STUDIES ON EPIBRASSINOLIDE INDUCED AMELIORATION OF HIGH TEMPERATURE STRESS IN *BRASSICA JUNCEA* (L.) CZERN & COSS.

BY

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CERTIFICATE – I

This is to certify that this thesis entitled, "**Studies on epibrassinolide induced amelioration of high temperature stress in *Brassica juncea* (L.) Czern & Coss.**" submitted for the degree of **Master of Science** in the subject of **Plant Physiology** to the **Chaudhary Charan Singh Haryana Agricultural University, Hisar**, is a bonafide research work carried out by **Mr. Walke Mahadev Bapu** under my supervision and guidance and that no part of this thesis has been submitted for any other degree.

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CERTIFICATE – II

This is to certify that this thesis, entitled **"Studies on epibrassinolide induced amelioration of high temperature stress in *Brassica juncea* (L.) Czern & Coss."**, submitted by **Mr. Walke Mahadev Bapu** to the **Chaudhary Charan Singh Haryana Agricultural University, Hisar,** in partial fulfillment of the requirement for the degree of **Master of Science** in the subject of **Plant Physiology**, has been approved by the Student's Advisory Committee, after an oral examination on the same.

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"O Lord of incomparable might
The greatest teacher of creation
You are the father and worthy of adoration
By worshiping you through performances
Of one's natural duties
Does one attain perfection"… (GEETA)

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LIST OF ABBREVIATIONS

1.	EBR	-	Epibrassinolide
2.	HTS	-	High temperature stress
3.	EDTA	-	Ethylene diamine tetra acetic acid
4.	PVP	-	Polyvinyl pyrillidone
5.	H_2O_2	-	Hydrogen peroxide
6.	MDA	-	Malondialdehyde
7.	POX	-	Peroxidase
8.	CAT	-	Catalase
9.	CD	-	Critical difference
10.	RWC	-	Relative water content

CHAPTER – 1

INTRODUCTION

India is one of the largest among rapeseed mustard growing countries in the world ranking second in area and third in production after China and Canada. According to the 4th advance estimates as released on 21st July, 2009, the area under this crop in the country is 6.19 million hectares with a production of 7.37 million tonnes and productivity of 1190 kg/ha during 2008-09 (Anonymous, 2009). The production of rapeseed-mustard in India is lower than the world's average (1730 kg/ha) due to large number of factors *viz*. its cultivation under marginal conditions and losses caused by various biotic and abiotic stresses (Toor and Atwal, 2007). *Brassica juncea* (L.) (Czern & Coss.) (Indian mustard) commonly known as *raya* is one of the most important oilseed crop of rapeseed-mustard group and grown in northern and north east part of India.

Abiotic stresses such as drought, salinity, extreme temperature and chemical toxicity are serious threats to agriculture and significantly diminish the plant productivity. High temperature stress is the most important stress which can strike crop at any time and affects the growth and development. High temperature is known to affect membrane linked processes due to alteration in membrane fluidity and permeability (Alfanso *et al.*, 2001; Sangwan *et al*., 2002). At cellular level, high temperature causes metabolic disturbances, disruption of cellular homeostasis, depletion of respiratory substrates, reduction of photosynthetic activity, denaturation of proteins, inactivation of enzymes and damage to cellular structures (Narwal *et al*., 2007). Active oxygen species produced under temperature stress bring about heat induced oxidative stress (Larkindale and Knight, 2003), which cause denaturation of functional and structural proteins (Smirnoff, 1998) and activates cell signalling pathway (Knight and Knight, 2001; Zhu, 2001 and 2002) and cellular responses such as production of stress proteins, upregulation of antioxidants and accumulation of compatible solutes (Zhu *et al*., 1997; Cushman and Bohnert, 2000). The molecular expression to heat stress in plants is seen in the form of production of some new proteins. These proteins are known as heat shock proteins (HSPs). These are responsible for repairing other heat-damaged proteins (Bond *et al.*, 1988; Pelham, 1988). These have no regular chemical composition and disappear when the stress gets away (Zhang *et al*., 1997). These proteins act as molecular chaperons to protect cellular damage against irreversible heat induced denaturation and facilitate refolding of heat induced damaged proteins (Boston, *et al.,* 1996).

These abiotic stresses severely affect every aspect of physiology and biochemistry of plant and cause a rapid and excessive accumulation of reactive oxygen species (ROS). These reactive oxygen species *viz.* superoxide radical ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), hydroxyl radical (OH^-) and singlet oxygen ($^1O_2$) together constitute the oxidative stress. These overproduced ROS under stress conditions react directly with lipids, proteins and nucleic acids and cause lipid peroxidation mediated membrane injury, protein degradation, enzyme inactivation, pigment bleaching and disruption of DNA strands (Liebler *et al.*, 1986; Davis, 1987).

The H_2O_2 is then detoxified either by catalase/peroxidase or in ascorbate glutathione cycle (Asada, 1999; Mittler, 2002). Catalase (CAT) reduces H_2O_2 into H_2O and O_2, whereas peroxidase (POX) decomposes H_2O_2 by oxidation of co-substrate such as phenolic compounds (Gaspar *et al.*, 1991). The detoxification/removal of H_2O_2 by ascorbate-glutathione cycle involves oxidation and reduction of ascorbate and glutathione through ascorbate peroxidase (APX) and glutathione reductase (GR) action (Foyer and Halliwell, 1976; Noctor and Foyer, 1998).

Brassica is an important oilseed crop of winter season and its early sowing implies many important advantages. Firstly, early harvest of *Brassica* is desirable to avoid disease infestation and aphid attack that normally coincides with the flowering stage. Secondly, shattering of siliquae can be avoided during the time of harvest when crop encounters high temperature. As *Brassica* being a crop of arid and semi arid region, its sowing depends upon rain. Due to early rains, may a times the farmers sow the crop early in the season on the conserved moisture. But high temperature prevailing at the time of sowing reduces seed germination and causes seedling mortality. Therefore crop is to be resown many a times before a final successful crop is taken. This causes a lot of economic loss to farmers. Crop faces high temperature at terminal phase too when the crop is near physiological maturity and are exposed to heat stress. The crop gets forced maturity and the seeds size gets shriveled. Due to global warming, the problem of high temperature stress is aggravating. This necessitates the identification of thermotolerant genotypes (Sharma *et al.*, 2007).

Brassinosteroids are a class of plant polyhydroxysteroids that are ubiquitously distributed in the plant kingdom. The biologically active plant steroid brassinolide was first discovered in the pollen of western rape in 1979 (Wilen *et al.*, 1995). The occurrence of brassinosteroids has been demonstrated in almost every part of plants, such as pollen, flower buds, fruits, seeds, vascular cambium, leaves, shoots and roots. These steroidal compounds

occur in free form and conjugated to sugars and fatty acids. To this date, about 70 brassinosteroids have been isolated from plants (Bajguz and Tretyn, 2003). Brassinosteroids are required for normal development of plants. Studies on higher plants suggest that they play a critical role in a range of developmental processes, e.g. stem and root growth, floral initiation, and the development of flowers and fruits (Hayat and Ahmad, 2003 a, b; Sasse, 2003). They have also played pivotal role in stress responses (Clouse and Sasse, 1998).

In India, no systematic work has been done on the possibility of epibrassinolide induced amelioration of high temperature stress in *Brassica*. Very scanty information is available on this aspect. Keeping in view the problem, the research was taken with the following objectives:

I. To study the effect of epibrassinolide on germination, seedling growth and seedling mortality under high temperature stress.

II. To study the effect of epibrassinolide on physiological and biochemical parameters under high temperature stress.

III. To study the effect of epibrassinolide on amelioration of high temperature stress at terminal stage.

CHAPTER-2

REVIEW OF LITERATURE

Stress is being defined as any environmental factor capable of inducing a potentially injurious response in plants (Cowan, 1994). High temperature is one of the major environmental factors affecting plant growth and productivity. Plant responses to heat stress are diverse and include many physiological and biological factors, such as disturbed synthesis of chlorophyll pigments (Chernysheva and Chernysheva, 1979; Volkova and Koshkin, 1984; Moffat *et al*., 1990), protein denaturation, lipid peroxidation, reduction in membrane stability and efficiency of photosynthesis (Jagtap and Bhargava, 1995; Gong *et al*., 1997; Kurganova *et al*., 1997). High temperature also causes stomatal closure and internal water deficits (Kolb and Robberecht, 1996); reduction in carbohydrate accumulation (Lafta and Lorenzen, 1995) and increase in respiratory rate (Al-Khatib and Paulsen, 1989).

In the events of excessive temperature, generation of reactive oxygen species (ROS) such as super oxide ($O_2^{\bullet-}$), singlet oxygen ($^1O_2$) hydrogen peroxide (H_2O_2) and hydroxyl radical (OH^-) takes place, which have greater toxicity potential on biomolecule, and membrane stability (Scandalios, 1993). Chloroplats, mitochondria and peroxisomes are important intracellular generators of activated oxygen species (Salin, 1988; Del-Rio *et al*., 1991) and the cell membrane is the primary site of injury by elevated levels of harmful oxidative molecules (Guitteridge, 1988) due to their ability to cause damage to membrane lipids. Activated oxygen species also denature the protein, chlorophyll and nucleic acids, thus, disrupting the homeostasis of the organism (Scandalios, 1993). The consequences of all these can ultimately lead to plant death as a result of growth arrest and molecular damage.

Plant growth regulators have also been reported to play significant role in alleviating effect of heat stress (Biddington and Robinson, 1993; Dhaubhadel *et al*., 1999). Ethylene has significant role in negating the effect of heat stress (Biddington and Robinson, 1991; 1993). Brassinosteroids have been explored for stress-protective properties in plants against a number of stresses like chilling (Dhaubhadel *et al*., 1999), salt (Ozdemir *et al*., 2004), heat (Dhaubhadel *et al*., 2002) and heavy metals (Bajguz, 2000; Janeczko *et al*., 2005). However, it is unclear whether brassinosteroids are involved in the modulation of plant responses to oxidative stresses. The influence of brassinosteroids on the response of the antioxidative enzymes of plants under stress conditions has been studied recently (Cao *et al*., 2005; Hayat *et al*., 2007 a). The available data show that the changes induced in the activity of antioxidative enzymes by brassinosteroids differed with plant species and with stress conditions (Ozdemir *et al*., 2004; Almeida *et al*., 2005; Hayat *et al*., 2007 b). These hormones are also known to change in plants responding to biotic stress, e.g. pathogen infection (Krishna, 2003).

Brassica being a *rabi* crop of arid and semi arid region, its sowing depends upon rain. Due to tropical environment of India, the effect of heat stress is very much harmful especially when the crop is sown early on the conserved moisture. High temperature prevailing at that time reduces seed germination and causes seedling mortality, which is the crucial stage of plant establishment. Therefore crop is to be resown many a times before a final successful crop is taken. This causes a lot of economic loss to farmers. Due to global warming the problem of high temperature stress is aggravating. Crop faces high temperature at terminal phase too when the crop is near physiological maturity and are exposed to heat stress. The crop gets forced maturity and the seeds size gets shriveled. In India, no systematic work has been done on the possibility of epibrassinolide induced amelioration of high temperature stress in *Brassica*.

The available literature on the effect of brassinosteroids in amelioration of high temperature stress has been reviewed in the present chapter under the following headings:

2.1 Germination

Germination of lettuce seed was inhibited at high temperature (Gonai *et al*., 2004). Brassinosteroids are plant hormones with significant growth-promoting activity. In addition to growth promotion, brassinosteroids also influence various other developmental processes like seed germination, rhizogenesis, flowering, senescence, abscission and maturation. Now it is well established that brassinosteroids also promote seed germination. Brassinolide, 24-epibrassinolide and 28-homobrassinolide promoted the germination of groundnut seeds

(Vardhini and Rao, 1996). Brassinosteroids not only promoted seed germination, but also reversed the inhibitory effect of abscisic acid (Vardhini and Rao, 1997). When seeds of *Triticum aestivum* cv. HD-2204 were soaked in graded concentration (10^{-10}, 10^{-8} or 10^{-6} M) of 28-homobrassinolide (HBR) for 8 h., the treatment significantly increased the per cent germination. Out of the three concentrations, 10^{-10} and 10^{-8} M proved best (Hayat and Ahmad, 2003 a). The ability of brassinosteroids to promote seed germination was also observed in the case of *Brassica napus* (Chang and Cai, 1988) and tomato (Vardhini and Rao, 2000).

It was also reported that when seeds of *B. juncea* treated with different concentrations (10^{-7}, 10^{-9} and 10^{-11} M) of 24-epibirassinolide for 8 hrs were subjected to various concentrations (0, 25, 50 and 100 mg l^{-1}) of heavy metals (Zn, Mn, Co and Ni), seedling growth was improved by 24-epibirassinolide treatments under heavy metal stress (Sharma and Bhardwaj, 2007). 28-homobrassinolide and 24-epibrassinolide were very effective in increasing the percentage of germination and seedling growth of sorghum under osmotic stress (Vardhini and Rao, 2003) and water stress (Vardhini and Rao, 2005). In case of tomato plant, it was reported that 24-epibrassinolide (1 μM) inhibit seed germination (Dhaubhadel *et al*., 1999).

2.2 Seedling growth

Growth is one of the best indices for evaluating plant response to environmental stress. The inhibition of seedling growth was observed in *Brassica* species after heat shock at 45 and 55°C (Kaur *et al*., 2009). Stimulation of growth is considered as an important physiological role of brassinosteroids in plants. The initial studies with brassinolide were concentrated around its ability to induce cell elongation, swelling, curvature and splitting of the second internode and such activity is called 'brassin activity'. Hypocotyl elongation of Packchoi (*Brassica chinensis*) by brassinosteroids was reported (Wang *et al.,* 1993) and application to apical 3-mm region gave the best effects. More vigorous root system, higher dry weight and length in homobrassinolide treated rice seedlings was reported (ShanNa *et al.,* 1997).

It was also revealed that seedling growth of *Brassica juncea* L. cv. PBR 91 was improved by 24-epibrassinolide treatments (10^{-7}, 10^{-9} and 10^{-11} M) under heavy metal stress (Sharma and Bhardwaj, 2007). In case of salt stress, 28-homobrassinolide (10^{-10}, 10^{-8} or 10^{-6} M) increases the growth of root and shoot in *Brassica juncea* seedlings. The negative effect generated by the lowest concentration (50 mM) of the salt was completely neutralized

by 10^{-8} M 28-homobrassinolide (Hayat *et al.,* 2006). Brassinosteroids inhibited root elongation in seedlings of *Brassica napus* (Dhaubhadel *et al*., 1999) and *Arabidopsis* (Clouse *et al*., 1993). Dhaubhadel *et al*., (1999) reported that, in 24-epibrassinolide treated seedlings, the portion of the hypocotyl just above the growth medium was typically swollen and appeared white. No significant differences were observed in the lengths of the hypocotyl and epicotyl regions between 24-epibrassinolide treated and untreated plants, but some curling of the hypocotyls was seen in 24-epibrassinolide treated plants.

2.3 Seedling mortality

Various workers have studied the role of plant growth regulators/hormones in alleviating effect of heat stress. Dhaubhadel *et al*., (1999) observed the role of hormones on thermo tolerance of *Brassica napus* and tomato seedlings. They found that seedling grown in presence of 24-epibrassinolide were more tolerant to lethal heat treatment than control. Epibrassinolide treatment increases the basic thermotolerance of *A. thaliana* seedlings (Kagale *et al*., 2007). Also in tomato, exposure of plants to 45°C for 3 hrs completely killed untreated plants, while 1 μM EBR application was found to be most effective for survival of tomato plants at lethal temperature (Singh and Shano, 2005).

2.4 Relative water content (RWC)

Relative water content is considered as an alternative measure of plant water status reflecting the metabolic activity in tissue affected by various types of stresses (Weatherley, 1950). Foliage application with 0.1 mg L^{-1} 24-epibrassinolide significantly increases leaf water content and water potential of maize plants grown in the soil spiked with 550 mg kg^{-1} Mn (Wang *et al*., 2009). Relative water content and water use efficiency were significantly increased by 24-epibrassinolide and 28-homobrassinolide under aluminium stress in mung bean (Ali *et al*., 2008 a). Ali *et al*., (2008 b) reported that 24-epibrassinolide improved the relative water content in *Brassica juncea* under salinity and nickel stress. Results from brassinosteroids treatment in rice under drought stress revealed that brassinosteroids improved water use efficiency and leaf water status (Farooq *et al*., 2009). However, leaves with heat acclimation pretreatment kept lower decrease in RWC than those without heat acclimation pretreatment in turfgrass species (Xu *et al*., 2006). RWC decreased during drought stress and heat stress but returned to normal levels following recovery (Wang and Huang, 2004).

2.5 Membrane stability

High temperature stress induces the membrane leakage in heat sensitive crop species whereas the heat stress resistant/tolerant crops tend to maintain the stability of membranes

even under period of heat stress. Loss in membrane integrity occurred in rape when exposed to heat stress (Melakeselam *et al*., 1999). Luo *et al*. (1996) observed the electrolyte leakage in Chinese cabbage under heat stress. Heat acclimation and salicylic acid pre-treatment reduced the electrolyte leakage from the seedlings of *Brassica* species compared to heat shocked ones (Kaur *et al*., 2009).

Ali *et al*., (2008 b) reported that 24-epibrassinolide improved the membrane stability index in *Brassica juncea* under salinity and nickel stress. Results from brassinosteroids treatment in rice under drought stress revealed that brassinosteroids improved membrane properties (Farooq *et al*., 2009). However, leaves with heat acclimation pretreatment maintained higher membrane thermostability than those without heat acclimation pretreatment in turfgrass species (Xu *et al*., 2006). Electrolyte leakage increased during drought stress and heat stress but returned to normal levels following recovery (Wang and Huang, 2004).

