



# Exogenous brassinosteroid and jasmonic acid improve drought tolerance in *Brassica rapa* L. genotypes by modulating osmolytes, antioxidants and photosynthetic system

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## Abstract

**Key message** Exogenously supplied BR and JA help KS101 and KBS3 genotypes of *Brassica rapa* to alleviate drought stress by modifying osmolyte concentration, levels of antioxidant enzymes and photosynthetic system.

**Abstract** Oilseed plants are susceptible to drought stress and a significant loss in yield has been reported during recent decades. Thus, it is imperative to understand the various underlying drought response mechanisms in *Brassica* oilseed plants to formulate the sustainable strategies to protect the crop yield under water-limiting conditions. Phytohormones play a key role in fine-tuning various regulatory mechanisms for drought stress adaptation in plants, and the present study explores the response of several physiological stress markers by exogenous supplementation of 24-epibrassinolide (EBL) and jasmonic acid (JA) on two genotypes of *Brassica rapa*, KS101 and KBS3 under drought stress conditions. The exogenous application of BR and JA, separately or in combination, significantly alleviated the drought stress by improving photosynthetic rate, photosynthetic pigments, stomatal conductance, transpiration rate and antioxidant defence. We observed that concentration of different osmolytes increased and membrane damage significantly reduced by the application of BR and JA. The overall activity of antioxidant enzymes POD, CAT, GR, APX and CAT elevated during all the treatments, be it stress alone or in combination with BR and JA, compared to the control. However, we observed that the BR was much better in mitigating the drought stress compared to JA. Thus, the present study suggests that BR and JA supplementation improves the performance of *B. rapa* on exposure to drought stress, which hints at the critical role of BR and JA in improving crop productivity in drought-prone areas.

**Keywords** Brassinosteroids · Drought stress · Jasmonic acid · Antioxidants · Osmolytes

## Abbreviations

KC/KS101C	KS-101 control
KD/KS101D	Drought-treated KS-101 genotype
KDB/KS101DB	Drought-treated KS-101 genotype with exogenously supplied BR
KJD/KS101JD	Drought-treated KS-101 genotype with exogenously supplied JA
KJDB/KS101JDB	Drought-treated KS-101 genotype with exogenously supplied JA and BR
BC/KBS3C	KBS-3 control
BD/KBS3D	Drought-treated KBS-3 genotype

BDB/KBS3DB	Drought-treated KBS-3 genotype with exogenously supplied BR
BJD/KBS3JD	Drought-treated KBS-3 genotype with exogenously supplied JA
BJDB/KBS3JDB	Drought-treated KBS-3 genotype with exogenously supplied JA and BR

## Introduction

Plant growth and development is hampered by several abiotic stresses which are caused by a number of ecological influences (Ma et al. 2020). Drought stress is described as a combination of insufficient rainfall and low soil moisture. It is a major environmental obstacle that plants in arid and semi-arid ecosystems are often exposed to (Fang et al. 2015). Drought overweighs all other “crop production affecting factors” by hampering the plant growth at its all-developmental

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stages, thereby decreasing crop productivity and incurring substantial economic loss worldwide (Fang et al. 2015; Farooq et al. 2009). A recent report by Climate Change Science Program mentions that the drought is inevitable in many parts of the world since the average temperature is expected to increase by 1.8–4.0 °C in 2100 (IPCC 2018).

Phytohormones, besides being important in various physiological mechanisms, are also key components for stress tolerance in plants (Shahzad et al. 2018). In fact, exogenous application of hormones leads to the rapid, although transient alteration of genome-wide transcriptome in plants. In *Arabidopsis*, exogenous hormonal supply for less than 1 h led to the changed expression of about 10–300 genes by upregulation and downregulation of an equal number of genes (Goda et al. 2008). Therefore, it becomes important to investigate the relationship between plant cell physiology and phytohormones, as well as the role of plant hormones in modulation of osmoprotectants under drought stress. Among phytohormones, brassinosteroid (BR) isolated from *Brassica* pollens have been known to regulate the temperature, salt, water, pesticide and other environmental cues in many plants (Shahzad et al. 2018) by regulating several osmolytes (Parvez et al. 2020). Although under stress conditions many biological processes in plants are controlled by BR signaling, BRL3 (BRI1-LIKE receptor) is believed to control the biogenesis of important osmolytes (Fàbregas et al. 2018). Jasmonates (JAs) represent another class of important phytohormones having important role under abiotic stress conditions. Under a number of stressful conditions, JA controls gene regulation, secondary metabolism and protein biosynthesis (Ahmad et al. 2018). Its increased endogenous levels indicate its significance in abiotic stress tolerance, while exogenous supplementation significantly improves plant growth in response to various environmental cues. JA has been shown in various studies to increase the production of non-enzymatic antioxidants including proline (Kaya et al. 2019; Shan et al. 2015).

Osmoprotectants are low molecular weight molecules that protect the membranes/proteins from osmotic shock and maintain the homeostatic osmotic balance in plant cells under drought stress (McNeil et al. 1999). Various abiotic stresses including drought stress induce the formation and buildup of several osmolytes such as sugars, glycine betaine (GB), proline (Pro) and proteins/free amino acids, which increase the cellular osmotic potential (Ramazan et al. 2021; Ozturk et al. 2020). Since Pro acts as a buffer to resolve lower water capacity, its accumulation serves as a significant indicator of diverse stress response mechanisms, particularly in plants grown under drought-stressed conditions (Jaleel et al. 2007). Drought causes plants to produce reactive oxygen species (ROS) namely,  $O_2^-$ ,  $^1O_2$  and  $H_2O_2$ , injuring living tissues/macromolecules (DNA, lipids, proteins and carbohydrates), thereby leading to rapid induced

programmed cell death (PCD) (McNeil et al. 1999). Plants, on the other hand, have evolved complex antioxidant defence system such as superoxide dismutase (SOD) and enzymes of the Halliwell–Asada pathway to quench deleterious ROS (Alam et al. 2019). Importantly, regulating enzymatic activity apparently seems to be the key process in plant resistance to environmental perturbations (Noctor and Foyer 1998).

Growth, development, yield and crop quality of *Brassica* crops in arid, semi-arid and Mediterranean regions get severely affected by drought stress (Zhang et al. 2014). In terms of abiotic stress responses in *Brassica* crops, Chinese cabbage (*B. rapa*) is the most studied species, with genome-wide analyses indicating that different plant hormones play role in different drought stress responses. However, systemic profiling of different phytohormones has yet to be investigated (Paul et al. 2016; Saha et al. 2016). We believe that in *Brassica* crops, certain plant hormones surely play their part in the drought tolerance. On these lines, our research offers an analysis of BR and jasmonic acid in two varieties of *Brassica rapa*. The outcome of hormonal profiles was explained in relation to morphological, physiological and biomolecular parameters.

## Materials and methods

### Plant material, growth conditions and hormonal treatments

Seeds for two genotypes of *Brassica rapa* used in this investigation, viz. KS-101 and Shalimar Sarson-3 (KBS3) were obtained from Sher-i-Kashmir University of Agricultural Science and Technology, Srinagar. Earthen pots with autoclaved soil, sand and vermiculite (2:1:1 w/w/w) were used for seed germination and kept under controlled conditions of the plant growth chamber (Blue Star-NKL-750) set at  $25 \pm 1$  °C, light intensity of  $200 \text{ mol photons m}^{-2} \text{ s}^{-1}$  and 60–80% of relative humidity. Plants from each variety were distributed into the following five sets—(1) set of control plants with all optimum environmental conditions, (2) set of drought-stressed plants, (3) set of drought-stressed plants with exogenously applied BR, (4) set of drought-stressed plants with exogenously applied JA and (5) set of drought-stressed plants with exogenously applied BR (0.01  $\mu\text{M}$ ) as well as JA (10  $\mu\text{M}$ ). The 21-day-old seedlings of *B. rapa* were exposed to water deficit stress. Plants were exogenously supplied with a foliar spray of BR and JA during the drought period. Plant tissue was harvested after 10 days of drought stress for several physio-biochemical as well as antioxidant enzyme assays. Plants collected at the end of drought stress were either used afresh or stored for further analysis at  $-80$  °C. For biochemicals and antioxidant enzymes, the homogenate was extracted and stored at  $-80$  °C.

## Estimation of morphological parameters

At the end of the drought period, morphological parameters such as plant height, shoot length, root length, petiole length, leaf length and leaf breadth were measured. These values were calculated using a scale and expressed in centimetres.

