

# Interactive Effects of Melatonin and Salicylic Acid on *Brassica Napus* Under Drought Condition

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## Research Article

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# Abstract

## Aims

Understanding the interactive actions of melatonin and salicylic acid in counteracting the negative effects of drought is imperative for increasing growth and yield of canola. Here we discuss the interactive effects of exogenously applied melatonin (MET) and salicylic acid (SA) on morphology, physiology, and biochemical characteristics of drought stressed canola plants.

## Methods

Two cultivars of canola (Super canola and Faisal canola) were used. MET (0.1  $\mu$ M) and SA (0.50mM) were applied in two ways, i.e., as seed priming and as foliar spray. Plants were exposed to severe drought stress of 45% field capacity at reproductive stage.

## Results

Subjecting canola cultivars to drought caused significant reduction in shoot length (19%), plant fresh (15%) and dry (17%) biomass, yield (30%), photosynthetic attributes (43%), meanwhile enhanced free amino acids (20%), total soluble sugars (5%), and different antioxidant enzymes like catalase (27%), peroxidase (23%) and superoxide dismutase (20%) Seed priming, and foliar application of MET and SA resulted in significant increase in growth parameters and yield attributes of both canola cultivars via improving photosynthetic attributes and activity of compatible solutes and antioxidant enzyme systems. Priming of seeds with MET as well as MET priming combined with SA foliar spray were the most effective treatments of all, showing 22% increase in plant height, 46% in fresh and 40% in dry biomass and yield components (49%) of canola cultivars at either drought stress or normal irrigation.

## Conclusions

The combined application of MET and SA has the potential to enhance canola plants growth under drought.

## Introduction

As the world population which is currently about 7 billion is expected to reach 9 billion by 2050, food security to support this much population is threatened to a larger extent by different types of environmental stresses (Khan et al. 2020). Among these, drought is a severe problem for the agriculture of Pakistan, affecting crop growth and yield. More than 1.2 billion hectares of agricultural land of the world are affected by drought stress (Cui et al. 2017; Urban et al. 2017). A limited supply of irrigation water is the main factor responsible for the low yield and production of crops in Pakistan (Liang et al.

2019). Water stress shows its effect by reducing root growth, seed number, and size of leaves as well as stomatal opening (Kamran et al. 2018). It also delays flowering and fruiting in plants thus reducing their productivity (Cui et al. 2017; Naeem et al. 2018; Osakabe et al. 2014; Xu et al. 2016). Canola (*Brassica napus*) is normally grown in Pakistan in areas that depend upon rainwater for good crop growth, so the availability of water remains a major limiting factor for plant growth and development in these rainfed areas (Tariq et al. 2019).

Brassica crops commonly grown as oilseed crops in Pakistan include Rapeseed (*B. rapa* and *B. napus*) and mustard (*B. juncea*). Among these, mustard is traditionally used in Pakistan, however, its oil is not preferred in the preparation of vegetable oil or ghee due to the presence of very high amounts (40-70%) of erucic acid in oil, which is not suitable for human consumption (Onemli 2014). The annual edible oil requirement of Pakistan is about 1.95 million tonnes, of which only 18% is obtained from the local resources and the rest 88% is imported (Mustafa et al. 2017). *B. napus*, being indigenous species, has all the potentials to overcome the problems of low local oil production. It contains 44-46% of good quality oil, with its meal having 38-40% of protein contents rich in lysine, methionine, and cystine amino acids (Onemli 2014).

The yield of crops in most areas of Pakistan is drastically affected by drought. Canola is cultivated in high rainfall regions and shows low production in areas with low rainfall (Ahmadi and Bahrani 2009; Tariq et al. 2019). To overcome drought, plant cells show different mechanisms which help them to survive under mild water stress (Osakabe et al. 2013). Among these, the production of plant growth regulators plays a vital role in plants' resistance against drought. Plant resistance to drought is found to be significantly boosted by exogenous application of these plant growth regulating substances (Peleg and Blumwald 2011).

Melatonin and Salicylic acid are two important plant growth regulators. Melatonin (N-acetyl-5-methoxytryptamine), being a relatively 'new' compound discovered in plants, has become a focus of plant researchers due to its discovery in numerous plants and tremendous potentials as a regulator of many physiological processes of plants. Melatonin application not only promotes plant growth and development but it also induces stress resistance in plant cells (Li et al. 2017; Liang et al. 2019; Martinez et al. 2018; Reina et al. 2018; Wang et al. 2016). Salicylic acid is involved in the regulation of developmental processes in plants. It is a phenolic compound that regulates plants' responses to biotic and abiotic stresses (Khan et al. 2013; Miura and Tada 2014). Many physiological processes in plants like photosynthesis, nitrogen metabolism, synthesis of glycine betaine, and production of antioxidants are regulated by salicylic acid, providing plants resistance against abiotic stresses (Anwar et al. 2013; Khan et al. 2014; Miura and Tada, 2014).

Present research work aimed to evaluate the response of two *B. napus* cultivars to drought stress concerning their growth, yield, and biochemical responses. In particular, our goal was to investigate the potential of the combined application of melatonin and salicylic acid, via pre-sowing seed treatment and

foliar spray, in inducing drought resistance and mitigating the drought stress effects on two canola cultivars.

## Materials And Methods

### Experimental setup

To check the efficacy of melatonin (MET) and salicylic acid (SA), potential plant growth regulators, in reducing the adversity of drought stress on the growth of canola (*Brassica napus* L.), exogenous application of these compatible solutes as seed priming, and foliar spray methods were employed in soil culture pot experiment. An experimental area of the department of Botany, PMAS Arid Agriculture University, Rawalpindi was used for the experiment. Two canola cultivars, namely Super canola (V1) and Faisal canola (V2) were used. Seeds were attained from National Agriculture Research Center Islamabad, Pakistan.

Before sowing, seeds were surface sterilized with 10% Clorox. Potassium, Phosphorous, and nitrogen fertilizers were applied to the soil prior to seed sowing. Soil analysis was also carried out before starting the experiment. Two plant growth regulators, Melatonin (Sigma-Aldrich, Lot# SLBZ6359) and Salicylic acid (Sigma-Aldrich, Lot# STBH3314) were used in present study. MET (0.1 $\mu$ M) and SA (0.5mM) were applied in two different ways, firstly, seed priming was done for 24 hours using 0.1 $\mu$ M concentration of MET, 0.5mM concentration of SA, and a combined seed priming treatment of 0.1 $\mu$ M MET and 0.5mM SA, later foliar spray of MET, SA and MET plus SA combined was used at adult growth stages in both control and water stressed plants. 4 hours seed soaking in distilled water (for control) was also done, before sowing. Drought stress was established at 45% field capacity 60 days after sowing, and the plants were grown in the 2020-2021 growing season. The treatments application included controlled irrigation (T<sub>0</sub>), MET priming (T<sub>1</sub>), MET foliar spray (T<sub>2</sub>), SA priming (T<sub>3</sub>), SA foliar spray (T<sub>4</sub>), MET plus SA combined priming (T<sub>5</sub>), MET plus SA combined foliar spray (T<sub>6</sub>), MET priming combined with SA foliar spray (T<sub>7</sub>), SA priming combined with MET foliar spray (T<sub>8</sub>). 10 days after the imposition of drought, data for different photosynthetic attributes of each plant was recorded by using portable infrared gas analyzer (IRGA). Leaf samples were then collected for further physiological and biochemical analysis.

### Morphological and yield attributes

Data for different morphological parameters, i.e., Plant height (cm), root length (cm), plant fresh and dry biomass (g), and yield attributes i.e., No. of siliqua per plant, No. of seeds per siliqua was recorded.