2.6 Free proline content

Among amino acids, proline responds most sensitively to stress conditions (Dubey, 1997). Osmoprotectants such as glycinebetaine, proline and total soluble sugars help the plant to overcome stress conditions. Hossain *et al.*, (1995) while working on Chinese cabbage hybrids reported more increase in proline content in heat tolerant hybrids of Chinese cabbage than heat sensitive hybrids under heat stress. Comparing the heat sensitive, moderately heat tolerant and heat tolerant cultivars of Chinese cabbage accumulation of proline was observed in proportion to tolerance in cultivars in Chinese cabbage under heat stress (Ye *et al.*, 1996).

Brassinosteroids enhanced the level of free proline under cadmium stress (Hayat *et al.,* 2007 a) and nickel stress in *Brassica juncea* (Alam *et al*., 2007). Also, the proline content was significantly increased by 24-epibrassinolide and 28-homobrassinolide under aluminium stress in mung bean (Ali *et al*., 2008 a). However, the content of proline increased in the *Brassica juncea* plants grown under copper stress and/ or raised from treatment with 28-homobrassinolide (Fariduddin *et al.,* 2009). Under osmotic stress condition, 28-homobrassinolide and 24-epibrassinolide enhanced levels of soluble proteins and free proline in sorghum (Vardhini and Rao, 2003). Results from brassinosteroids treatment in rice under drought stress revealed that brassinosteroids improve production of free proline (Farooq *et al*., 2009).

2.7 Malondialdehyde (MDA)

Malondialdehyde content is an indicator of peroxidation of the cellular membrane lipids, and it has been widely believed that lipid peroxidation has been responsible for

membrane deterioration during most type of stresses (Smirnoff, 1993). It is the final product of degradation of unsaturated fatty acids during lipid peroxidation. Gong *et al*. (1997) observed an increase in level of MDA under heat stress in maize seedlings. Bhattacharjee and Mukherjee (2003) observed high accumulation of MDA content in *A. lividus* seedlings under high temperature stress.

The oxidative stress caused by excess Mn, as reflected by the increase in malondialdehyde (MDA) content was greatly decreased by 24-epibrassinolide treatment in maize crop (Wang *et al*., 2009). Results from brassinosteroids treatment in rice under drought stress revealed that brassinosteroids declined the malondialdehyde content (Farooq *et al*., 2009). However, leaves with heat acclimation pretreatment kept lower membrane lipid peroxidation than those without heat acclimation pretreatment in turfgrass species (Xu *et al*., 2006). Lipid peroxidation increased during drought stress and heat stress but returned to normal levels following recovery (Wang and Huang, 2004). Decrease in MDA level in wheat seedlings during recovery from salt stress was observed by Mandhania *et al*. (2006).

2.8 H_2O_2 content

Hydrogen peroxide is a natural toxic metabolite, which results in increased permeability of the cell membranes by attacking membrane lipids. It is formed in peroxisomes as the product of photorespiration. The formation of $O_2^{\bullet-}$ radicals and hydrogen peroxide have been reported to increase under various environmental stresses. Bhattacharjee (2003) and Bhattacharjee and Mukherjee (2006) reported an increase in accumulation of H_2O_2 in *A. lividus* seedlings, Nagesh and Devraj (2008) in French bean and Ma *et al*. (2008) in apple leaves at high temperature stress. However, there was reduction in the concentration of H_2O_2 in both susceptible and tolerant genotypes during recovery from salt stress in wheat seedlings (Mandhania *et al*., 2006).

The oxidative stress caused by excess Mn, as reflected by the increase in H_2O_2 was greatly decreased by 24-epibrassinolide treatment in maize crop (Wang *et al*., 2009). However, H_2O_2 content increased significantly in copper treated *Brassica juncea* plants and decreased in plants given 28-homobrassinolide treatment (Fariduddin *et al.,* 2009). Results from brassinosteroids treatment in rice under drought stress revealed that brassinosteroids declined the H_2O_2 production (Farooq *et al*., 2009). However, leaves with heat acclimation pretreatment kept lower accumulation of H_2O_2 than those without heat acclimation pretreatment in turfgrass species (Xu *et al*., 2006).

2.9 Peroxidase (POX)

Peroxidase is another group of non chloroplastic enzymes that detoxifies H_2O_2 in the cytosolic part of cell and is non specific in utilizing electron donor for oxidation of H_2O_2. A number of peroxidase enzymes play a significant role in response to environmental stresses (Heinze *et al*., 1997). Thermal stress which causes physiological injuries in plants is related to peroxidase activity (Chaitanya *et al*., 2001 and Mazorra *et al*., 2002). Peroxidase activity increased significantly in *Brassica* species (Kaur *et al*., 2009) under high temperature stress.

The influence of 24-epibrassinolide on some enzymatic antioxidants in tomato leaf disc under high temperature (40°C) was reported (Mazorra *et al*., 2002). 24-epibrassinolide increased the activity of peroxidase in respond to high temperature in tomato leaves. Brassinosteroids enhanced the level of peroxidase under cadmium stress (Hayat *et al.,* 2007 a) and nickel stress in *Brassica juncea* (Alam *et al*., 2007), Mn stress in maize (Wang *et al*., 2009) and aluminium stress in mung bean (Ali *et al*., 2008 a). However, the activity of peroxidase increased in the *Brassica juncea* plants grown under copper stress and/ or raised from treatment with 28-homobrassinolide (Fariduddin *et al.,* 2009). It was also reported that heat acclimation and salicylic acid pre-treatment increased the activity of peroxidase in seedlings of *Brassica* species compared to heat shocked ones (Kaur *et al*., 2009).

2.10 Catalase (CAT)

Catalase is one of the hydrogen peroxide detoxifying enzymes. Although it is found abundantly in plant tissue but is absent in chloroplast and most of the catalase activity is associated with peroxisomes where it removes the hydrogen peroxide formed during photorespiration (Foyer and Mullineaux, 1994). Therefore, in plants CAT is often considered a peroxisomal marker enzyme because of its presence in these organelles (Corpas *et al*., 1993). Plants have isoenzymes of catalase and CAT-1 and CAT-2 are associated with peroxisomes, whereas CAT-3 is associated with mitochondria (Qureshi *et al*., 2007). Chaitanya *et al*. (2001) found significantly higher CAT activity in tolerant genotypes of mulberry cultivars under heat stress. Catalase activity increased during drought stress but returned to normal levels following recovery (Mittler and Zillinskas, 1994). Heat acclimation and salicylic acid pre-treatments increased the activity of catalase in seedlings of *Brassica* species compared to heat shocked ones (Kaur *et al*., 2009).

It was reported that brassinosteroids treatment enhanced the catalase activity in tomato (Vardhini and Rao, 2004). When maize (*Zea mays*) seedlings treated with brassinolide were subjected to water stress, the activities of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), as well as ascorbic acid and carotenoids

contents increased (Li *et al*., 1998). On the other hand, brassinosteroids enhanced the activity of CAT under osmotic stress conditions in sorghum (Vardhini and Rao, 2003). Rice seedlings exposed to saline stress and treated with brassinosteroids showed a significant increase in the activities of CAT, SOD, glutathione reductase (GR) and a slight increase in APX (Mazzafera *et al*., 2003). Under high temperature (40°C), activity of catalase was increased by 24-epibrassinolide in tomato leaf disc (Mazorra *et al*., 2002). Brassinosteroids enhanced the level of catalase under cadmium stress (Hayat *et al.,* 2007 a) and nickel stress in *Brassica juncea* (Alam *et al*., 2007), Mn stress in maize (Wang *et al*., 2009) and aluminium stress in mung bean (Ali *et al*., 2008 a). However, the activity of catalase was increased in the *Brassica juncea* plants grown under copper stress and/ or raised from treatment with 28-homobrassinolide (Fariduddin *et al.,* 2009).

2.11 Gaseous exchange

The photosynthetic efficiency of plant determines its dry matter production on which the biological yield depends (Yoshida 1981). The reduction in photosynthesis may occur due to (i) low diffusion of CO_2 into the chloroplast; (ii) alteration in the structure and function of organelles of photosynthesis, and (iii) change in light and dark reactions of photosynthesis (Ehleringer and Bjorkman, 1977; Bohnert *et al*., 1995; Hasegawa *et al*., 2000; Sonia, 2004; Cha-um *et al*., 2006; Taiz and Zeiger, 2006).

High temperature reduces the photosynthesis by inhibiting Rubisco activation in cotton (Crafts-Brandner and Law, 2000). Foliage application with 0.1 mg L^{-1} 24-epibrassinolide significantly increases chlorophyll content and photosynthetic rate of maize plants grown in the soil spiked with 550 mg kg^{-1} Mn (Wang *et al*., 2009). In wheat, inhibitory effect of salt stress on photosynthetic rate was ameliorated significantly by the exogenous application of 24-epibrassinolide (Muhammad *et al.*, 2008). Chlorophyll content and the rate of photosynthesis were significantly increased by 24-epibrassinolide and 28-homobrassinolide under aluminium stress in mung bean (Ali *et al*., 2008 a). Prakash *et al*., (2008) reported that when sesame plants were sprayed with 0.25, 0.5 and 1.0 ppm homobrassinolide at the vegetative, flowering and capsule formation stages (30, 45 and 60 DAS), net assimilation rate was increased with the application of 1.0 ppm homobrassinolide thrice. Results from brassinosteroids treatment in rice under drought stress revealed that brassinosteroids improved net CO_2 assimilation (Farooq *et al*., 2009). Photosynthesis inhibiting effect of herbicide terbutryn was ameliorated by 24-epibrassinolide increasing photosynthetic CO_2 assimilation in *Vicia faba* (Pinol and Simon, 2009).

Photosynthetic rate has a close relation with transpiration rate. Reduction in transpiration rate was reported in spring wheat as a result of epibrassinolide spraying (Prusakova *et al*., 2000).

Stomatal conductance to the diffusion of CO_2 is commonly used as an indicator of crop water status and photosynthetic activity (Muchow, 1985). The plant hormone brassinosteroids increased the stomatal conductance in *Vigna radiata* **(**Fariduddin *et al*., 2003; Fariduddin *et al*., 2006) and soybean **(**Donc *et al*., 2008).

2.12 Yield and yield attributes

Russo and Diaz-Perez (2005) reported decrease in yield of pepper under heat stress. Temperature greater than 27°C, in a growth cabinet, have resulted in floral sterility and yield loss in *Brassica juncea* L (Morrison and stewart, 2002). A number of plant growth regulators have been successfully exploited to enhance growth and yield. Brassinosteroids, a new class of hormone, improves production of watermelon (Wang *et al*., 1994), spring barley (Kabashnikova *et al*., 1998), tomato (Vardhini and Rao, 2001) and seed yield in groundnut (Vardhini and Rao, 1998). In sesamum plant, the application of 28-homobrassinolide enhanced the total dry matter production, number of capsules per plant, number of seeds per capsule, capsule weight and 1000-seed weight (Prakash *et al*., 2007).

Prakash *et al.* (2008) reported that when sesame plants were sprayed with 0.25, 0.5 and 1.0 ppm homobrassinolide at the vegetative, flowering and capsule formation stages (30, 45 and 60 DAS) morphological parameters such as plant height, number of leaves, number of branches and leaf area index (LAI) increased with increasing number of sprays and dosage of spray compared to the control and water sprays. The dry matter production increased at a faster rate in plants those were treated twice and thrice with 1.0 ppm homobrassinolide. The oil content was significantly enhanced in the plants treated with homobrassinolide which were treated thrice. Yield per plant were also increased significantly due the increase in morphological, physiological and biochemical parameters.

The application of 24-epibrassinolide increased the dry matter weight and grain yields in wheat crop (Hnilicka *et al*., 2007). Treatment with epibrassinolide at 10^{-8} M in buckwheat resulted in increased number of flowers and shoots, and increased yield by 48.1% (Ezhov *et al*., 1999). Danilevich (2005) reported the greatest increases in productivity (by 32.9%) and 1000-seed weight (by 0.4 g) by epibrassinolide treatment in cabbage. Spraying fenugreek plant with brassinosteroids at 50 ppm in combination with Fe at 100 ppm resulted in the highest values of number of pods/ plant, number of seeds per pod, pod length, 1000-seed weight, seed yield, oil contents and harvest index (Farahat, 2002). Increase in the

number of branches, number of pods and seeds as well as their weights in grams per plant have been reported in faba bean plants as a response to brassinosteroids treatment (El Gazzar *et al*., 2000). Foliar application of 0.4 ppm brassinosteroid at preflowering and pod development increased seed and oil yield in mustard (Kumawat *et al.*, 1997). Also 28-homobrassinolide has been used to increase the productivity of mustard (Ramraj *et al*., 1997). Significant increase in number of pods per plant and seed yield per plant was reported in mustard as a result of 28-homobrassinolide spraying (Hayat *et al*., 2000; Hayat *et al*., 2001).

Under different stress condition also brassinosteroids improves the yield of *Brassica juncea* plants, like salt stress (Hayat *et al*., 2006); water deficit stress (Kumawat *et al*., 1997) and saline stress (Hayat *et al*., 2007 b).

CHAPTER - 3

MATERIALS AND METHODS

1 Materials

1.1 Plant Material

Seeds of variety RH-8812 (Laxmi) of *Brassica juncea* procured from oilseeds section, Department of Genetics and Plant Breeding were used for the present study.

1.2 Chemicals

All the chemicals used during the present course of investigations were of analytical grade and purchased from either Sisco Research Laboratories (Mumbai), E-Merck or Sigma Aldrich Chemical Co. (USA).

EBR was dissolved in absolute ethanol to achieve a stock solution of 10 mM. The stock solution was stored at -20^{O}C. Dilutions were made to 10^{-6}, 10^{-8} or 10^{-10} M in double distilled de ionized water as and when needed.

2 Methods

Expt. 1(a): Effect of epibrassinolide on germination and seedling growth at different temperatures

Epibrassinolide (EBR) concentrations: 0, 10^{-6}, 10^{-8}, 10^{-10} M

Temperature (s): 25, 30, 35°C

Relative humidity: 70%

Raising the seedlings:

Seeds of variety RH-8812 (Laxmi) were sown in trays having sandy loam soil. Each tray was filled with 7 kg soil which was previously homogenized with enough water to bring the soil to field capacity (150 ml water/kg soil). Each tray was uniformly marked into 4-5 rows and 6-7 spots in each row as per requirement. Five seeds were sown at each spot. Three trays were used as three replications. Seedlings were allowed to grow at optimum

temperature (25±0.5°C), relative humidity (70%) and 16 hrs light, 8 hrs dark cycle for five days.

Treatments: Seeds were soaked in 0, 10^{-6}, 10^{-8}, 10^{-10} M epibrassinolide for 2 hrs and the seedlings were grown at 25±0.5, 30±0.5 or 35±0.5°C in seed germinator at 70% relative humidity .

Observations: Following observations were recorded 5 days after sowing (5 DAS):

Per cent germination

Per cent germination was calculated as follows:

$$\textbf{Per cent germination} = \frac{\text{Number of seed germinated}}{\text{Total number of seeds sown}} \times 100$$

Speed of germination

Speed of germination was calculated as follows:

$$\textbf{Speed of germination} = \frac{D1}{1} + \frac{D2}{2} + \text{------} + \frac{Dn}{n}$$

Where,

D1: Number of seedling germinated on 1st day

D2: Number of seedling germinated on 2nd day

Dn: Number of seedling germinated on nth day

Root length (cm)

Root length (cm) of seedlings was measured with the help of scale.

Shoot length (cm)

Shoot length (cm) of seedling was measured from soil surface to shoot apex with help of scale.

Seedling dry weight (mg seedling^{-1})

Seedlings were taken out carefully from the tray after washing away the sand adhering to roots with copious of tap water. Ten seedlings were dried in labeled paper envelopes in an oven at 80°C for 72 hrs and then their dry weights (g) were determined with the help of an electronic balance.

Expt. 1(b): Effect of epibrassinolide on seedling mortality at high temperature

Raising the seedlings: Seedlings were raised in seed germinator at 25±0.5°C and 70% relative humidity as in expt.1 (a).

Treatments: Seeds were soaked in 0, 10^{-6}, 10^{-8}, 10^{-10} M epibrassinolide for 2 hrs. Five days after sowing (DAS), the seedlings were exposed to high temperature (45±0.5°C).

Observations: Time taken to 50% seedling mortality (when 50% hypocotyls hooked down and dried to thread like structure and did not revive after withdrawing high temperature) was noted.

Expt.2: Effect of epibrassinolide on physiological and biochemical parameters under high temperature stress

Raising the seedlings: Seedlings were raised in seed germinator at 25±0.5°C and 70% relative humidity as in expt.1 (a).

Treatments:

- First set was allowed to grow at optimum temperature (25±0.5°C) as control.
- Second set was sprayed with epibrassinolide (10^{-8} M and 10^{-10} M) on 5^{th} day and allowed to grow at optimum temperature (25±0.5°C).
- Third set was allowed to grow at optimum temperature (25±0.5°C) for 5 days followed by exposure to high temperature (45±0.5°C) for 2 hrs on 6^{th} day.
- Fourth set was exposed to 32±0.5°C (for 24 hrs) on 5^{th} day followed by exposure to high temperature (45±0.5°C) for 2 hrs on 6^{th} day.
- Fifth set was sprayed with epibrassinolide (10^{-8} M and 10^{-10} M) on 5^{th} day followed by exposure to high temperature (45±0.5°C) for 2 hrs on 6^{th} day.

Following observations were recorded (in hypocotyl portion) immediately after high temperature treatment and on 6 and 24 hrs after recovery at 25±0.5°C.