## Estimation of pigments

Chlorophyll and carotenoid content estimation was done by Holden's (1961) method using UV–Vis spectrophotometer (Shimadzu-1800). Anthocyanin estimation was done by using the method of Nakata and Ohme-Takagi (2014).

## Estimation of proline and glycine betaine

Estimation of Pro and GB content was done by following the protocol of Bates et al. (1973) and Grieve et al. (1983), respectively.

## Estimation of total phenolic content

The protocol of Apak et al. (2008) was used to estimate the phenolic content in the plant tissue. Leaf tissue (50 mg) was homogenised in 80% acetone and centrifuged for 10 min at 10,000g. 1 mL Folin–Ciocalteu's reagent and 2 mL distilled water were applied to 100  $\mu$ L of the supernatant and vigorously shaken. 20% sodium carbonate was added to this mixture, and the volume was increased with distilled water to 10 mL. At 750 nm, the absorbance was measured using UV–Vis spectrophotometer (Shimadzu, Japan).

## Estimation of total soluble sugar

The total soluble sugar was calculated as in Dey (1990). A UV–Vis spectrophotometer was used to read the absorbance at 485 nm (Shimadzu-1800). Sugar concentration was measured using a standard curve.

## Estimation of malondialdehyde

MDA was estimated by following the method of Hodges et al. (1999). OD was measured at 532 nm and 600 nm using UV–Vis spectrophotometer (Shimadzu-1800). Histochemical approach was also followed to analyse lipid peroxidation qualitatively through Schiff's reagent as in Awasthi et al. (2018).

## Estimation of relative water content

FW (fresh weight), TW (turgid weight) and DW (dry weight) of leaves were calculated to estimate relative water content (RWC). The RWC was then calculated by the equation:

$$\text{RWC} = \frac{[\text{FW} - \text{DW}]}{[\text{TW} - \text{DW}]} \times 100.$$

## Estimation of photosynthetic parameters

To calculate the different photosynthetic parameters, an infra-red gas analyser (IRGA) (LICOR-6400XT) was used. At the end of the drought period, from 9 am to 11 am, various photosynthetic parameters were measured, including photosynthetic rate, water usage efficiency, stomatal conductance and transpiration rate.

## DAB assay

Histochemical detection of hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) was done by following the protocol of Kumaret al. (2014). 3, 3'-Diaminobenzidine (DAB) was used as the chromogenic substrate which gets oxidised by  $\text{H}_2\text{O}_2$  and produces a reddish brown precipitate.

## Estimation of total soluble protein and antioxidant enzyme activity

Leaf tissue was crushed in 100 mM and 7.8 pH sodium phosphate buffer. After centrifugation for 20 min at 10,000 rpm at 4 °C, OD was measured from the supernatant at 260 nm and 280 nm for protein estimation by using UV–Vis spectrophotometer (Shimadzu-1800) (Chen et al. 2015). The remaining supernatant was utilised for estimating the enzyme activities of SOD, CAT, POD, GR and APX.

## Estimation of superoxide dismutase (SOD) activity

The activity of SOD was measured using the Zhang et al. (2005) protocol. The reaction mixture contained 0.05 M phosphate buffer (pH 7.8), 750  $\mu$ mol/L NBT, 20  $\mu$ mol/L riboflavin, 130 mM methionine, 100 mol/L EDTA- $\text{Na}_2$  and 0.05 mL of enzyme extract. The reaction mixture tubes were exposed to twin 15 W fluorescent lamps for 50 s, while the control set was kept in the dark for the same time. 560 nm wavelength was used to check the optical density using a UV–Vis spectrophotometer (Shimadzu-1800) (Simonian et al. 2006).

## Estimation of peroxidase (POD) activity

POD activity was accessed by following the protocol of Chen and Zhang (2016). The reaction mixture comprised 100 mM phosphate buffer, 0.2% guaiacol, 30%  $\text{H}_2\text{O}_2$  and 0.05 ml of enzyme extract. To calculate POD activity, the absorbance was read at 470 nm after every 15 s for 1 min by using a UV–Vis spectrophotometer (Shimadzu-1800).

## Estimation of catalase (CAT) activity

The protocol of Zhang et al. (2005) was used to calculate the CAT activity. 2.25 mL sodium phosphate buffer (pH 7.8), 1.5 mL deionised water, 0.45 mL 0.1 M H<sub>2</sub>O<sub>2</sub> and 0.3 mL enzyme extract made up the reaction mixture. The decline in optical density at 240 nm was used to estimate the CAT activity.

## Estimation of glutathione reductase (GR) activity

To assess the GR activity, Halliwell and Foyer (1978) was followed. 0.5 mM glutathione (oxidised), 0.15 mM NADPH, 3 mM MgCl<sub>2</sub> in 50 mM Tris (pH 7.5) and 0.06 mL enzyme extract were included in the reaction mixture. The oxidation of NADPH by glutathione at 340 nm was then monitored using a UV–Vis spectrophotometer to evaluate GR activity (Shimadzu-1800).

## Estimation of ascorbate peroxidase (APX) activity

The activity of APX was estimated by following the method of Harb et al. (2015). The reaction mixture with pH 7.0 potassium phosphate buffer (25 mM), 0.1 mM EDTA, 0.25 mM ascorbate and H<sub>2</sub>O<sub>2</sub> of 1 mM was mixed with 0.04 mL of enzyme extract. Enzyme activity was measured by observing the decline in absorbance at 290 nm.

## Statistical analysis

With three replicates, the experiments were carried out in a fully randomised design. SPSS software (Version 21) was used to analyse the data. Data shown are the means (sum of three replicates) and standard deviations. Vertical bars signify standard deviation and, on the top, different letters represent significant difference among the means according to Tukey's test ( $P < 0.05$ ). Correlation between variables, hierarchical cluster analysis (HCA) and principal component analysis (PCA) were conducted in R version 4.0.3 (R Core Team 2020).

## Results

### Effect of drought on morphological characteristics

Drought stress affected both elongation and expansion growth in both the cultivars of *B. rapa*. KJD and BJDB displayed up to 63% and 59% increase in overall plant height compared to the control plants (KC and BC), while in KD and BD it increased by 4% and 1.5% only when compared to the control. Root growth increased in the JA-supplemented drought-stressed plants (360% in KJD and

463% in BJDB) than the BR-supplied drought-stressed plants (54% in BDB and 1% in KDB). During drought stress, root length in both KD and BD increased up to 20% and 24% compared to the control KC and BC. Stem length was significantly reduced in JA- and BR-treated plants, but KJD and KJDB displayed reduction in stem length, while in case of KD and KDB stem length was increased by 18 and 5%. Petiole length was decreased during all the treatments in the KS101 and KBS3 genotypes, except in KD in which it was increased up to 8%. The leaf length and leaf breadth in both the cultivars were decreased during all the treatments when compared to control (see Fig. 1, Table 1).

## Photosynthetic pigments

The total chlorophyll content increased during all the treatments except KD and BJD, where it declined by 15 and 24% compared to control plants (KC and BC). We observed an increase in the total chlorophyll content in both the BR- and JA-treated plants during drought as illustrated in Fig. 2a. The maximum chlorophyll content was observed in KDB and BDB which increased by 17 and 13%. Analysis of KJD, BJD, KJDB and BJDB revealed an increase of 5, 3, 8 and 5%, respectively, compared to the control plants.