### Gas exchange parameters

Infrared gas analyzer (LCA-4 ADC) was used for the estimation of gas exchange characteristics. These measurements were carried out from 10:00 to 14.00 hours. Measurement of photosynthetic rate, transpiration rate, water use efficiency and stomatal conductance were made using the young leaf.

## Biochemical Attributes

Bates et al. (1973) method was followed for estimation of protein contents. 0.2g of fresh leaf material were mixed with 10ml of phosphate buffer. 0.5ml of this extract was then added with 3ml of bio-red dye and 0.5ml of distilled water. Sample absorbance was checked at 595nm. Total amino acids were estimated according to the methodology given by Sohail et al. (2018). 0.2g of fresh leaf material was mixed with 10ml of phosphate buffer. 1ml of this extract was then added with 1ml of ninhydrin and 1ml of pyridine solution. Obtained mixture was then boiled at 100 °C for 30 mins and sample absorbance was checked at 570nm by using Cecil 2021 Photo spectrometry. Dubois et al. (1956) methodology was used for this purpose. 0.2g of fresh leaf material was mixed with 10ml of 80% ethanol. After filtration, 0.5ml of filtrate was mixed with 0.5ml of distilled water and 1ml of 18% phenol solution. After keeping the mixture as such for an hour, 2.5ml of sulphuric acid was added. Absorbance was noted at 490nm.

## Antioxidant Enzymes

Fresh leaf material was grounded in liquid nitrogen, crushed with pestle mortar, and powdered material was stored at -80°C for further use. Methodology given by Aebi (1984) was used to check catalase activity. Reaction mixture contained 20µl of enzymes extract mixed with 50mM of potassium phosphate buffer (pH 6.8), 1mM EDTA and 15mM H<sub>2</sub>O<sub>2</sub>. Catalase activity was measured by decrease in H<sub>2</sub>O<sub>2</sub> in 1 min at the absorbance range of 240 nm.

Methodology given by Nakano and Asada (1981) was used to check peroxidase activity. Reaction mixture was prepared by dissolving 5 mM H<sub>2</sub>O<sub>2</sub>, 15 mM guaiacol and 40mM phosphate buffer having pH 6.8. H<sub>2</sub>O<sub>2</sub> was added and absorbance at 470nm was recorded for 1 minute. SOD activity was estimated according to the methodology given by Sohail et al. (2018). 0.5g of plant material was grinded in sodium phosphate buffer. Centrifugation for 15 minutes at 15000rpm was then performed. 0.1ml of extract was mixed with 0.1ml riboflavin and 3ml SOD buffer. Another test tube set was taken having 0.1ml riboflavin and 3ml SOD buffer, without plant extract. Plant extract containing test tubes was placed under fluorescent lamp to initiate the reaction while the 2nd set of test tubes was kept in dark. Absorbance of both sets at 560 nm wavelength was recorded.

## Statistical Analysis

The experiment was executed in a Completely Randomized Design (CRD) with two factors (variety and treatment) with 3 replications for each treatment. Pearson's correlation co-efficient and principal component analysis was plotted by using statistical program "R (v 4.0.4)" (R Development Core Team 2020), and Costat-v6.303 (Cohort software, Monterey, CA, USA) was used to execute Duncan's multiple range test (DMRT) at  $p \leq 0.005$  significance level for comparison of means (Kim 2014; Steel et al. 1997).

# Results

## Morphological Attributes

Drought stress affected different morphological attributes of plants, and 19 percent reduction was recorded in shoot lengths of both canola cultivars as compared to control (Fig. 1-a). The application of MET and SA caused a significant increase in plant height in both normal and stressed conditions. MET priming appeared to be most effective treatment of all under both normal and drought-stressed conditions, as an increase of 22% in plant height was recorded in MET primed ( $T_1$ ), super canola plants compared with non-treated drought-stressed plants (Fig. 1-a). Similarly, plant height was improved by 17% in plants treated with MET priming combined with foliar spray of SA ( $T_7$ ). Shoot fresh and dry biomass also show a declining trend when plants were exposed to drought and a reduction of about 14% in fresh biomass and 17% in dry weight was recorded (Fig. 1-c, d). Application of plant growth regulators proved beneficial in reducing the negative effects of drought shoot fresh and dry biomass. ME priming showed better results among all treatments where about 46% better fresh biomass and 40% better dry biomass was recorded in both canola cultivars under drought stress (Fig. 1-c, d). These results were statistically non-significant from the plants treated with combined application of melatonin priming and salicylic acid foliar spray. Overall, V1 showed better growth under drought than V2.

### **Photosynthetic Attributes**

Photosynthetic rate showed a significant reduction (43%) after canola cultivars were exposed to drought. Seed priming treatments with plant growth regulators improved the photosynthetic rate of both cultivars under normal as well as stressed conditions (Fig. 2). Seed priming treatment with MET ( $T_1$ ) caused a significant increase of 43% in photosynthetic rate, followed by those plants which were treated with combined ME and SA ( $T_5$ ) priming (Fig. 2-a). A similar sort of drought stress caused reduction in transpiration rate (73%) and stomatal conductance (55%) was recorded in both canola cultivars (Fig. 2-b, c). Seed priming treatment with MET ( $T_1$ ) and SA ( $T_3$ ) showed better results and improved transpiration rates and stomatal conductance under drought in both canola cultivars (Fig. 2-b, c). Foliar spray of SA ( $T_4$ ) and combined foliar treatment of SA and MET ( $T_6$ ) slightly improved all photosynthetic attributes in stressed environment, but their impact was not as significant as of all other treatments involved. Among the cultivars, V1 showed better results than V2.

### **Water Relations**

Declining trend was recorded in water and osmotic potential values of canola plants after exposure to drought stress (Fig. 3-a, b). Seed priming and foliar spray application of both plant growth regulators improved water potential and osmotic potential under both controlled and stressed conditions. Maximum water and osmotic potential values under normal and drought-stressed environments were recorded in MET primed plants ( $T_1$ ), followed by combined treatment of MET priming and SA foliar spray ( $T_7$ ). Combined foliar spray treatment ( $T_6$ ) of both growth regulators didn't show as significant effect on these parameters as shown by other treatments involved (Fig. 3-a, b).

### **Bio-Chemical Attributes**

An increase in compatible solutes concentration i.e., soluble proteins, free amino acids, and total soluble sugars were recorded after drought stress treatment in canola plants (Fig. 4). An increase of 15% was recorded in protein contents under drought stress, compared to water-treated plants. These Proteins contents were further increased by MET and SA application, and the maximum increase was recorded in MET primed (T1) plants (Fig. 4-a), where an increase of 25% in protein contents was recorded under drought, compared to non-treated drought-stressed plants (T0). Similarly, about 20% more free amino-acid contents were recorded in drought stress, as plants accumulate more amino acids to cope with drought stress. Amino-acid contents were further enhanced by seed priming treatment of MET combined with foliar spray of SA (T7), where about 34% more amino-acid activity was found compared with non-treated (T0) water-stressed plants (Fig. 4-b). A slight increase (5%) was recorded in total soluble sugar contents of drought-stressed cultivars, compared to well-watered plants. Hormonal priming treatment further enhanced these contents, and MET priming treatment combined with SA foliar spray (T7) proved to be most effective under drought stress. Foliar spray of SA (T4) and combined foliar treatment of SA and MET (T6) slightly improved all biochemical attributes under water deficit conditions, but their impact was not as significant as of all other treatments involved (Fig. 4-c). Among the cultivars, V1 showed better biochemical activity than V2.