Relative water content (RWC)

The seedlings were sampled and hypocotyl portion was cut from the seedlings. Soil was removed with the help of a soft brush. Then the hypocotyls were weighed immediately to take their fresh weight. These hypocotyls were then kept in Petri dishes containing fix measured amount of distilled water for 3 hrs. Hypocotyls (fully turgid) were then filter dried and weighed again. These were kept in oven at 85°C for 72 hrs till a constant dry weight was obtained. These observations were used to calculate RWC (%) according to the formula given by Weatherley (1950).

$$\textbf{RWC (\%)} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Turgid weight} - \text{Dry weight}} \times 100$$

Free proline content

Proline content was estimated by using the method of Bates *et al*. (1973).

Reagents

1. 3% aqueous sulphosalicylic acid (w/v)
2. Acid ninhydrin (prepared by dissolving 1.25 g ninhydrin in 30 ml glacial acetic acid and 20 ml 6.0 M O-phosphoric acid until dissolved).
3. Toluene

Extraction

Three hundred mg of hypocotyls were homogenized in 5 ml of 3 per cent sulphosalicylic acid and then centrifuged at 5000 rpm for 15 minutes and supernatant was taken.

Procedure

Two ml of supernatant was taken and 2.0 ml reagent acid ninhydrin + 2.0 ml acetic acid was added. This mixture was then kept in boiling water bath for 1 hr at 100°C and thereafter reaction was terminated by keeping tubes in ice-bath. Then 4.0 ml of toluene was added. After vigorous shaking, the upper organic phase was taken after attainment of room temperature and absorbance was recorded at 520 nm by using blank.

A standard curve was prepared by using graded concentration of proline in 3% sulphosalicylic acid. The proline content was expressed as mg g^{-1} fresh weight.

Membrane stability (% Injury)

The assessment of membrane stability was done by the procedure of Dionisio-Sese and Tobita (1998).

Procedure

The 150 mg hypocotyls were kept in 20 ml vials containing 10 ml deionized water at 25°C. After five hours, the electrical conductivity (EC) of the surrounding solution was measured by the Water Analysis Kit (Naina, India Ltd., NDC 732) and designated as EC_a. Then the samples were kept in boiling water bath for 20 minutes to achieve total killing of the tissue. After cooling, the EC of the solution was again measured and designated as EC_b. The per cent injury was calculated as follows:

$$\textbf{\% Injury} = \frac{EC_a}{EC_a + EC_b} \times 100$$

Non-enzymatic studies (MDA and H_2O_2)

Preparation of extract

For the extraction of MDA and H_2O_2, 1.0 g hypocotyl each from 0 M, 10^{-8} M and 10^{-10} M epibrassinolide treated seedlings was taken and ground in 5 ml of chilled 0.8 N $HClO_4$ and centrifuged at 10,000 rpm for 25 minutes. The clear supernatant was decanted carefully and was used for further estimation.

Estimations

Malondialdehyde (MDA) content

Malondialdehyde was estimated according to the method of Heath and Packer (1968).To 0.5 ml of supernatant was added 2.3 ml of 20% (w/v) trichloroaceticacid (TCA) containing 0.5% thiobarbituric acid. The mixture was heated in a water bath at 95°C for 30 minutes and quickly cooled in ice bath. The absorbance was recorded at 532 nm and the value of non-specific absorption at 600 nm was subtracted from it. The concentration of malondialdehyde was calculated using the extinction coefficient of 155 mM^{-1} cm^{-1}.

H_2O_2 content

Hydrogen peroxide was estimated by the method of Sinha (1972). To 0.4 ml extract, 0.6 ml of 0.1 M phosphate buffer (pH 7.0) and 3 ml mixture of 5% (w/v) potassium dichromate and glacial acetic acid (1:3, v/v) was added. The mixture was heated for 10 minutes in a boiling water bath. Colour of solution changed to green due to the formation of chromic acetate. After cooling, absorbance was recorded at 570 nm against the reagent blank without sample extract. The quantity of H_2O_2 was determined from the standard curve of H_2O_2 (20-180 μmoles).

Enzymatic studies (Peroxidase and Catalase)

Preparation of enzyme extract

One gram of hypocotyls were homogenized in a pre-chilled pestle and mortar with 4 ml of 0.1 M potassium phosphate buffer (pH 7.0) containing 1% PVP, 0.1 mM EDTA and 20% glycerol, 0.5 mM ascorbate was included in the extraction buffer of ascorbate peroxidase. The homogenate was centrifuged at 12,000 rpm for 30 minutes in refrigerated centrifuge. The supernatant was carefully decanted and used for the assay of different enzymes.

Estimations

Peroxidase

The procedure of Siegel and Siegel (1969) was followed for estimating peroxidase activity. Three ml of reaction mixture contained 0.1 M phosphate buffer (pH 7.0), 0.1 mM guaiacol, 0.1 mM H_2O_2 and 50 µl cell-free extract. Reaction was started with the addition of H_2O_2 and increase in the absorbance at 470 nm was recorded for 2 minutes. The activity was calculated using the extinction coefficient value of 22.6 mM^{-1} cm^{-2} for guaiacol. One unit of enzyme activity was equivalent to one µmol of H_2O_2 oxidized.

Catalase

The activity of catalase was estimated according to the procedure described by Aebi (1984). The reaction mixture in final volume of 3 ml, contained 0.1 M phosphate buffer (pH 7.0), 10 mM H_2O_2 and 50 µl of cell-free extract. Reaction was initiated with the addition of H_2O_2 and enzyme activity was determined by following the consumption of H_2O_2 at 240 nm for 2 minutes. The enzyme activity was calculated using the extinction coefficient value of 39.4 mM^{-1} cm^{-1} for H_2O_2. One unit of enzyme activity corresponded to one µmol H_2O_2 consumed during the reaction.

Expt. 3: Effect of epibrassinolide on gaseous exchange, yield and yield attributes under high temperature stress at terminal stage

Gaseous exchange

Normal (28th October, 2009) and late (12th November, 2009) field sown *Brassica juncea* (variety RH- 8812), was sprayed (on 5 randomly selected plants per treatment) with epibrassinolide (10^{-8} and 10^{-10} M) along with water spray as control at "flowering cessation stage (83 DAS)". Gaseous exchange studies (rate of photosynthesis, transpiration and stomatal conductance) were done using Infra red gas analyzer (IRGA) (ADC Bioscientific Ltd., model LCi) after 24 and 48 hrs of spray between 11:00 AM to 12:30 PM.

Max. temperature (°C), relative humidity (%) and sun shine (hrs) on the day of spray and observation taken in normal sown (Date of spray: 18th January, 2010) and late sown (Date of spray: 2nd February, 2010):

Particulars	Max. temperature (°C)	Relative Humidity (%)		Sun shine (hrs)
		Morning	Evening	

Day of Spray	14.6 (24.2)	100 (100)	79 (50)	3.1 (8.3)
24 hrs after spray	15.2 (23.4)	100 (100)	74 (41)	5.4 (7.6)
48 hrs after spray	15.4 (25.2)	100 (85)	70 (47)	5.1 (7.3)

N.B: Figures in parenthesis represent corresponding parameter value for late sown.

Yield and its attributes

The following data of yield and its attributing characters were recorded at the time of harvesting.

Plant height (cm)

Number of branches plant^{-1}

Number of siliquae plant^{-1}

1000 seed weight (g)

Number of seeds siliqua $^{-1}$

Seed yield (g plant^{-1})

Oil per cent (using Soxwet method) (A. O. A. C. 1960)

3 Statistical analysis

Data were analyzed using complete randomized design (CRD) for two factors or three factors. Treatments were compared using critical difference (CD) at 5 per cent level of significance.

CHAPTER – 4

EXPERIMENTAL RESULTS

The present investigations were carried out to study the possibility of epibrassinolide induced amelioration of high temperature stress in *Brassica juncea* (L.) Czern & Coss. The observations recorded during the investigations are presented under the following heads:

1. Effect of epibrassinolide on germination, seedling growth and seedling mortality under high temperature stress

1.1 Germination and seedling growth at different temperatures

1.1.1 Per cent germination

Irrespective of temperature used (25, 30 or 35°C); soaking seeds with 10^{-6} M EBR resulted in a reduction in seed germination over control. The reduction in germination with 10^{-6} M EBR was gradually overcome with decreasing concentrations of EBR used (from 10^{-8} M to 10^{-10} M) i.e. maximum reduction in germination was at 10^{-6} M, followed by 10^{-8} M and 10^{-10} M respectively (Table 1 and Fig. 1).

At 30°C, germination in control and 10^{-6} M was at par with 25°C but it was lesser than at 25°C when seeds were soaked in 10^{-8} and 10^{-10} M.

At 35°C, irrespective of hormonal concentration used there was drastic reduction in germination over 25°C and 30°C. On mean basis, in temperature treatments, germination decreased with increase in temperature from 25°C to 35°C but there was drastic reduction when temperature was increased from 30 to 35°C whereas, in EBR treatments, 10^{-6} M EBR reduced germination over control but this reduction tended to overcome with lowering of EBR concentration.

1.1.2 Speed of germination

Irrespective of temperature, there was drastic reduction in speed of germination when seeds were soaked in 10^{-6} M EBR solution but it was improved with lowering concentrations of EBR viz: 10^{-8} M and 10^{-10} M respectively. At 25°C, the reduction in speed of germination by 10^{-6} M was partially overcome by 10^{-8} M and 10^{-10} M but it was still lesser than control (Table 2 and Fig. 2).

At 30°C, speed of germination in control was at par with 25°C, but at 30°C, soaking seeds in 10^{-6} M EBR had higher speed of germination than at 25°C. It was at par with application of 10^{-8} M and 10^{-10} M EBR.

At 35°C, there was a drastic reduction in speed of germination over 25 or 30°C with all EBR applications. On mean basis, in temperature treatments, speed of germination at 25°C was statistically at par with 30°C but it decreased drastically with increase in temperature

from 30 to 35°C, whereas in EBR treatments, 10^{-6} M EBR reduced speed of germination over control. This reduction tended to overcome with lowering of EBR concentration but still could not match control even with 10^{-10} M EBR application.

Table 1. Effect of EBR and temperature on germination percentage in *Brassica juncea*

EBR	Temperature (^{O}C)			
	25^{O}C	**30^{O}C**	**35^{O}C**	**Mean**
Control	96.65 **(84.06)**	96.65 **(84.06)**	26.67 **(31.11)**	**(66.41)**
10^{-6} M	56.67 **(48.83)**	53.33 **(46.92)**	06.67 **(15.00)**	**(36.92)**
10^{-8} M	86.67 **(69.32)**	63.33 **(52.81)**	10.00 **(18.20)**	**(46.77)**
10^{-10} M	96.65 **(82.73)**	77.78 **(62.09)**	16.67 **(23.98)**	**(56.27)**
Mean	**(71.24)**	**(61.47)**	**(22.07)**	
CD at 5%	EBR	3.87		
	Temperature	3.35		
	EBR × Temperature	6.71		

N.B: Figures in parenthesis represent transformed value

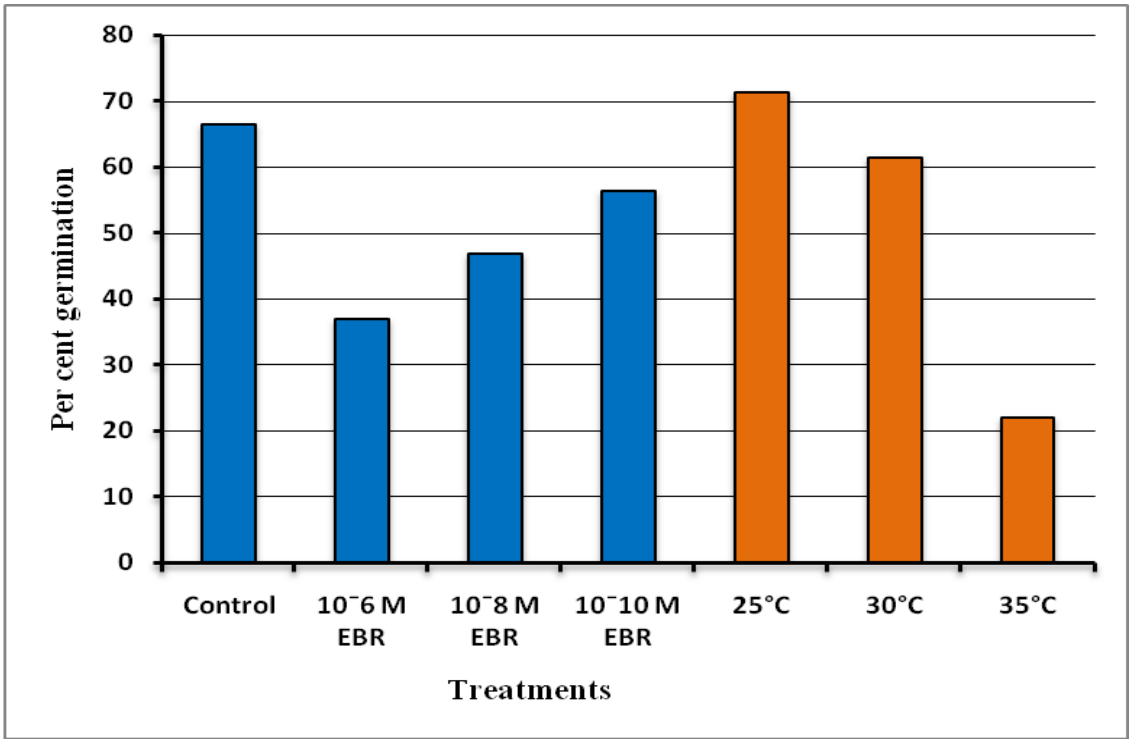

Fig. 1: Effect of EBR and temperature on germination percentage (Mean values) in *Brassica juncea*

Table 2. Effect of EBR and temperature on speed of germination in *Brassica juncea*

EBR	Temperature (^{O}C)			
	25^{O}C	**30^{O}C**	**35^{O}C**	**Mean**
Control	2.12	2.15	0.45	**1.57**
10^{-6} M	0.85	1.30	0.24	**0.80**
10^{-8} M	1.46	1.49	0.38	**1.11**
10^{-10} M	1.55	1.55	0.42	**1.17**
Mean	**1.50**	**1.62**	**0.37**	
CD at 5%	EBR	0.20		
	Temperature	0.17		

	EBR × Temperature	0.34		

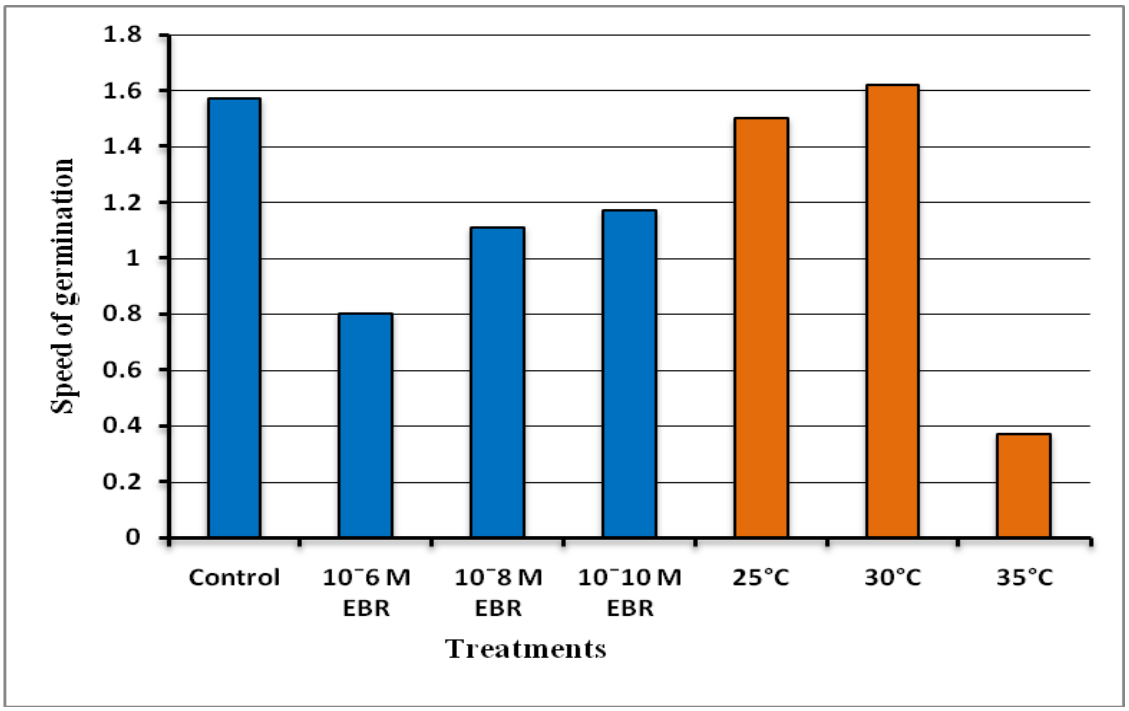

Fig. 2: Effect of EBR and temperature on speed of germination (Mean values) in *Brassica juncea*

1.1.3 Root length (cm)

Both at 25 and 30°C, root length in control was statistically at par with all EBR concentrations used but when temperature increased to 35°C, root length decreased significantly in 10^{-6} M EBR over control whereas root length with 10^{-8} M and 10^{-10} M were at par with control (Table 3 and Fig. 3).

On the mean basis, in temperature treatments, a significant decrease in root length was observed at 30 and 35°C over 25°C. The decrease was more prominent at 35°C temperature.

In EBR treatments a significant decrease in root length was observed at higher concentration (10^{-6} M) however, in 10^{-8} M and 10^{-10} M it was at par with control.

