) and incubated in a 100 °C water bath for 30 min. Then it was placed in an ice-water bath. Finaly, To determine the MDA used from the absorbance at 532 nm followed by correction for the non-specific absorbance at 600 nm and calculated from the extinction coefficient 155 $mM^{-1}cm^{-1}$ [83].

2.9. Data processing

All data (mean ± standard error) were analyzed by SAS 9.4 software for windows. The brown and Forsythe's test indicated that the variance was homogeneity among different years. The differences between the mean of main and interactions values obtained using different treatments were compared using the Least Significant Difference (LSD) test ($p < 0.05$) and General Linear Model (GLM) procedure, respectively.

3. Results

3.1. Pigments

Photosynthetic pigments (Chl a, Chl b and Total Chl) were significantly affected by the interaction of IR, FS (Table 1); and 13.7, 11.2 and 13.0 % were lower in 2018-19 than in 2019-20 in this study (Table 2). Under withholding irrigation, the combination of lower B ($B_{0.5}$) plus BR (0.5 and 1) increased Chl a, Chl b and Total Chl (26.8, 8.1 and 17.3 %) at flowering (WI_1) and (23.8, 6.7 and 15.3 %) at seed filling stage (WI_2) compared to the control, respectively (Table 3). Carotenoid content was 28 % higher in the 2018-19 than in the 2019-20 (Table 2). At withholding irrigation conditions, the Car content increased (58 % at WI_1) significantly compared with control. Further, the maximum of Car was observed at the combination of B plus BR (in particular $B_{0.5} + BR_1$) (Table 4).

3.2. Proline content

Three way interaction (IR × FS × Y) for proline content was significant (Table 1). In both years, the lowest and highest proline concentrations were obtained under normal and withholding irrigation at flowering stage conditions, respectively [Fig. 2(A and B)]. In both years, under withholding irrigation at (B: WI_1), the highest accumulation of proline in leaves was obtained with the combined application of $B_{0.5} + BR_{0.5}$ by about 5–9 %. In addition, in the absence of irrigation during the seed filling stage (C: WI_2) in the first year, combined application of $B_{0.5} + BR_{0.5}$, $B_{0.5} + BR_1$ and BR_1μM had higher proline content than other foliar application, while in the second year, combined application of $B_{0.5} + BR_{0.5}$ had remarkable improvement on proline accumulation compared with other treatments, in the same conditions (Fig. 2C).

Table 1

Analysis of variance (*F* values) on phytochemical properties of camelina affected by irrigation regime and foliar spray during the 2018-19 and 2019-20 growing seasons.

Effect	df	Chl a[a]	Chl b	Total Chl	Car	Pro	Ant	TPC	TFC	SOD	CAT	POD	APX	MDA
Year (Y)	1	1.60**	0.64**	4.3**	7.28**	4.11**	5.05**	0.01^{ns}	0.06^{ns}	0.06^{ns}	2.40**	0.28^{ns}	0.002^{ns}	0.4^{ns}
IR	2	8.04**	1.60**	16.72**	5.00**	407.72**	0.77**	11.56**	3.55**	511.67**	607.86**	76.67**	47.90**	63.55**
IR × Y	2	0.05^{ns}	0.03^{ns}	0.05^{ns}	0.02^{ns}	1.08**	0.005^{ns}	0.07^{ns}	0.001^{ns}	0.002^{ns}	0.001^{ns}	0.02^{ns}	0.07^{ns}	0.02^{ns}
FS	9	0.35**	0.10**	0.75**	0.41**	1.70**	0.007*	2.24**	0.34**	1.46**	0.50**	0.37**	0.35**	0.24**
IR × FS	18	0.04**	0.03*	0.12**	0.06^{ns}	0.28**	0.001^{ns}	0.22**	0.03^{ns}	0.48**	0.43**	0.01^{ns}	0.04^{ns}	0.10**
FS × Y	9	0.04^{ns}	0.08^{ns}	0.01^{ns}	0.05^{ns}	0.03^{ns}	0.001^{ns}	0.08^{ns}	0.006^{ns}	0.003^{ns}	0.001^{ns}	0.02^{ns}	0.001^{ns}	0.009^{ns}
IR × FS × Y	18	0.03^{ns}	0.03^{ns}	0.08^{ns}	0.01^{ns}	0.17*	0.0003^{ns}	0.18*	0.002^{ns}	0.003^{ns}	0.002^{ns}	0.01^{ns}	0.009^{ns}	0.004^{ns}
CV (%)		9.18	11.52	7.69	16.15	2.34	2.13	2.66	3.73	1.34	2.02	4.20	5.85	6.82

*, significant at $P < 0.05$.

**, significant at $P < 0.01$.

† IR = irrigation regime, FS = foliar spray.

[a] Chl a = chlorophyll *a*, Chl b = chlorophyll *b*, Car = carotenoid, Pro = proline, Ant = Anthocyanin, TPC = total phenol content, TFC = total flavonoid content, SOD = super oxide dismutase, CAT = catalase, POD = peroxidase, APX = ascorbate peroxidase, MDA = malonyldealdehyde. ns, not significant at $P \geq 0.05$.

Table 2
Main effects of the year on Chl a, Ch b, Total Chl, Car, Ant and CAT of camelina during 2018-19 and 2019-20 growing seasons.

treat	year	
	2018–19	2019–20
Chl a	1.31 ± 0.02^{b}	1.49 ± 0.03^{a}
Chl b	1.07 ± 0.02^{b}	1.19 ± 0.03^{a}
Total Chl	2.38 ± 0.05^{b}	2.69 ± 0.05^{a}
Car	1.41 ± 0.10^{a}	1.01 ± 0.08^{b}
Ant	2.42 ± 0.04^{b}	2.76 ± 0.05^{a}
CAT	13.00 ± 0.05^{a}	12.76 ± 0.04^{b}

Values (means ± standard error) with the same letter are not significantly different according to LSD ($P < 0.05$).

Table 3
A two-way interaction effect of irrigation regime (I) and foliar spray (F) on chlorophyll pigments (Chl a, Chl b and Total Chl), Superoxide dismutase (SOD), Catalase (CAT) activities and Malondialdehyde (MDA) content of camelina.