### **Antioxidant activity**

Antioxidant levels i.e., Peroxidase (POD), Superoxide Dismutase (SOD), and catalase (CAT) activity increased in drought exposed canola plants, compared to well-watered plants (Fig. 5). An increase of 23% in POD levels was observed in drought-stressed canola cultivars. Application of plant growth regulators further enhanced POD contents under both normal and stressed conditions. Maximum results were shown by combined treatment of MET as seed priming and SA as foliar spray (T7) under both normal and drought stress conditions (Fig. 5-a). These results were statistically non-significant from MET priming treated plants (T1). Similarly, an increase of 20% in SOD contents was recorded in drought-stressed canola plants. MET priming combined with SA foliar spray (T7) further enhanced these contents and about 17% increase under normal and 28% increase under stressed conditions was recorded (Fig. 5-b). CAT contents also showed a significant increase (27%) after drought exposure (Fig. 5-c). MET priming (T1) further increased catalase contents by 35% under stressed environment and by 14% under normal conditions. Foliar spray of SA (T4) and combined foliar treatment of SA and MET (T6) slightly improved SOD, POD and CAT levels in stressed environment, but their impact was not as significant as of all other treatments involved (Fig. 5-a, b, c). Among the cultivars, V1 showed better antioxidant activity than V2.

### **Yield Attributes**

Yield attributes of canola showed significant reduction after exposure to drought stress, where about 30% less siliqua per plant and 18% and 30% lower number of grains per siliqua were recorded in Super and Faisal canola respectively, as compared to control (Fig. 6-a, b). However, a considerable increase (49 and 47%) in siliqua per plant and an increase of 40 and 56% in the number of grains per siliqua was recorded in Super (V1) and Faisal canola (V2) respectively, after treating plants with MET and SA under drought.

Maximum no. of siliqua per plant under drought conditions were shown by MET priming treated plants (T1), followed by plants treated with SA (T3) priming (Fig. 6-a). Similarly, the maximum number of grains per siliqua was recorded in those plants which were treated with MET priming combined with foliar spray of SA (T7) in a stressed environment (Fig. 6-b). Foliar spray of SA (T4) and combined foliar treatment of SA and MET (T6) slightly improved plant yield attributes in both controlled and stressed environment, but their impact was not as significant as of all other treatments involved. Among the cultivars, V1 showed a slightly better yield than V2.

### **Relationship and multivariate analysis**

In order to verify the relationship between different studied attributes, we have plotted Pearson correlation and principal component analysis (PCA-biplot) (Fig. 7-8). This analysis disclosed correlation (positive and negative) among various parameters of canola cultivars under drought and normal conditions. It has been noticed that drought stress imposed significant impact on all the parameters. Fig. 7(a-b) showed significantly positive correlations among biochemical attributes, enzymatic antioxidant, and plant water relations both under drought and control conditions. On the other side, biochemical parameters showed a negative correlation with root length and stomatal conductance. Plant height and fresh weights were positively impacted by water relation attributes, but transpiration rate showed negative relationship with them. Overall transpiration rate (E) had negatively correlation with all other attributes under control conditions but showed almost zero relationship with number of grain per siliqua and number of seed per plant, while photosynthetic rate had shown positive correlation both under drought as well as controlled conditions due to application of melatonin and salicylic acid (Fig. 7a, b).

Our Pearson's correlation among various attributes of both canola varieties have been further validated by PCA-Biplot. Fig. 8 represents PCA-Biplot of different attributes in canola plants subjected to drought stress with foliar and primed seed treatments of growth regulators. Principal component analysis executed the degree of association between variables under drought and controlled environment. Both dimensions of PCA i.e., Dim-1 and Dim-2 jointly elaborated 82.6% variability in the dataset. They showed obvious and marked separation of attributes under stressed and non-stressed conditions. Dim-1 showed 44.9%, while Dim-2 observed 37.7% variation to the total variance. There was a clear separation in studied parameters under normal and drought stress. Biochemical and antioxidant traits showed more relationship and impact under drought while growth attributes showed better relation in controlled conditions (Fig. 8).

## **Discussion**

Keeping in view the drastic effect of drought stress on plant growth, the present study was conducted to check out the efficacy of plant growth regulators (MET and SA) in enhancing morphological, biochemical, and physiological attributes of canola cultivars grown under drought stress conditions. The role of hormones in enhancing plant growth under stressed environments is a well-documented phenomenon.

Water stress is the major limiting factor responsible for the reduced growth and yield of crops (Sattar et al. 2021). Results obtained from our study showed all the growth attributes i.e., shoot and root length, plant fresh and dry biomass, as well as yield parameters, showed significant reduction after exposure to water stress in both canola cultivars. These results are in agreement with those obtained by Abd Elhamid et al. (2016); Dawood et al. (2019); Elewa et al. (2017) and Sadiq et al. (2018). Dawood and Sadak (2014) observed that canola growth and yield showed significant reduction after exposure to drought which might be due to the production of ROS in plant cells during stress. Drought negatively affects cell elongation, cell turgor, and cell volume, which together cause a decrease in plant height (Sadak et al. 2020). Similarly, reduction in shoot water contents, inhibition of cell division, and disturbance of plant water relations occur during drought, affecting all growth attributes of crops (Alam et al. 2014; Sattar et al. 2021). The negative effects of drought on fresh and dry biomass of shoot and roots of canola might be due to reduced rates of photosynthesis in drought exposed plants (Haq et al. 2014). Reduced yield in canola cultivars might be due to the reduced photosynthetic rate under stress conditions. Reduced photosynthesis in drought-stressed canola leaves leads to lower accumulation of carbohydrates in mature leaves, resulting in their lower rates of transport to developing organs, so the reduction in yield results (Sadak et al. 2020). However, the reduction in growth and yield of canola caused by drought is significantly reduced by seed priming treatment with melatonin, as well as priming and foliar spray of salicylic acid. Melatonin caused enhanced growth and yield is reported by various researchers (Cui et al. 2017; Huang et al. 2019; Kabiri et al. 2018; Ye et al. 2016).

Melatonin acts both as a growth promotor (Arnao and Hernandez-Ruiz 2019) as well as a protector against abiotic stresses (Ahmad et al. 2019; Liang et al. 2019). Yield enhancing role of melatonin can be attributed to its role in enhancing photosynthesis, thus increasing translocation to sink from source organs. Melatonin application further causes ion homeostasis and stimulates vegetative growth, leading to increased seed yield in plants (Çolak 2018; El-Awadi et al. 2017; Li et al. 2017). These positive actions of melatonin on canola growth and yield proves its significance as an endogenous hormone, working in trace amounts (Zhang et al. 2015). SA application also resulted in much increase in morphological and physiological attributes under drought, which may be attributed to its role in increased uptake of nutrients and more photosynthetic rate in drought stressed plants (Abdelaal et al. 2020). Significant increase in yield is recorded in SA treated plants as well under drought, as SA has major role in flower formation, leading to more production of grains. Similar sort of results was recorded by Abdelaal (2015), who observed significant increase in seed weight and pod number of faba beans under drought after SA application. This increase in yield in SA treated plants in stressed environments could be due to water reserving actions of SA in plant cells, which results in increased enzymatic actions in stressed conditions resulting in better metabolism and yield (Pirasteh et al. 2015).

Among the different adaptive mechanisms shown by plants to cope with water stress, the closure of stomata to reduce water loss is the primary one. This resulted in a reduced photosynthetic rate under drought stress due to decreased stomatal conductance (Ahmad et al. 2021). Recent research work on melatonin on different crop species shows that melatonin increases plant resistance to drought by improving stomatal functions, where reopening of stomata is recorded under drought after application of

exogenous melatonin at optimum levels (Ahmad et al. 2021; Fereiduni et al. 2019; Sharma et al. 2020). Present research work showed a significant reduction in net photosynthetic rate and stomatal conductance in both canola cultivars after exposure to drought stress. Seed priming with melatonin and salicylic acid increased photosynthetic rate and stomatal conductance, both under control and stressed conditions. Leaf relative water content was also significantly increased by hormonal treatment in a stressed environment. Foliar spray of salicylic acid in combination with melatonin priming also showed statistically significant results, signifying the synergetic effect of these growth regulators on photosynthetic attributes under stressed conditions. Our results are in line with those obtained by Cui et al. (2017), who observed similar sort of melatonin effects on photosynthetic rate and stomatal conductance while working on stress-exposed wheat plants.