Table 3. Effect of EBR and temperature on root length (cm) in seedlings of *Brassica juncea*

EBR	Temperature (^{O}C)			
	25^{O}C	**30^{O}C**	**35^{O}C**	**Mean**
Control	8.3	7.5	7.2	**7.7**
10^{-6} M	8.5	7.6	4.9	**7.0**
10^{-8} M	8.4	8.1	6.8	**7.8**
10^{-10} M	8.9	8.4	7.0	**8.1**
Mean	**8.5**	**7.9**	**6.5**	
CD at 5%	EBR	0.7		
	Temperature	0.6		
	EBR × Temperature	1.1		

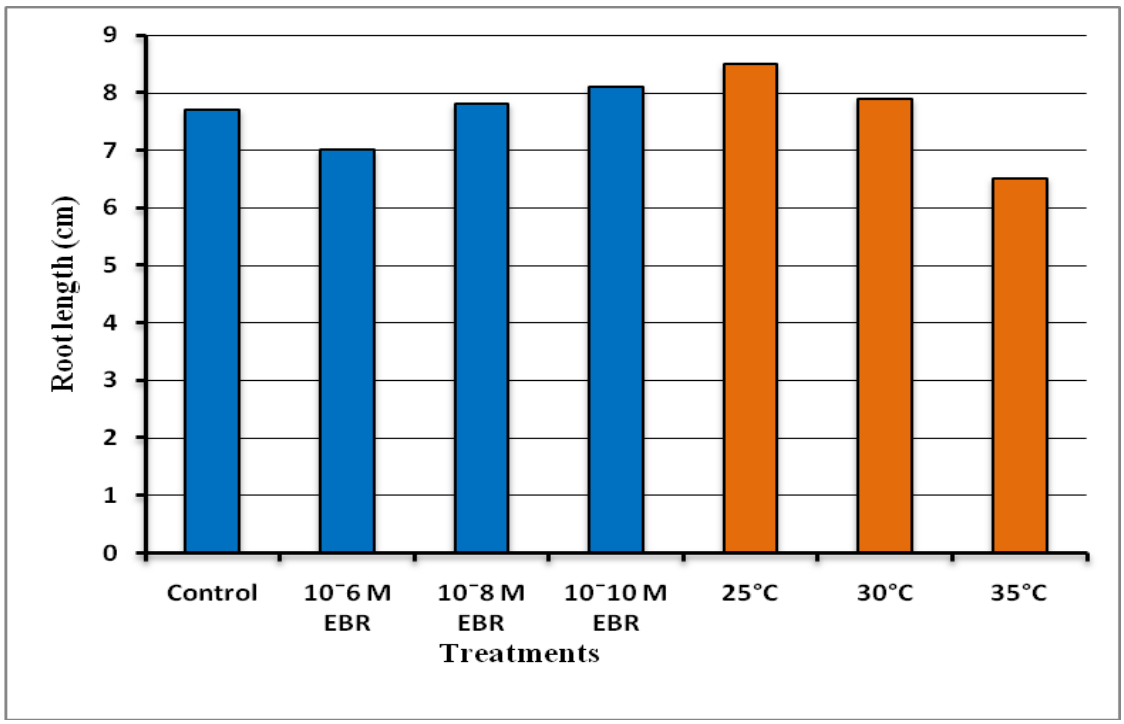

Fig. 3: Effect of EBR and temperature on root length (cm) (Mean values) in seedlings of *Brassica juncea*

1.1.4 Shoot length (cm)

Irrespective of temperature (25, 30 or 35°C), the shoot length in all EBR hormonal concentrations used remained unchanged over their respective controls. Though, the mean shoot length at 25°C was at par with 30°C, it decreased drastically when temperature increased from 30 to 35°C, whereas in EBR treatments, irrespective of EBR concentration used, the shoot length was at par with control (Table 4 and Fig. 4). The interaction results between EBR × Temperature for shoot length were found to be non significant.

1.1.5 Seedling dry weight (mg/seedling)

A significant decrease in seedling dry weight (on mean basis) was observed when temperature was increased from 25 to 30°C and then to 35°C. The minimum seedling dry weight was observed at 35°C (Table 5 and Fig. 5).

The mean seedling dry weight increased significantly over control in all EBR concentrations used. The increase was at par in all concentrations used. The interaction results between EBR × Temperature for seedling dry weight were non significant.

Table 4. Effect of EBR and temperature on shoot length (cm) in seedlings of *Brassica juncea*

EBR	**Temperature (^{O}C)**			
	25^{O}C	**30^{O}C**	**35^{O}C**	**Mean**
Control	3.8	3.8	3.3	**3.7**
10^{-6} M	3.9	3.6	3.3	**3.6**
10^{-8} M	3.8	3.6	3.3	**3.6**
10^{-10} M	3.8	3.7	3.4	**3.6**
Mean	**3.8**	**3.7**	**3.3**	
CD at 5%	EBR	N.S.		
	Temperature	0.2		
	EBR × Temperature	N.S.		

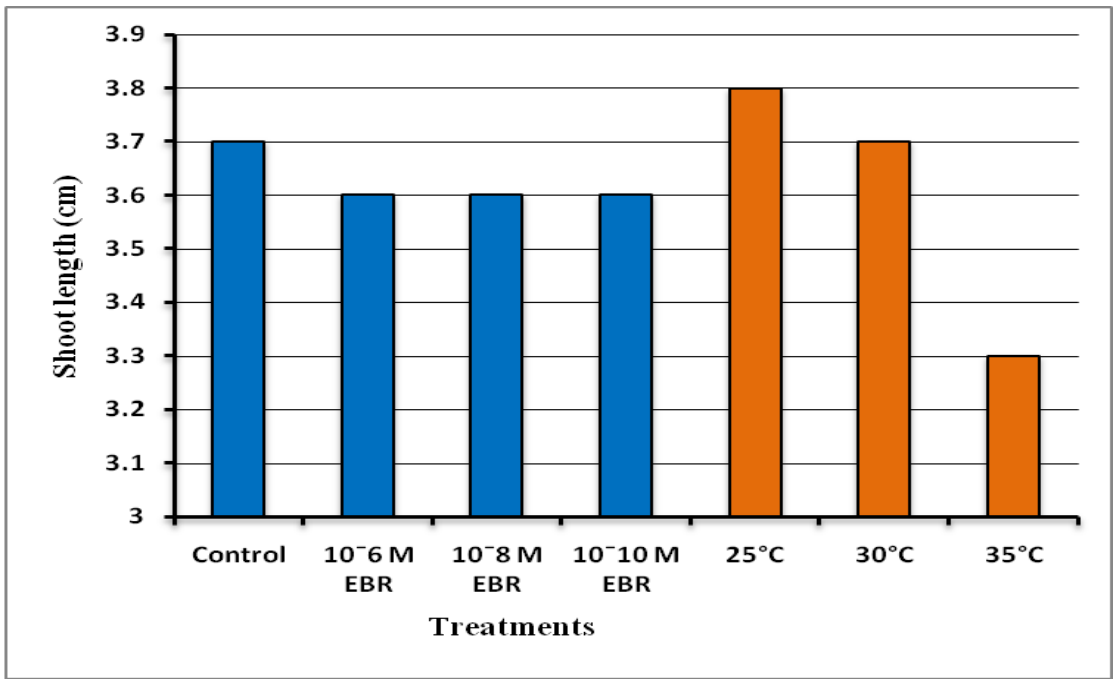

Fig. 4: Effect of EBR and temperature on shoot length (cm) (Mean values) in seedlings of *Brassica juncea*

Table 5. Effect of EBR and temperature on seedling dry weight (mg/seedling) in *Brassica juncea*

EBR	Temperature (^{O}C)			
	25 ^{O}C	**30 ^{O}C**	**35 ^{O}C**	**Mean**
Control	10.46	9.34	7.78	**9.20**
10^{-6} M	11.63	10.40	9.33	**10.46**
10^{-8} M	11.75	10.01	8.63	**10.13**
10^{-10} M	11.83	10.00	8.57	**10.13**
Mean	**11.42**	**9.94**	**8.58**	
CD at 5%	EBR	0.64		
	Temperature	0.56		
	EBR × Temperature	N.S.		

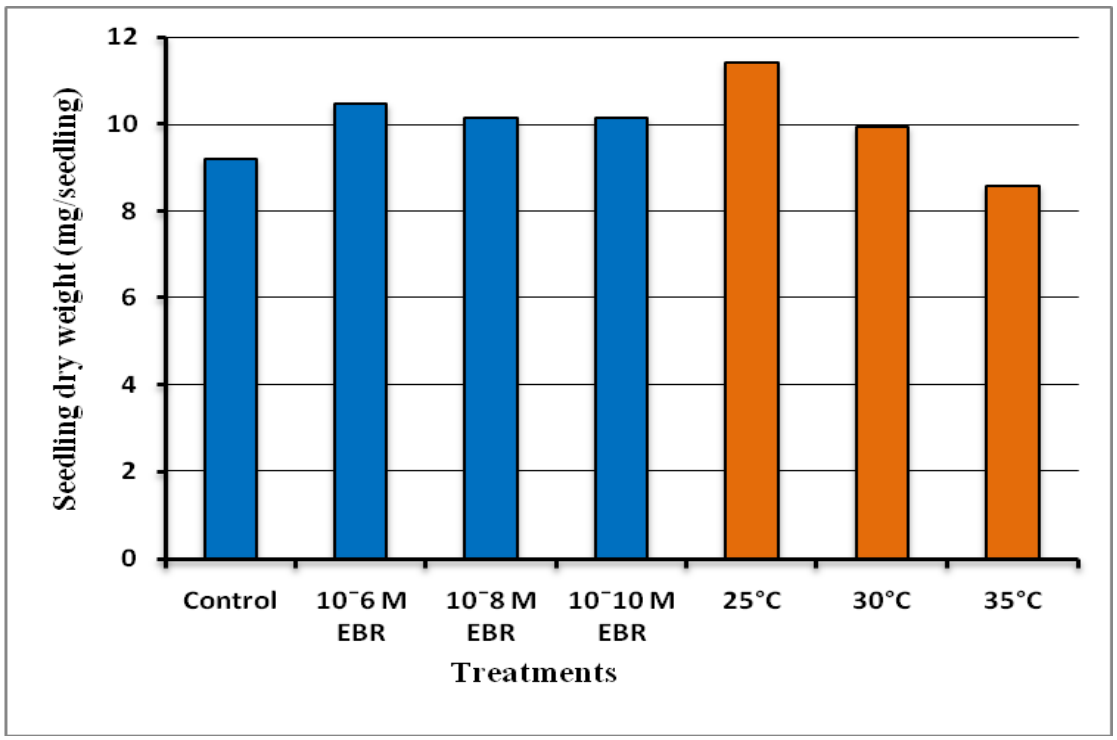

Fig. 5: Effect of EBR and temperature on seedling dry weight (mg/seedling) (Mean values) in *Brassica juncea*

1.2 Seedling mortality at high temperature

High temperature treatment initially causes bending (hypocotyl hook), followed by seedling mortality at hook portion. Data (Table 6 and Fig. 6) reveal the time taken to 50% seedling mortality at high temperature. Significant increase (delay) in time taken to 50% seedling mortality was recorded in EBR treated seedlings over control. Increase was more prominent at 10^{-8} M and 10^{-10} M than 10^{-6} M concentration of EBR. Effect of 10^{-8} M and 10^{-10} M was at par with each other.

Table 6. Effect of EBR on time taken to 50% seedling mortality in *Brassica juncea* under high temperature stress

EBR	**Time taken to 50% seedling mortality** (minutes)
Control	187
10^{-6} M	205
10^{-8} M	222
10^{-10} M	223
CD at 5%	13

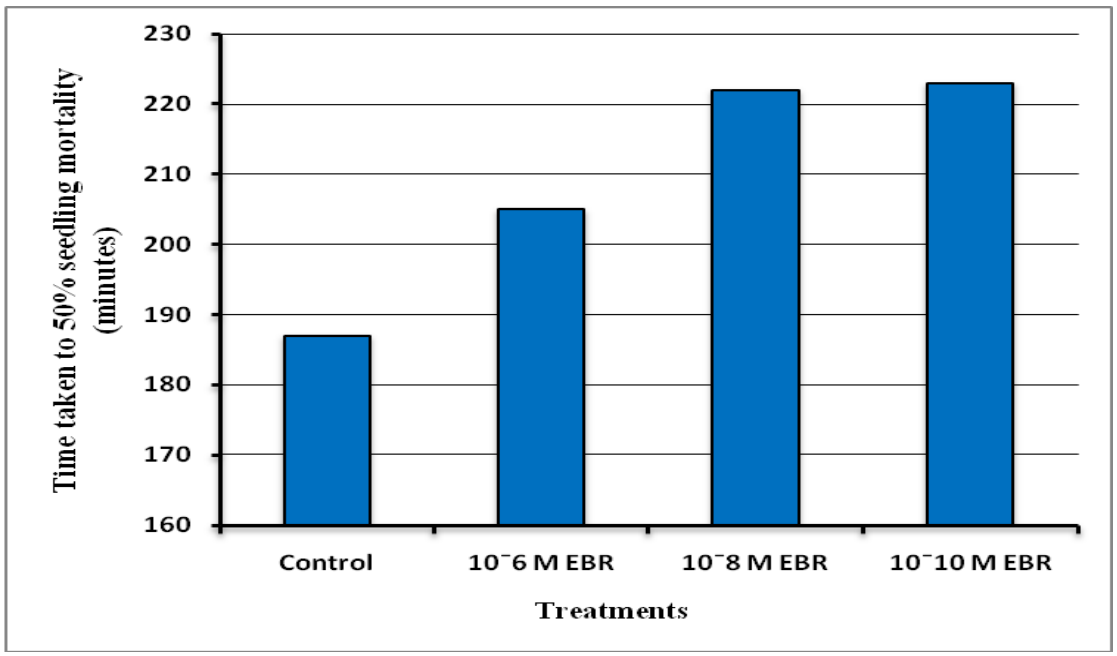

Fig. 6: Effect of EBR on time taken to 50% seedling mortality in *Brassica juncea* under high temperature stress

2. Effect of epibrassinolide on physiological and biochemical parameters under high temperature stress

2.1 Relative water content (RWC)

The changes in RWC during high temperature (45°C) stress acclimation (32°C followed by 45°C), EBR spray and recovery in hypocotyl of *Brassica juncea* seedlings has been presented in Table 7 and Fig. 7.

Compared to at optimum temperature (25°C), when seedlings were exposed to high temperature stress (HTS), the RWC decreased significantly. When seedlings were first acclimated at 32°C and then exposed to high temperature stress (HTS) at 45°C, the decrease in RWC was lesser in acclimated seedlings. RWC increased significantly after 6 or 24 hrs recovery from HTS. The recovery was more in acclimated seedlings over non acclimated seedlings. In case of acclimation, RWC increased significantly over 45°C after 6 hrs recovery but content still remained lower than at 25°C. Maximum possible recovery took place in 6 hrs and there was no further recovery up to 24 hrs.

An overall (on mean basis), though there was an increase in RWC with both the concentrations of EBR used, 10^{-10} M EBR was more effective in increasing the relative water content.

2.2 Free proline content

The data presented in Table 8 and Fig. 8 reveal changes in free proline content during high temperature stress (45°C), acclimation (32°C followed by 45°C), EBR spray and recovery in hypocotyl of *Brassica juncea* seedlings.

The free proline content (on the overall mean basis) increased significantly under HTS as well as after acclimation however; increase was higher after acclimation as compared to that under normal temperature (25°C). Free proline content decreased significantly after 6 hrs and 24 hrs recovery in case of HTS as well as acclimation but contents remained higher over the 25°C. Higher free proline content was observed during recovery in case of acclimation as compared to HTS.

Both the concentrations of EBR resulted in an increase in free proline content however lowest diluted concentration (10^{-10} M) was more effective.

2.3 Membrane stability (% Injury)

The change in per cent injury as judged by measuring leakage of ions during high temperature stress (45°C), after acclimation, EBR spray and recovery in hypocotyl of *Brassica juncea* seedlings has been presented in Table 9 and Fig. 9.

The per cent injury increased drastically (on the overall mean basis) under HTS as well as after acclimation however, increase was higher under HTS as compared to that under normal temperature (25°C). The per cent injury decreased significantly after 6 hrs and 24 hrs recovery in case of HTS as well as acclimation but value remained higher over the 25°C.

Higher per cent injury was observed during recovery in case of HTS as compared to acclimation.

Spray with EBR significantly decreased per cent injury as compared to control however; decrease was more in 10^{-10} M concentration.

Table 7. Effect of EBR, temperature, acclimation and recovery period on relative water content (%) in hypocotyl of *Brassica juncea*

EBR	Temperature / Recovery period							
	25^{O}C	**45^{O}C**	**Recovery**		**32^{O}C 45^{O}C**	**Recovery**		**Mean**
			6 hrs	**24 hrs**		**6 hrs**	**24 hrs**	
Control	84.38	70.45	71.64	73.59	72.43	74.30	75.33	**74.59**
10^{-8} M	89.07	75.55	76.60	79.77	77.70	82.09	83.13	**80.56**
10^{-10} M	90.00	81.41	82.14	86.47	85.10	87.93	88.77	**85.98**
Mean	**87.82**	**75.80**	**76.79**	**79.94**	**78.41**	**81.44**	**82.41**	
CD at 5%	EBR	1.39						
	Temperature / Recovery	2.13						
	EBR × Temperature / Recovery	N.S.						