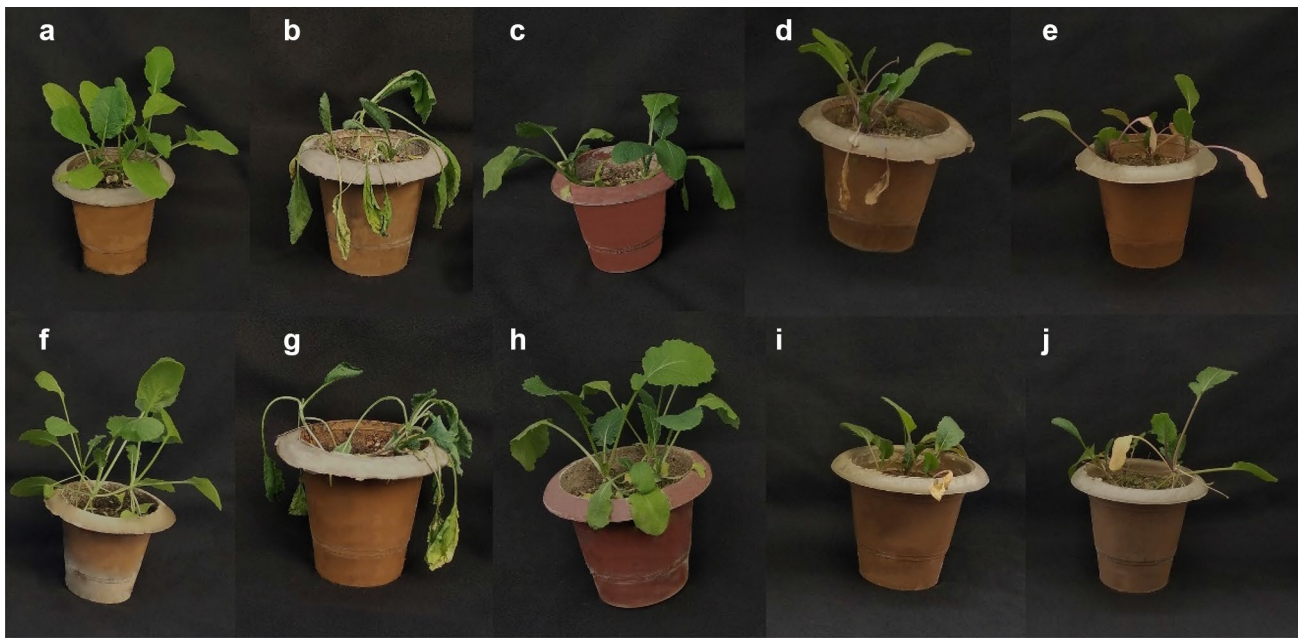
The carotenoid content increased significantly in all the treatments; however, the increase was more in KD and BD than the other treatments in comparison to the control (Fig. 2c).

Anthocyanin shows a significant increase of 28 and 19% in case of KDB and BDB. However, it increased only by 12 and 5% in KD and BD. Anthocyanin content was also increased to various extents in other treatments as depicted in Fig. 2b.

## Photosynthetic parameters

Drought stress resulted in reduced photosynthesis in both cultivars. KD and BD displayed reduction in photosynthesis by 86 and 89%, while in KJDB and BJDB the minimal reduction of 21% was observed (Fig. 3a). Drought stress reduced the transpiration rate in both the genotypes. We observed significant decline in transpiration rate in KD and BD by 90 and 89%, respectively, when compared to KC and BC. However, transpiration rate increased by 31% in the BJDB genotype when treated with both BR and JA as compared to its control (Fig. 3b). Stomatal conductance was reduced in both KD and BD by 84 and 54%, respectively, but increased by 6 and 52% in BJD and BJDB (Fig. 3c). Water use efficiency was highest in KJDB and BDB (Fig. 3d).





**Fig. 1** The effect of drought stress with and without the combination of phytohormones. **(a)** Control plants (KC), **(b)** drought plants without the exogenous application of phytohormones (KD), **(c)** drought plants with brassinosteroid treatment (KDB), **(d)** drought plants with JA treatment (KJD), **(e)** drought plants with exogenously supplied

Br and JA treatment (KJDB), **(f)** control plants (BC), **(g)** plants during drought without phytohormone treatment (BD), **(h)** plants with drought and exogenously supplied brassinosteroid (BDB), **(i)** plant with drought and JA treatment (BJD), **(j)** plants during drought with exogenously supplied JA and Br (BJDB) (colour figure online)

**Table 1** Effect of different treatments on the morphological parameters of KS101 and KBS3 genotypes (in cm)

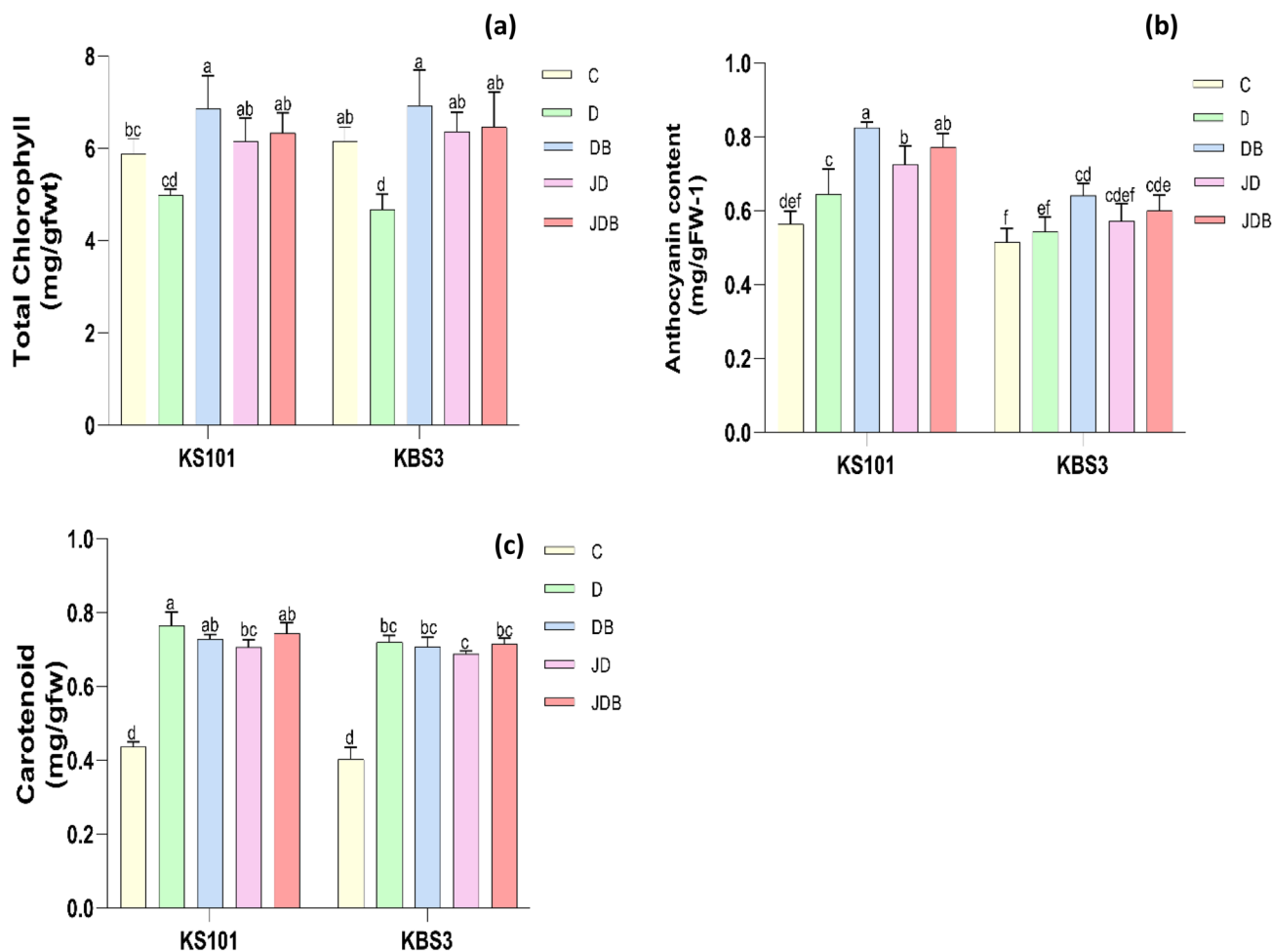
	Plant Height	Stem Length	Root Length	Petiole Length	Leaf Length	Leaf Breadth
KC	20.11	3.56	4.70	5.77	6.38	3.66
KD	20.93	4.20	5.64	6.21	5.72	3.58
KDB	20.56	3.73	4.69	5.67	5.70	2.92
KJD	32.83	2.50	21.61	4.24	3.97	2.61
KJDB	26.06	2.59	15.11	3.72	3.97	2.04
BC	19.82	3.69	3.89	7.56	5.24	4.04
BD	20.90	3.62	4.81	6.12	4.82	2.83
BDB	19.89	2.77	5.99	5.98	4.93	2.54
BJD	27.68	2.21	19.34	3.06	4.06	1.72
BJDB	31.50	2.60	21.87	3.63	3.62	2.11

### Effect of drought stress on osmolytes

Drought stress led to the elevation of glycine betaine by 11 and 22% in KD and BD, respectively. Maximum levels of GB were observed in KDB (34%) and BDB (36%), indicating that BR induced glycine betaine formation in drought-stressed genotypes of *B. rapa*. Similarly, JA too led to the increase in glycine betaine concentration in both the genotypes (Fig. 4a).

The proline content increased significantly in all the treatments in comparison to control; however, the percentage increase was more in BR- and JA-treated plants than the simple drought-stressed plants (Fig. 4b).

