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ORIGINAL ARTICLE

Effect of 24-Epibrassinolide on Lipid Peroxidation and Proline in three *Brassica* species under temperature stress.

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Abiotic stresses, such as temperature, drought and salinity are serious threats to agriculture. Temperature stress is the primary cause of crop loss worldwide, reducing average yields for most major crop plants. In present study role of 24-epibrassinolide in three *Brassica* species (*B. carinata, B. juncea* and *B. napus*) on lipid peroxidation and proline under temperature stress was investigated. Seeds were given temperature treatments (4, 14, 24, 34 and 44°C) for 5 hours alone or in combination with EBR (10^{-11}, 10^{-9} and 10^{-7} M). Temperature stress whether low and high causes stress in terms of lipid peroxidation. High temperature causes more stress as compared with low temperature stress, as level of temperature stress rises increase in the membrane damage was observed. However the seeds treated with the EBR shows positive effect as there is decrease in the lipid peroxidation in terms of MDA content. Accumulation of proline was also observed in all temperature stress in all three *Brassica* species. Application of EBR at all concentrations causes significant increase in the proline content as compared with control and untreated.

Key words: Brassica, Temperature stress, Proline, Lipid Peroxidation, Epibrassinolide

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Brassinosteroids (BRs) are essential for various growth and developmental processes in plants and also protect plants from a variety of environmental stresses. Exogenous application of BRs induces a wide range of physiological effects, including promotion of cell elongation and division, cell expansion, seed germination, fruit development and stress responses (Bajguz and Hayat 2009; Montoya *et al*., 2005). Brassinosteriods were necessary for normal physiological processes in plants which were confirmed by using the inhibitors of brassinosteriods synthesis and mutants insensitive to brassinosteriods and defective in their synthesis (Bishop and Yokota, 2001). BRs were involved in modulation of various type of stress by up regulation of antioxidative enzyme activities and osmoprotectant in various plant species (Dhaubhadel *et al*., 2002, Nunez *et al*., 2003 and Ozdemir *et al*., 2004). Accumulation of free proline in response to various environmental stresses is observed in many plants (Schafleitner *et al*., 2007). Proline plays adaptive roles in stress tolerance by acting as osmolyte for osmotic adjustment, also stabilizes membranes, and scavenges free radicals (Kishore *et al*., 2005; Verbruggen and Hermans, 2008). Effect of epibrassinolide under different types of stress such as salinity, drought, and heavy metal stress on the proline content were well documented by many workers (Anuradha and Rao 2007; Houimli *et al.,* 2010) There are very few reports on the accumulation of proline under temperature stress. Chu *et al.*, (1974) observed slight increase in proline under high temperature stress in barley and radish. Lv *et al.* (2011) showed that proline accumulation is harmful to plants under heat stress as due to lower survival rate, higher ROS and MDA levels in HspP5CS lines in Arabidopsis. Under temperature stress, generation of reactive oxygen species occurs which affects the membrane permeability and lipid peroxidation (Wahid *et al*., 2007). Measurement of lipid peroxidation is the direct index to study the damage due to oxidative stress caused due to temperature stress. *Brassica* is an important oilseed crop of winter season and temperature stress causes limitations on their germination and yield (Kaur *et al*., 2009). Literature available is scanty to define the role of epibrassinolide on the proline level in *Brassica* under temperature stress. So in present study effect of the epibrassinolide on the proline content and lipid peroxidation in three *Brassica* species were investigated under low and high temperature stress (4, 14, 24, 34 and 44°C).

MATERIALS AND METHODS

Plant material and treatment conditions

Seeds of *Brassica spp*. (*B.juncea, B.carinata and B.napus*) were procured from Punjab Agriculture University, Ludhiana. Seeds were surface sterilized by 0.01% $HgCl_2$ for 10 min and then rinsed three times with sterile water. Seed were soaked in constant volumes of DW and EBR (10^{-11}, 10^{-9} and 10^{-7} M) for 5hrs alone or in combination with temperature 4, 14, 24, 34 and 44°C for pre-sowing treatment. After pre-sowing treatment, uniform seeds from every application were sown in 10 cm Petri dishes covered with two sheets of filter paper moistened with 5 mL of distilled water and placed in growth chamber in controlled conditions. Plants were sampled on the 8th day after sowing for measuring the proline content and TBARS assay. All the experiments were repeated three times under same conditions.

Determination of Proline Content

Proline content was determined by the method of Bates *et al*. (1973). Samples of 0.5 g were homogenized in 6 ml 3% sulfosalicylic acid, and the homogenate was centrifuged at 3000 g for 25 min. The supernatant was treated with acid ninhydrin, boiled for 1 h, and then absorbance at 520 nm was recorded with L-proline as standard. Proline content was expressed as µg/gmFW.