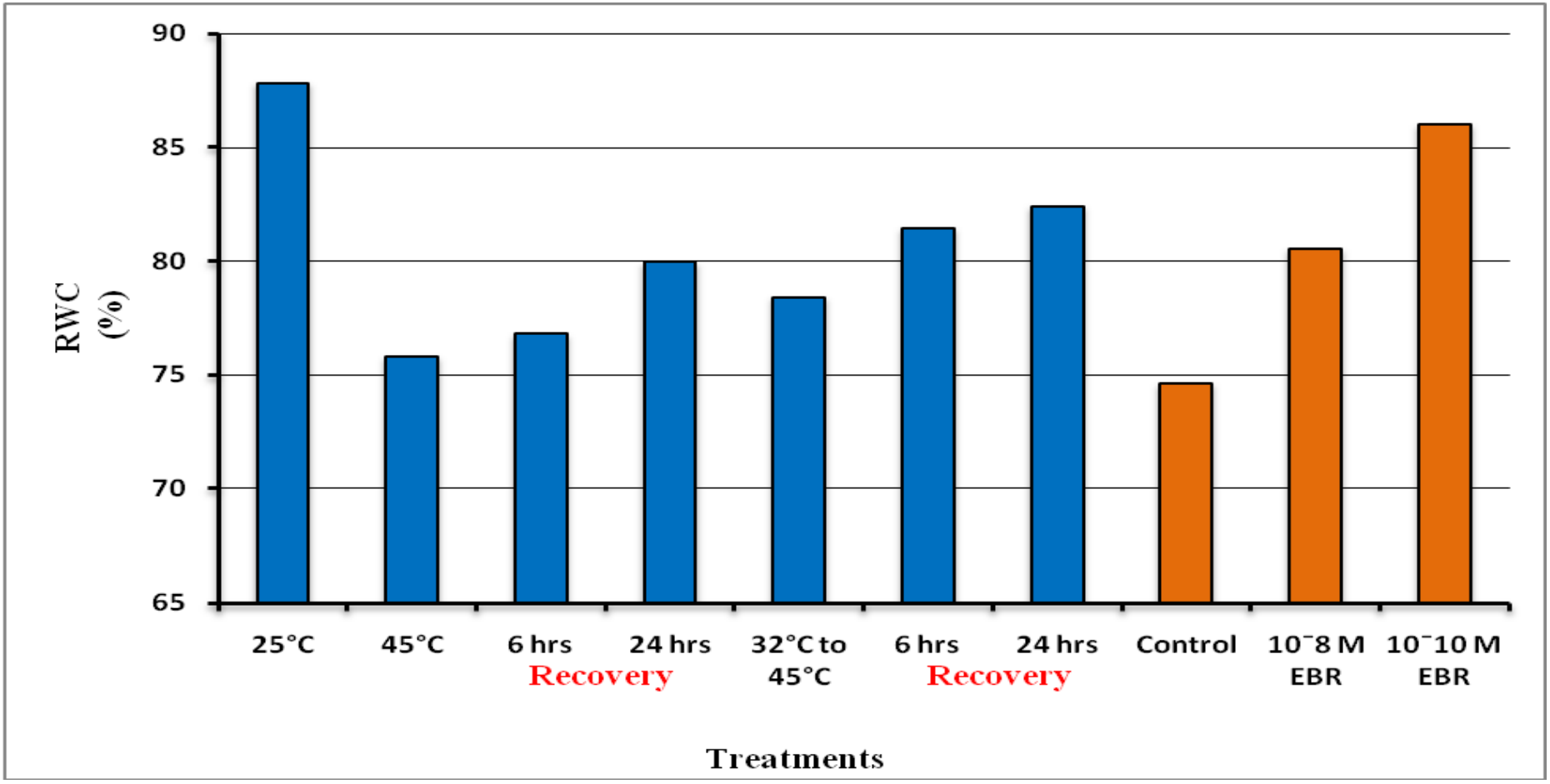

Fig. 7: Effect of EBR, temperature, acclimation and recovery period on relative water content (%) (Mean values) in hypocotyl of *Brassica juncea*

Table 8. Effect of EBR, temperature, acclimation and recovery period on free proline content (mg g^{-1} fresh weight) in hypocotyl of *Brassica juncea*

EBR	Temperature / Recovery period							
	$25^{O}C$	$45^{O}C$	Recovery		$32^{O}C$ $45^{O}C$	Recovery		Mean
			6 hrs	24 hrs		6 hrs	24 hrs	
Control	44.45	58.55	50.16	47.28	65.00	55.26	50.33	**53.00**
10^{-8} M	45.18	70.21	63.94	59.03	82.28	77.02	70.00	**66.81**
10^{-10} M	45.81	85.14	81.50	78.19	101.07	97.19	93.45	**83.20**
Mean	**45.15**	**71.30**	**65.20**	**61.50**	**82.79**	**76.49**	**71.26**	
CD at 5%	EBR	2.41						
	Temperature /Recovery	3.68						
	EBR × Temperature/ Recovery	6.37						

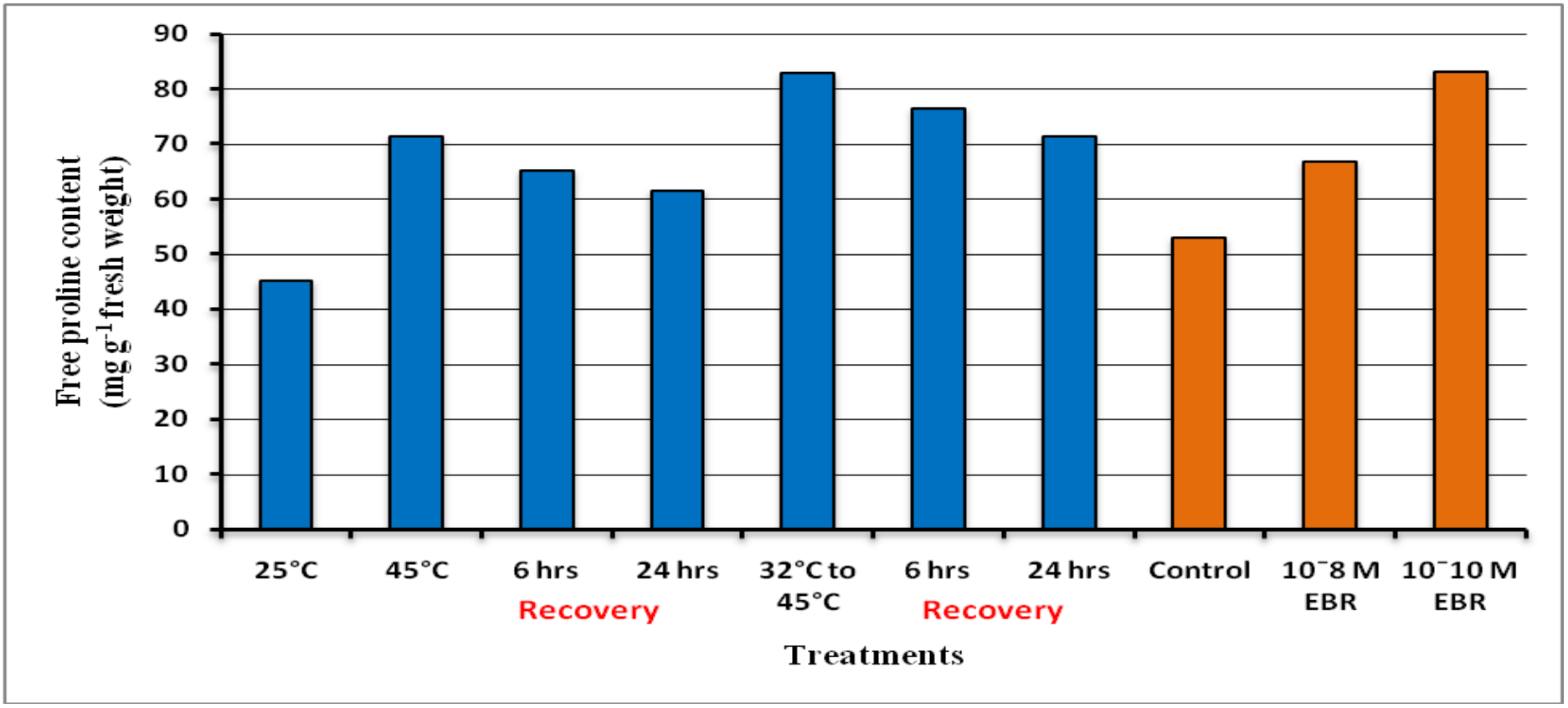

Fig. 8: Effect of EBR, temperature, acclimation and recovery period on free proline content (mg g^{-1} fresh weight) (Mean values) in hypocotyl of *Brassica juncea*

Table 9. Effect of EBR, temperature, acclimation and recovery period on membrane stability (% injury) in hypocotyl of *Brassica juncea*

EBR	Temperature/ Recovery period							
	25^{O}C	**45^{O}C**	**Recovery**		**32^{O}C**	**Recovery**		**Mean**
			6 hrs	**24 hrs**	**45^{O}C**	**6 hrs**	**24 hrs**	
Control	12.53	24.07	23.04	22.29	22.38	21.28	20.45	**20.86**
10^{-8} M	12.27	22.41	19.17	17.21	20.03	18.45	15.85	**17.91**
10^{-10} M	12.02	20.73	17.29	15.27	18.31	16.22	13.87	**16.25**
Mean	**12.27**	**22.40**	**19.83**	**18.26**	**20.24**	**18.65**	**16.72**	
CD at 5%	EBR	0.97						
	Temperature/ Recovery	1.48						
	EBR × Temperature/ Recovery	N.S.						

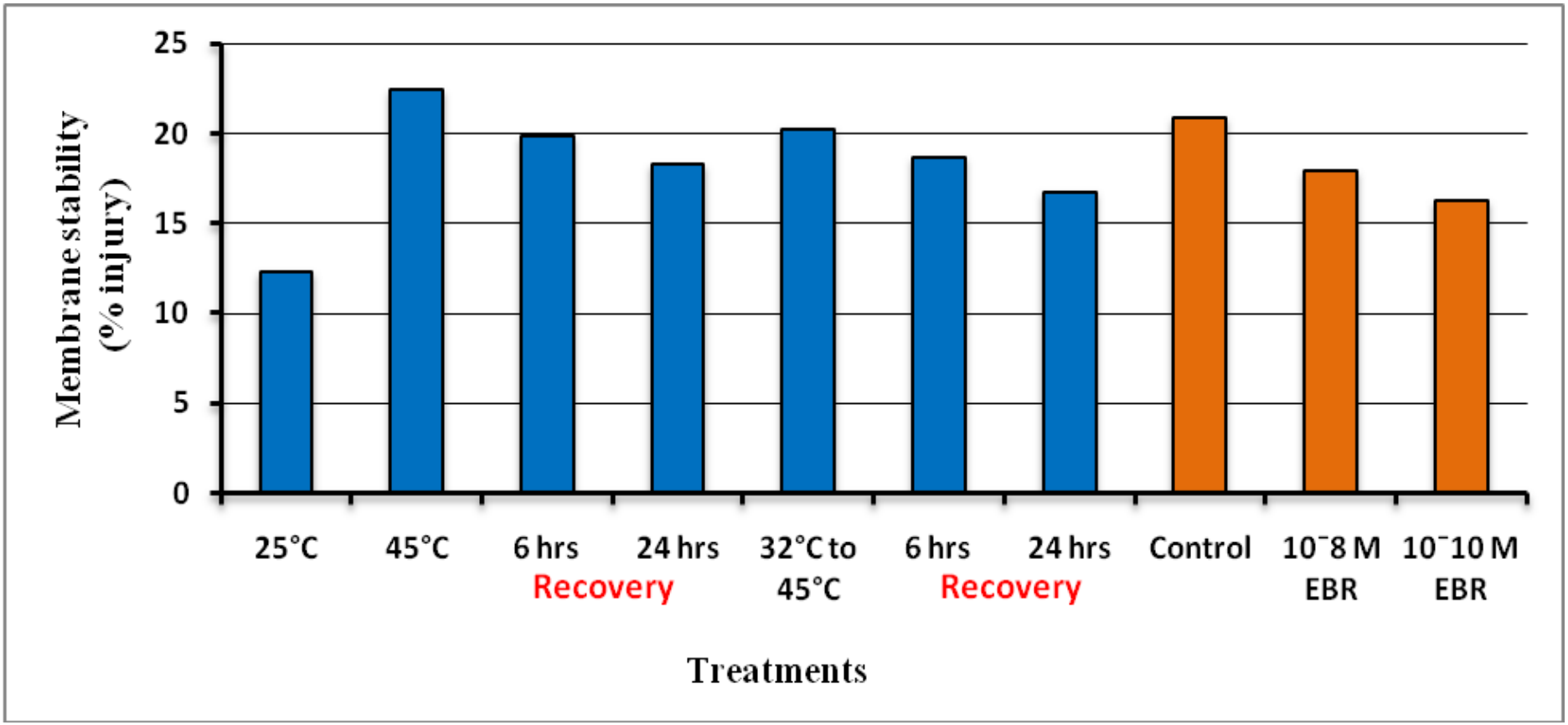

Fig. 9: Effect of EBR, temperature, acclimation and recovery period on membrane stability (% injury) (Mean values) in hypocotyl of *Brassica juncea*

2.4 Malondialdehyde (MDA) content

Malondialdehyde, a product of lipid peroxidation has been considered as an indicator of degree of oxidative damage. Lipid peroxidation as judged by the formation of malondialdehyde (MDA) during high temperature stress (45°C), after acclimation, EBR spray and recovery in hypocotyl of *Brassica juncea* seedlings has been presented in Table 10 and Fig. 10.

The MDA content (on the overall mean basis) increased under HTS as well as after acclimation however; increase was more under HTS as compared to that under normal temperature (25°C). It decreased significantly after 6 hrs and 24 hrs recovery in case of HTS as well as acclimation but value remained higher over the 25°C. Content was higher during recovery in case of HTS as compared to after acclimation.

Both concentrations of EBR showed significant decrease in MDA content however, the decrease was higher in 10^{-10} M.

2.5 H_2O_2 content

The results presented in Table 11 and Fig. 11 demonstrate the change in H_2O_2 content during high temperature stress (45°C), acclimation (32°C followed by 45°C), after EBR spray and recovery in hypocotyl of *Brassica juncea* seedlings.

On the overall mean basis compared to at 25°C, the H_2O_2 content increased significantly under HTS. When seedlings were first acclimate at 32°C and then exposed to HTS (45°C), the H_2O_2 content decreased over 45°C. H_2O_2 content decreased significantly after 6 hrs and 24 hrs recovery in case of HTS, while after 6 hrs in case of acclimated seedlings but value remained higher over the 25°C. Content was higher during recovery in case of HTS as compared to acclimation.

The significant decrease in H_2O_2 content was observed in both the concentrations of EBR as compared to control but decrease was more in 10^{-10} M.

2.6 Peroxidase

The differential effect of HTS (45°C), acclimation (32°C followed by 45°C), EBR and recovery period on peroxidase activity in hypocotyl of *Brassica juncea* seedlings has been shown in Table 12 and Fig. 12.

On the overall mean basis the peroxidase activity increased significantly under HTS as well as acclimation however, increase was higher under acclimation as compared to that under normal temperature (25°C). It decreased significantly after 24 hrs recovery in case of

HTS as well as acclimation but content remained higher over the 25°C. Higher peroxidase activity was observed after recovery in case of acclimation as compared to HTS.

Table 10. Effect of EBR, temperature, acclimation and recovery period on MDA content (nmoles g^{-1} fresh weight) in hypocotyl of *Brassica juncea*

EBR	Temperature / Recovery period							
	$25^{O}C$	$45^{O}C$	Recovery		$32^{O}C$ $45^{O}C$	Recovery		Mean
			6 hrs	24 hrs		6 hrs	24 hrs	
Control	3.44	8.30	7.90	7.18	7.17	6.37	5.71	**6.58**
10^{-8} M	3.88	7.33	6.97	5.43	6.27	5.44	5.04	**5.77**
10^{-10} M	3.87	6.70	5.95	5.00	5.26	4.80	4.06	**5.09**
Mean	**3.73**	**7.44**	**6.94**	**5.87**	**6.24**	**5.54**	**4.94**	
CD at 5%	EBR	0.32						
	Temperature / Recovery	0.49						
	EBR × Temperature / Recovery	0.85						