Exposure to abiotic stress results in excessive production of ROS inside plant tissues, which causes breakdown and peroxidation of membrane lipids (Kar 2011). For maintaining cell homeostasis, plants show different resistive mechanisms against this ROS-caused oxidative damage under abiotic stresses. Activation of many enzymes like SOD, CAT, and POD occurs which inhibit further production of ROS in plant cells (Imran et al. 2021; Khan et al. 2020; Yildiztugay et al. 2017). SOD first converts  $O_2^-$  to  $H_2O_2$ , which is then broken down to water molecules by the action of POD and CAT (Hu et al. 2016; Imran et al. 2021;). Findings of present research work show declined enzymatic antioxidants levels in drought-stressed canola cultivars. However, pre-sowing seed treatments with melatonin enhanced enzymatic antioxidants activity under controlled conditions, and combined application of Melatonin as seed priming and SA as foliar spray showed a marked increase in CAT, POD, and SOD concentrations under water stress conditions. This might be attributed to the fact that melatonin application reduces  $H_2O_2$  levels and electrolyte leakage, caused by drought stress Li et al. (2015). Melatonin application on one hand stimulates antioxidant enzymes activity, and at the same time, it maintains intracellular  $H_2O_2$  at a constant level (Cui et al. 2017; Imran et al. 2021;). A similar sort of findings was recorded by Shi et al. (2015) while working on Bermuda grass, where the exogenous application of  $100\mu\text{m}$  melatonin increased ROS accumulation and caused their removal by increasing antioxidant concentrations. The present study also signifies the importance of foliar application of salicylic acid in enhancing ROS scavenging via stimulated antioxidant action, in combination with pre-sowing melatonin application. SA treated plants showed increased enzymatic antioxidant activities under stress, as SA boosts plant resistance to stress by reducing oxidative stress, resulting in better plant growth under drought (Mutlu et al. 2016). Our study, thus, confirms that these two plant growth regulators act in a synergetic way to boost plant resistance against drought stress by enhancing antioxidant enzyme activity.

Defensive responses in plants to drought stress include accumulation of low molecular weight, osmotically active compounds called compatible solutes which include soluble proteins, free amino acids, and total soluble sugars (Ahmad et al. 2021; Ibrahim et al. 2020; Liu et al. 2018; Zhao et al. 2021). The present study showed an increase in levels of these compatible solutes under drought. Seed priming application of melatonin caused a further significant increase in soluble proteins, amino acids, and soluble sugars levels compared with non-treated drought-stressed plants, highlighting the significance of

melatonin in coping with drought. Enhanced synthesis of amino acids and proteins stabilize cellular structures and maintain osmotic pressure under drought (Meng et al. 2014; Zhong et al. 2018; Zhao et al. 2021). An increase in proteins and amino acids by melatonin application shows that melatonin stimulates the synthesis of additional compatible compounds which ensure membrane stability at stress times (Georgiadou et al. 2018; Zhao et al. 2021). Similarly, plant stress resistance is enhanced by stability in protein production, as most proteins are those enzymes that are metabolically active (Sun et al. 2020). Although much reduction in photosynthetic rate is observed in drought-stressed plants, an important resistive mechanism in plants to drought is the accumulation of soluble sugars. Different researchers working on different crop plants exposed to drought recorded an increase in total soluble sugar contents (Elewa et al. 2017; Ezzo et al. 2018; Sadak and Bakry 2020). Elevated levels of soluble carbohydrates maintain turgor and stabilize cellular membranes by removing ROS (Hosseini et al. 2014; Sadak and Bakry 2020). The present study showed that the exogenous application of both plant growth regulators increased drought resistance in both canola cultivars. Melatonin application as seed priming alone as well as in combination with foliar spray of salicylic acid further enhanced soluble sugar contents in drought-stressed canola. Melatonin acts as a natural antioxidant, directly removing ROS, thus stabilizing cellular membranes.

## Conclusion

Present research work has proved that exogenous application of melatonin and salicylic acid has the potential to induce drought tolerance in canola plants, though melatonin appeared to be much more effective than salicylic acid. The combined application of melatonin priming, and salicylic acid foliar spray showed a significant effect on plant growth and can be very effective in inducing drought resistance in canola cultivars. Among the cultivars, Super canola (V1) showed much better growth and yield than Faisal canola (V2), so Super canola can be a better choice in drought affected areas and can be used for future studies.

## Declarations

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

**Data availability** Not applicable

**Declaration of competing interest:** Not applicable

**Author contribution** NI Conceived the Idea and designed the experiment; NR: Set and performed the experiment, took data, wrote first draft; MA: Writing, Review, editing; NIR: analyzed the data, and helps in interpretation of results; GS: Critically revised the MS and provide technical support. All authors approved the final version of the manuscript.