I × F	Chl a (mg/g FW)	Chl b (mg/g FW)	Toltal Chl (mg/g FW)	SOD (U mg/protein)	CAT(ΔA 240/mg protein)	MDA (μM MDA/gFW)
I_1F_1	1.51 ± 0.04^{d}	$1.24 \pm 0.04^{b\text{-}e}$	2.76 ± 0.08^{d}	11.29 ± 0.07^{mn}	8.94 ± 0.07^{gh}	0.81 ± 0.01^{h}
I_1F_2	1.51 ± 0.05^{d}	$1.25 \pm 0.04^{a\text{-}e}$	2.77 ± 0.08^{d}	11.28 ± 0.06^{n}	8.93 ± 0.06^{gh}	0.81 ± 0.01^{h}
I_1F_3	1.85 ± 0.05^{bc}	$1.29 \pm 0.06^{a\text{-}c}$	3.14 ± 0.10^{bc}	$11.46 \pm 0.06^{k\text{-}n}$	8.96 ± 0.07^{gh}	0.77 ± 0.01^{h}
I_1F_4	1.75 ± 0.09^{c}	$1.29 \pm 0.14^{a\text{-}c}$	3.04 ± 0.14^{c}	$11.51 \pm 0.09^{j\text{-}m}$	$9.64 \pm 0.08f$	0.78 ± 0.01^{h}
I_1F_5	1.85 ± 0.06^{bc}	$1.28 \pm 0.04^{a\text{-}d}$	3.13 ± 0.09^{bc}	$11.55 \pm 0.06^{j\text{-}l}$	8.77 ± 0.07^{h}	0.80 ± 0.01^{h}
I_1F_6	1.86 ± 0.05^{bc}	1.33 ± 0.06^{ab}	3.20 ± 0.10^{bc}	11.60 ± 0.06^{jk}	8.82 ± 0.07^{h}	0.79 ± 0.03^{h}
I_1F_7	2.06 ± 0.04^{a}	1.40 ± 0.07^{a}	3.46 ± 0.09^{a}	$11.37 \pm 0.06^{l\text{-}n}$	9.20 ± 0.07^{g}	0.77 ± 0.01^{h}
I_1F_8	1.97 ± 0.05^{ab}	$1.35 \pm 0.05^{a\text{-}b}$	3.32 ± 0.09^{ab}	$11.38 \pm 0.06^{k\text{-}n}$	9.21 ± 0.10^{g}	0.78 ± 0.00^{h}
I_1F_9	1.84 ± 0.05^{bc}	$1.27 \pm 0.04^{a\text{-}d}$	3.11 ± 0.07^{bc}	11.70 ± 0.06^{j}	9.79 ± 0.10^{f}	0.80 ± 0.02^{h}
I_1F_{10}	1.83 ± 0.07^{bc}	$1.28 \pm 0.06^{a\text{-}d}$	3.11 ± 0.13^{bc}	11.93 ± 0.06^{i}	9.83 ± 0.09^{f}	0.80 ± 0.02^{h}
I_2F_1	$1.19 \pm 0.06^{f\text{-}h}$	1.11 ± 0.04^{ef}	2.31 ± 0.09^{fg}	16.24 ± 0.08^{ef}	14.60 ± 0.08^{de}	$2.52 \pm 0.04^{c\text{-}e}$
I_2F_2	1.20 ± 0.06^{fg}	1.11 ± 0.05^{ef}	2.31 ± 0.11^{fg}	16.24 ± 0.07^{ef}	14.60 ± 0.06^{de}	$2.53 \pm 0.05^{c\text{-}e}$
I_2F_3	1.27 ± 0.05^{ef}	$1.13 \pm 0.04^{d\text{-}f}$	2.40 ± 0.10^{fg}	16.54 ± 0.06^{d}	$14.69 \pm 0.08^{c\text{-}e}$	$2.50 \pm 0.04^{d\text{-}f}$
I_2F_4	$1.06 \pm 0.04^{g\text{-}j}$	$0.95 \pm 0.06^{g\text{-}i}$	$2.01 \pm 0.10^{i\text{-}k}$	$16.20 \pm 0.06^{e\text{-}g}$	14.56 ± 0.10^{de}	2.60 ± 0.16^{cd}
I_2F_5	1.39 ± 0.05^{de}	$1.13 \pm 0.04^{d\text{-}f}$	2.52 ± 0.07^{ef}	17.11 ± 0.08^{a}	15.03 ± 0.12^{ab}	2.36 ± 0.04^{fg}
I_2F_6	1.49 ± 0.07^{d}	$1.16 \pm 0.04^{b\text{-}f}$	2.65 ± 0.07^{de}	17.18 ± 0.09^{a}	15.07 ± 0.11^{a}	2.36 ± 0.04^{fg}
I_2F_7	1.51 ± 0.08^{d}	$1.20 \pm 0.04^{c\text{-}f}$	2.71 ± 0.12^{de}	17.07 ± 0.06^{ab}	15.09 ± 0.07^{a}	2.31 ± 0.05^{g}
I_2F_8	1.47 ± 0.05^{d}	$1.17 \pm 0.05^{c\text{-}f}$	2.65 ± 0.07^{de}	17.09 ± 0.06^{ab}	15.20 ± 0.13^{a}	2.30 ± 0.05^{g}
I_2F_9	1.26 ± 0.06^{ef}	1.09 ± 0.04^{fg}	2.35 ± 0.07^{fg}	16.87 ± 0.10^{bc}	$14.93 \pm 0.19^{a\text{-}c}$	$2.42 \pm 0.07^{e\text{-}g}$
I_2F_{10}	1.30 ± 0.06^{ef}	1.11 ± 0.04^{ef}	2.42 ± 0.11^{fg}	16.87 ± 0.12^{bc}	$14.99 \pm 0.20^{a\text{-}c}$	$2.42 \pm 0.06^{e\text{-}g}$
I_3F_1	$1.05 \pm 0.05^{h\text{-}j}$	$1.03 \pm 0.05^{f\text{-}h}$	$2.08 \pm 0.09^{h\text{-}j}$	16.01 ± 0.08^{gh}	14.49 ± 0.07^{de}	2.90 ± 0.04^{b}
I_3F_2	1.04 ± 0.04^{ij}	$1.03 \pm 0.05^{f\text{-}h}$	$2.08 \pm 0.09^{h\text{-}j}$	$16.02 \pm 0.07^{f\text{-}h}$	14.48 ± 0.07^{de}	2.90 ± 0.06^{b}
I_3F_3	0.95 ± 0.05^{jk}	0.84 ± 0.05^{i}	1.80 ± 0.08^{k}	16.32 ± 0.15^{h}	14.52 ± 0.12^{de}	2.94 ± 0.01^{ab}
I_3F_4	0.82 ± 0.08^{k}	0.67 ± 0.06^{j}	1.50 ± 0.14^{l}	15.95 ± 0.05^{h}	14.45 ± 0.15^{e}	3.07 ± 0.05^{a}
I_3F_5	$1.18 \pm 0.14^{f\text{-}i}$	$1.05 \pm 0.04^{f\text{-}h}$	2.24 ± 0.18^{gh}	17.03 ± 0.06^{ab}	14.58 ± 0.11^{de}	$2.45 \pm 0.04^{d\text{-}g}$
I_3F_6	$1.18 \pm 0.08^{f\text{-}i}$	$1.04 \pm 0.07^{c\text{-}f}$	$2.23 \pm 0.15^{g\text{-}i}$	17.15 ± 0.06^{a}	14.62 ± 0.16^{de}	$2.43 \pm 0.05^{e\text{-}g}$
I_3F_7	1.30 ± 0.09^{ef}	$1.10 \pm 0.06^{a\text{-}e}$	2.40 ± 0.10^{fg}	16.58 ± 0.14^{d}	$14.77 \pm 0.08^{b\text{-}d}$	$2.43 \pm 0.00^{e\text{-}g}$
I_3F_8	1.26 ± 0.05^{ef}	1.07 ± 0.07^{fg}	2.33 ± 0.10^{fg}	16.65 ± 0.12^{cd}	14.62 ± 0.24^{de}	$2.45 \pm 0.02^{d\text{-}g}$
I_3F_9	1.02 ± 0.06^{j}	0.91 ± 0.04^{hi}	1.93 ± 0.08^{jk}	16.01 ± 0.04^{gh}	14.52 ± 0.09^{de}	2.67 ± 0.05^{c}
I_3F_{10}	$1.06 \pm 0.09^{g\text{-}j}$	0.91 ± 0.04^{hi}	1.98 ± 0.11^{jk}	15.97 ± 0.03^{h}	14.53 ± 0.10^{de}	2.67 ± 0.07^{c}

I_1 = well irrigation from emergence until the end of growing season, I_2 = withholding irrigation from flowering to silique formation, and I_2 = withholding irrigation from silique formation to harvest; F_1 = non-sprayed, F_2 = distilled water, F_3= $B_{0.5\ \%}$, F_4= $B_{1\ \%}$, F_5= $BR_{0.5\ \mu M}$, F_6= $BR_{1\ \mu M}$, F_7= $B_{0.5\ \%}$ + $BR_{0.5\ \mu M}$, F_8=$B_{0.5\ \%}$ + $BR_{1\ \mu M}$, F_9= $B_{1\ \%}$ + $BR_{0.5 \mu M}$, F_{10}= $B_{1\ \%}$+ $BR_{0.5 \mu M}$.
Values (means ± standard error) with the same letter are not significantly different according to LSMEANS multiple comparison procedure ($P < 0.05$).

3.3. Total phenol, flavonoid and anthocyanin

The content of total phenol was affected by a tree way interaction of IR × FS × Y (Table 1). Under normal irrigation, combination application of B and BR ($B_{0.5}$+$BR_{0.5}$, $B_{0.5}$+BR_1, B_1+$BR_{0.5}$ and B_1+BR_1) and BR single applications ($BR_{0.5}$ and BR_1) increased significantly total phenol content [Fig. 3 (A)]. Under WI_1, application of $B_{0.5}$+$BR_{0.5}$ and $B_{0.5}$+BR_1 had the greatest effect (6.78–10.26 % increase) on the accumulation of phenol in camellia leaves in both years and In the 2018-19 it was more than the 2019-20 [Fig. 3 (B)]. Also, under WI_2 in the first year, the highest amount of total phenol was recorded using $B_{0.5}$+$BR_{0.5}$, $B_{0.5}$+BR_1 and BR_1. However, in the second year of application of $B_{0.5}$+$BR_{0.5}$ and $BR_{0.5}$ had the greatest effect on increasing total phenol [(Fig. 3 (C)].

The TFC in leaves of camelina was affected by IR and FS and increased (by ≈ 5–6 %) significantly under-withholding irrigation conditions (Tables 1 and 4). Among foliar application treatments, $B_{0.5}$ + $BR_{0.5}$ and $B_{0.5}$ + BR_1 application had the highest significant effect on flavonoid content that was 4.60–5.27 % higher compared to the control. Further, the B_1 application did not differ in the TFC

Table 4

Effect of irrigation regime (IR) and foliar spray (FS) treatments on carotenoid (Car), anthocyanin (Anth), total flavonoid content (TFC), peroxidase activity (POD) and ascorbate peroxidase (APX) of camelina.

	Car (mg/g FW)	Anth (mg/g FW)	TFC (mg/g DW)	POD (ΔA 470/mg protein)	APX (ΔA 290/mg protein)
Irrigation regime					
W_0	0.87 ± 0.03^{b}	2.52 ± 0.02^{b}	7.28 ± 0.03^{b}	5.61 ± 0.03^{b}	3.88 ± 0.03^{c}
W_1	1.37 ± 0.04^{a}	2.72 ± 0.02^{a}	7.73 ± 0.03^{a}	7.65 ± 0.03^{a}	5.60 ± 0.03^{a}
W_2	1.38 ± 0.04^{a}	2.53 ± 0.02^{b}	7.67 ± 0.04^{a}	7.48 ± 0.04^{a}	5.13 ± 0.04^{b}
Foliar spray					
control	1.01 ± 0.08^{e}	2.57 ± 0.04^{bc}	7.39 ± 0.07^{d}	6.76 ± 0.22^{de}	4.70 ± 0.18^{f}
dw	1.04 ± 0.07^{e}	2.57 ± 0.04^{bc}	7.39 ± 0.06^{d}	6.76 ± 0.22^{de}	4.71 ± 0.18^{ef}
$B_{0.5}$	1.14 ± 0.09^{cd}	2.60 ± 0.04^{ab}	7.50 ± 0.09^{cd}	$6.81 \pm 0.23^{c\text{-}e}$	$4.82 \pm 0.18^{c\text{-}f}$
B_1	1.05 ± 0.06^{de}	2.56 ± 0.04^{bc}	7.38 ± 0.04^{d}	6.72 ± 0.22^{e}	$4.71 \pm 0.15^{d\text{-}f}$
$BR_{0.5}$	1.28 ± 0.09^{b}	2.61 ± 0.04^{a}	$7.61 \pm 0.10^{a\text{-}c}$	$7.09 \pm 0.24^{a\text{-}b}$	$4.93 \pm 0.21^{a\text{-}c}$
BR_1	1.27 ± 0.10^{b}	2.62 ± 0.05^{a}	$7.62 \pm 0.08^{a\text{-}c}$	7.11 ± 0.24^{a}	$4.89 \pm 0.19^{b\text{-}e}$
$B_{0.5}$+$BR_{0.5}$	1.41 ± 0.09^{a}	$2.60 \pm 0.04^{a\text{-}c}$	7.73 ± 0.08^{ab}	$7.00 \pm 0.23^{a\text{-}c}$	5.06 ± 0.19^{ab}
$B_{0.5}$+BR_1	1.44 ± 0.09^{a}	$2.59 \pm 0.04^{a\text{-}c}$	7.78 ± 0.08^{a}	7.04 ± 0.23^{ab}	5.11 ± 0.20^{a}
B_1+$BR_{0.5}$	1.20 ± 0.08^{bc}	2.60 ± 0.04^{ab}	$7.55 \pm 0.05^{b\text{-}d}$	$6.90 \pm 0.21^{b\text{-}e}$	$4.90 \pm 0.17^{b\text{-}d}$
B_1+$BR_{0.5}$	1.23 ± 0.08^{bc}	2.60 ± 0.04^{ab}	$7.62 \pm 0.08^{a\text{-}c}$	$6.93 \pm 0.23^{a\text{-}d}$	$4.87 \pm 0.16^{b\text{-}f}$

WI_0 = well irrigation from emergence until the end of growing season, WI_1 = withholding irrigation from flowering to silique formation, and WI_2 = withholding irrigation from silique formation to harvest.