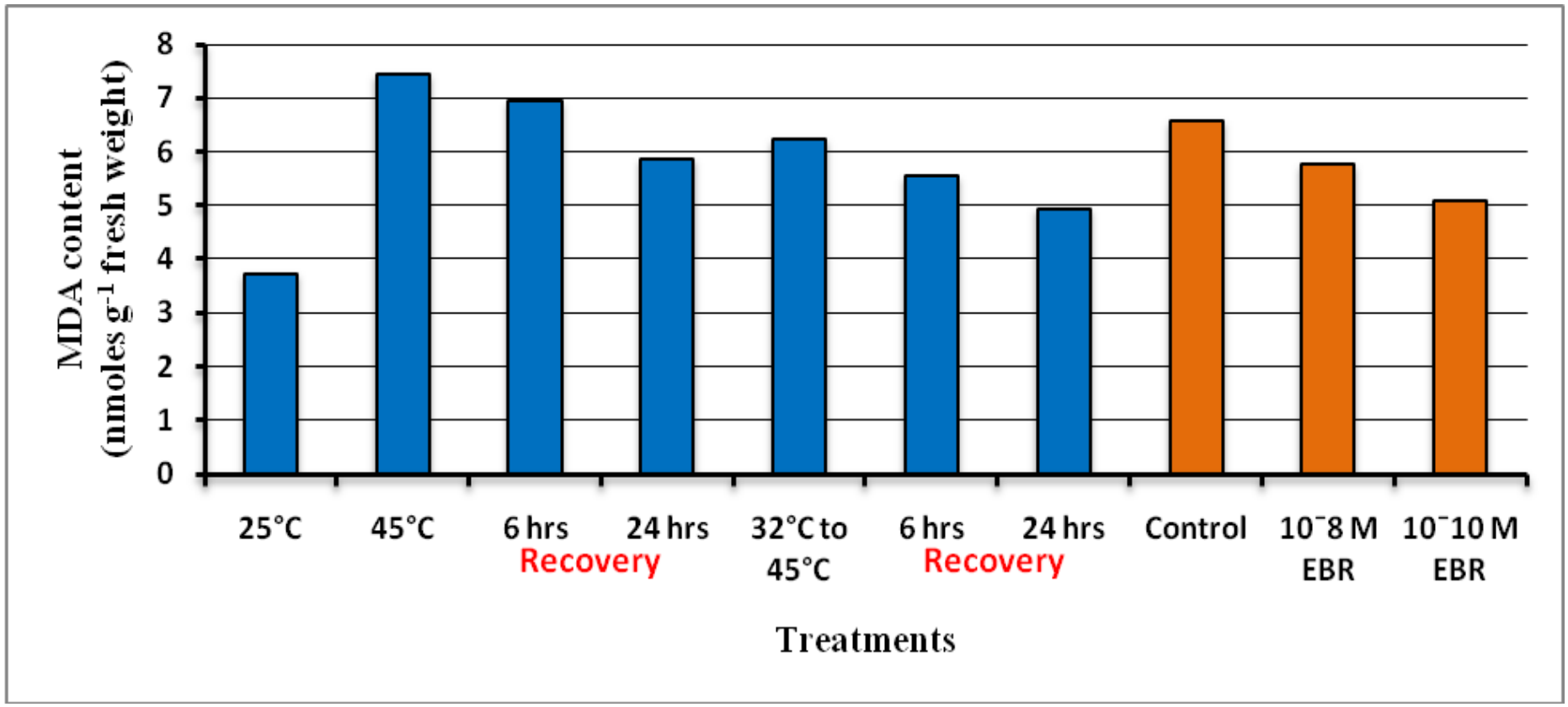

Fig. 10: Effect of EBR, temperature, acclimation and recovery period on MDA content (nmoles g^{-1} fresh weight) (Mean values) in hypocotyl of *Brassica juncea*

Table 11: Effect of EBR, temperature, acclimation and recovery period on H_2O_2 content (µmole g^{-1} fresh weight) in hypocotyl of *Brassica juncea*

EBR	Temperature / Recovery period							
	25^OC	**45^OC**	**Recovery**		**32^OC 45^OC**	**Recovery**		**Mean**
			6 hrs	**24 hrs**		**6 hrs**	**24 hrs**	
Control	58.45	81.19	76.25	70.11	76.57	73.36	69.33	**72.18**
10^{-8} M	56.86	75.55	66.33	62.25	70.53	65.34	61.49	**65.48**
10^{-10} M	55.14	72.15	61.23	59.13	65.82	59.09	57.03	**61.37**
Mean	**56.82**	**76.30**	**67.94**	**63.83**	**70.97**	**65.93**	**62.62**	
CD at 5%	EBR	2.55						
	Temperature / Recovery	3.90						
	EBR × Temperature / Recovery	N.S.						

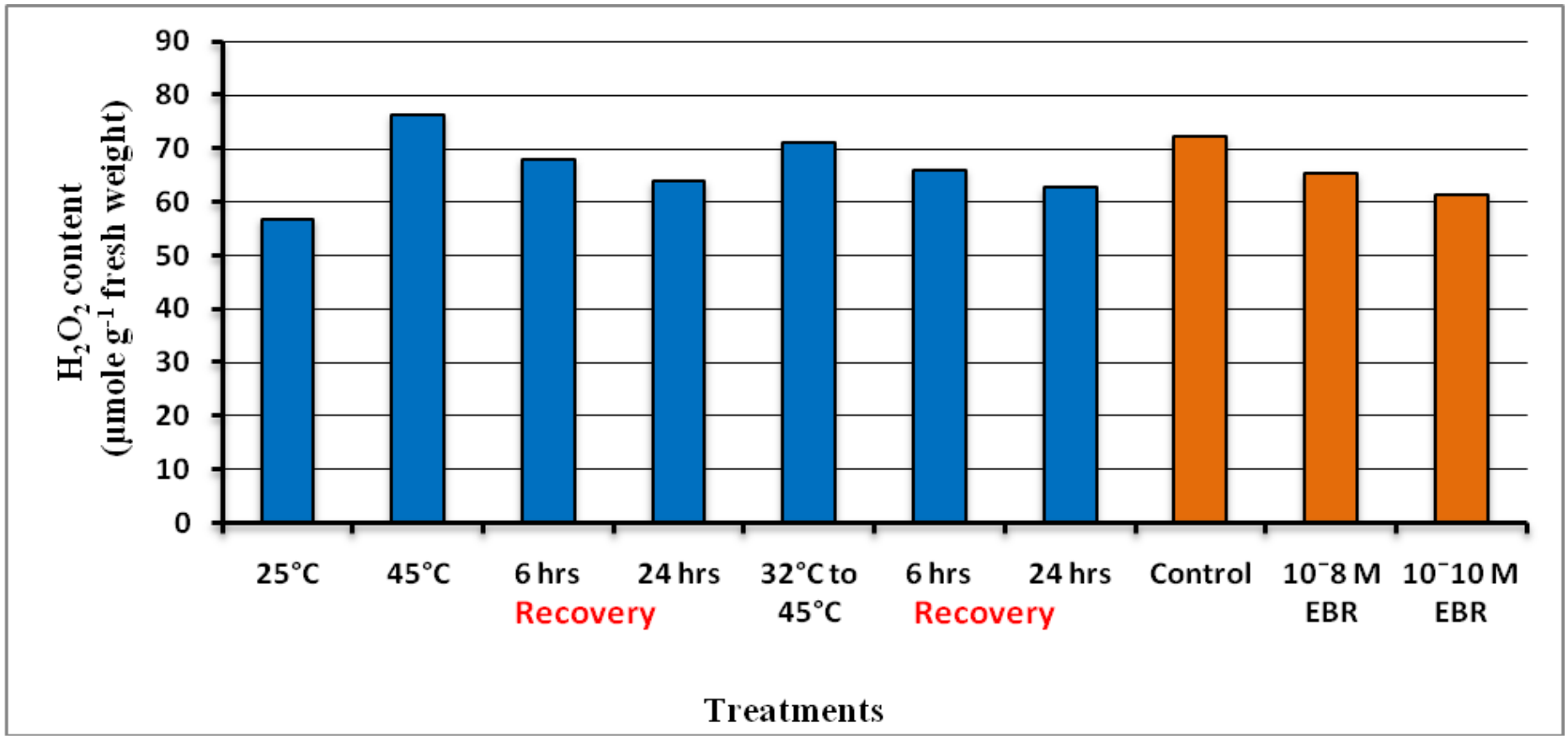
90
80
70
60
50
40
30
20
10
0
H_2O_2 content
(µmole g^{-1} fresh weight)
25°C
45°C
6 hrs
Recovery
24 hrs
32°C to
45°C
6 hrs
Recovery
24 hrs
Control
10^{-8} M
EBR
10^{-10} M
EBR
Treatments

Fig. 11: Effect of EBR, temperature, acclimation and recovery period on H_2O_2 content (µmole g^{-1} fresh weight) (Mean values) in hypocotyl of *Brassica juncea*

Table 12. Effect of EBR, temperature, acclimation and recovery period on activity of peroxidase (units g^{-1} fresh weight min^{-1}) in hypocotyl of *Brassica juncea*

EBR	Temperature / Recovery period							
	25^OC	45^OC	Recovery		32^OC 45^OC	Recovery		Mean
			6 hrs	24 hrs		6 hrs	24 hrs	
Control	10.22	13.70	12.28	11.24	14.77	13.65	12.91	**12.68**
10^{-8} M	9.01	16.47	15.18	14.52	18.12	16.76	16.04	**15.16**
10^{-10} M	9.41	17.65	16.89	16.02	20.19	18.94	18.04	**16.73**
Mean	**9.55**	**15.94**	**14.78**	**13.92**	**17.69**	**16.45**	**15.66**	
CD at 5%	EBR	0.97						
	Temperature / Recovery	1.48						
	EBR × Temperature / Recovery	N.S.						

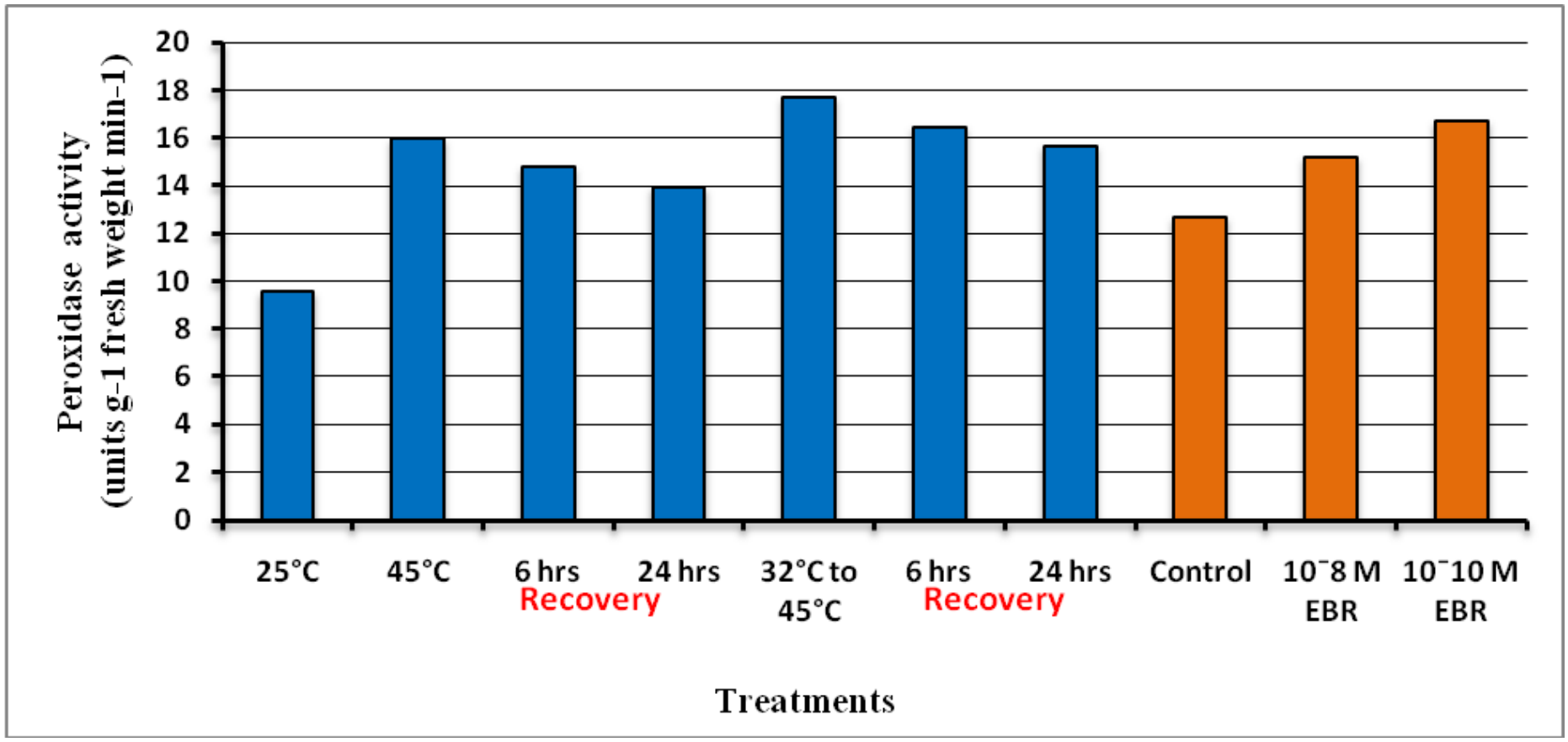
Peroxidase activity
(units g-1 fresh weight min-1)
20
18
16
14
12
10
8
6
4
2
0
25°C
45°C
6 hrs
Recovery
24 hrs
32°C to
45°C
6 hrs
Recovery
24 hrs
Control
10¯8 M
EBR
10¯10 M
EBR
Treatments

Fig. 12: Effect of EBR, temperature, acclimation and recovery period on activity of peroxidase (units g^{-1} fresh weight min^{-1}) (Mean values) in hypocotyl of *Brassica juncea*

The increase in peroxidase activity was observed in both the concentrations of EBR as compared to control but increase was more in 10^{-10} M.

2.7 Catalase

The data presented in Table 13 and Fig. 13 reveal change in catalase activity during high temperature stress (45°C), acclimation (32°C followed by 45°C), after EBR spray and recovery in hypocotyl of *Brassica juncea* seedlings.

Compared to under normal temperature (25°C), the catalase activity increased significantly under HTS as well as acclimation. There was non significant increase in catalase activity under acclimation over high temperature stress. It decreased significantly after 24 hrs recovery in case of HTS as well as acclimation but content remained higher over the 25°C. Higher catalase activity was observed during recovery in case of acclimation as compared to HTS.

Both concentrations of EBR showed significant increase in catalase activity however, 10^{-10} M was more effective in this regard.

3. Effect of epibrassinolide on gaseous exchange, yield and yield attributes under high temperature stress at terminal stage

3.1 Gaseous exchange studies: The results on rate of photosynthesis, rate of transpiration and rate of stomatal conductance have been explained on the mean basis.

3.1.1 Rate of photosynthesis

Spray of both the concentrations of EBR increased the photosynthetic rate over control, but the increase was significantly higher in plants sprayed with 10^{-10} M concentration. The rate of photosynthesis increased significantly higher after 48 hrs spray than after 24 hrs. spray (Table. 14 b)

The rate of photosynthesis declined significantly under late sown condition as compared to normal sown.

3.1.2 Rate of transpiration

Spray of EBR increased the transpiration rate as compared to control while increase was more in plants sprayed with 10^{-10} M concentration. Transpiration rate increased more at 48 hrs after EBR spray as compared to at 24 hrs. (Table 15 b).

The significant increase in transpiration rate was observed under late sown condition as compared to normal sown.

3.1.3 Rate of stomatal conductance

Application of EBR increased the rate of stomatal conductance as compare to control however; the increase was more in plants sprayed with 10^{-10} M concentration. Rate of stomatal conductance increased more at 48 hrs after EBR spray as compared to at 24 hrs.

The rate of stomatal conductance increased significantly under late sown condition as compared to normal sown (Table 16 b).

Table 13. Effect of EBR, temperature, acclimation and recovery period on activity of catalase (μ moles H_2O_2 decomposed g^{-1} fresh weight min^{-1}) in hypocotyl of *Brassica juncea*

EBR	Temperature / Recovery period							
	25^OC	45^OC	Recovery		32^OC 45^OC	Recovery		Mean
			6 hrs	24 hrs		6 hrs	24 hrs	
Control	278.32	325.81	300.81	291.40	330.70	306.52	296.17	**304.25**
10^{-8} M	297.98	365.84	346.80	339.10	373.76	350.77	345.47	**345.68**
10^{-10} M	309.50	403.14	383.16	376.99	420.84	396.66	391.84	**383.16**
Mean	**295.27**	**364.93**	**343.59**	**335.83**	**375.10**	**351.32**	**344.49**	
CD at 5%	EBR	18.58						
	Temperature / Recovery	28.38						
	EBR × Temperature / Recovery	N.S.						