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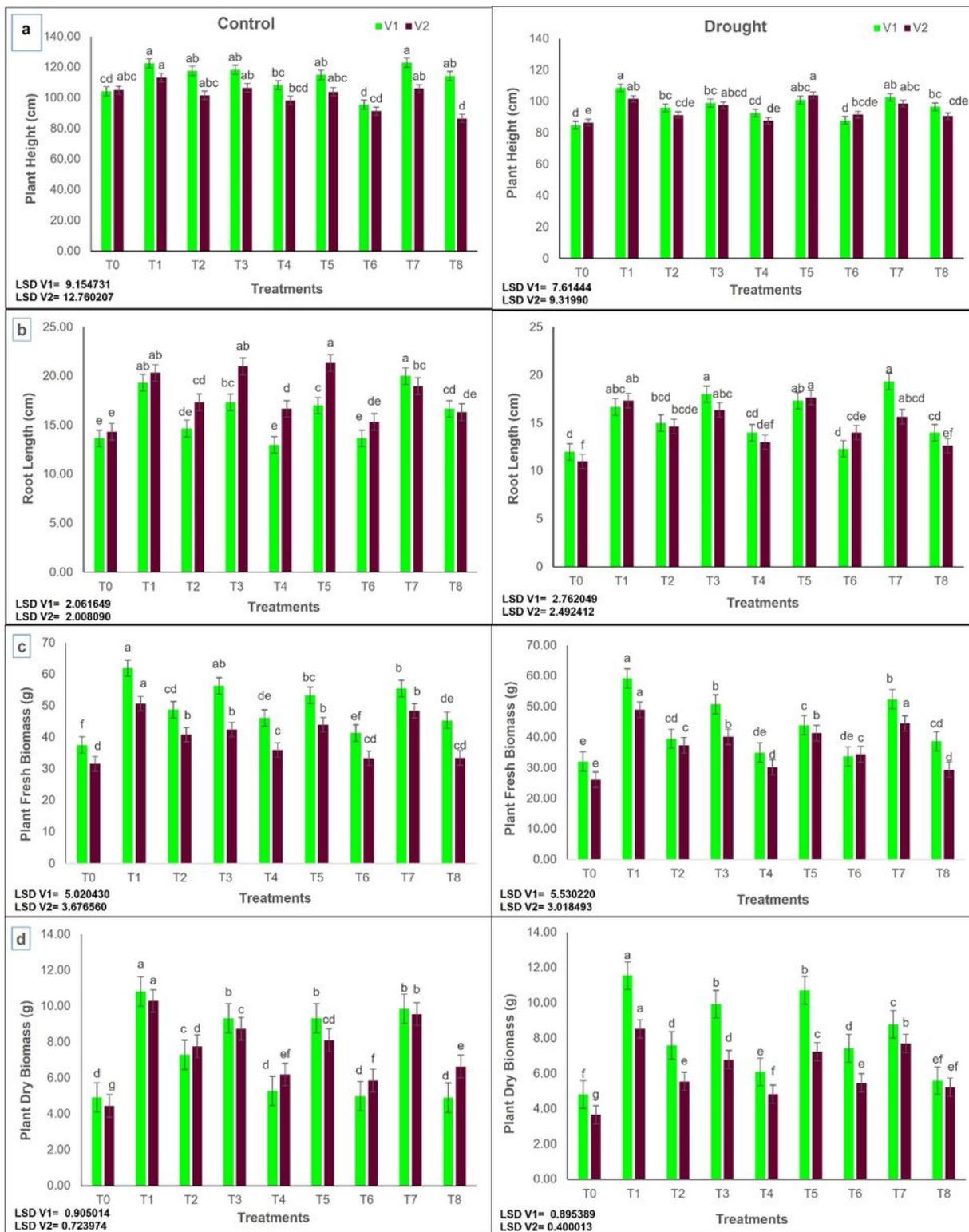
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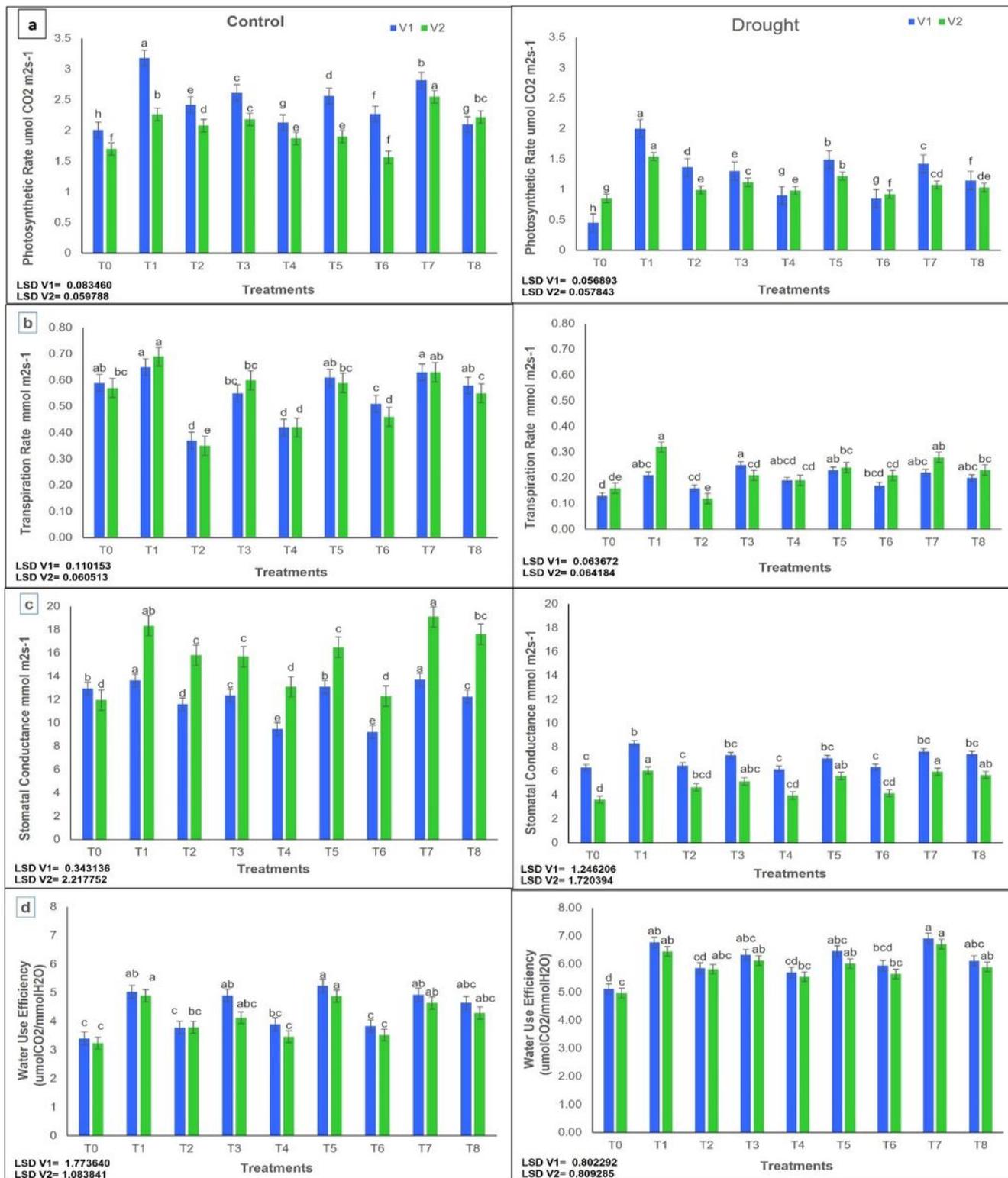
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## Figures