Values (means ± standard error) with the same letter are not significantly different according to LSD ($P < 0.05$).

compared with other foliar application treatments and no was significant differences with the control (Table 4).

Anthocyanin accumulation was influenced by IR, FS and Y in this study, and also was smaller 14 % in 2018-19 than in 2019-20 (Tables 1 and 2). Water stress at the flowering stage (WI_1), increased significantly (about 8 %) anthocyanin content compared to the well irrigation (WI_0). Among BR and B application, the highest and lowest anthocyanin content was obtained at BR_1 and B_1 single application, respectively (Table 4).

3.4. *Antioxidant enzymes activity*

IR and FS interactions have a significant influence on the amount of SOD and CAT activity and also there was the main effect of years on CAT activity. As well as, POD and APX activity was affected by only the main effects of IR and FS in this study (Table 1). CAT activity was higher (by ≈ 2 %) in 2018-19 than in 2019-20. Under well irrigation (WI_0), the lowest SOD and CAT activity were observed at foliar application treatments in this study. However, BR single application ($BR_{0.5}$ and BR_1) and combination with B (B_1 + $BR_{0.5}$ and B_1 + BR_1) and also B_1, B_1+$BR_{0.5}$ and B_1+ BR_1 were shown to have similar effects in increasing on SOD and CAT activity in such conditions. Further, at WI_1, foliar application of single BR (0.5 and 1 μM) and $BR_{0.5}$ and $B_{0.5}$ increased SOD (5.3–5.7 %) and CAT (4.10 %) activity compared to the control, respectively. But single B (B_1) application decreased the activity of SOD and CAT compared to the control in such conditions. Under withholding irrigation at seed filling stage (WI_2), the highest SOD activity was recorded for BR (1 and 0.5 μM) application while, under such conditions, no significant differences were detected in CAT among foliar application (Table 3). Finally, withholding irrigation at WI_1 and WI_2 increased the POD and APX activity by 33–36 % and 32–44 % compared to the normal irrigation, respectively. In addition, a single application of $BR_{0.5}$ and a combined application of $B_{0.5}$ + BR_1 had more positive effects on POD and APX activity compared with control, respectively while, B_1 single application showed a negative effect on POD and APX activity compared to other foliar application treatments (Table 4).

3.5. *Malondialdehyde (MDA) content*

Irrigation regimes, foliar spray and their interaction also influenced malondialdehyde (MDA) content in camelina leaves (Table 1). Withholding irrigation at two growing stages significantly increased the MDA value in camelina plants. Further, foliar application mitigated MDA levels under-withholding irrigation conditions. Under well irrigation (WI_0), there were no significant differences among foliar spray treatments. In addition, when plants exposed to water deficiency (WI_1 and WI_2), the highest MDA levels were obtained at B_1 application under WI_2 condition that was 6 % higher compared to untreated-stress plants and there was no significant difference with $B_{0.5}$ treatment. However, at WI_1, a combination of $B_{0.5}$ + BR_1 declined the MDA value by about 8.73 % compared to the other foliar application treatments (Table 3).

4. Discussion

4.1. *Photosynthetic pigments*

Photosynthetic pigments were directly or indirectly influenced by phytohormones and micronutrients status in plant tissue [84,85]. According to our findings B and BR synergistically improved photosynthetic pigments in camelina plants. In contrast, withholding irrigation reduced all photosynthetic pigments except Car content that was increased in drought conditions (Tables 3 and 4). However,

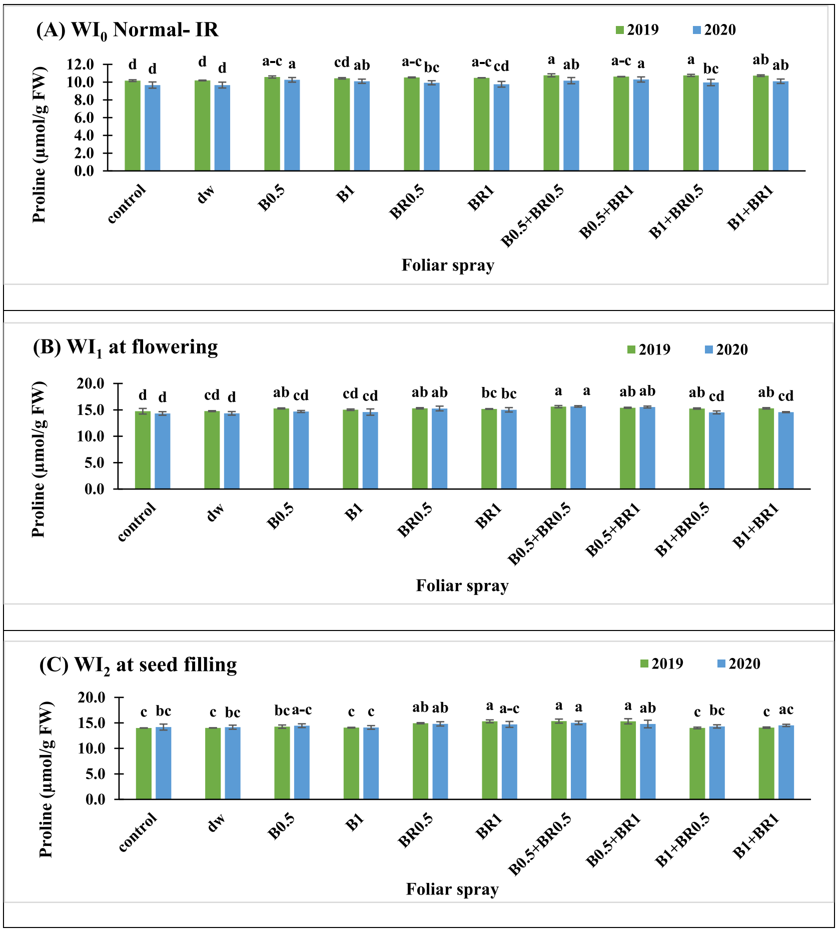

Fig. 2. A three-way interaction effect of irrigation regime (A: WI_0 = well irrigation from emergence until the end of growing season, B: WI_1 = withholding irrigation from flowering to silique formation, and C: WI_2 = withholding irrigation from silique formation to harvest) foliar spray (conrol = non-sprayed, dw = distilled water, $B_{0.5}$ = 0.5 %, B_1 = 1 %, $BR_{0.5}$ = 0.5 µM, BR_1 = 1 µM, $B_{0.5}$+$BR_{0.5}$ = 0.5 % B and 0.5 µM BR, $B_{0.5}$+BR_1 = 0.5 % B and 1 µM BR, B_1+$BR_{0.5}$ = 1 % B and 0.5 µM BR, B_1+$BR_{0.5}$ = 1 % B and 1 µM BR) and year on proline content of camelina during the 2019 and 2020 growing seasons. Values (means ± standard error) with the same letter are not significantly different according to LSMEANS multiple comparison procedure ($P < 0.05$).

BR (0.5 and 1 µM) application in combination with B at a lower dose (B 0.5 %) improved the chlorophyll (Chl a, Chl b and Total Chl) content under-withholding irrigation, while B_1 application resulted in a negative effect on chlorophyll pigments in such conditions. Researches showed that BR as a signaling compound could recovery in chlorophyll contents by promoting modulation of transcription and translation processes which finally improves chlorophyll biosynthesis [86,87]. In addition, it has also been hypothesized that brassinosteroids increase the rate of photosynthesis by affecting processes such as stomatal conductance, which accelerates carbon dioxide uptake. This hypothesis is supported by the studies of Hu et al. [88], who reported that the BR-induced performance in CO_2 uptake under drought conditions was mainly due to stomatal events. Also, Barros Junior et al. [89], studying *Eucalyptus orophylla*, found that BR treatment increased the rate of photosynthesis and stomatal conductance under drought stress conditions. Kolomeichuk et al. [90] described that pretreatment of barley plants with HBL (28-hemobrassinolide) improved the efficiency of photosynthetic pigments by reducing the effects of water deficit. It was also observed that the B treatments ($B_{0.5}$, B_1, B_1 + $BR_{0.5}$ and B_1 + BR_1) had a minimum effect on all photosynthetic pigments (Chl a, Chl b, Total Chl and Car) compared with other foliar applications (Tables 3 and 4). The decrease of this pigments in B_1 concentration may be due to the degradation of chloroplast and conflict in the electron supply chain of two photosystem reaction center (PSI and PSII), that result in the production of the ROS under such conditions [91–93]. Similar to our results, Shah et al. [94] documented that the B excess inhibits chlorophyll and carotenoid contents in the *Citrus sinensis*.

4.2. Proline content

Proline is a known beneficial solute compound, which plays an important role in an osmotic potential under water deficit [95–97]. Further, BR is a phytohormone that synthesizes proline in plant cells under water shortage [98]. It has been demonstrated that BR is responsible for stimulating Δ1-pyrroline-S-Carboxylate synthase, which is the key enzyme of the proline biosynthetic pathway [65].