Drought stress led to the increase in total soluble sugars; however, drought stress along with BR supplement led to maximum elevation, as we observed in KDB (32%), BDB (42%). The slight increase in the sugar content was found in KD and BD which increased by 23 and 22% in KC and BC. Total soluble sugars also increased in drought-stressed



**Fig. 2** Change in photosynthetic pigments in the KS101 and KBS3 genotypes: **(a)** total chlorophyll, **(b)** anthocyanin content, **(c)** carotenoid content. Data shows the mean  $\pm$  SD of three or more replicates and vertical bars signify standard deviation and, on the top, different letters represent significant difference among the means according to

Tukey's test ( $P < 0.05$ ). In legends, C represents KC and BC, D represents KDB and BDB, DB represents KDB and BDB, JD represents KJDB and BJDB, JDB represents KJDB and BJDB; this will be followed throughout the manuscript (colour figure online)

plants supplemented with JA, KJD, BJD, KJDB and BJDB (Fig. 4c).

### Change in protein content in response to drought stress and exogenous supply of BR and JA

Protein content decreased significantly by 79 and 77% in KD and BD in response to drought in both the genotypes. However, in case of KDB and BDB only 12 and 7% decrease in protein content was observed. At the same time, decrease in the protein content in KJD, BJD, KJDB and BJDB was less than that of KD and BD (Fig. 4d).

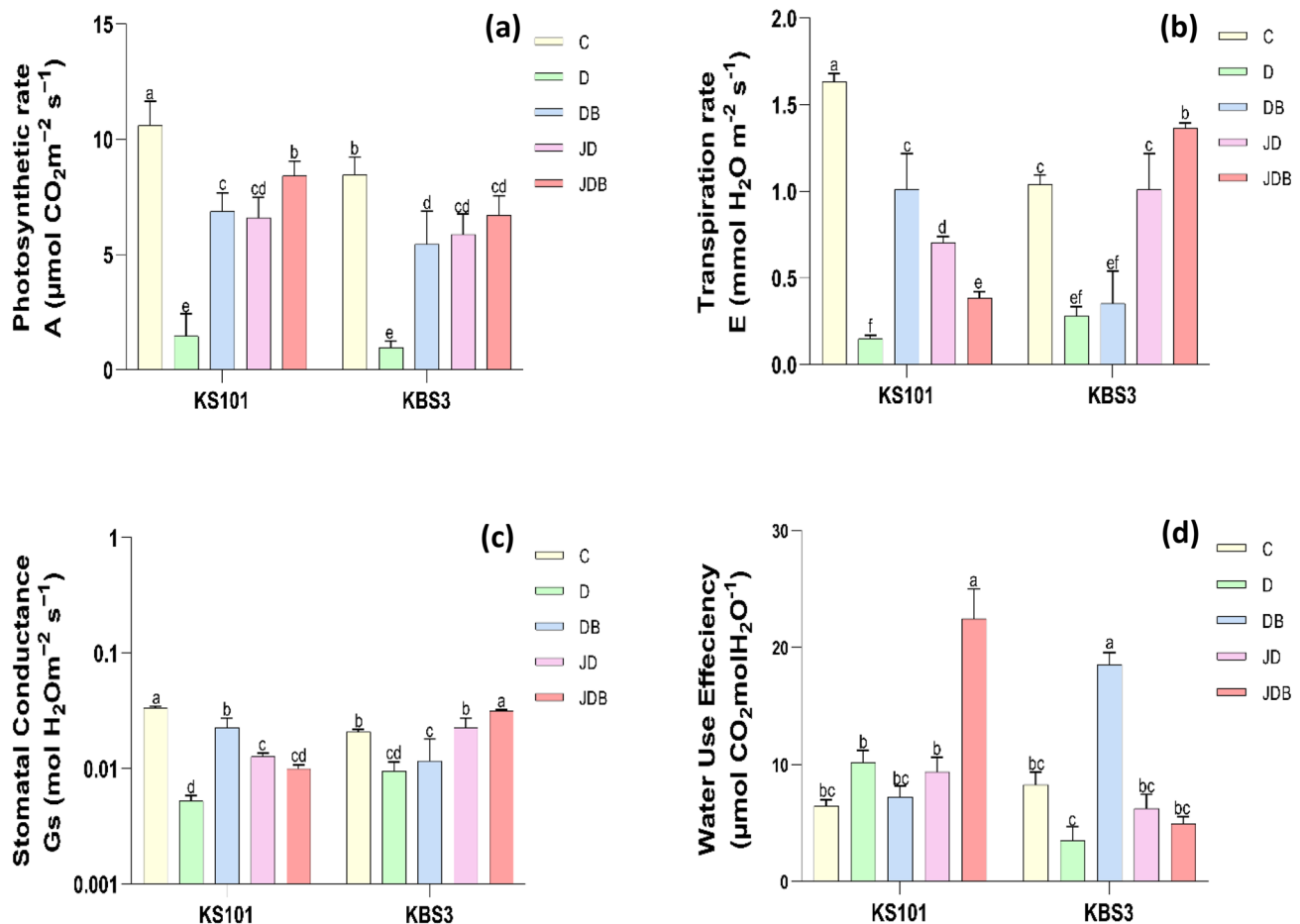
### Lipid peroxidation

All the treatments displayed a slight increase in the MDA content as compared to the control during the drought

stress. We observed a highest increase in MDA content by 2% and 7% in KD and BD, while only slight increase of the MDA content by 0.01 and 0.3% was observed in KDB and KJD. The increase in other cases was also much less than that of KD and BD as can be clearly seen in Fig. 5a–j and Fig. 6a.

### Relative water content

Relative water content changed in all the treatments as illustrated in Fig. 6b. In both genotypes of *B. rapa*, RWC reduced during the drought treatment compared to their control. RWC significantly decreased in both KD and BD by 58% and 52%; however, it increased by 23, 27, 66, 55, 40 and 86% in KJD, BJD, BJDB, KDB, BDB and KJDB respectively compared to KD and BD as depicted in Fig. 6b.



**Fig. 3** Change in photosynthetic parameters in KS101 and KBS3 genotypes. (a) Photosynthetic rate. (b) Transpiration rate. (c) Stomatal conductance. (d) Water use efficiency. Data show the mean  $\pm$  SD of three or more replicates and vertical bars signify standard deviation

tion and, on the top, different letters represent significant difference among the means according to Tukey's test ( $P < 0.05$ ) (colour figure online)

### Change in phenolic content

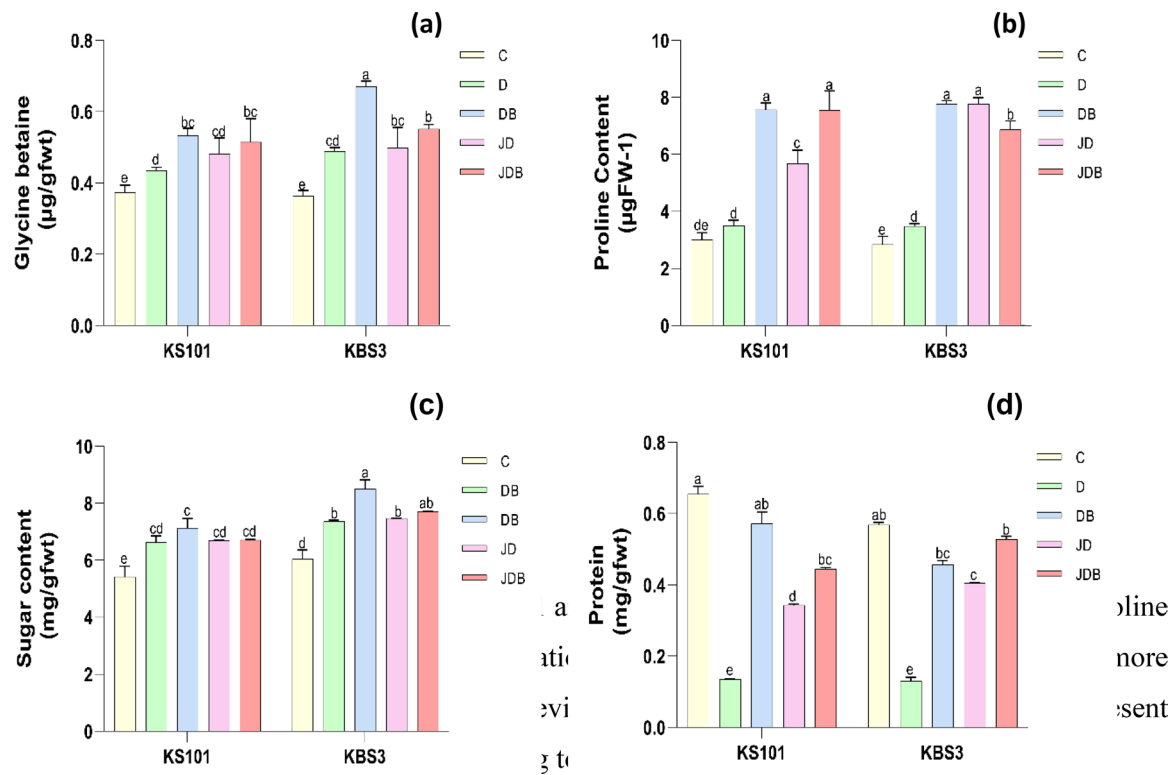
Phenolic content showed a significant increase during all the treatments except in BJD where it decreased significantly by 65% compared to BC. The highest phenolic content was found in KJD (237%) and KDB (179%), followed by BJDB (112%). The phenolic content in both KD and BD elevated significantly by 75% (Fig. 6c).