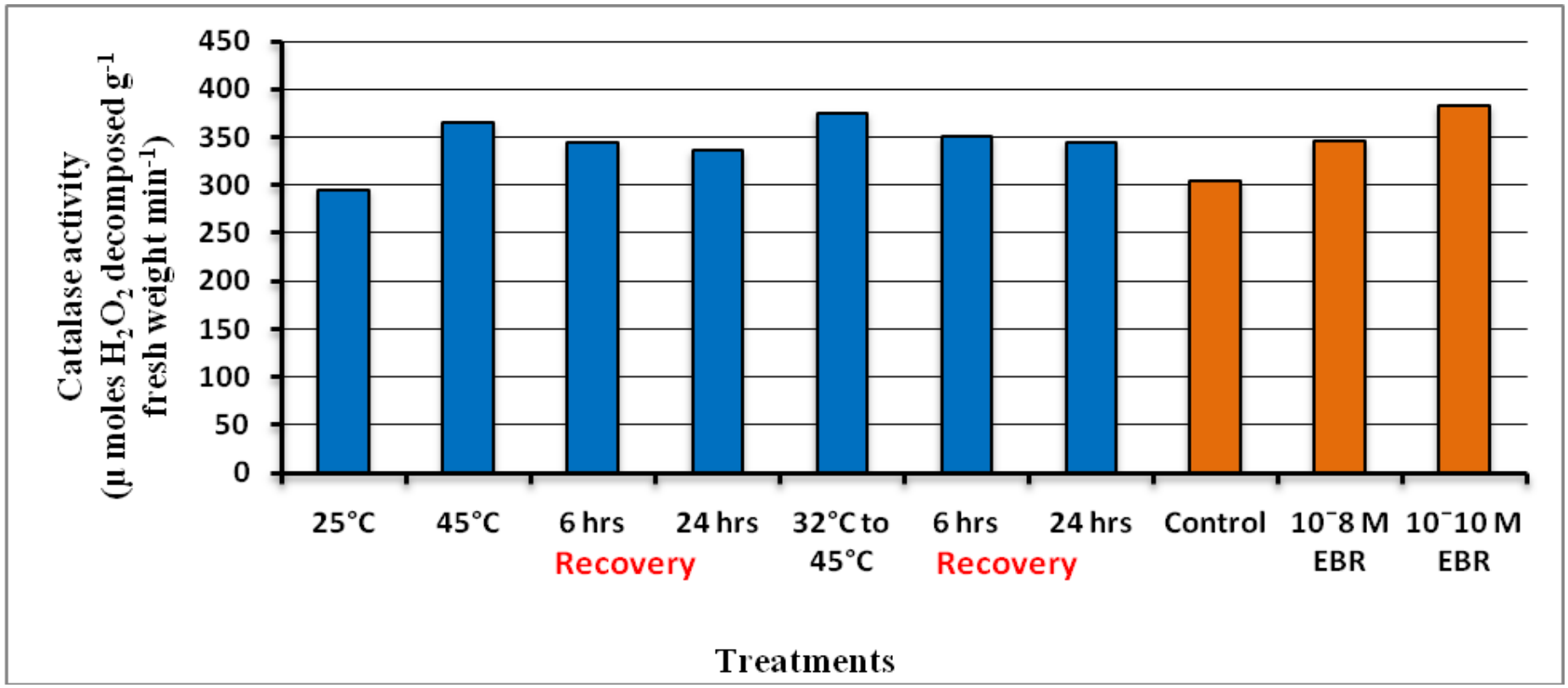

Fig. 13: Effect of EBR, temperature, acclimation and recovery period on activity of catalase (µ moles H_2O_2 decomposed g^{-1} fresh weight min^{-1}) (Mean values) in hypocotyl of *Brassica juncea*

Table 14 a. Effect of EBR, time of sowing and hours after EBR treatment on photosynthetic rate (µmol m^{-1} s^{-1}) in leaves of *Brassica juncea*

EBR	Hrs after EBR Treatment	Time of sowing	
		Normal sown	***Late sown***
Control	**24 hrs**	18.26	12.73
	48 hrs	18.32	12.66
10^{-8} M	**24 hrs**	20.86	14.82
	48 hrs	23.12	16.59
10^{-10} M	**24 hrs**	24.11	17.46
	48 hrs	25.88	19.86

Table 14 b. Mean analysis table for photosynthetic rate

EBR × Hrs after EBR treatment (mean values)				**EBR × Time of sowing (mean value)**				**Hrs after EBR treatment × Time of sowing (mean value)**			
EBR	**24 hrs**	**48 hrs**	**Mean**	**EBR**	***Normal sown***	***Late sown***	**Mean**	**Factor**	***Normal sown***	***Late sown***	**Mean**
Control	15.50	15.49	**15.49**	**Control**	18.29	12.70	**15.49**	**24 hrs**	21.08	15.00	**18.04**
10^{-8} M	17.84	19.86	**18.85**	**10^{-8} M**	21.99	15.70	**18.85**	**48 hrs**	22.44	16.37	**19.41**
10^{-10} M	20.79	22.87	**21.83**	**10^{-10} M**	25.00	18.66	**21.83**				
Mean	**18.04**	**19.41**		**Mean**	**21.76**	**15.69**		**Mean**	**21.76**	**15.69**	
CD at 5%	EBR	Hrs after EBR treatment	Time of sowing	EBR × Hrs after EBR treatment		EBR × Time of sowing		Hrs after EBR treatment × Time of sowing		EBR × Hrs after EBR treatment × Time of sowing	

	0.58	0.47	0.47	0.81	N.S.	N.S.	N.S.

Table 15 a. Effect of EBR, time of sowing and hours after EBR treatment on transpiration rate (mmol m^{-1} s^{-1}) in leaves of *Brassica juncea*

EBR	Hrs after EBR Treatment	Time of sowing	
		Normal sown	***Late sown***
Control	**24 hrs**	2.37	2.84
	48 hrs	2.41	2.81
10^{-8} M	**24 hrs**	2.72	3.43
	48 hrs	2.91	3.65
10^{-10} M	**24 hrs**	3.63	4.45
	48 hrs	3.8	4.67

Table 15 b. Mean analysis table for transpiration rate

EBR × Hrs after EBR treatment (mean values)				**EBR × Time of sowing (mean value)**				**Hrs after EBR treatment × Time of sowing (mean value)**			
EBR	**24 hrs**	**48 hrs**	**Mean**	**EBR**	***Normal sown***	***Late sown***	**Mean**	**Factor**	***Normal sown***	***Late sown***	**Mean**
Control	2.61	2.61	**2.61**	**Control**	2.39	2.83	**2.61**	**24 hrs**	2.91	3.57	**3.24**
10^{-8} M	3.07	3.28	**3.18**	**10^{-8} M**	2.81	3.54	**3.18**	**48 hrs**	3.04	3.71	**3.38**
10^{-10} M	4.04	4.24	**4.14**	**10^{-10} M**	3.72	4.56	**4.14**				
Mean	**3.24**	**3.38**		**Mean**	**2.97**	**3.64**		**Mean**	**2.97**	**3.64**	
CD at 5%	EBR	Hrs after EBR treatment	Time of sowing	EBR × Hrs after EBR treatment		EBR × Time of sowing		Hrs after EBR treatment × Time of sowing		EBR × Hrs after EBR treatment × Time of sowing	

	0.08	0.07	0.07	0.11	0.11	N.S.	N.S.

Table 16 a. Effect of EBR, time of sowing and hours after EBR treatment on stomatal conductance (mol m^{-1} s^{-1}) in leaves of *Brassica juncea*

EBR	Hrs after EBR Treatment	Time of sowing	
		Normal sown	*Late sown*
Control	**24 hrs**	0.31	0.47
	48 hrs	0.34	0.45
10^{-8} M	**24 hrs**	0.41	0.63
	48 hrs	0.47	0.69
10^{-10} M	**24 hrs**	0.55	0.82
	48 hrs	0.62	0.88

Table 16 b. Mean analysis table for stomatal conductance

EBR × Hrs after EBR treatment (mean values)				EBR × Time of sowing (mean value)				Hrs after EBR treatment × Time of sowing (mean value)			
EBR	**24 hrs**	**48 hrs**	**Mean**	**EBR**	***Normal sown***	***Late sown***	**Mean**	**Factor**	***Normal sown***	***Late sown***	**Mean**
Control	0.39	0.40	**0.39**	**Control**	0.33	0.46	**0.39**	**24 hrs**	0.42	0.64	**0.53**
10^{-8} M	0.52	0.58	**0.55**	**10^{-8} M**	0.44	0.66	**0.55**	**48 hrs**	0.48	0.67	**0.58**
10^{-10} M	0.68	0.75	**0.72**	**10^{-10} M**	0.58	0.85	**0.72**				
Mean	**0.53**	**0.58**		**Mean**	**0.45**	**0.66**		**Mean**	**0.45**	**0.66**	

CD at 5%	EBR	Hrs after EBR treatment	Time of sowing	EBR × Hrs after EBR treatment	EBR × Time of sowing	Hrs after EBR treatment × Time of sowing	EBR × Hrs after EBR treatment × Time of sowing
	0.03	0.02	0.02	0.04	0.04	N.S.	N.S.

3.2 Harvest data

3.2.1 Number of branches plant^{-1}

The data in Table 17 show that compared to normal sown crop, in late sown, the number of primary, secondary and tertiary branches decreased significantly which lead to decrease in total branches plant.$^{-1}$

Exogenous application of EBR (10^{-8} M and 10^{-10} M) had no effect on number of primary and secondary branches plant^{-1} however, number of tertiary branches increased significantly, resulting thereby the total branches plant^{-1} increased significantly in EBR treated plants as compared to control.

3.2.2 Plant height (cm)

A significant decrease in plant height was observed in late sown crop as compared to normal sown. Application of EBR had no effect on the plant height as compared to control plants (Table 18).

3.2.3 Number of siliquae plant^{-1}

Number of siliquae plant^{-1} decreased in late sown crop as compared to normal sown (Table 18). EBR spray treatment increased the total number of siliquae plant^{-1} as compared to control however; the increase was more in a plant sprayed with 10^{-10} M concentration. The increase in number of siliquae plant^{-1} with application of EBR was primarily because of development of new tertiary branches and new siliquae there upon.

3.2.4 1000 seed weight (g)

Table 18 indicates that like plant height number of siliquae plant^{-1} the 1000 seed weight too significantly decreased in late sown crop as compared to normal sown.

Exogenous application of EBR significantly increased 1000 seed weight as compared to control however; increase was more in plants sprayed with 10^{-10} M concentration.

3.2.5 Number of seeds siliqua^{-1}

Number of seeds siliqua^{-1} too decreased in late sown crop as compared to normal sown (Table 18).

EBR spray treatment increased the number of seeds siliqua^{-1} as compared to control however; increase was more in a plant sprayed with 10^{-10} M concentration.

3.2.6 Seed yield (g plant^{-1})

Seed yield being the cumulative effect of number of siliquae plant^{-1} , number of seeds siliqua^{-1}, 1000 seed weight too decreased significantly in late sown crop as compared to normal sown (Table 19 and Fig. 14).

Exogenous application of EBR enhanced the seed yield as compared to control however; the increase was more in a plant sprayed with 10^{-10} M concentration.

On the mean basis there was 30.74% reduction in seed yield plant^{-1} in late sown over timely sown. One spray of 10^{-10} M EBR increased seed yield plant^{-1} by 13.6% over control.

Table 17. Effect of EBR and time of sowing on number of primary, secondary, tertiary and total branches plant^{-1} in *Brassica juncea*

EBR	No. primary branches			No. secondary branches			No. tertiary branches			Total branches		
	Normal sown	***Late sown***	**Mean**	***Normal sown***	***Late sown***	**Mean**	***Normal sown***	***Late sown***	**Mean**	***Normal sown***	***Late sown***	**Mean**
Water	7.7	6.7	**7.2**	18.7	13.0	**15.8**	13.3	6.0	**9.7**	39.7	25.7	**32.7**
10^{-8} M	8.3	7.0	**7.7**	19.7	14.7	**17.2**	15.3	8.7	**12.0**	43.3	30.3	**36.8**
10^{-10} M	8.3	7.3	**7.8**	21.3	15.7	**18.5**	19.3	10.7	**15.0**	49.0	33.7	**41.3**
Mean	**8.1**	**7.0**		**19.9**	**14.4**		**16.0**	**8.4**		**44.0**	**29.9**	
CD at 5%	EBR	N.S.		EBR	N.S.		EBR	1.8		EBR	2.3	
	Time of sowing	0.7		Time of sowing	4.7		Time of sowing	1.4		Time of sowing	1.9	
	EBR × Time of sowing	N.S.		EBR × Time of sowing	N.S.		EBR × Time of sowing	N.S.		EBR × Time of sowing	N.S.	

Table 18. Effect of EBR and time of sowing on plant height (cm), number of siliquae plant^{-1}, 1000 seed weight (g) and number of seeds siliqua^{-1} in *Brassica juncea*

EBR	Plant height (cm)			Number of siliquae plant^{-1}			1000 seed weight (g)			Number of seeds siliqua^{-1}		
	Normal sown	***Late sown***	**Mean**	***Normal sown***	***Late sown***	**Mean**	***Normal sown***	***Late sown***	**Mean**	***Normal sown***	***Late sown***	**Mean**
Water	196.7	164.0	**180.3**	219.7	175.0	**197.3**	5.10	4.30	**4.70**	12.5	11.3	**11.9**
10^{-8} M	197.0	165.3	**181.2**	231.3	183.0	**207.7**	5.47	4.62	**5.05**	12.6	11.7	**12.2**
10^{-10} M	199.7	167.7	**183.7**	252.3	205.3	**228.8**	5.81	4.93	**5.32**	12.6	11.5	**12.0**
Mean	**197.8**	**165.7**		**234.4**	**187.8**		**5.46**	**4.62**		**12.6**	**11.5**	
CD at 5%	EBR	N.S.		EBR	8.4		EBR	0.26		EBR	N.S.	
	Time of sowing	9.4		Time of sowing	6.9		Time of sowing	0.22		Time of sowing	0.8	
	EBR × Time of sowing	N.S.		EBR × Time of sowing	N.S.		EBR × Time of sowing	N.S.		EBR × Time of sowing	N.S.	

The increase in seed yield was both on normal sown and late sown conditions. Though the increase in seed yield was numerically higher in late sown than normal sown, the differences were non significant.

3.2.7 Oil per cent

A significant decrease in oil per cent was observed in late sown crop as compared to normal sown.

EBR spray treatment increased the oil per cent as compared to control. The increase in oil per cent with application of 10^{-8} M and 10^{-10} M EBR over control was 1.65 and 4.23 per cent respectively (Table 19 and Fig. 14).

Table 19. Effect of EBR and time of sowing on seed yield (g) plant^{-1} and oil per cent in *Brassica juncea*

EBR	Seed yield (g plant^{-1})			Oil per cent		
	Normal sown	***Late sown***	**Mean**	***Normal sown***	***Late sown***	**Mean**
Control	11.23	7.60	**9.42**	36.87	32.07	**34.47**
10^{-8} M	11.77	8.27	**10.02**	37.97	34.27	**36.12**
10^{-10} M	12.60	8.80	**10.70**	40.47	36.93	**38.70**
Mean	**11.87**	**8.22**		**38.43**	**34.42**	
CD at 5%	EBR	0.55		EBR	1.42	
	Time of sowing	0.45		Time of sowing	1.16	
	EBR × Time of sowing	N.S.		EBR × Time of sowing	N.S.	

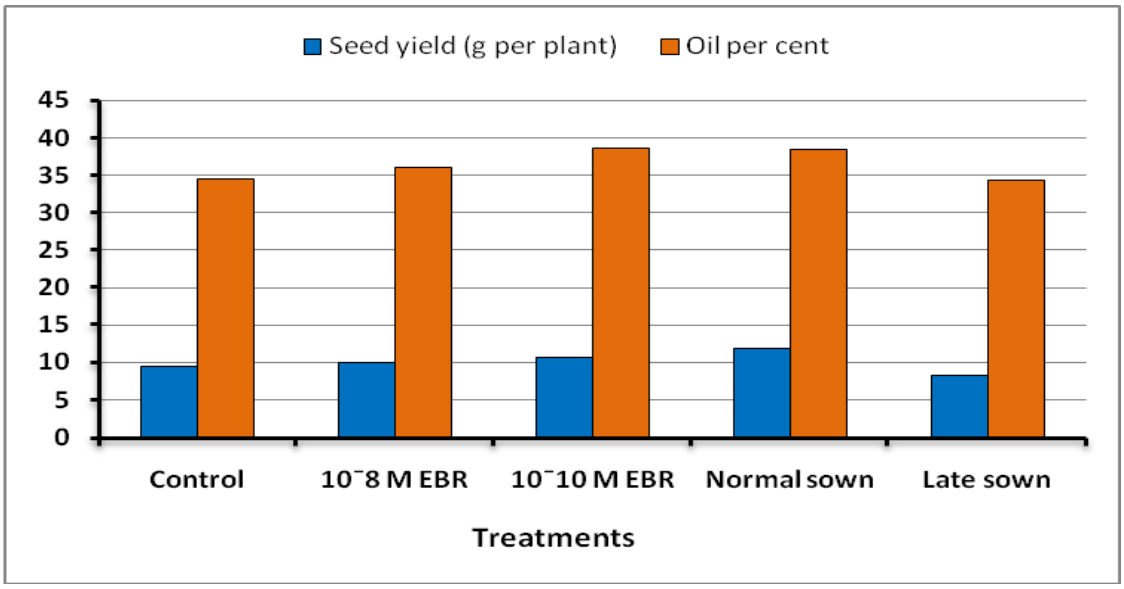

Fig. 14: Effect of EBR and time of sowing on seed yield (g plant^{-1}) and oil per cent (Mean values) in *Brassica juncea*

CHAPTER-5

DISCUSSION

High temperature is one of the major environmental factors affecting plant growth and productivity. Plant responses to heat stress are diverse and include many physiological and biological factors. Plant growth regulators have also been reported to play significant role in alleviating effect of heat stress. A thorough perusal of the literature indicates the paucity of systematic work on brassinosteroids induced physiological and biochemical responses of *Brassica juncea* to high temperature stress. Attempts were also made to probe into the brassinosteroids induced physiological and biochemical basis for injury/tolerance of *Brassica juncea* Var. RH 8812 (Laxmi) under high temperature stress. The results obtained in the present investigation have been discussed herewith in the light of recent literature available on the subject with the following heads:

5.1 Germination

In present work, on the mean basis, germination decreased with increase in temperature but there was drastic reduction when temperature was increased from 30 to 35°C (Table 1). The results presented are in agreement with those reported by Gonai *et al*., (2004) in lettuce. Similarly on an overall/ on the mean basis speed of germination at 25°C was at par with 30°C but it decreased drastically with increase in temperature to 35°C (Table 2), this might be due to delayed seed germination at high temperature.

On an overall 10^{-6} M EBR reduced germination (Table 1 and Fig. 1) and speed of germination (Table 2 and Fig. 2) over control but this reduction tended to overcome with lowering of EBR concentration from 10^{-6} M to 10^{-10} M. The present results are in consistence with the earlier findings in tomato (Dhaubhadel *et al*., 1999). Also, inhibitory action of EBR (10^{-6} M) on germination might lead to reduced speed of germination over control.

5.2 Seedling growth

When seedlings were grown at 25°C, 30°C and 35°C, it was observed that high temperature stress decreased the seedling growth of *Brassica juncea* in terms of root length,

shoot length and seedling dry weight (Table 3-5 and Fig. 3-5). Decrease in seedling growth under heat stress has been reported in *Brassica* (Kaur *et al*., 2009).

However, soaking treatment with EBR (10^{-6} M, 10^{-8} M and 10^{-10} M) further decreased root length and shoot length as compared to control, but seedling dry weight was significantly increased in 10^{-6} M EBR concentration over control and it was at par with 10^{-8} M and 10^{-10} M. Decrease in root length and shoot length over control was more with increasing concentration of EBR. These results are in agreement with the observation of Dhaubhadel *et al*., (1999) who observed that, in 24-epibrassinolide treated seedlings, the portion of the hypocotyl just above the growth medium was typically swollen. No significant differences were observed in the lengths of the hypocotyl and epicotyl regions between 24-epibrassinolide treated and untreated plants, but some curling of the hypocotyls were seen in 24-epibrassinolide treated plants.