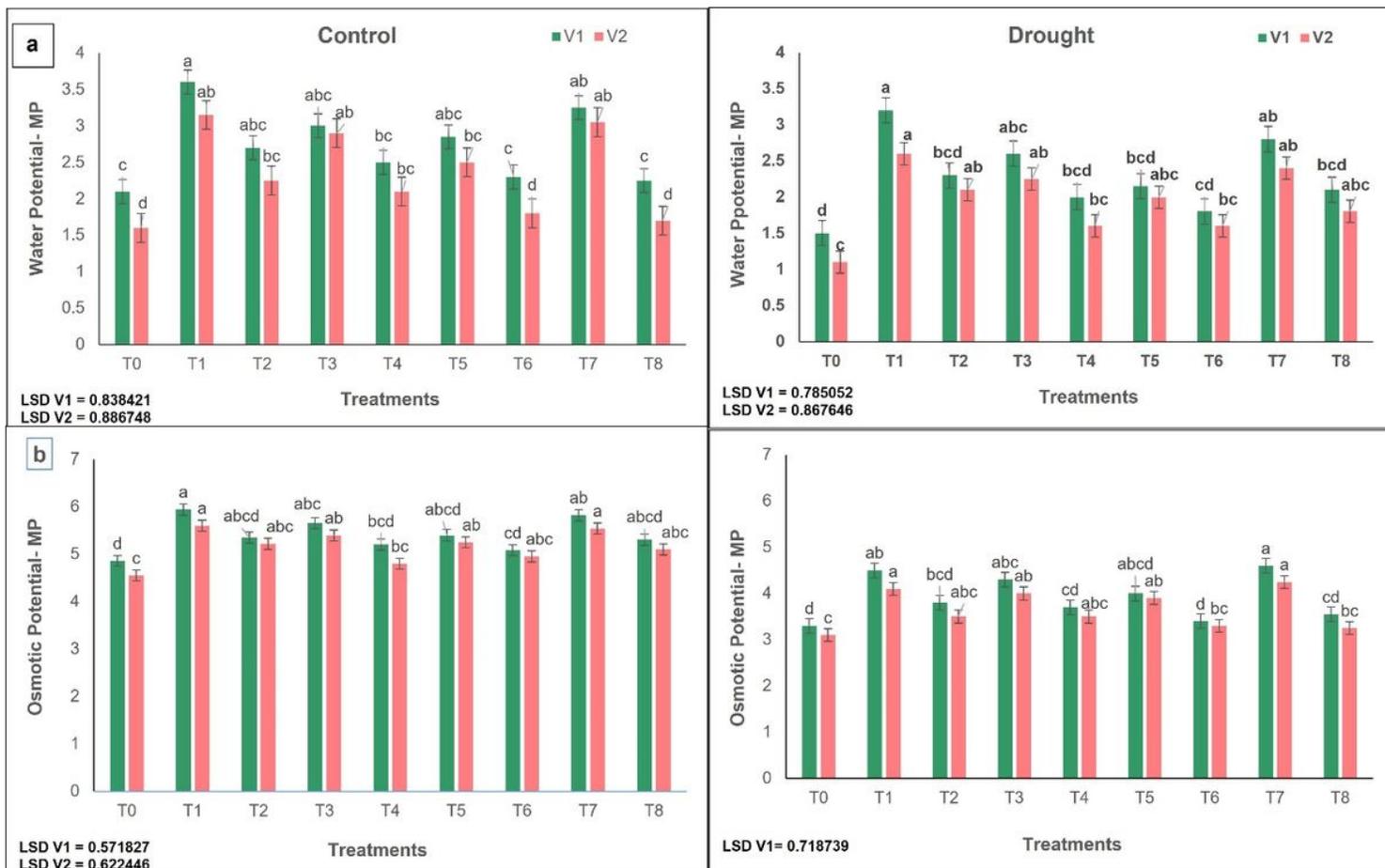
**Figure 1**

Effect of different treatments of priming and foliar spray of Melatonin and Salicylic acid on plant height (a), Root Length (b), Plant Fresh Biomass (c), and Plant Dry Biomass (d) of two canola cultivars under normal and drought stress. Small letter above each bar shows statistical differences at  $p < 0.05$



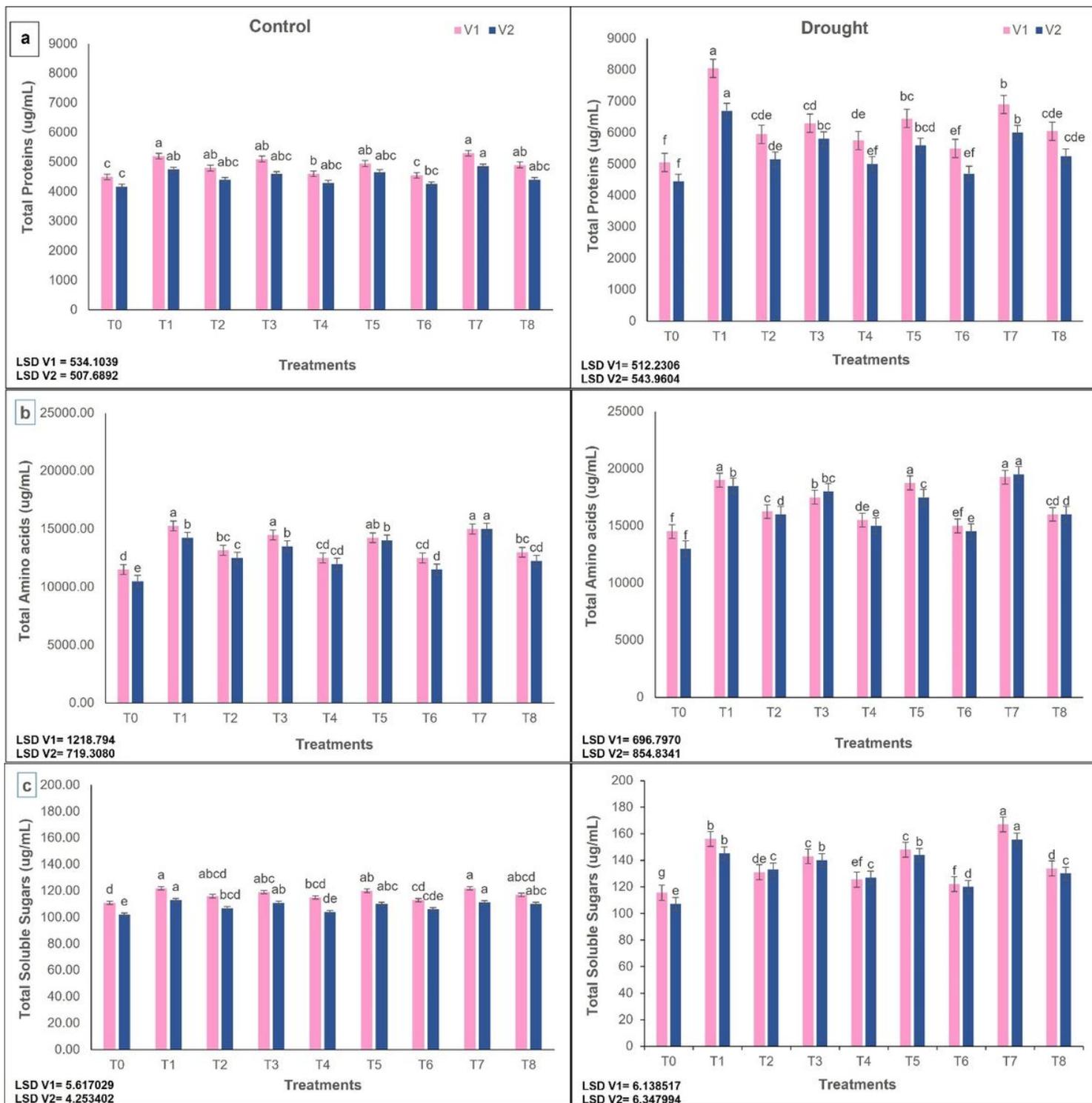
**Figure 2**

Effect of different treatments of priming and foliar spray of Melatonin and Salicylic acid on Photosynthetic Rate (a), Transpiration Rate (b), Stomatal Conductance (c) and Water Use Efficiency (d) of two canola cultivars under normal and drought stress. Small letter above each bar shows statistical differences at  $p < 0.05$