### Change in antioxidant enzyme activities

The overall activity of antioxidant enzymes POD, CAT and GR increased during all the treatments, be it stress alone or in combination with BR and JA, compared to the control. APX activity reduced in case of BJD by 4% and its highest activity was observed in KDB (42%) and BDB (43%). In the case of KD and BD, APX activity increased by 1 and 0.1%.

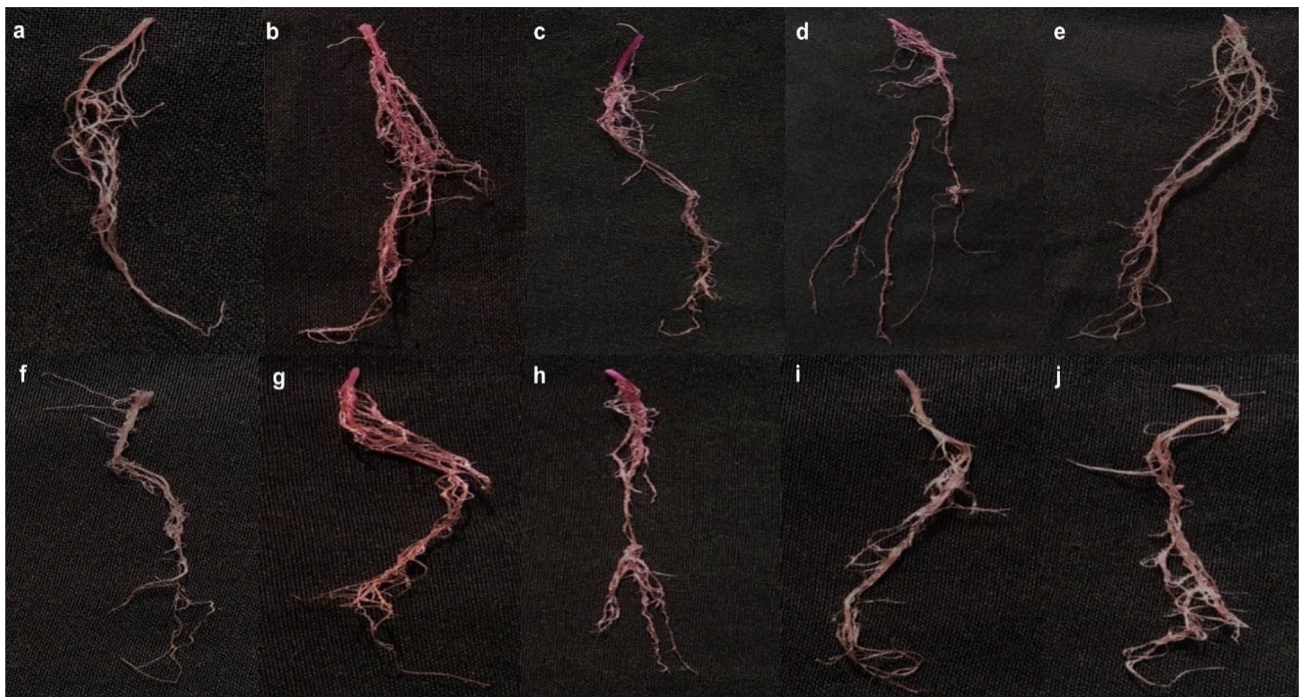
The APX activity in the case of KJDB, KJD and BJDB also increased during drought stress as shown in Fig. 7a.

SOD activity increased significantly by 239- and 244-fold in KDB and BDB, while in the case of KD and BD its activity increased only by 102 and 70% when compared to the control. Its activity was also increased by 202 and 187% in KJDB and BJDB (Fig. 7c). Maximum CAT activity was found in KDB (210%) and BDB (264%), while its minimum activity was observed in KD (23%), BD (11%) and KJD (23%) (Fig. 7b). In case of KDB and BDB, GR activity was increased significantly by 271 and 312% and that of KJD and BJD was increased by 25 and 45% as compared to KC and BC (Fig. 7d). Increased POD activity by 384 and 323% during the drought was found in KDB and BDB and its enzymatic activity was only 0.7, 0.9 and 6% in KD, KJD and BJD (Fig. 7e).



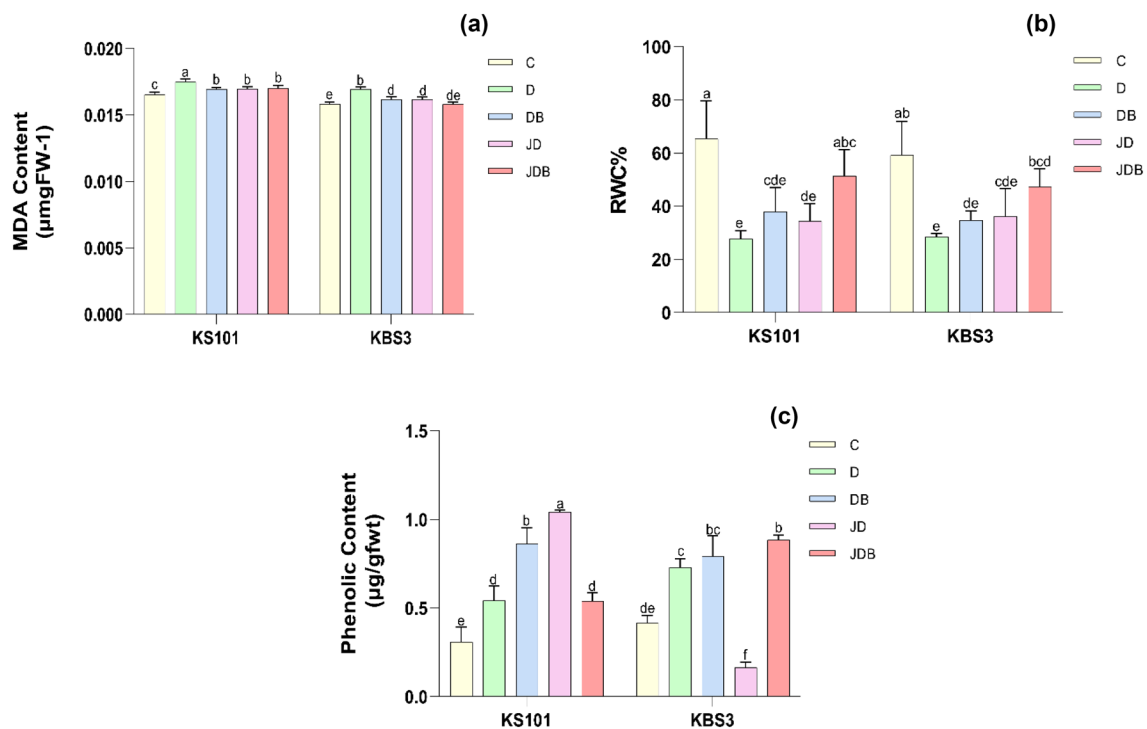
**Fig. 4** Change in osmolytes and protein in KS101 and KBS3 genotypes. **(a)** Glycine betaine, **(b)** proline content, **(c)** sugar content. **(d)** Protein concentration. Data show the mean  $\pm$  SD of three or more

replicates and vertical bars signify standard deviation and, on the top, different letters represent significant difference among the means according to Tukey's test ( $P < 0.05$ ) (colour figure online)