Determination of Lipid peroxidation

Lipid peroxidation was measured in terms of malondialdehyde (MDA) content determined by thiobarbituric acid (TBA) reaction by Heath and Packer (1968). MDA content was expressed as nM/FW.

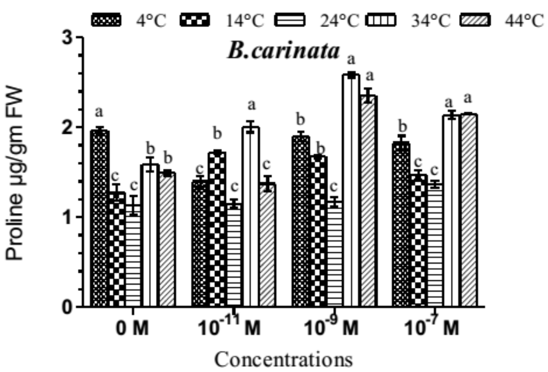
Statistical analysis

Analysis of variance was been carried out for all data using GraphPad Software. The means were compared by Bonferroni multiple comparisons post hoc test to study the significance at 5% level of probability. Standard error of the replicates was also calculated.

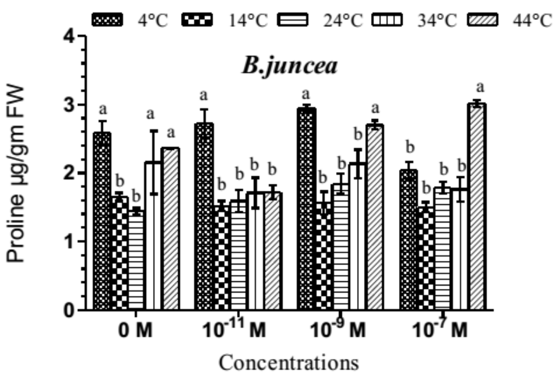
RESULTS

Proline is accumulated in many plant species under stress. Accumulation of proline amongst the *Brassica* species studied differed significantly in response to the temperature stress. As seen in *B.carinata* application of EBR induces accumulation of proline at all concentrations but not significantly as compared with control seedlings. In low and high temperature stress, proline was accumulated more (1.96 & 1.49 µg/gm FW) as compared with untreated control seedlings (1.13 µg/gm FW). With the application of EBR at all concentrations in combination with temperature stress, more proline was accumulated as compared with temperature stress alone (Fig. 1a). In *B.juncea* application of EBL causes same effect on the proline content. As temperature stress increases, accumulation of proline observed. Both temperature extremes low and high cause's accumulation of proline (2.58 & 2.36 µg/gm FW) as compared with control seedlings (1.44 µg/gmFW). Maximum amount of proline was observed in the 10^{-7} M conc. in combination with 44°C temperature stress (Fig. 1b). In *B.napus* as temperature shifts from lower to higher side, proline accumulates more. In the control groups, maximum proline accumulated in 44°C temperature stress (2.12 µg/gm FW). Application of EBR alone and with combination of temperature adds more proline in the seedlings. Maximum proline (2.75µg/gmFW) recorded in the 10^{-11} M conc. along with 34°C temperature stress (Fig. 1c).

1a



1b



1c

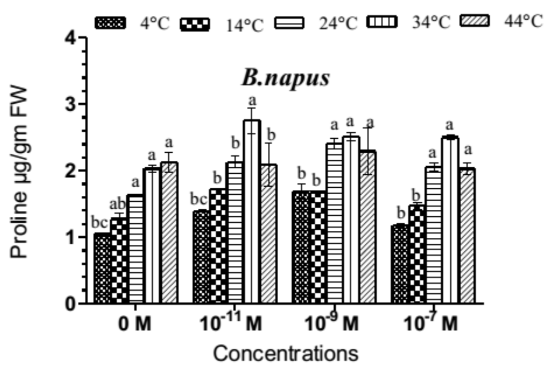


Figure 1. Effect of EBR on Proline content in 7-day-old *Brassica* spp. seedlings under temperature stress a) *B.carinata* b) *B.juncea* c) *B.napus*. *Bar* represents the SE. Different letters (*a, b, c*) are significantly different (Bonferroni multiple comparisons test, p≤ 0.05)