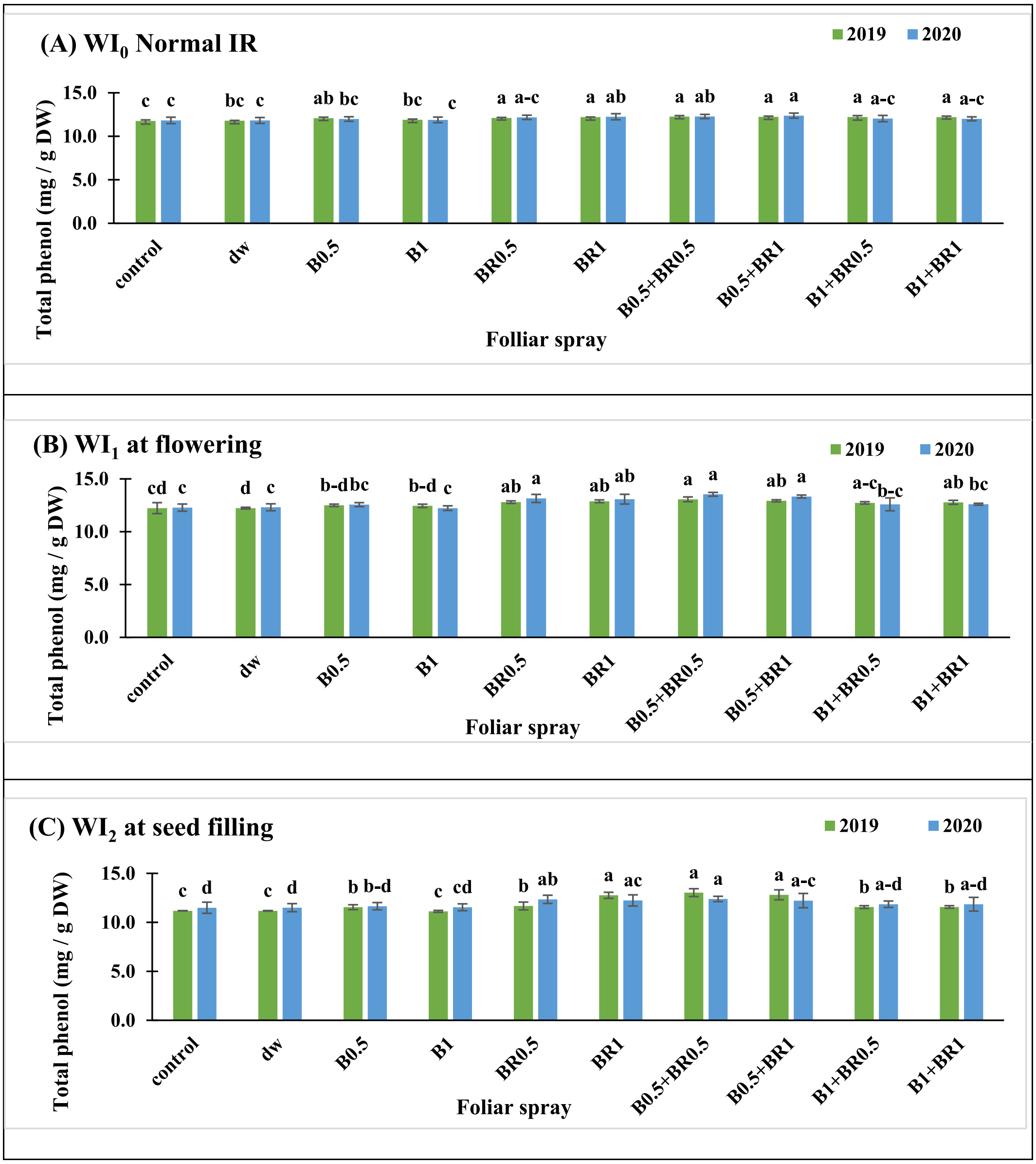

Fig. 3. A three-way interaction effect of irrigation regime (A: WI_0 = well irrigation from emergence until the end of growing season, B: WI_1 = withholding irrigation from flowering to silique formation, and C: WI_2 = withholding irrigation from silique formation to harvest) foliar spray (conrol = non-sprayed, dw = distilled water, $B_{0.5}$ = 0.5 %, B_1 = 1 %, $BR_{0.5}$ = 0.5 μM, BR_1 = 1 μM, $B_{0.5}$+$BR_{0.5}$ = 0.5 % B and 0.5 μM BR, $B_{0.5}$+BR_1 = 0.5 % B and 1 μM BR, B_1+$BR_{0.5}$ = 1 % B and 0.5 μM BR, B_1+$BR_{0.5}$ = 1 % B and 1 μM BR) and year on total phenol content of camelina during the 2019 and 2020 growing seasons. Values (means ± standard error) with the same letter are not significantly different according to LSMEANS multiple comparison procedure ($P < 0.05$).

Accordingly, brassinosteroids have been identified in various studies as an important factor in stimulating proline accumulation under water deficit conditions [99–101]. According to our results, under water stress condition, a significant increase in the prolin content of the camelina leaves was observed in two years. Also, the application of different foliar spray treatments (B and BR) was different in both years. According to the results, the BR_1 application in 2018-19 and combination of BR_1 with $B_{0.5}$ in 2019-20 increased proline accumulation under-withholding irrigation at flowering stage (WI_2); However, BR application could not influence proline accumulation under normal irrigation (Fig. 2). Recently, many studies have revealed that the BR application in abiotic stress increase proline levels of the plants, which protect plant cells against ROS accumulation under stressful conditions [102–104]. Consistent with our results, Kolomeichuk et al. [90] showed that brassinosteroids applied to barley plants under normal irrigation conditions had no significant effect on proline levels, whereas pretreatment of plants with BRs followed by drought stress significantly inhibited proline accumulation induced by water deficit.

4.3. Secondary metabolites

Among secondary metabolites of plants, phenolic, flavonoid, terpenoid and anthocyanin compounds are phytochemical compounds that are generally found in various organs of plants [105,106]. Secondary metabolites are produced in various plants in interaction with internal factors and environmental conditions [107,108]. For example, concentrartion of phenolic [109,110] and flavonoid [110,111] increased during water deficiency in various plant species which that similar to this study. When exposed to drought stress, Camelina plants responded to drought by increasing secondary metabolite levels. Additionally, our results revealed that BR application (alone and combined with a low level of B) enhanced the secondary metabolites (Ant, TFC and TPC) content in camelina leaves (Table 4 and Fig. 3). Therefore, one of the protective effects of brassinosteroids (alone and in combination with boron) under drought conditions is the significant activation of secondary metabolites. This is may be due to essential role of BR in modify the expression of genes involved in secondary metabolites biosynthesis under stress conditions [112,113]. Sharma et al. [114] suggested that the application of 24-EBL increases phenolic compounds of black mustard leaves under saline conditions. Further, the finding of Aghdam et al. [115] indicated that brassinosteroid increases the activity of phenylalanine ammonia-lyase (PAL) enzyme and causes the production of phenolic compounds. Furthermore, BR increases the key enzymes of phenylpropanoid metabolism pathways like PAL, cinnamate-4-hydroxylase (C4H), and 4-coumarate-CoA ligase (4CL), leading to the attenuation of oxidative stress [116]. Previously reported studies confirmed this effect of BR, which enhances PAL activity and flavonoid content, leading to the decrease of oxidative stress-induced ROS accumulation in cucumber plants [117]. Generally, brassinosteroid is known as a phytohormone that has the potential to increase secondary metabolites in plants [118]. In black mustard (*Brassica nigra*), 24-epibrassinolide application promoted the secondary metabolites, especially the content of flavonoids and anthocyanins [119]. Treatment of rice plants with brassinosteroid under salt stress conditions, caused a significant increase in the total phenol and flavonoid content compared to the untreated plants [120].

4.4. Antioxidant enzymes

Types of ROS are generated under stressful situation including drought [121] and boron toxicity [122]. Among the ROS, hydroxyl radicals (·OH) were converted to Hydrogen peroxide (H2O2) by SOD and then H_2O_2 converted into dioxygen (O_2) and water (H_2O) by catalase and peroxidase [123,124]. On the other hand, the way of applying drought stress and brassinosteroid treatment has different results in the activation of antioxidant enzymes. For example, treatment of tomatoes with brassinosteroids, followed by drought stress increased catalase content [125], while treatment of plants during the stress period was associated with inhibition of catalase activity [126]. The results of other studies also well prove the increase in the activity of superoxide dismutase and peroxidase enzymes under stress conditions [72,99]. The activity of different enzymatic and nonenzymatic antioxidants by BR application may be related to an important role of BR in antioxidant gene expression and also acts as a signaling compound in plant tissues [127,128]. In other plants, cowpea [129] and tomato [130] showed that BR application increased the SOD, CAT, APX and POD enzyme activities and counteracted the harmful effects of excess ROS under water stress condition. Kolomeichuk et al. [90] also reports that the Positive application of HBL in barley caused a significant increase in SOD and CAT activity and a decrease in POD activity compared to the control under drought stress conditions.

Simultaneously, in this study, brassinosteroid neutralized the damaging effects of high boron level (B_1) and drought stress. BRs can reduce the level of ROS by modulating major oxidant compounds [112,131] and increase tolerance to abiotic stress including drought [129,132] and heavy metal [133,134]. Our findings revealed that SOD and CAT activity decreased by a high supply of B concentration at withholding irrigation conditions (WI_1 and WI_2), while increased these enzyme activities at normal irrigation (WI_0) conditions (Table 3). These results in contrast to the finding of Liu et al. [54] who observed that the glutathione reductase activity increased in watermelon (*Citrullus lanatus*) plants under the co-combination of B toxicity and drought stress conditions. Many studies have reported the enhancement of antioxidant enzymes, such as SOD, guaiacol peroxidase (GPO) and ascorbate peroxidase (APX) activity in wheat [129], and catalase, peroxidase, SOD in mango [135] results from B toxicity. In similar, Shah et al. [94] reported that CAT, POD and SOD activities decreased significantly at both B deficiency and excess. In current study BR application (alone and combined with low B) increased SOD, CAT, POD and APX activity (Tables 3 and 4). Similar reports were observed in *Arabidopsis thaliana* (L.) Heynh, Surgun et al., 2016 [136], who demonstrated that 24-epibrassinolide ameliorate the negative effects of B excess by increasing antioxidant capacity and decreasing B accumulation.

4.5. Membrane lipid peroxidation

In the present study, drought stress caused a significant increase in MDA content, and this increase was intensified by the addition of boron treatments ($B_{0.5}$ and B_1) at the seed filling stage, while under normal irrigation the application of B did not affect MDA content (Table 3). An increase in ROS under water-limited conditions, results in membrane lipid peroxidation, dysfunction of enzymes, electrolyte leakage and alleviate photosynthetic rate [137]. Shah et al. [94] reported that B excess can increase MDA contents in citrange orange plants. Further, our results revealed that BR application reduced the MDA levels in camelina leaves (Table 3). The decrease in MDA level by BR application may be due to the modulating antioxidant production (CAT, POD, SOD, APX, etc.) and decrease the oxidative degradation in cellular organelles [138,139]. It is also well known that BR has a remarkable impact on the characteristics of the cell membrane viscosity [140–142]. Recently, Nie et al. [130] revealed that BR decreased the electrolyte leakage and MDA content under drought stress in tomato plants. In a similar study, brassinosteroid-treated soybean plants exposed to drought stress showed a significant decrease in MDA content [143]. Additionally, BR in combination with B decreased MDA content at withholding irrigation conditions in our study (Table 3). Previous studies have shown that the BR and Zn combination under water stress could lead to decline in H_2O_2 and MDA contents in tomato seedlings [144]. Avalbaev et al. [100] observed that Pretreatment of wheat plants with BR under stress conditions with PEG6000 significantly reduced lipid peroxidation and electrolyte leakage.