**Fig. 5** Lipid peroxidation of roots during drought stress in *Brassica rapa* KS101 and KBS3 genotypes. **(a)** KC, **(b)** KD, **(c)** KDB, **(d)** KJD, **(e)** KJDB, **(f)** BC, **(g)** BD, **(h)** BDB, **(i)** BJD, **(j)** BJDB (colour figure online)





**Fig. 6** Change in different biochemical parameters in KS101 and KBS3 genotypes, (a) MDA content (b) relative water content, (c) phenolic content. Data show the mean  $\pm$  SD of three or more repli-

cates and vertical bars signify standard deviation and, on the top, different letters represent significant difference among the means according to Tukey's test ( $P < 0.05$ ) (colour figure online)

## ROS accumulation in leaves

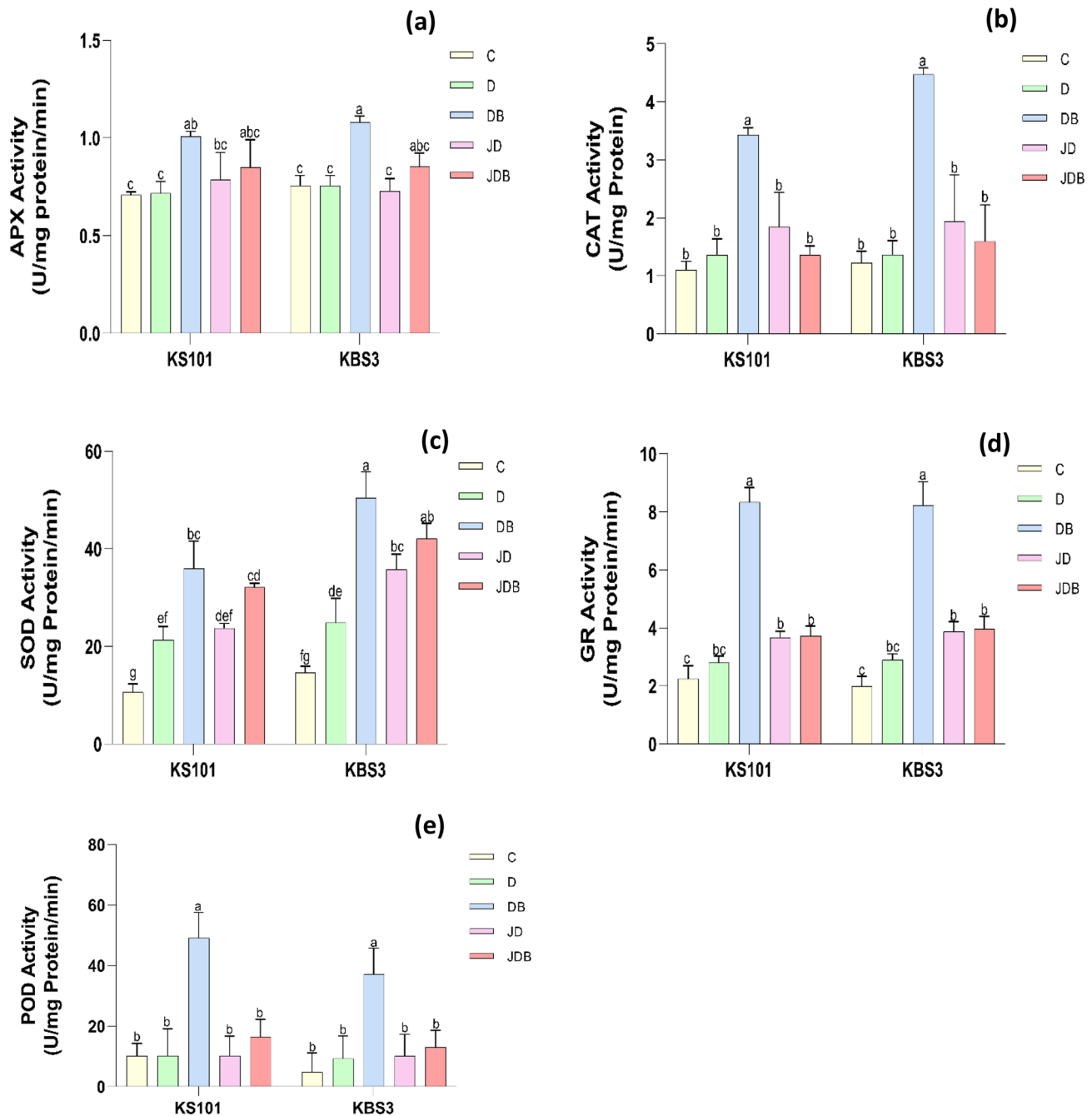
We assessed the accumulation of ROS by allowing DAB–H<sub>2</sub>O<sub>2</sub> reaction in leaves which resulted in the formation of localised brown pigment after the leaves were decolourised. Drought-treated plants had noticeably more brown pigmentation than control as well as BR- and JA-treated plants (Fig. 8a–j).

## Statistical analysis

Hierarchical cluster analysis (HCA) and principal component analysis (PCA) have shown that the exogenous application of BR and JA results in strong correlations among the vectors such as SOD, POD, CAT, GR, APX, carotenoids, phenolics, GB, proline, sugar and total chlorophyll in KS101 genotype (Fig. 9). Further, in the KBS3 genotype, a strong correlation was found among the vectors such as SOD, POD, CAT, GR, APX, GB, phenolics and sugar (Fig. 10). Further, cluster analysis has shown that the BR- and JA-applied plants form clusters which are close to control plants. However, the drought-stressed plants form a separate cluster distant from the other treatments (Figs. 9, 10).

## Discussion

Drought stress was induced in two genotypes of *B. rapa* in 21-day-old seedling stage by withholding water supply which created water deficit in these seedlings. We observed decreased fresh and dry weights along with reduced RWC in leaves of drought-stressed plants. According to several research reports, the most common impacts of drought on plants is the decrease in plant biomass and RWC (Mahmood et al. 2012). Drought stress greatly influences plant–water relationship, resulting in osmotic stress in plants which finally results in reduction in growth and affects plant development (Xu et al. 2014). However, the research studies have revealed that this reduction in growth and osmotic stress under drought can be improved by the exogenous supplementation of hormones such as BR and JA. Plant height, leaf length, FW and DW declined considerably in both the KS101 and KBS3 genotypes under drought stress conditions during the present study. Further, it was observed that the exogenous application of JA and BR counters the drought stress significantly. In addition, it was found that the exogenous supplementation of drought-stressed plants with the two hormones was effective in improving the RWC of plants (Fig. 6b). Several other studies have also revealed that BR and JA are effective agents in restoring the water content in plants during

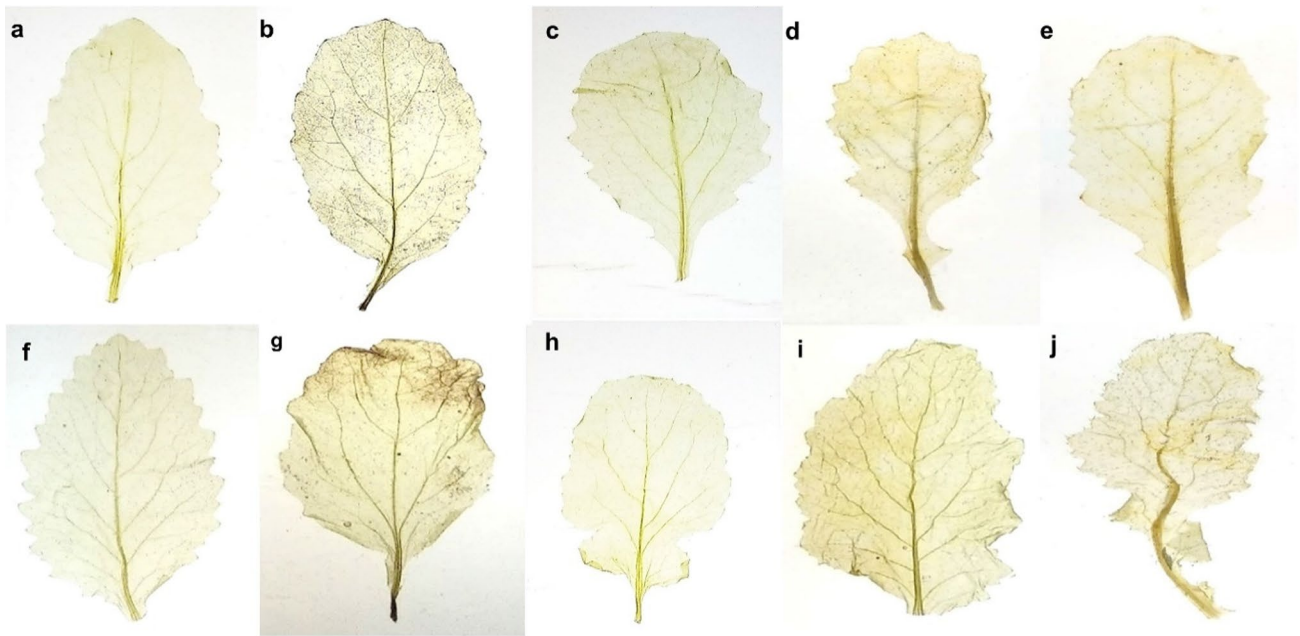


**Fig. 7** Change in the antioxidant enzyme activity in KS101 and KBS3 genotypes. **(a)** APX activity, **(b)** catalase activity, **(c)** SOD activity, **(d)** GR activity, **(e)** POD activity. Data show the mean  $\pm$  SD of three or more replicates and vertical bars signify standard deviation and, on the top, different letters represent significant difference among the means according to Tukey's test ( $P < 0.05$ ) (colour figure online)