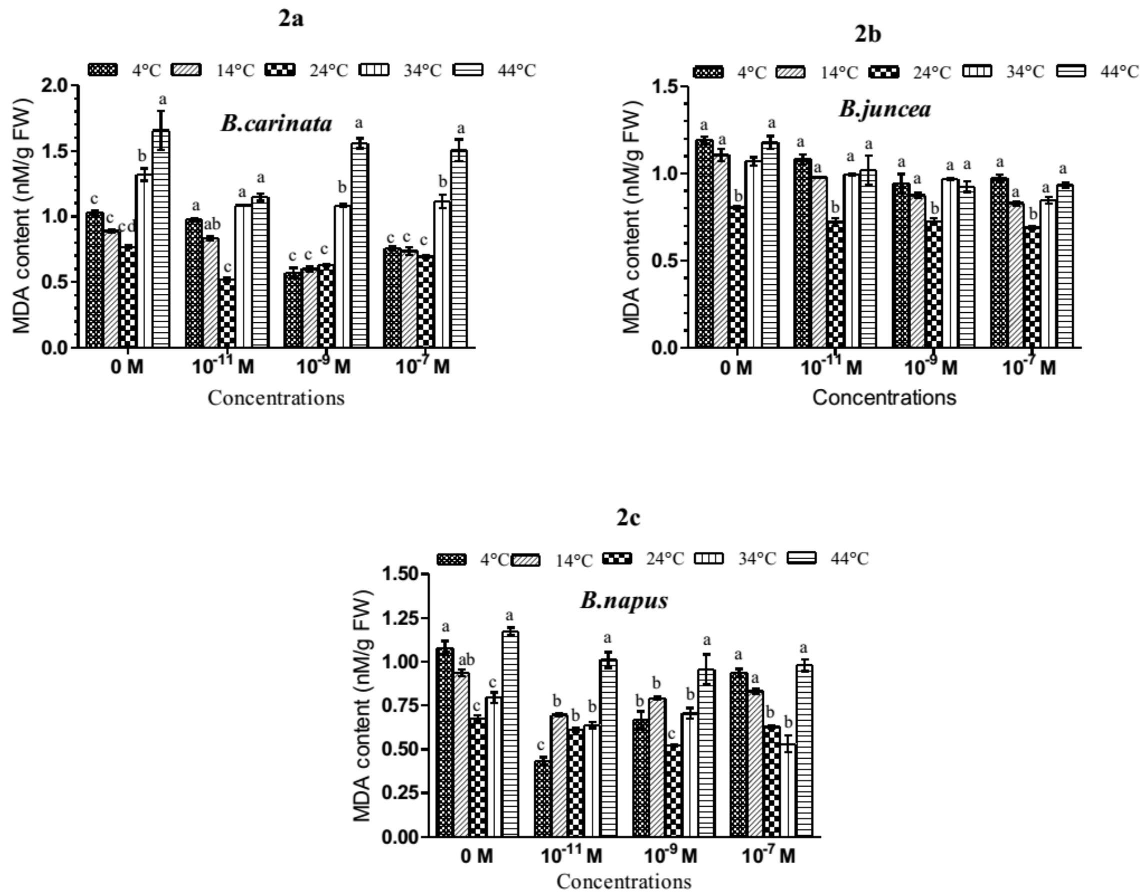


Figure 2. Effect of EBR on MDA content in 7-day-old *Brassica* spp. seedlings under temperature stress a) *B. carinata* b) *B. juncea* c) *B .napus*. *Bar* represents the SE. Different letters (*a, b,c*) are significantly different (Bonferroni multiple comparisons test, p≤ 0.05)

Accumulation of MDA is due to increase in ROS production under various biotic and abiotic stress. Membrane destabilization is generally attributed to lipid peroxidation. In *Brassica* species studied, accumulation of MDA was observed as due to both low and high temperature stress. In *B.carinata*, MDA content increased with the increase in temperature stress but decreased with EBR treatments. Maximum content of MDA (1.656 nM/g FW) was observed in 44°C temperature stress while minimum content (0.518 nM/g FW) was observed in 10^{-9} M EBR (Fig. 2a). In *B.juncea* both low and high temperature stress causes peroxidation of membrane lipids. MDA content was almost same in 4°C and 44°C temperature stress i.e (1.193 & 1.178 nM/g FW). EBR application causes decrease in the MDA content at all concentrations. Minimum MDA content was observed in 10^{-9} M EBR. in 4°C temperature while concentration of 10^{-7} M was best in 44°C temperature stress(Fig. 2b). In *B. napus* 4°C and 44°C temperature cause stress as increase in MDA content (1.141 & 1.139 nM/g FW) was observed as compared with control seedlings (0.677 nM/g FW). In 4°C stress minimum MDA content (0.433 nM/g FW) was observed in 10^{-11} M conc. while in 44°C temperature it was 10^{-9} M concentration (0.956 nM/g FW) (Fig. 2c).