5. Conclusions

Foliar spray of BR and B (mainly at concentrations of $B_{0.5} + BR_{0.5}$ and $B_{0.5} + BR_1$) can synergically reduction of oxidative damage and improved physiological traits of camelina including photosynthetic pigments, antioxidant system activities, secondary metabolites and proline under drought stress and helped camelina plants with the adverse effects of drought stress. Moreover, the combination of BR and B decreased MDA levels by improving the antioxidant defense system; Which can be considered a suitable agricultural technique to combat drought stress damage. In addition, BR application alone at two levels ($BR_{0.5}$ and BR_1) improved the studied traits under drought stress and performed better in reducing drought stress compared to boron alone treatments ($B_{0.5}$ and B_1). In general boron application functions as a double-edged sword. This is evidenced by a significant decrease in all physiological treatments (except for MDA content) at high B levels under drought conditions, while B in low level had no adverse effect on the measured traits in such conditions. Finally, further experiments are needed to investigate the effect of different levels of brassinosteroids and boron on the biochemical and physiological properties and nutrient uptake of camelina under water deficit conditions.

CRediT authorship contribution statement

Sajjad Aghdasi: Writing – original draft, Visualization, Project administration, Investigation, Data curation. **Majid AghaAlikhani:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Seyed Ali Mohammad Modarres-Sanavy:** Software, Formal analysis. **Danial Kahrizi:** Validation, Resources.

Comprehensive ethics statement

(1) This article is a detailed report of a real research, written as a Ph.D. dissertation in the field of crop physiology. (2) The basic data of the research are shown in the article, and public access to details is possible only upon request from the responsible author. (3) In cases where the works and words of others have been used to interpret the results or express the history of the research, they have been properly referenced. (4) This article is free from plagiarism in any possible form, and accuracy and honesty have been the basis for its writing. (5) No artificial intelligence has been used in writing this article. (6) No animal or human samples have been examined in this research. (7) The authors of this article have no conflicts of interest with other individuals or organizations.

Data availability statement

Data will be made available on request. For requesting data, please write to the corresponding author.

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Corresponding author recently helped the journal (HELIYON) for review the following manuscript: HELIYON-D-24-02596 on March 29, 2024. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] E.A. Waraich, Z. Ahmed, R. Ahmad, M.Y. Ashraf, M.S. Naeem, Z. Rengel, Camelina sativa, a climate proof crop, has high nutritive value and multiple-uses: A review, Aust. J. Crop Sci. 7 (2013) 1551–1559.
[2] Z. Ahmed, E.A. Waraich, A. Rashid, M. Shahbaz, Morpho-physiological and biochemical responses of camelina (*Camelina sativa* crantz) genotypes under drought stress, Int. J. Agric. Biol. 19 (2017).
[3] L. Gao, C.D. Caldwell, Y. Jiang, Photosynthesis and growth of camelina and canola in response to water deficit and applied nitrogen, Crop Sci. 58 (2018) 393–401.
[4] M. Leclère, M.-H. Jeuffroy, A. Butier, C. Chatain, C. Loyce, Controlling weeds in camelina with innovative herbicide-free crop management routes across various environments, Ind. Crops Prod. 140 (2019) 111605.

[5] P. Čanak, A.M. Jeromela, B. Vujošević, B. Kiprovski, B. Mitrović, B. Alberghini, E. Facciolla, A. Monti, F. Zanetti, Is drought stress tolerance affected by biotypes and seed size in the emerging oilseed crop Camelina? Agronomy 10 (2020) 1856.

[6] S. Bansal, T.P. Durrett, Camelina sativa: an ideal platform for the metabolic engineering and field production of industrial lipids, Biochimie 120 (2016) 9–16.

[7] K. Ghamkhar, J. Croser, N. Aryamanesh, M. Campbell, N. Kon'kova, C. Francis, Camelina (*Camelina sativa* (L.) Crantz) as an alternative oilseed: molecular and ecogeographic analyses, Genome 53 (2010) 558–567.

[8] F. Zanetti, B. Alberghini, A.M. Jeromela, N. Grahovac, D. Rajković, B. Kiprovski, A. Monti, Camelina, an ancient oilseed crop actively contributing to the rural renaissance in Europe. A review, Agron. Sustain. Dev. 41 (2021) 1–18.

[9] D.J. Hunsaker, A.N. French, K.R. Thorp, Camelina water use and seed yield response to irrigation scheduling in an arid environment, Irrig. Sci. 31 (2013) 911–929.

[10] R.H. Lohaus, D. Neupane, M.A. Mengistu, J.K.Q. Solomon, J.C. Cushman, Five-Year Field Trial of Eight *Camelina sativa* cultivars for biomass to be used in biofuel under irrigated conditions in a semi-arid climate, Agronomy 10 (2020) 562.

[11] B.R. Moser, S.F. Vaughn, Evaluation of alkyl esters from Camelina sativa oil as biodiesel and as blend components in ultra low-sulfur diesel fuel, Bioresour. Technol. 101 (2010) 646–653.

[12] M. Berti, R. Gesch, C. Eynck, J. Anderson, S. Cermak, Camelina uses, genetics, genomics, production, and management, Ind. Crops Prod. 94 (2016) 690–710.

[13] H.Y. Sintim, V.D. Zheljazkov, A.K. Obour, A. Garcia y Garcia, Managing harvest time to control pod shattering in oilseed camelina, Agron. J. 108 (2016) 656–661.

[14] M. Larsson, Cultivation and processing of *Linum usitatissimum* and *Camelina sativa* in southern scandinavia during the roman iron age, Veg. Hist. Archaeobot. 22 (2013) 509–520.

[15] R. Šoštarić, A. Alegro, V. Hržak, Z. Stančić, H. Küster, Plant Remains from an Early Iron Age Well at Hajndl, vol. 33, Coll. Antropol., Slovenia, 2009, pp. 1295–1301.

[16] M.J. Stolarski, M. Krzyżaniak, J. Kwiatkowski, J. Tworkowski, S. Szczukowski, Energy and economic efficiency of camelina and crambe biomass production on a large-scale farm in north-eastern Poland, Energy 150 (2018) 770–780.

[17] P. V Mauri, D. Mostaza, A. Plaza, J. Ruiz-Fernandez, J. Prieto, A. Capuano, Variability of camelina production in the center of Spain in two years of cultivation, a new profitable and alternative crop, in: Proc. 27th Eur. Biomass Conf. Exhib. Lisbon, Port., 2019, pp. 27–30.

[18] R.W. Gesch, J.M. Johnson, Water use in camelina–soybean dual cropping systems, Agron. J. 107 (2015) 1098–1104.

[19] M. Kheiri, J. Kambouzia, S. Rahimi-Moghaddam, S.M. Moghaddam, L. Vasa, H. Azadi, Effects of agro-climatic indices on wheat yield in arid, semi-arid, and sub-humid regions of Iran, Reg. Environ. Chang. 24 (2024) 10.

[20] X. Yu, L. Zhang, T. Zhou, X. Zhang, Long-term changes in the effect of drought stress on ecosystems across global drylands, Sci. China Earth Sci. 66 (2023) 146–160.

[21] J. Zhang, J. Ding, X. Hou, P. Wu, Quantifying drought response sensitivity and spatial and temporal heterogeneity of vegetation in arid and semi-arid regions, Int. J. Remote Sens. 44 (2023) 1665–1683.

[22] J. Prasad, N. Akila, C.S. Bharathi, S. Alagudurai, C.A.R. Rao, B.M.K. Raju, K. V Rao, A.V.M.S. Rao, R. Rejani, D.B. V Ramana, Assessment of adaptation practices for risk minimization to drought in semi-arid environments, Clim. Risk Manag. 42 (2023) 100563.

[23] A. Wadood, A. Hameed, S. Akram, M. Ghaffar, Unraveling the impact of water deficit stress on nutritional quality and defense response of tomato genotypes, Front. Plant Sci. 15 (2024) 1403895.

[24] S. Mehravi, M. Hanifei, A. Gholizadeh, M. Khodadadi, Water deficit stress changes in physiological, biochemical and antioxidant characteristics of anise (*Pimpinella anisum* L.), Plant Physiol. Biochem. 201 (2023) 107806.

[25] Y. Baba, A. Cimen, A. Birinci Yildirim, A. Ucar Turker, How does water stress affect the bioaccumulation of galanthamine and lycorine, growth performance, phenolic content and defense enzyme activities in summer snowflake (*Leucojum aestivum* L.)? Physiol. Mol. Biol. Plants (2024) 1–16.

[26] R.A. Shawon, B.S. Kang, S.G. Lee, S.K. Kim, H.J. Lee, E. Katrich, S. Gorinstein, Y.G. Ku, Influence of drought stress on bioactive compounds, antioxidant enzymes and glucosinolate contents of Chinese cabbage (*Brassica rapa*), Food Chem. 308 (2020) 125657.

[27] S. Sachdev, S.A. Ansari, M.I. Ansari, Oxidative stress triggered damage to cellular biomolecules, in: React. Oxyg. Species Plants Right Balanc, Springer, 2023, pp. 45–59.

[28] D. Mondal, D. Shukla, N.K. Vishvakarma, R. Prabha, V. Dixit, Cellular responses against abiotic stress-induced reactive oxygen species, in: Stress Biol. Photosynth. Org. Mol. Insights Cell. Responses, Springer, 2024, pp. 107–120.

[29] M.F. Seleiman, W.M. Semida, M.M. Rady, G.F. Mohamed, K.A. Hemida, B.A. Alhammad, M.M. Hassan, A. Shami, Sequential application of antioxidants rectifies ion imbalance and strengthens antioxidant systems in salt-stressed cucumber, Plants 9 (2020) 1783.