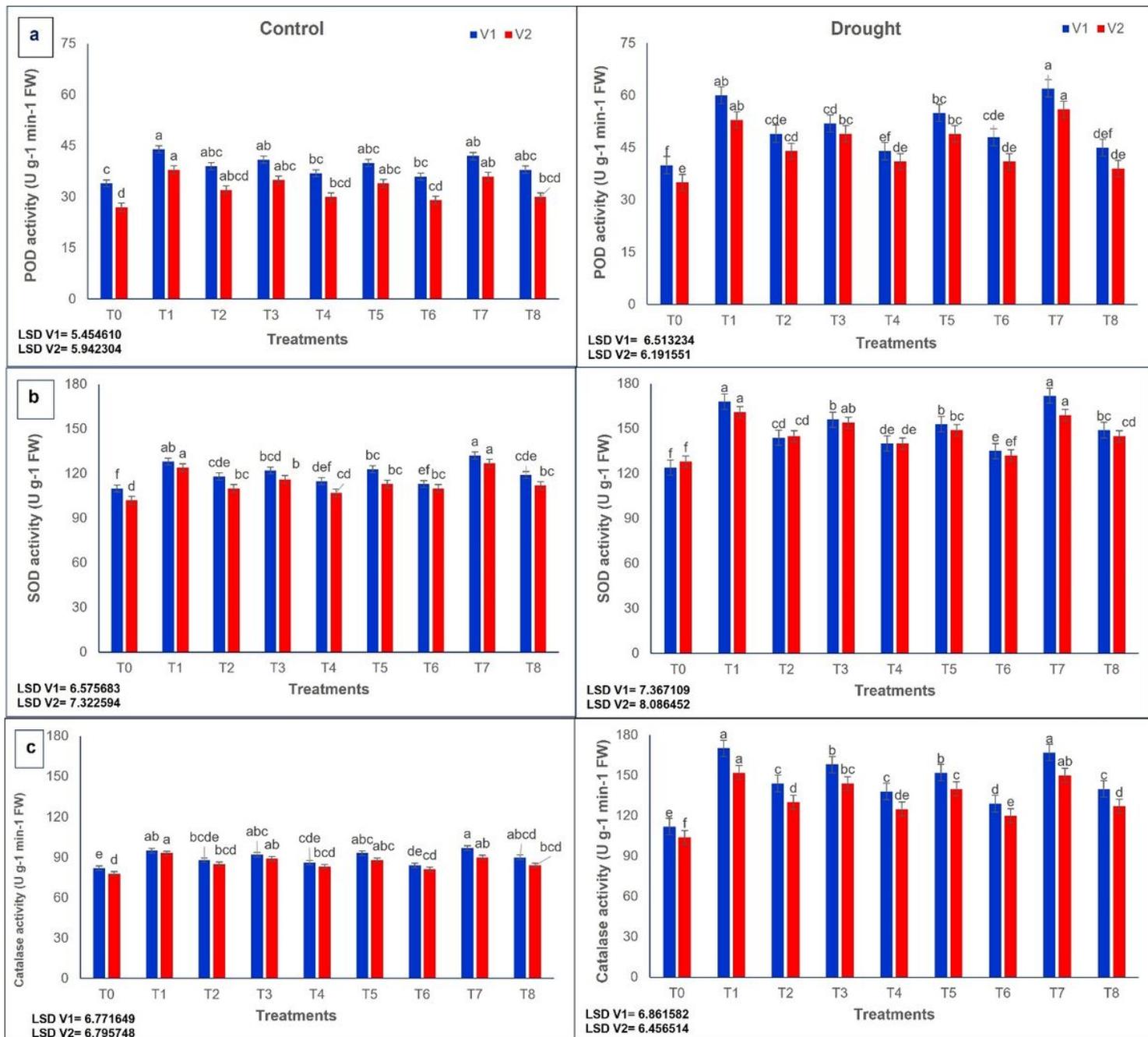
**Figure 3**

Effect of different treatments of priming and foliar spray of Melatonin and Salicylic acid on Water Potential (a) and Osmotic Potential (b) of two canola cultivars under normal and drought stress. Small letter above each bar shows statistical differences at  $p < 0.05$



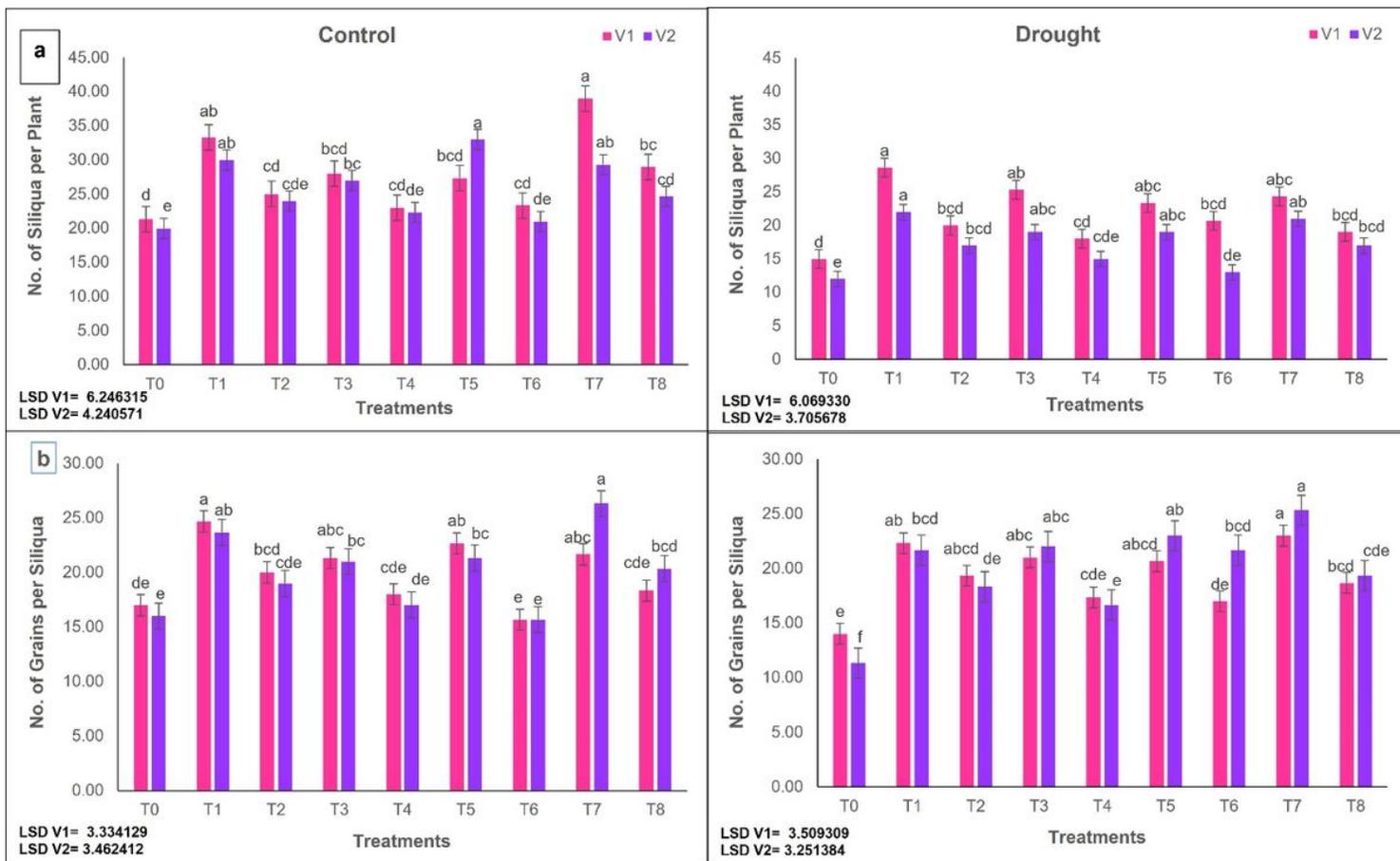
**Figure 4**

Effect of different treatments of priming and foliar spray of Melatonin and Salicylic acid on Total Proteins (a), Total Amino acids (b), and Total Soluble Sugars (c) of two canola cultivars under normal and drought stress. Small letter above each bar shows statistical differences at  $p < 0.05$