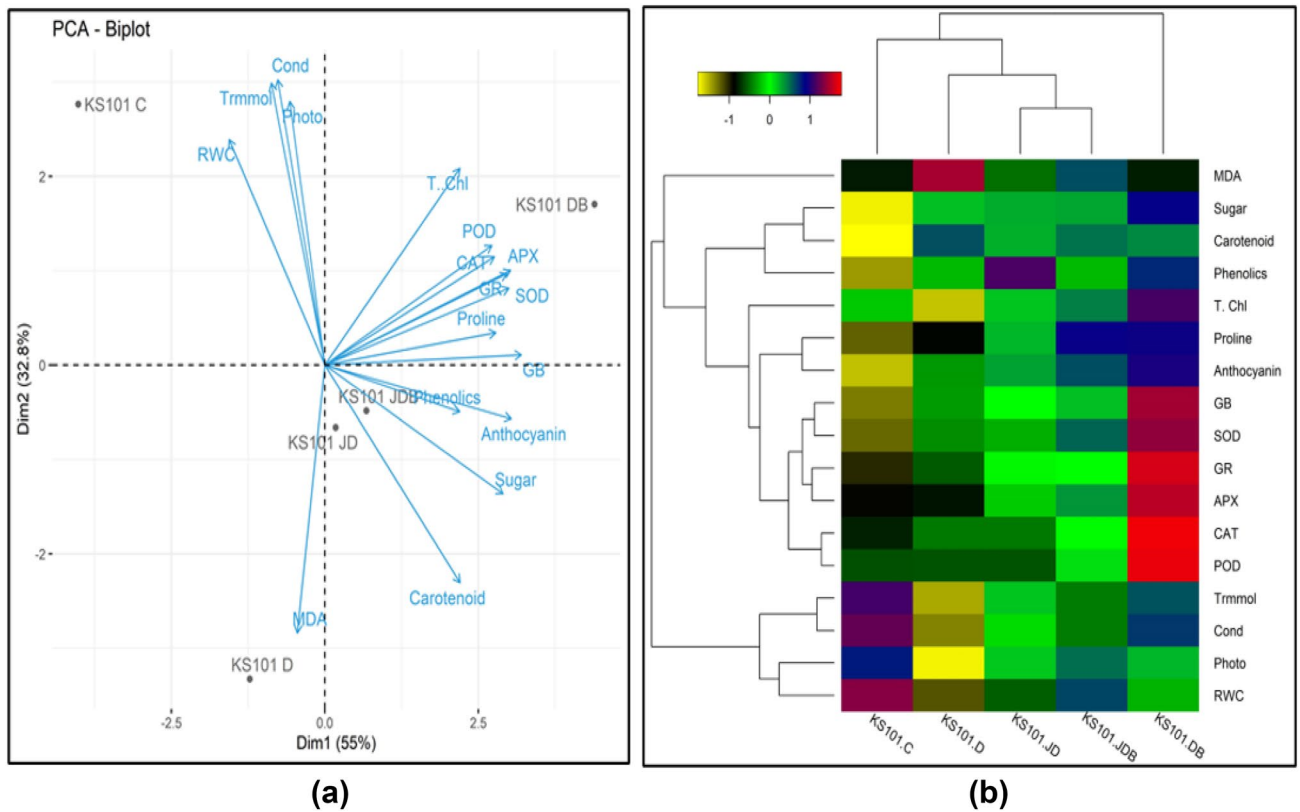
tion and, on the top, different letters represent significant difference among the means according to Tukey's test ( $P < 0.05$ ) (colour figure online)

water shortage (Alam et al. 2014). The drought stress has also resulted in increased growth of roots than the control plants. This root length was further increased with the exogenous application of JA and BR. However, the increased root length was more significantly increased by JA than the BR treatment. Crops grown in drought stress

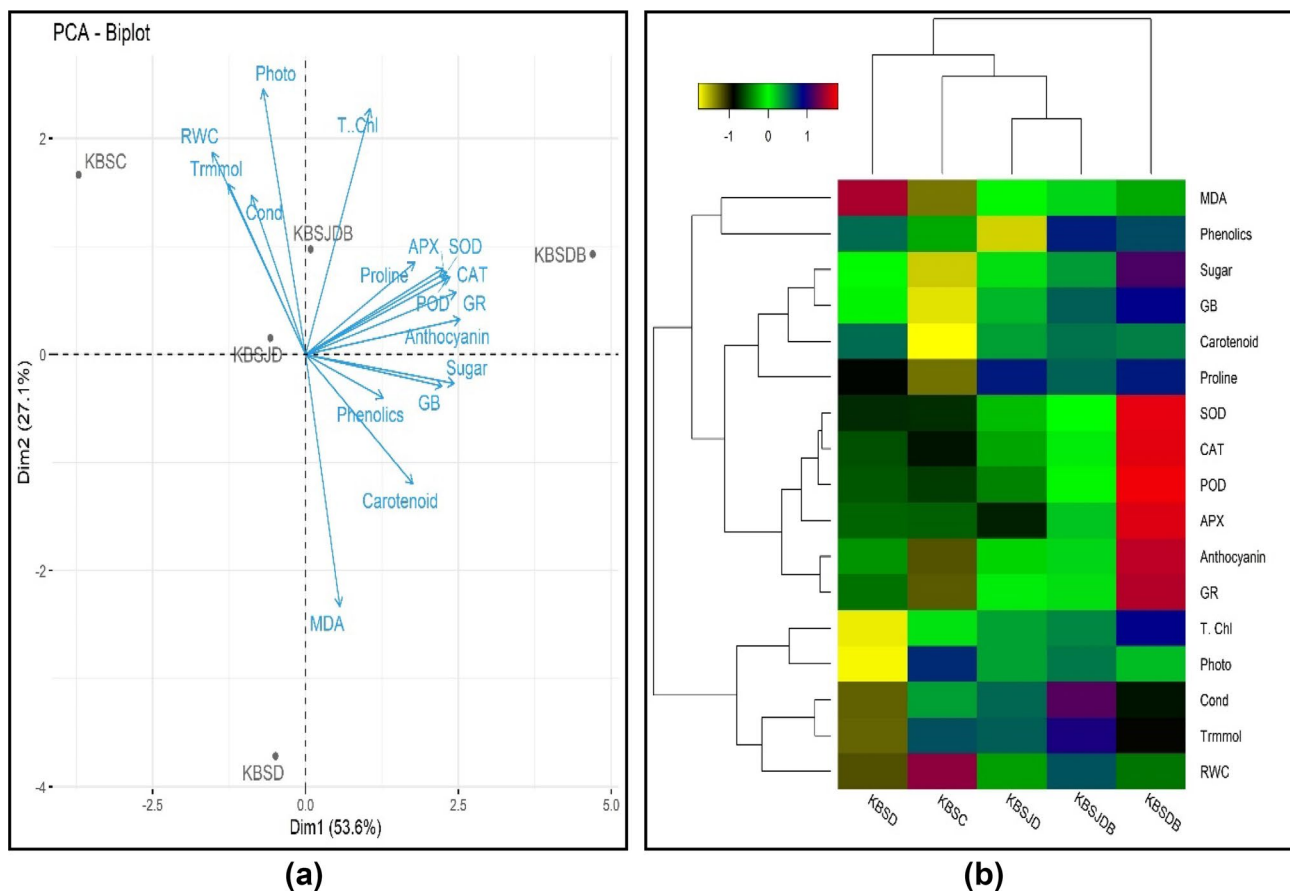
require a large root system to absorb more soil water and alleviate drought stress (Ehdaie et al. 2012), because JA transitory buildup is required for abscisic acid-regulated increase in root length during drought stress (Ollas et al. 2013). Higher root dispersion at depth in the subsurface layers is thought to be a potential characteristic for plant



**Fig. 8** DAB assay showing the generation of H<sub>2</sub>O<sub>2</sub> in leaves. (a) KC, (b) KD, (c) KDB, (d) KJD, (e) KJDB, (f) BC, (g) BD, (h) BDB, (i) BJD, (j) BJDB (colour figure online)



**Fig. 9** (a) Principal component analysis, (b) heatmap showing physio-biochemical changes in the KS101 genotype (colour figure online)



**Fig. 10** (a) Principal component analysis, (b) heatmap showing physio-biochemical changes in the KBS3 genotype (colour figure online)

adaptation to water stress, enhancing the subsoil profile's water extraction capability for grain filling and enhanced grain production (Ehdaie et al. 2012).