[30] F.A. Sattar, B.T. Hamooh, G. Wellman, M.A. Ali, S.H. Shah, Y. Anwar, M.A.A. Mousa, Growth and biochemical responses of potato cultivars under in Vitro lithium chloride and mannitol simulated salinity and drought stress, Plants 10 (2021) 924.

[31] A.R. Reddy, K.V. Chaitanya, M. Vivekanandan, Drought-induced responses of photosynthesis and antioxidant metabolism in higher plants, J. Plant Physiol. 161 (2004) 1189–1202.

[32] M. Ozturk, B. Turkyilmaz Unal, P. García-Caparrós, A. Khursheed, A. Gul, M. Hasanuzzaman, Osmoregulation and its actions during the drought stress in plants, Physiol. Plant. 172 (2021) 1321–1335.

[33] D. Neupane, J.K.Q. Solomon, E. Mclennon, J. Davison, T. Lawry, Camelina production parameters response to different irrigation regimes, Ind. Crops Prod. 148 (2020) 112286.

[34] S. Aghdasi, M. AghaAlikhani, S.A.M. Modarres-Sanavy, D. Kahrizi, Exogenously used boron and 24-epibrassinolide improved oil quality and mitigate late-season water deficit stress in camelina, Ind. Crops Prod. 171 (2021) 113885.

[35] F. Hosseini Sanehkoori, H. Pirdashti, E. Bakhshandeh, Effect of environmental factors on *Camelina sativa* seed germination and emergence, Acta Physiol. Plant. 45 (2023) 4.

[36] M. Nazim, M. Ali, X. Li, S. Anjum, F. Ahmad, U. Zulfiqar, K. Shahzad, W. Soufan, Unraveling the synergistic effects of microbes and selenium in alleviating drought stress in *Camelina sativa* L, Plant Stress 9 (2023) 100193.

[37] S.A.G. Nejad, H. Etesami, The importance of boron in plant nutrition, Met. Plants Adv. Futur. Prospect. (2020) 433–449.

[38] M. Brdar-Jokanović, Boron toxicity and deficiency in agricultural plants, Int. J. Mol. Sci. 21 (2020) 1424.

[39] A. Asad, F.P.C. Blamey, D.G. Edwards, Dry matter production and boron concentrations of vegetative and reproductive tissues of canola and sunflower plants grown in nutrient solution, Plant Soil 243 (2002) 243–252.

[40] A.Q. Dinh, A. Naeem, K.H. Mühling, Growth and distribution of boron in oilseed rape (*Brassica napus* L.) as affected by boron supply, Plants 11 (2022) 2746.

[41] B. Pommerrenig, A. Junker, I. Abreu, A. Bieber, J. Fuge, E. Willner, M.D. Bienert, T. Altmann, G.P. Bienert, Identification of rapeseed (*Brassica napus*) cultivars with a high tolerance to boron-deficient conditions, Front. Plant Sci. 9 (2018) 1142.

[42] P. Vera-Maldonado, F. Aquea, M. Reyes-Díaz, P. Cárcamo-Fincheira, B. Soto-Cerda, A. Nunes-Nesi, C. Inostroza-Blancheteau, Role of boron and its interaction with other elements in plants, Front. Plant Sci. 15 (2024) 1332459.

[43] M.A. Wimmer, T. Eichert, Mechanisms for boron deficiency-mediated changes in plant water relations, Plant Sci. 203 (2013) 25–32.

[44] V. Gimeno, I. Simón, M. Nieves, V. Martínez, J.M. Cámara-Zapata, A.L. García, F. García-Sánchez, The physiological and nutritional responses to an excess of boron by Verna lemon trees that were grafted on four contrasting rootstocks, Trees (Berl.) 26 (2012) 1513–1526.

[45] A. González-Fontes, Why boron is an essential element for vascular plants, New Phytol. 226 (2020) 1228–1230.

[46] M.A. Wimmer, I. Abreu, R.W. Bell, M.D. Bienert, P.H. Brown, B. Dell, T. Fujiwara, H.E. Goldbach, T. Lehto, H. Mock, Boron: an essential element for vascular plants, New Phytol. 226 (2019) 1232–1237.

[47] V.M. Shorrocks, The occurrence and correction of boron deficiency, Plant Soil 193 (1997) 121–148.

[48] M. Landi, E. Degl'Innocenti, A. Pardossi, L. Guidi, Antioxidant and photosynthetic responses in plants under boron toxicity: a review, Am. J. Agric. Biol. Sci. 7 (2012) 255–270.

[49] J.J. Camacho-Cristóbal, M.T. Navarro-Gochicoa, J. Rexach, A. González-Fontes, M.B. Herrera-Rodríguez, Plant response to boron deficiency and boron use efficiency in crop plants, in: Plant Micronutr. Use Effic., Elsevier, 2018, pp. 109–121.
[50] C.F. You, A.J. Spivack, J.M. Gieskes, R. Rosenbauer, J.L. Bischoff, Experimental study of boron geochemistry: implications for fluid processes in subduction zones, Geochim. Cosmochim. Acta. 59 (1995) 2435–2442.
[51] S. Simón-Grao, M. Nieves, J.J. Martínez-Nicolás, J.M. Cámara-Zapata, M. Alfosea-Simón, F. García-Sánchez, Response of three citrus genotypes used as rootstocks grown under boron excess conditions, Ecotoxicol. Environ. Saf. 159 (2018) 10–19.
[52] M. Landi, T. Margaritopoulou, I.E. Papadakis, F. Araniti, Boron toxicity in higher plants: an update, Planta 250 (2019) 1011–1032.
[53] M. Hrmova, M. Gilliham, S.D. Tyerman, Plant transporters involved in combating boron toxicity: beyond 3D structures, Biochem. Soc. Trans. 48 (2020) 1683–1696.
[54] C. Liu, Z. Dai, J. Xia, C. Chang, H. Sun, Combined effect of salt and drought on boron toxicity in *Puccinellia tenuiflora*, Ecotoxicol. Environ. Saf. 157 (2018) 395–402.
[55] C.J. Yang, C. Zhang, Y.N. Lu, J.Q. Jin, X.L. Wang, The mechanisms of brassinosteroids' action: from signal transduction to plant development, Mol. Plant 4 (2011) 588–600.
[56] P. Jiroutova, J. Oklestkova, M. Strnad, Crosstalk between brassinosteroids and ethylene during plant growth and under abiotic stress conditions, Int. J. Mol. Sci. 19 (2018) 3283.
[57] H. Ye, S. Liu, B. Tang, J. Chen, Z. Xie, T.M. Nolan, H. Jiang, H. Guo, H.-Y. Lin, L. Li, RD26 mediates crosstalk between drought and brassinosteroid signalling pathways, Nat. Commun. 8 (2017) 14573.
[58] G.J. Ahammed, X. Li, A. Liu, S. Chen, Brassinosteroids in plant tolerance to abiotic stress, J. Plant Growth Regul. 39 (2020) 1451–1464.
[59] H. Manghwar, A. Hussain, Q. Ali, F. Liu, Brassinosteroids (BRs) role in plant development and coping with different stresses, Int. J. Mol. Sci. 23 (2022) 1012.
[60] W. Ahmad Lone, N. Majeed, U. Yaqoob, R. John, Exogenous brassinosteroid and jasmonic acid improve drought tolerance in *Brassica rapa* L. genotypes by modulating osmolytes, antioxidants and photosynthetic system, Plant Cell Rep. 41 (2022) 603–617.
[61] N. Fàbregas, F. Lozano-Elena, D. Blasco-Escámez, T. Tohge, C. Martínez-Andújar, A. Albacete, S. Osorio, M. Bustamante, J.L. Riechmann, T. Nomura, Overexpression of the vascular brassinosteroid receptor BRL3 confers drought resistance without penalizing plant growth, Nat. Commun. 9 (2018) 1–13.
[62] Y. Tang, J. Zhang, L. Wang, H. Wang, H. Long, L. Yang, G. Li, J. Guo, Y. Wang, Y. Li, Water deficit aggravated the inhibition of photosynthetic performance of maize under mercury stress but is alleviated by brassinosteroids, J. Hazard Mater. 443 (2023) 130365.
[63] L.R. dos Santos, L. de S. Paula, Y.C. Pereira, B.R.S. da Silva, B.L. Batista, A.A. Alsahli, A.K. da S. Lobato, Brassinosteroids-mediated amelioration of iron deficiency in soybean plants: beneficial effects on the nutritional status, photosynthetic pigments and chlorophyll fluorescence, J. Plant Growth Regul. 40 (2021) 1803–1823.
[64] P. Yang, M. Azher Nawaz, F. Li, L. Bai, J. Li, Brassinosteroids regulate antioxidant system and protect chloroplast ultrastructure of autotoxicity-stressed cucumber (*Cucumis sativus* L.) seedlings, Agronomy 9 (2019) 265.
[65] I. Sharma, P.K. Pati, R. Bhardwaj, Effect of 24-epibrassinolide on oxidative stress markers induced by nickel-ion in Raphanus sativus L, Acta Physiol. Plant. 33 (2011) 1723–1735.
[66] A. Zhang, J. Zhang, J. Zhang, N. Ye, H. Zhang, M. Tan, M. Jiang, Nitric oxide mediates brassinosteroid-induced ABA biosynthesis involved in oxidative stress tolerance in maize leaves, Plant Cell Physiol. 52 (2011) 181–192.
[67] S. Hayat, G. Khalique, A.S. Wani, M.N. Alyemeni, A. Ahmad, Protection of growth in response to 28-homobrassinolide under the stress of cadmium and salinity in wheat, Int. J. Biol. Macromol. 64 (2014) 130–136.
[68] W. Lv, L. Sui, X. Yan, H. Xie, L. Jiang, C. Geng, Q. Li, X. Yao, Y. Kong, J. Cao, ROS-dependent Atg4 upregulation mediated autophagy plays an important role in Cd-induced proliferation and invasion in A549 cells, Chem. Biol. Interact. 279 (2018) 136–144.
[69] Z. Jia, R.F.H. Giehl, R.C. Meyer, T. Altmann, N. von Wirén, Natural variation of BSK3 tunes brassinosteroid signaling to regulate root foraging under low nitrogen, Nat. Commun. 10 (2019) 1–13.
[70] C. Zhang, M. He, S. Wang, L. Chu, C. Wang, N. Yang, G. Ding, H. Cai, L. Shi, F. Xu, Boron deficiency-induced root growth inhibition is mediated by brassinosteroid signalling regulation in Arabidopsis, Plant J. 107 (2021) 564–578.
[71] X. Chen, S.M. Smith, S. Shabala, M. Yu, Phytohormones in plant responses to boron deficiency and toxicity, J. Exp. Bot. 74 (2023) 743–754.
[72] W. Ahmad Lone, N. Majeed, U. Yaqoob, R. John, Exogenous brassinosteroid and jasmonic acid improve drought tolerance in *Brassica rapa* L. genotypes by modulating osmolytes, antioxidants and photosynthetic system, Plant Cell Rep. 41 (2022) 603–617.
[73] N.A.K. Sima, H. Jabbari, A. Ebadi, M.R. Ghaffari, P. Koobaz, Comparative analysis of exogenous hormone application on contrasting canola (*Brassica napus* L.) genotypes under drought stress conditions, J. Soil Sci. Plant Nutr. 24 (2024) 308–317.
[74] A. Mokhtassi-Bidgoli, M. AghaAlikhani, M. Nassiri-Mahallati, E. Zand, J.L. Gonzalez-Andujar, A. Azari, Agronomic performance, seed quality and nitrogen uptake of Descurainia sophia in response to different nitrogen rates and water regimes, Ind. Crops Prod. 44 (2013) 583–592.
[75] D.I. Arnon, Copper enzymes in isolated chloroplasts. Polyphenoloxidase in Beta vulgaris, Plant Physiol 24 (1949) 1.
[76] M.M. Bradford, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, Anal. Biochem. 72 (1976) 248–254.
[77] C.N. Giannopolitis, S.K. Ries, Superoxide dismutases: I. Occurrence in higher plants, Plant Physiol. 59 (1977) 309–314.
[78] I. Cakmak, W.J. Horst, Effect of aluminium on lipid peroxidation, superoxide dismutase, catalase, and peroxidase activities in root tips of soybean (*Glycine max*), Physiol. Plant. 83 (1991) 463–468.
[79] Y. Nakano, K. Asada, Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts, Plant Cell Physiol. 22 (1981) 867–880.
[80] L.S. Bates, R.P. Waldren, I.D. Teare, Rapid determination of free proline for water-stress studies, Plant Soil 39 (1973) 205–207.
[81] Z. Ahmad, E.A. Waraich, C. Barutcular, H. Alharby, A. Bamagoos, F. Kizilgeci, et al., Enhancing drought tolerance in *Camelina sativa* L. and canola (*Brassica napus* L.) through the application of selenium, Pakistan J. Bot. 52 (2020) 1927–1939.
[82] M. Hara, K. Oki, K. Hoshino, T. Kuboi, Enhancement of anthocyanin biosynthesis by sugar in radish (*Raphanus sativus*) hypocotyl, Plant Sci. 164 (2003) 259–265.
[83] C.H.R. De Vos, H. Schat, M.A.M. De Waal, R. Vooijs, W.H.O. Ernst, Increased resistance to copper-induced damage of the root cell plasmalemma in copper tolerant Silene cucubalus, Physiol. Plant. 82 (1991) 523–528.
[84] M.A. Ayub, Z. Ahmad, W. Umar, Z. ur R. Farooqi, A.A. Waris, H. Fatima, M. Nadeem, I. Iftikhar, Accumulation, partitioning, and bioavailability of micronutrients in plants and their crosstalk with phytohormones, Plant Growth Regul. Signal. under Stress Cond (2021) 39–73.
[85] P.R. Divte, P. Yadav, A.B. Pawar, V. Sharma, A. Anand, R. Pandey, B. Singh, Crop response to iron deficiency is guided by cross-talk between phytohormones and their regulation of the root system architecture, Agric. Res. (2021) 1–14.
[86] J. Honnerová, O. Rothová, D. Holá, M. Kočová, L. Kohout, M. Kvasnica, The exogenous application of brassinosteroids to *Zea mays* (L.) stressed by long-term chilling does not affect the activities of photosystem 1 or 2, J. Plant Growth Regul. 29 (2010) 500–505.
[87] M. Zhao, L. Yuan, J. Wang, S. Xie, Y. Zheng, L. Nie, S. Zhu, J. Hou, G. Chen, C. Wang, Transcriptome analysis reveals a positive effect of brassinosteroids on the photosynthetic capacity of wucai under low temperature, BMC Genom. 20 (2019) 1–19.
[88] W. Hu, X. Yan, Y. Xiao, J. Zeng, H. Qi, J.O. Ogweno, 24-Epibrassinosteroid alleviate drought-induced inhibition of photosynthesis in *Capsicum annuum*, Sci. Hortic. (Amsterdam) 150 (2013) 232–237.
[89] U.O. Barros Junior, M.D.R. Lima, A.A. Alsahli, A.K.S. Lobato, Unraveling the roles of brassinosteroids in alleviating drought stress in young *Eucalyptus urophylla* plants: implications on redox homeostasis and photosynthetic apparatus, Physiol. Plant. 172 (2021) 748–761.
[90] L. V Kolomeichuk, O.K. Murgan, E.D. Danilova, M. V Serafimovich, V.A. Khripach, R.P. Litvinovskaya, A.L. Sauchuk, D. V Denisiuk, V.N. Zhabinskii, V. V Kuznetsov, Effects of lactone-and ketone-brassinosteroids of the 28-homobrassinolide series on barley plants under water deficit, Plants 13 (2024) 1345.
[91] X. Wu, X. Lu, M. Riaz, L. Yan, C. Jiang, Boron toxicity induced specific changes of cell ultrastructure and architecture of components in leaf center and tip of trifoliate orange [*Poncirus trifoliata* (L.) Raf.], J. Environ. Manage. 246 (2019) 426–433.