DISCUSSION

Exposure of temperature stress to *Brassica* can be easily detected by the elevated levels of lipid peroxidation in terms of MDA content. Present findings suggest the protective role of epibrassinolide under temperature stress both low and high by elevating the level of proline and by decreasing the MDA content. Production of lipid peroxides in the all three *Brassica* species were markedly different and increases with the increase in temperature stress. In our results high temperature causes more stress in the terms of MDA content in all three *Brassica* species. The increases in MDA content under heat stress suggests as due to decreased activities of antioxidant enzymes and decreased activities of antioxidant enzymes could contribute to damage of cell membranes (Liu and Huang, 2000). As *Brassica* are winter crops so it is more prone to high temperature stress as compared with low temperature stress. Russo *et al*. (2010) reported the negative effects of temperature stress in *Brassica* species as it was clearly observed that high temperature causes more stress in terms of final germination. This was clearly revealed in our results that high temperature causes more stress as compared with low temperature stress. EBR causes decrease in the total MDA content at all concentrations as compared with the control and as well as under temperature stress. EBR modulates the antioxidant defense system under various types of abiotic stress and biotic stress (Bajguz and Hayat 2009). In our findings EBR clearly protect the *Brassica* under both low and high temperature stress which is in parallel with the results of various workers in different plants (Fuji and Saka, 2001; Kagale *et al*., 2007; Gonza´lez-Olmedo *et al*., 2005; Manish *et al*., 2010). Dhaubhadel *et al*. (1999) reported the positive effect of epibrassinolide in *Brassica napus* seedlings which leads to an increase in the basic thermo tolerance. It also resulted in higher accumulation of four major classes of heat-shock proteins which suggest that the EBR treatment limits the loss of some of the components of the translational apparatus and cellular and membrane protein during heat stress. Ali *et al*. 2008 also showed that EBL causes significant increased growth in *Brassica juncea* which is clearly due to increases in antioxidant defence system which protects the plant from oxidative damage. Accumulation of proline under osmotic stress and drought stress were reported by various workers in different plants (Durgaprasad *et al*., 1996; Madan *et al*., 1995; Wang *et al*., 2011). Accumulation of proline under stress in different plant species has been correlated with stress tolerance, and its concentration has been shown to be generally higher in stress-tolerant plants (Verbruggen and Hermans, 2008). In our findings accumulation of more proline were observed in all three *Brassica* species as compared with the control seedlings. Treatment of EBR causes further increase in the proline content as compared with control and treated seedlings. Rizhsky *et al*. (2004) reported that heat stress causes decrease in the accumulation of proline but we observed that more proline is accumulated in response to high temperature stress in all three species which was due to the protective role of the proline for enzymes and cellular structure. Proline being act as osmolytes also reported in scavenging free radicals which were generated due to oxidative damage (Ashraf and Foolad, 2007). Radyukina *et al*. (2011) reported the antioxidant action of proline during UV-B irradiation in Salvia which was mediated by the proline dependent stimulation of antioxidant enzymes, including SOD which is a key enzyme of the cell antioxidant system. Javadian *et al.* (2010) reported the accumulation of free proline in different cultivars of wheat under low temperature stress which suggests its protective role in low temperature stress also to cope up with the stress and maintain the osmotic potential also. Our results under low temperature stress also show the positive accumulation of proline in *B. juncea* and *B. carinata* as compared with control seedlings. Similar results were observed by Esra *et al*. (2010), proline content increased in stem and leaf of pepper which was activated by cold stress and correlation between freezing tolerance and an increase of proline concentration after exposure to low temperatures. In contrast to this decrease in proline content under low temperature stress was observed only in *B. napus*. As proved EBR acts as anti-stress compound it protects the plant from stress by modulating the accumulation of more proline in various plants under different types of abiotic stress. Ozdemir *et al.* (2004) reported the accumulation of proline content in salt stress rice seedlings in combination with EBR, which shows the positive role of EBL in modulating the proline level. Exogenously applied proline protects *Vigna radiata* seedlings against lipid peroxidation, due to low temperature stress as it stabilizes membranes during chilling and may also function as a source of nitrogen and carbon (Posmyk and Janas, 2007). However there were no clear reports regarding the accumulation of proline under temperature stress in combination with the epibrassinolide treatment. From these results, it may be concluded that EBR protects the *Brassica* species under both low and high temperature stress. Indeed it reduces the negative effects of oxidative damage also and as well as protects the cell membrane by inducing more accumulation in the proline content in all three species. These results suggest the important protective role of epibrassinolide in *Brassica* which should be confirmed with more lab and field trials.

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