The abiotic stress environments lead to overproduction of ROS resulting in oxidative stress. The DAB assay showed that the drought stress leads to production of  $H_2O_2$  which was reduced by the application of BR and JA. MDA, an indicator of oxidative stress and membrane impairment in plants, is formed when unsaturated fatty acids in the phospholipid layer of membranes experience peroxidation owing to increased production of ROS inside cells (Martinez et al. 2018). The increased MDA content under drought stress compared to control genotypes (KC and BC) indicates membrane lipid peroxidation and, thus, membrane integrity is lost. Various studies have shown that the exogenous supplementation of BR and JA decreases the levels of MDA due to production of various osmolytes which scavenge the ROS and prevent peroxidation of lipids (Mir et al. 2018). The histochemical analysis of roots also revealed that the MDA levels were decreased in the BR- and JA-applied plants (Fig. 5a–j).

Drought stress leads to decrease in photosynthetic pigments such as chlorophyll, carotenoid and anthocyanins in

different crop plants. Furthermore, it negatively impacts the photosynthesis due to ROS-induced oxidation of photosynthetic pigments, impaired biosynthesis of pigments, destruction of chlorophyll structure and stomatal closure (Banks and botany 2018). Our study revealed that under drought stress conditions, the chlorophyll content reduces to a large degree than in the control plants in both KS101 and KBS3 genotypes of *B. rapa* (Fig. 2a). Reduced activity of the enzymes—proto-chlorophyllide reductase and aminolevulinic acid dehydratase (ALA-dehydratase)—both important components in chlorophyll biogenesis, could explain the decrease in chlorophyll material (Padmaja et al. 1990). A similar trend was observed for carotenoids which decreased under drought stress compared to the control set of both the genotypes of *B. rapa*. Supplementation of both BR and JA reduced the reduction in the pigment concentration due to drought stress irrespective of the genotype. However, the positive impact of BR was more as compared to JA, showing that BR has a more dominant role in drought stress compared to JA. Both the hormones have indeed significant positive impact on photosynthetic pigments (Mahmood et al. 2012). Carotenoids are known to prevent chlorophyll molecules from photo-oxidative damage (Prasad et al. 2005).



Janeczko et al. (2007) revealed that BR application results in augmented carotenoid content when applied to drought-stressed plants. The anthocyanin contents showed a significant increase under water deficit conditions than the control in both KS101 and KBS3 genotypes of *B. rapa*. Further, it was observed that the exogenous application of BR and JA increased the anthocyanin content in both the genotypes. However, it was found the BR treatment was more effective in improving the anthocyanin content in both the genotypes (Fig. 2b). Previous studies also show that exogenous supply of BRs upregulates the anthocyanin content by increasing the expression of BR genes (Luan et al. 2013). The photosynthetic investigation analysed by IRGA revealed that the rate of photosynthesis, water use efficiency, conductance of stomata and the rate of transpiration reduced considerably due to drought stress. It has been found that drought conditions affect photosynthesis and growth by disturbing the water exchange properties and leaf gas exchange attributes (Farooq et al. 2010). Nevertheless, the exogenous supply of BR and JA improves these attributes significantly (Fig. 3) and this corroborates with the research study by (Behnamnia et al. 2009) which showed that BR and JA had positive impact under drought stress in normalising the water use efficiency, photosynthetic rate, stomatal conductance and transpiration rate. By stabilising membrane structures under stressful conditions, BRs improve photosynthetic rate and carbon absorption in PSII antennae (Farooq et al. 2010), as well as increase efficiency in light utilisation and dissipation of excessive excitation energy (Hu et al. 2013).

Plants amass diverse non-toxic compounds such as sugars, proline, GB, phenolics and other compounds that help them withstand osmotic stress (Jan et al. 2018). Many studies reveal that proline content upsurges by several folds in response to drought stress, not only for osmotic equilibrium but also for free radical scavenging, cellular structure stability and redox potential (Alam et al. 2013). Further it was found that the exogenous application of JA and BR augmented the proline levels in all treatments in both the genotypes (4A). In our study, the concentration of GB increased significantly in reaction to water deficit. Further, in response to exogenous supplementation of BR and JA, the GB increased to a larger extent even more than the plants facing drought. However, the increase in GB was higher in BR-applied plants than the JA-applied ones. GB has been found to provide plants with abiotic stress tolerance by activating LOX (lipid peroxidase) and FAD (fatty acid desaturase), which preserve the stability of membrane or mediate the regulation of various genes (Karabudak et al. 2014). Sugars are main molecules present in the cells under drought stress and uphold osmotic balance in plants (Fariduddin et al. 2013). The present study observed that, although the water deficit elevated the levels of soluble sugars in both the genotypes, exogenous application of BR and JA further increased the

soluble sugar content to larger extent (Fig. 4c). However, the increase in sugars was found more in BR-applied plants than the JA-applied ones, similar to proline and GB. The PCA and HCA analysis has shown that strong correlation exists among the different osmolytes under DS, and the application of BR and JA further strengthens this correlation. In this study, BR and JA stimulated the synthesis of phenolics, which are important for DS tolerance in several plants (Anuradha et al. 2007; Wahid and Ghazanfar 2006; Wahid 2007). However, the increase was more in JA-applied plants. Showing the role of JA in upregulating phenolics under DS owing to increased PAL (phenylalanine ammonia lyase) activity (Fig. 6c) (Yoshida et al. 2020). The phenolics have phenol ring which plays significant role in the stabilisation of membrane during drought stress and creates a network of ROS scavenging in plants (Farooq et al. 2009).

Exogenous supplementation of BR and JA has been shown in several studies to upregulate the levels of antioxidant enzymes in drought-stressed plants. SOD is one of the first antioxidant enzymes against oxidative stress, converting  $O_2^-$  to  $O_2$  and  $H_2O_2$  (Cruz de Carvalho 2008), which is then decomposed in the cytosol and peroxisomes by APX and CAT, respectively (Zhao et al. 2018). Under DS, the exogenous supplementation of BR and JA results in strong correlations among antioxidant enzymes such as SOD, POD, CAT, GR, and APX (Fig. 7). Several studies have revealed that BRs and JA can activate the transcription of various genes coding for antioxidant enzymes and increase the activity of key enzymes of the antioxidant pathway, i.e. SOD, APX, POD, GR and CAT (Alam et al. 2014; Vardhini et al. 2010).

## Conclusion

Plants are vulnerable to drought stress, and significant yield reductions have been documented in recent decades; therefore, understanding the many underlying drought response mechanisms in plants is critical. Exogenous treatment of BR and JA, either separately or together, considerably reduced the effect of drought stress by increasing the photosynthetic rate, photosynthetic pigments, stomatal conductance, transpiration rate and antioxidant defence. The application of BR and JA resulted in a rise in the concentration of various osmolytes and antioxidants such as sugars, glycine betaine (GB), proline (Pro), proteins/free amino acids and SOD, APX, POD, GR and CAT, which increase the cellular osmotic potential, ROS scavenging and a considerable reduction in membrane damage. We conclude that the exogenous supply of BR and JA has significant role in alleviating drought stress in KS101 and KBS3 genotypes of *B. rapa*. However, BR has more effective role in imparting drought tolerance to both the genotypes of *B. rapa* compared to JA.

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**Author contribution** WA, NM and UY did all the experiments, analysis of data and manuscript writing. RJ conceived the idea, supervised the work and finalised the manuscript.

**Declaration**

**Conflict of interest** The authors declare no conflict of interest.

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