[92] J. Huo, B. Song, M. Riaz, X. Song, J. Li, H. Liu, W. Huang, Q. Jia, W. Wu, High boron stress leads to sugar beet (*Beta vulgaris* L.) toxicity by disrupting photosystem II, Ecotoxicol. Environ. Saf. 248 (2022) 114295.
[93] Ö. Arslan, Boron Toxicity Impacts on Photosystem II Photochemical Efficiency of Sage (*Salvia Officinalis*), Bot. Serbica., vol. 46, 2022, pp. 113–119.
[94] A. Shah, X. Wu, A. Ullah, S. Fahad, R. Muhammad, L. Yan, C. Jiang, Deficiency and toxicity of boron: alterations in growth, oxidative damage and uptake by citrange orange plants, Ecotoxicol. Environ. Saf. 145 (2017) 575–582.
[95] M.E. Alvarez, A. Savouré, L. Szabados, Proline metabolism as regulatory hub, Trends Plant Sci. 27 (2022) 39–55.
[96] U.K. Ghosh, M.N. Islam, M.N. Siddiqui, X. Cao, M.A.R. Khan, Proline, a multifaceted signalling molecule in plant responses to abiotic stress: understanding the physiological mechanisms, Plant Biol 24 (2022) 227–239.
[97] V. Cacefo, A.F. Ribas, L.G.E. Vieira, Proline metabolism as a mechanism for the energy dissipation in VaP5CSF129A transgenic tobacco plants under water deficit, J. Plant Physiol. 283 (2023) 153964.
[98] Z. Chen, Z. Wang, Y. Yang, M. Li, B. Xu, Abscisic acid and brassinolide combined application synergistically enhances drought tolerance and photosynthesis of tall fescue under water stress, Sci. Hortic. (Amsterdam) 228 (2018) 1–9.
[99] R. Khan, X. Ma, Q. Hussain, M. Asim, A. Iqbal, X. Ren, S. Shah, K. Chen, Y. Shi, Application of 2, 4-epibrassinolide improves drought tolerance in tobacco through physiological and biochemical mechanisms, Biology 11 (2022) 1192.
[100] A. Avalbaev, V. Fedyaev, A. Lubyanova, R. Yuldashev, C. Allagulova, 24-Epibrassinolide reduces drought-induced oxidative stress by modulating the antioxidant system and respiration in wheat seedlings, Plants 13 (2024) 148.
[101] C. Sukpitak, S. Munné-Bosch, K. Seraypheap, Brassinosteroids alleviate postharvest water deficit stress in cut Dendrobium'Khao Sanan'orchid through modulation of protective mechanisms, osmotic regulation, and endogenous hormonal levels, Postharvest Biol. Technol. 211 (2024) 112832.
[102] M. Yusuf, T.A. Khan, Q. Fariduddin, Interaction of epibrassinolide and selenium ameliorates the excess copper in *Brassica juncea* through altered proline metabolism and antioxidants, Ecotoxicol. Environ. Saf. 129 (2016) 25–34.
[103] W. Wu, Q. Zhang, E. Ervin, Z. Yang, X. Zhang, Physiological mechanism of enhancing salt stress tolerance of perennial ryegrass by 24-epibrassinolide, Front. Plant Sci. 8 (2017) 1017.
[104] S.K. Kohli, N. Handa, S. Bali, S. Arora, A. Sharma, R. Kaur, R. Bhardwaj, Modulation of antioxidative defense expression and osmolyte content by co-application of 24-epibrassinolide and salicylic acid in Pb exposed Indian mustard plants, Ecotoxicol. Environ. Saf. 147 (2018) 382–393.
[105] J.M. Al-Khayri, R. Rashmi, V. Toppo, P.B. Chole, A. Banadka, W.N. Sudheer, P. Nagella, W.F. Shehata, M.Q. Al-Mssallem, F.M. Alessa, Plant secondary metabolites: the weapons for biotic stress management, Metabolites 13 (2023) 716.
[106] H.S. Elshafie, I. Camele, A.A. Mohamed, A comprehensive review on the biological, agricultural and pharmaceutical properties of secondary metabolites based-plant origin, Int. J. Mol. Sci. 24 (2023) 3266.
[107] M. Nazari, F. Zarinkamar, V. Niknam, Changes in primary and secondary metabolites of *Mentha aquatica* L. exposed to different concentrations of manganese, Environ. Sci. Pollut. Res. 25 (2018) 7575–7588.
[108] L. Yang, K.-S. Wen, X. Ruan, Y.-X. Zhao, F. Wei, Q. Wang, Response of plant secondary metabolites to environmental factors, Molecules 23 (2018) 762.
[109] E.T. Albergaria, A.F.M. Oliveira, U.P. Albuquerque, The effect of water deficit stress on the composition of phenolic compounds in medicinal plants, South African J. Bot. 131 (2020) 12–17.
[110] U. Sarker, S. Oba, Drought stress enhances nutritional and bioactive compounds, phenolic acids and antioxidant capacity of Amaranthus leafy vegetable, BMC Plant Biol. 18 (2018) 1–15.
[111] S. Gao, Y. Wang, S. Yu, Y. Huang, H. Liu, W. Chen, X. He, Effects of drought stress on growth, physiology and secondary metabolites of Two Adonis species in Northeast China, Sci. Hortic. (Amsterdam) 259 (2020) 108795.
[112] M.A. Ahanger, R.A. Mir, M.N. Alyemeni, P. Ahmad, Combined effects of brassinosteroid and kinetin mitigates salinity stress in tomato through the modulation of antioxidant and osmolyte metabolism, Plant Physiol. Biochem. 147 (2020) 31–42.
[113] A. Sharma, M. Ramakrishnan, K. Khanna, M. Landi, R. Prasad, R. Bhardwaj, B. Zheng, Brassinosteroids and metalloids: regulation of plant biology, J. Hazard Mater. 424 (2022) 127518.
[114] A. Sharma, S. Thakur, V. Kumar, M.K. Kanwar, A.K. Kesavan, A.K. Thukral, R. Bhardwaj, P. Alam, P. Ahmad, Pre-sowing seed treatment with 24-epibrassinolide ameliorates pesticide stress in *Brassica juncea* L. through the modulation of stress markers, Front. Plant Sci. 7 (2016) 226602.
[115] M.S. Aghdam, M. Asghari, B. Farmani, M. Mohayeji, H. Moradbeygi, Impact of postharvest brassinosteroids treatment on PAL activity in tomato fruit in response to chilling stress, Sci. Hortic. (Amsterdam) 144 (2012) 116–120.
[116] Y. Wei, D. Zhou, J. Peng, L. Pan, K. Tu, Hot air treatment induces disease resistance through activating the phenylpropanoid metabolism in cherry tomato fruit, J. Agric. Food Chem. 65 (2017) 8003–8010.
[117] G.J. Ahammed, B.B. He, X.J. Qian, Y.H. Zhou, K. Shi, J. Zhou, J.Q. Yu, X.J. Xia, 24-Epibrassinolide alleviates organic pollutants-retarded root elongation by promoting redox homeostasis and secondary metabolism in *Cucumis sativus* L, Environ. Pollut. 229 (2017) 922–931.
[118] B. Ali, Z.A. Wani, M.I. Dar, Brassinosteroids: the phytohormones with potential to enhance the secondary metabolite production in plants, in: Biotechnol. Approaches to Enhanc. Plant Second. Metab., CRC Press, 2021, pp. 125–132.
[119] K. Ghassemi-Golezani, N. Hassanzadeh, M.-R. Shakiba, B. Esmaeilpour, Exogenous salicylic acid and 24-epi-brassinolide improve antioxidant capacity and secondary metabolites of *Brassica nigra*, Biocatal. Agric. Biotechnol. 26 (2020) 101636.
[120] R. Shahzad, P.W. Harlina, M. Ewas, P. Zhenyuan, X. Nie, P.P. Gallego, S. Ullah Khan, E. Nishawy, A.H. Khan, H. Jia, Foliar applied 24-epibrassinolide alleviates salt stress in rice (*Oryza sativa* L.) by suppression of ABA levels and upregulation of secondary metabolites, J. Plant Interact. 16 (2021) 533–549.
[121] X. Zhi, X. Bian, J. Yu, X. Xiao, B. Duan, F. Huang, Z. Jiang, G. Zhou, N. Ma, Comparative metabolomics analysis of tolerant and sensitive genotypes of rapeseed (*Brassica napus* L.) seedlings under drought stress, Agric. Water Manag. 296 (2024) 108797.
[122] M. Moustafa-Farag, H.I. Mohamed, A. Mahmoud, A. Elkelish, A.N. Misra, K.M. Guy, M. Kamran, S. Ai, M. Zhang, Salicylic acid stimulates antioxidant defense and osmolyte metabolism to alleviate oxidative stress in watermelons under excess boron, Plants 9 (2020) 724.
[123] T.S. Gechev, F. Van Breusegem, J.M. Stone, I. Denev, C. Laloi, Reactive oxygen species as signals that modulate plant stress responses and programmed cell death, Bioessays 28 (2006) 1091–1101.
[124] R. Mittler, ROS are good, Trends Plant Sci. 22 (2017) 11–19.
[125] M. Behnamnia, K. Kalantari, J. Ziaie, The effects of brassinosteroid on the induction of biochemical changes in *Lycopersicon esculentum* under drought stress, Turk. J. Botany. 33 (2009) 417–428.
[126] R. Sivakumar, G.K. Nandhitha, P. Chandrasekaran, P. Boominathan, M. Senthilkumar, Impact of pink pigmented facultative methylotroph and PGRs on water status, photosynthesis, proline and NR activity in tomato under drought, Int J Curr Microbiol App Sci. 6 (2017) 1640–1651.
[127] J. Zhou, J. Wang, X. Li, X.J. Xia, Y.H. Zhou, K. Shi, Z. Chen, J.Q. Yu, H2O2 mediates the crosstalk of brassinosteroid and abscisic acid in tomato responses to heat and oxidative stresses, J. Exp. Bot. 65 (2014) 4371–4383.
[128] M. Ghorbel, F. Brini, Role of brassinosteroids in regulating physiological and molecular aspects of plants under abiotic stress, in: Role Growth Regul. Phytohormones Overcoming Environ. Stress, Elsevier., 2023, pp. 197–233.
[129] J. V Lima, A.K.S. Lobato, Brassinosteroids improve photosystem II efficiency, gas exchange, antioxidant enzymes and growth of cowpea plants exposed to water deficit, Physiol. Mol. Biol. Plants 23 (2017) 59–72.
[130] S. Nie, S. Huang, S. Wang, Y. Mao, J. Liu, R. Ma, X. Wang, Enhanced brassinosteroid signaling intensity via SlBRI1 overexpression negatively regulates drought resistance in a manner opposite of that via exogenous BR application in tomato, Plant Physiol. Biochem. 138 (2019) 36–47.
[131] F. Basit, J. Tao, J. An, X. Song, M.S. Sheteiwy, P. Holford, J. Hu, I. Jośko, Y. Guan, Nitric oxide and brassinosteroids enhance chromium stress tolerance in *Glycine max* L.(Merr.) by modulating antioxidative defense and glyoxalase systems, Environ. Sci. Pollut. Res. 30 (2023) 51638–51653.
[132] N.B. Talaat, B.T. Shawky, A.S. Ibrahim, Alleviation of drought-induced oxidative stress in maize (*Zea mays* L.) plants by dual application of 24-epibrassinolide and spermine, Environ. Exp. Bot. 113 (2015) 47–58.