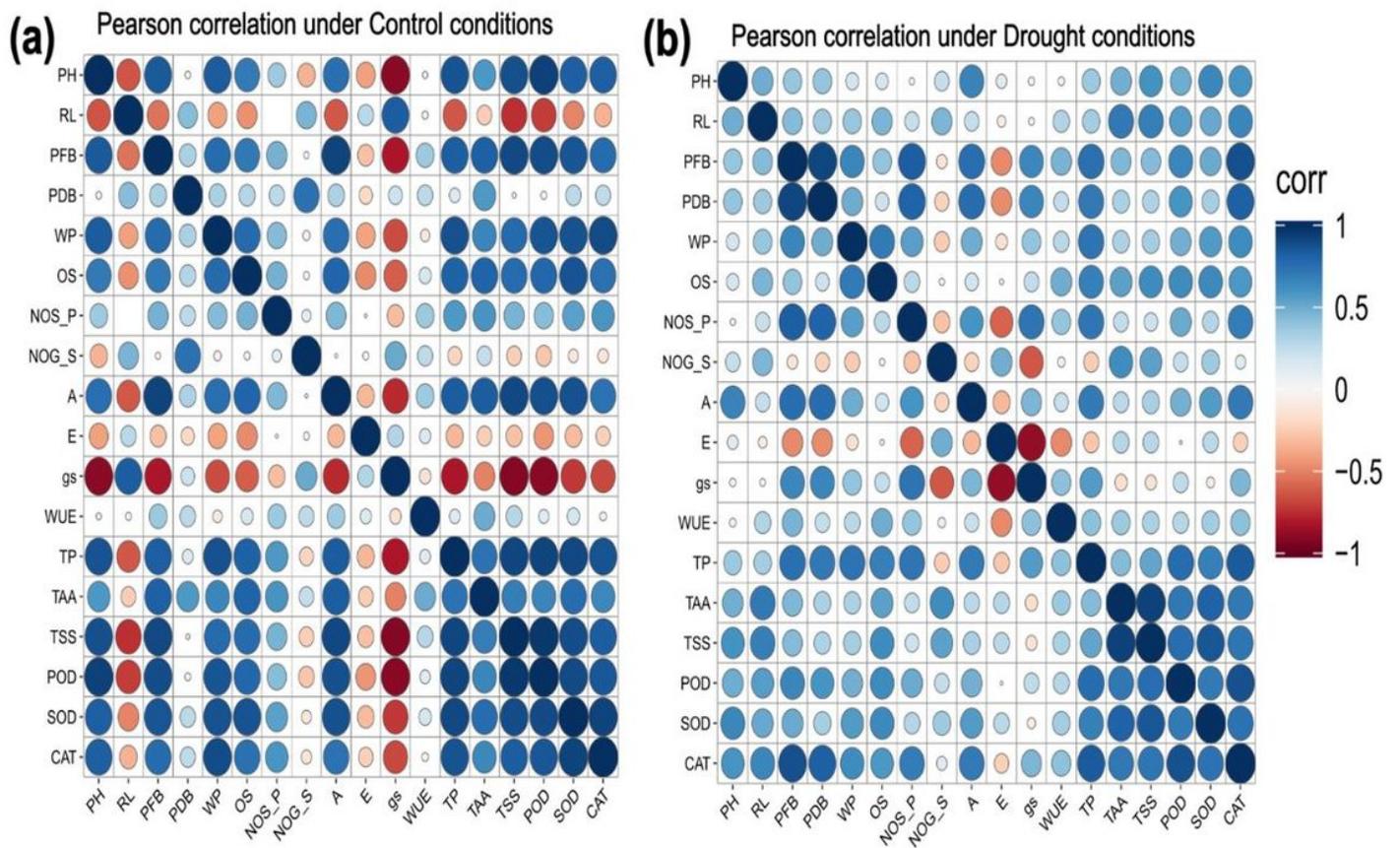
**Figure 5**

Effect of different treatments of priming and foliar spray of Melatonin and Salicylic acid on POD activity (a), SOD activity (b) and Catalase activity (c) of two canola cultivars under normal and drought stress. Small letter above each bar shows statistical differences at  $p < 0.05$



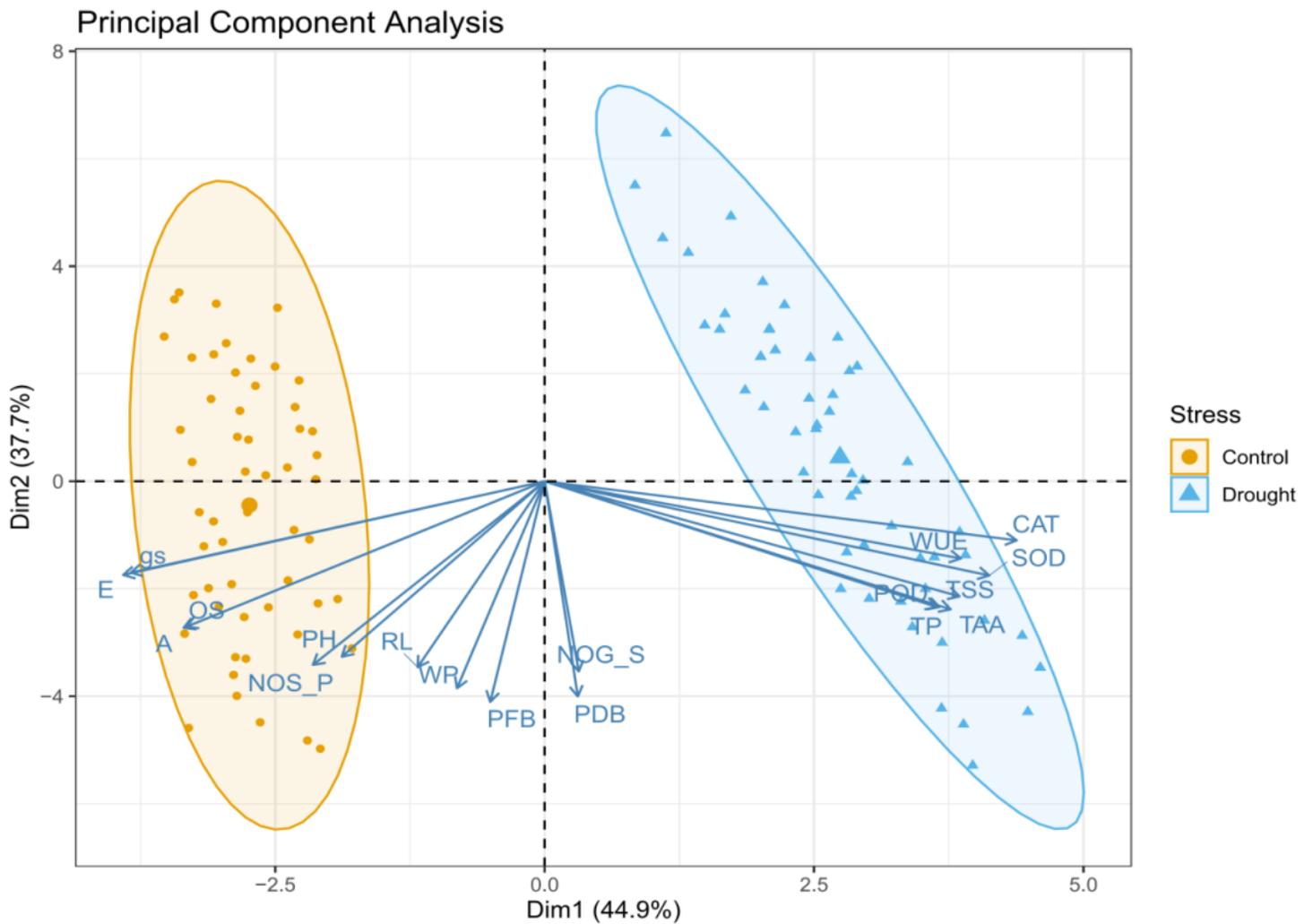
**Figure 6**

Effect of different treatments of priming and foliar spray of Melatonin and Salicylic acid on Number of Siliqua per plant (a) and Number of grains per Siliqua (b) of two canola cultivars under normal and drought stress. Small letter above each bar shows statistical differences at  $p < 0.05$



**Figure 7**

Pearson correlation under control (A), and drought (B) condition showing relationship and impact of different measured attributes on each other. PH: Plant Height; RL: Root Length; PFB: Plant Fresh Biomass; PDB: Plant Dry Biomass; WP: Water Potential; OS: Osmotic Potential; NOS-P: No. of Siliqua Per Plant; NOG-S: No. of Grains per Siliqua; A: Photosynthetic Rate; E: Transpiration Rate; gs: Stomatal Conductance; WUE: Water Use Efficiency; TP: Total Proteins; TAA: Total Amino acids; TSS: Total Soluble Sugars; POD: Peroxidase; SOD: Superoxide Dismutase; CAT: Catalase



**Figure 8**

Principal component analysis (PCA) showing the relationship between different attributes of canola cultivars under normal and drought stress condition. PH: Plant Height; RL: Root Length; PFB: Plant Fresh Biomass; PDB: Plant Dry Biomass; WP: Water Potential; OS: Osmotic Potential; NOS-P: No. of Siliqua Per Plant; NOG-S: No. of Grains per Siliqua; A: Photosynthetic Rate; E: Transpiration Rate; gs: Stomatal Conductance; WUE: Water Use Efficiency; TP: Total Proteins; TAA: Total Amino acids; TSS: Total Soluble Sugars; POD: Peroxidase; SOD: Superoxide Dismutase; CAT: Catalase