[133] B. Shahzad, M. Tanveer, Z. Che, A. Rehman, S.A. Cheema, A. Sharma, H. Song, S. ur Rehman, D. Zhaorong, Role of 24-epibrassinolide (EBL) in mediating heavy metal and pesticide induced oxidative stress in plants: a review, Ecotoxicol. Environ. Saf. 147 (2018) 935–944.

[134] J. Kour, S.K. Kohli, K. Khanna, P. Bakshi, P. Sharma, A.D. Singh, M. Ibrahim, K. Devi, N. Sharma, P. Ohri, Brassinosteroid signaling, crosstalk and, physiological functions in plants under heavy metal stress, Front. Plant Sci. 12 (2021) 608061.

[135] M.S. Ali, M.A. Elhamahmy, A.F. El-Shiekh, Mango trees productivity and quality as affected by boron and putrescine, Sci. Hortic. (Amsterdam) 216 (2017) 248–255.

[136] Y. Surgun, B. Çöl, B. Bürün, 24-Epibrassinolide ameliorates the effects of boron toxicity on *Arabidopsis thaliana* (L.) Heynh by activating an antioxidant system and decreasing boron accumulation, Acta Physiol. Plant. 38 (2016) 71.

[137] S. Talbi, M.C. Romero-Puertas, A. Hernández, L. Terrón, A. Ferchichi, L.M. Sandalio, Drought tolerance in a Saharian plant *Oudneya africana*: role of antioxidant defences, Environ. Exp. Bot. 111 (2015) 114–126.

[138] J. Praveena, S. Dash, L. Behera, G.R. Rout, Brassinosteroids: a multifunctional phytohormone of plant development and stress responses, Curr. J. Appl. Sci. Technol. 39 (2020) 174–196.

[139] F. Basit, M. Chen, T. Ahmed, M. Shahid, M. Noman, J. Liu, J. An, A. Hashem, A.-B. Fahad Al-Arjani, A.A. Alqarawi, Seed priming with brassinosteroids alleviates chromium stress in rice cultivars via improving ROS metabolism and antioxidant defense response at biochemical and molecular levels, Antioxidants 10 (2021) 1089.

[140] P. Sharma, R. Bhardwaj, Effects of 24-epibrassinolide on growth and metal uptake in *Brassica juncea* L. under copper metal stress, Acta Physiol. Plant. 29 (2007) 259–263.

[141] M. Shahbaz, M. Ashraf, H.-R. Athar, Does exogenous application of 24-epibrassinolide ameliorate salt induced growth inhibition in wheat (*Triticum aestivum* L.)? Plant Growth Regul. 55 (2008) 51–64.

[142] D. Kapoor, S. Bhardwaj, S. Gautam, A. Rattan, R. Bhardwaj, A. Sharma, Brassinosteroids in plant nutrition and heavy metal tolerance, in: Brassinosteroids Plant Dev. Biol. Stress Toler., 2022, pp. 217–235.

[143] D.G. dos S. Ribeiro, B.R.S. da Silva, A.K. da S. Lobato, Brassinosteroids induce tolerance to water deficit in soybean seedlings: contributions linked to root anatomy and antioxidant enzymes, Acta Physiol. Plant. 41 (2019) 82.

[144] A. Javadi, S. Khomari, B. Esmaeilpour, A. Asghari, Exogenous application of 24-epibrassinolide and nano-zinc oxide at flowering improves osmotic stress tolerance in harvested tomato seeds, Appl. Ecol. Environ. Res. 16 (2018).