5.3 Seedling mortality

Soaking seeds in EBR delayed seedling mortality over unsoaked control. Significant increase in time taken to 50% seedling mortality to the tune of 18, 35 and 36 minutes was recorded by soaking seeds in 10^{-6} M, 10^{-8} M and 10^{-10} M EBR respectively over control (Table 6 and Fig. 6). These results are in accordance with the earlier reports in *Brassica napus* and tomato seedlings (Dhaubhadel *et al*., 1999), *A. thaliana* seedlings (Kagale *et al*., 2007) and tomato plant (Singh and Shano, 2005). Delay of seedling mortality by EBR application is a significant achievement as this delay could escape seedling mortality.

5.4 Relative water content (RWC)

RWC decreased significantly under high temperature stress (45°C) over control (25°C). When seedlings were first acclimated at 32°C and then exposed to high temperature (45°C), RWC was higher than at 45°C. This suggests that 32°C is possibly an ideal temperature for high temperature acclimation.

RWC increased significantly after 24 hrs recovery in case of HTS, while in case of acclimation it recovered significantly after 6 hrs but still the RWC remained lower than at 25°C. After 24 hrs recovery, the RWC was in general higher in acclimated seedlings over non acclimated (HTS) seedlings (Table 7 and Fig. 7). The present results are consistent with the earlier findings of Kolb and Robberecht (1996) for high temperature, Xu *et al*., (2006) for heat acclimation and Wang and Huang (2004) for recovery after drought stress and heat stress.

The increase in RWC was observed in both the concentrations of EBR as compared to control but increase was more in 10^{-10} M (Table 7 and Fig. 7). Increase in relative water content in stressed seedlings with EBR application might probably be due to accumulation of inorganic or organic osmolytes, which make the surplus of water uptake and helps in maintenance of relative water content of tissue. The results presented are in agreement with those reported by Wang *et al*. (2009) in maize, Ali *et al*. (2008 a) in mung bean under aluminium stress, Ali *et al*. (2008 b) in *Brassica juncea* under salinity and nickel stress and Farooq *et al*. (2009) in rice under drought stress.

5.5 Free proline content

It is evident that stress metabolites are used as an indicator of the level of stress. In the present investigation, free proline content of the seedlings increased under high temperature stress (Table 8 and Fig. 8) however, high temperature acclimated seedlings showed more accumulation of free proline as compared to high temperature stress. Among amino acids, proline responds most sensitively to stress conditions (Dubey, 1997). Also, Hossain *et.al.*, (1995) while working on Chinese cabbage hybrids reported more increase in proline content in heat tolerant hybrids of Chinese cabbage than heat sensitive hybrids under heat stress. Comparing the heat sensitive, moderately heat tolerant and heat tolerant cultivars of Chinese cabbage, accumulation of proline was observed in proportion to tolerance in cultivars in Chinese cabbage under heat stress (Ye *et.al.*, 1996).

Application of EBR increases free proline content as compared to control (Table 8 and Fig. 8). Increased free proline content by brassinosteroids application was also reported under cadmium stress in *Brassica juncea* (Hayat *et al.,* 2007), under nickel stress in *Brassica juncea* (Alam *et al*., 2007), under aluminium stress in mung bean (Ali *et al*., 2008 a) and under drought stress in rice (Farooq *et al*., 2009).

5.6 Membrane stability

The membrane injury increased (Table 9 and Fig. 9) under high temperature stress, while high temperature acclimated seedlings showed less membrane injury as compared to high temperature stress. The present results of decrease in membrane stability under high temperature stress are in consistent with those of Melakeselam *et al*. (1999) and Luo *et al*. (1996). Kaur *et al*. (2009) reported that, heat acclimation pre-treatment reduced the electrolyte leakage from the seedlings of *Brassica* species compared with heat shocked ones. The decline in electrolyte leakage after recovery in the present study is also in accordance with the observation of Wang and Huang (2004) during drought stress and heat stress.

Spray with EBR significantly decreased per cent injury as compared to control (Table 9 and Fig. 9). The similar decrease in membrane injury with application of EBR was reported by Ali *et al*. (2008 b) in *Brassica juncea* under salinity and nickel stress and Farooq *et al*. (2009) in rice under drought stress.

5.7 Malondialdehyde (MDA) content

The MDA content increased under HTS however, increase was less after acclimation as compared to HTS (Table 10 and Fig. 10). It decreased significantly after 6 hrs and 24 hrs recovery in case of HTS as well as acclimation but value remained higher over the 25°C. Content was higher during recovery in case of HTS as compared to acclimation. The results presented are in agreement with those reported by Bhattacharjee and Mukherjee (2006) in *A. lividus* and Gong *et al*. (1997) in maize seedlings under heat stress. The decline in lipid peroxidation in terms of MDA content after recovery in the present study is also in accordance with the observation of Mandhania *et al*. (2006) during salt stress and Wang and Huang (2004) during drought stress and heat stress.

Both concentrations of EBR showed significant decrease in MDA content however, the decrease was higher in 10^{-10} M (Table 10 and Fig. 10). The results presented are in agreement with those reported by Wang *et al*. (2009) in maize crop and Farooq *et al*. (2009) in rice.

5.8 H_2O_2 content

The increase in H_2O_2 content was significantly higher under HTS, but increase was less after acclimation as compared to HTS (Table 11 and Fig. 11). It decreased significantly after 6 hrs and 24 hrs recovery in case of HTS and acclimation but in case of acclimation main recovery was in first 6 hrs, and there were non significant differences between 6 and 24 hrs recovery periods. Inspite of recovery, the H_2O_2 content remained higher over the 25°C. After recovery the H_2O_2 content was higher in HTS seedlings as compared to acclimated seedlings.

Hydrogen peroxide has been reported to increase in various plants in *A. lividus* (Bhattacharjee, 2003), french bean (Nagesh Babu and Devraj, 2008) and apple leaves (Ma *et al*. 2008) under high temperature stress. However, leaves with heat acclimation pre treatment kept lower accumulation of H_2O_2 than those without heat acclimation pretreatment in turfgrass species (Xu *et al*., 2006). On revival, decrease in H_2O_2 content over stressed condition was reported in wheat seedlings (Mandhania *et al*., 2006).

Significant decrease in H_2O_2 content with application of EBR (Table 11 and Fig. 11) is in agreement with the observation of Wang *et al*. (2009) in maize crop, Fariduddin *et al.* (2009) in *Brassica juncea* and Farooq *et al*. (2009) in rice.

5.9 Peroxidase

Peroxidase activity increased significantly under HTS, but increase was more after acclimation as compared to HTS (Table 12 and Fig. 12). Peroxidase activity decreased during recovery period. The results obtained in present investigations are in accordance with those observed by Kaur *et al*. (2009) in *B. juncea* where an increase in peroxidase activity was observed following heat stress and acclimation.

The increase in peroxidase activity was observed in both concentrations of EBR as compared to control (Table 12 and Fig. 12). These results are in agreement with the observation of Mazorra *et al*. (2002) in tomato under high temperature stress.

5.10 Catalase (CAT)

Catalase activity increased significantly under HTS, but increase was numerically more after acclimation treatment as compared to HTS (Table 13 and Fig. 13). It decreased significantly after recovery period. Increase in CAT activity under heat stress was also observed by Chaitanya *et al*. (2001) in mulberry cultivars. Heat acclimation pre-treatment increased the activity of catalase in seedlings of *Brassica* species compared to heat shocked ones (Kaur *et al*., 2009). During recovery, decrease in CAT activity was also observed by Mittler and Zilinskas (1994).

Both concentrations of EBR showed significant increase in catalase activity (Table 13 and Fig. 13). The similar increase in catalase activity with application of brassinosteroid was reported by Mazorra *et al*. (2002) in tomato, Hayat *et al.* (2007) in *Brassica juncea,* Wang *et al*. (2009) in maize and Ali *et al*. (2008 a) in mung bean.

5.11 Gaseous exchange

In late sown maximum air temperature on the day of EBR spray, 24 hrs and 48 hrs after EBR spray was 24.2, 23.4 and 25.2°C whereas in case of normal sown it was 14.6, 15.2 and 15.4°C respectively. Sunshine hours on the day of EBR spray, 24 hrs and 48 hrs after EBR spray, were 8.3, 7.6 and 7.3 in late sown and 3.1, 5.4 and 5.1 in case of normal sown respectively.

Compared to normal sown, the rate of photosynthesis declined significantly under late sown condition (Table 14 b). In present investigation decreased photosynthetic rate in late sown could be possibly due to high temperature stress (Crafts-Brandner and Law, 2000).

Application of EBR increased the photosynthetic rate as compared to control. Increase in photosynthetic rate, 48 hrs after EBR spray, was more than 24 hrs (Table 14 b). It might be due to *de novo* synthesis of chlorophyll or activation of some enzymes of photosynthesis during this period (Wang *et al*., 2009). In wheat inhibitory effect of salt stress on photosynthetic rate was ameliorated significantly by the exogenous application of 24-epibrassinolide (Muhammad *et al.*, 2008). Chlorophyll content and the rate of photosynthesis increased significantly by 24-epibrassinolide and 28-homobrassinolide under aluminium stress in mung bean (Ali *et al*., 2008 a).

The significant increase in rate of transpiration and stomatal conductance was observed under late sown condition as compared to normal sown (Table 15 b and 16 b). This increased rate of transpiration and stomatal conductance might be due to high air temperature as compared to normal sown.

Spray of EBR increased the rate of transpiration and stomatal conductance as compared to control. Rate of transpiration and stomatal conductance increased more at 48 hrs after EBR spray as compared to 24 hrs (Table 15 b and 16 b). Brassinosteroids has been reported to increase stomatal conductance in *Vigna radiata* **(**Fariduddin *et al*., 2003; Fariduddin *et al*., 2006) and soybean **(**Donc *et al*., 2008).

5.12 Yield and yield attributes

High temperature in late sown crop caused a significant reduction in the yield and its attributes over timely sown (Tables 17-19). Under high temperature stress, the height plant^{-1} (cm), total number of branches plant^{-1}, number of siliquae plant^{-1}, number of seeds siliqua^{-1}, 1000 seed weight (g), oil per cent and seed yield (g plant^{-1}) were adversely affected. The reduction in seed yield under high temperature stress in late sown was mainly because of reduction in number of siliquae plant^{-1} and 1000 seed weight. The decrease in number of siliquae in late sown might probably be due to reduced flower production and due to greater abortion of flowers. The present observations are similar to those of Russo and Diaz-Perez (2005) in pepper under heat stress and Morrison and Stewart (2002) who reported temperature greater than 27°C, in a growth cabinet, resulted in floral sterility and yield loss in *Brassica juncea* L.

In present investigation it was observed that under both late sown and normal sown conditions EBR increased the total number of branches plant^{-1}, number of siliquae plant^{-1}, 1000 seed weight (g), seed yield (g plant^{-1}) and oil per cent (Tables 17-19), while there was no effect on height plant^{-1}, number of seeds per siliqua, number of primary and secondary

branches plant^{-1}. As spray of EBR was done at "flowering cessation stage" and generally at this stage growth of plant had nearly stopped, might be due to this EBR had no effect on height plant^{-1}, number of primary and secondary branches plant^{-1}.

Spray of 10^{-10} M EBR increased seed yield plant^{-1} by 13.6% over control. The increase in seed yield was both in normal sown and late sown conditions. Though the increase in seed yield was numerically higher in late sown than normal sown, the differences were non significant. Increased seed yield (g plant^{-1}) might possibly be due to increase in number of siliquae plant^{-1} and 1000 seed weight (g).

EBR spray treatment increased the oil per cent as compared to control. The increase in oil per cent with application of 10^{-8} M and 10^{-10} M EBR over control was 1.65 and 4.23 per cent respectively (Table 19 and Fig. 14). Increased oil per cent might possibly be due to increased seed size. Similar response of brassinosteroids induced yield improvement in *Brassica juncea* was reported by Hayat *et al*. (2006) under salt stress and Hayat *et al*. (2007) under saline stress. Brassinosteroids induced improvement in oil content in *Brassica juncea* crop has been reported by Kumawat et *al*. (1997) under water deficit stress.

On an overall, there was 13.6% increase in seed yield plant $^{-1}$ and 4.23% increase in oil content with one spray of 10^{-10} M EBR (Table 19 and Fig. 14). This is a significant achievement from practical point of view.

Interaction of EBR with temperature was significant for germination percentage, speed of germination, root length, seedling dry weight, proline content and MDA content. It was non significant for shoot length, RWC, membrane stability, H_2O_2 content, peroxidase, catalase and harvest parameters. Non significant interaction indicates that EBR had a similar effect under temperature, recovery and acclimation and significant interaction reveals that EBR had a role under particular condition.

CHAPTER-6

SUMMARY AND CONCLUSION

The present investigation entitled "Studies on epibrassinolide induced amelioration of high temperature stress in *Brassica juncea* (L.) Czern & Coss." was conducted in the laboratory and field during *rabi* season of 2009-10. Three concentrations of EBR (10^{-6} M, 10^{-8} M and 10^{-10} M) were used to study various physiological and metabolic aspects of the problem under investigation and the results achieved are summarized as follow:

1. When seedlings were grown at 25°C, 30°C or 35°C, it was observed that 25°C is optimum temperature for germination and seedling growth. The germination, speed of germination and seedling growth of *Brassica juncea* in terms of root length, shoot length and seedling dry weight in general reduced when temperature was increased from 25°C to 30°C and 35°C.
2. Soaking seeds in EBR (10^{-6} M, 10^{-8} M or 10^{-10} M) for 2 hrs in general reduced germination and seedling growth (except seedling dry weight which increased with all concentrations of EBR used). Higher the concentration of EBR used, more was the reduction in germination and seedling growth.
3. When seeds soaked in EBR solutions (10^{-6} M, 10^{-8} M or 10^{-10} M) were grown at optimum temperature (25°C) for 5 days and then exposed to high temperature shock (45°C), EBR soaking in general delayed seedling mortality (in terms of time taken to 50% seedling mortality). 10^{-8} M and 10^{-10} M EBR concentrations were more effective in delaying seedling mortality over 10^{-6} M EBR.
4. When 5 days old seedlings were first temperature acclimated at 32°C for 24 hrs or sprayed with EBR (10^{-8} M and 10^{-10} M) before exposure to high temperature shock (for 2 hrs at 45°C), both the temperature acclimation and EBR pretreatments helped seedlings to recover and protect from heat stress by improving relative water content, reduced per cent injury and increased free proline content.
5. Acclimations to high temperature and EBR pre treatments resulted in an increase in enzymatic activity of Catalase, Peroxidase and also reduction in MDA and H_2O_2 content conferring thermotolerance to seedlings. Lower concentration of EBR (10^{-10} M) was most effective in this regard.
6. Two concentrations of EBR (10^{-8} M and 10^{-10} M) were sprayed on normal sown (28^{th} October, 2009) and late sown (12^{th} November, 2009) field grown plants to know their effect on gaseous exchange studies, seed yield and yield contributing parameters. Gaseous exchange studies were recorded at "flowering cessation stage (83 DAS)".

Compared to normal sown crop the rate of photosynthesis decreased significantly in late sown crop whereas, rate of transpiration and stomatal conductance increased.

7. Late sown crop in general exhibited 30.7% reduction in seed yield plant^{-1} over timely sown.
8. Spray of EBR to field grown plants in general improved rate of photosynthesis, transpiration and stomatal conductance. The increase was more on 48 hrs after its spray over 24 hrs. Lower concentration of EBR (10^{-10} M) was more effective in this regard.
9. Seed yield plant^{-1} increased by 13.6 per cent (mean of normal and late sown) over control with one spray of 10^{-10} M EBR. Though the increase in seed yield by EBR application over control was numerically higher in late sown than normal sown, the differences were non significant. Under both conditions EBR increased the total branches plant^{-1}, number of siliquae plant^{-1}, 1000 seed weight, seed yield (g plant^{-1}) and oil per cent, while there was no effect on plant height, number of seeds per siliqua, number of primary and secondary branches plant^{-1}. Among two concentrations of EBR used, the lower concentration (10^{-10} M) was more effective in improving yield and yield attributes.
10. EBR spray increased the oil per cent as compared to control. The increase in oil per cent with application of 10^{-8} M and 10^{-10} M EBR over control was 1.65 and 4.23 per cent respectively.

Conclusion

It can be concluded that the 25°C is optimum temperature for germination and seedling growth. Though all the EBR concentrations were inhibitory for germination and seedling growth (except for seedling dry weight), 10^{-10} M concentration was least inhibitory. Soaking seeds in 10^{-8} and 10^{-10} M EBR for 2 hrs significantly delayed the seedling mortality under high temperature stress. High temperature stress adversely affects the physiological and biochemical parameters in *B. juncea* seedlings, while EBR (10^{-8} and 10^{-10} M) and temperature acclimation improve it. High temperature stress at terminal stage in late sown reduces photosynthetic rate and seed yield, while EBR (10^{-8} and 10^{-10} M) increases these parameters. Lower concentration of EBR (10^{-10} M) in general was more effective in imparting thermotolerance in *B. juncea*.

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ABSTRACT

Title of Thesis	:	Studies on epibrassinolide induced amelioration of high temperature stress in *Brassica juncea* (L.) Czern & Coss.
Name of the Degree Holder	:	**Mr. Walke Mahadev Bapu**
Admission Number	:	2008BS115M
Title of the Degree	:	Master of Science
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Year of Award of Degree	:	2010
Major Subject	:	Plant Physiology
Total Number of Pages in Thesis	:	57
Number of Words in Abstract	:	313
Key words	:	***Brassica juncea*, Antioxidative enzymes, Epibrassinolide, Thermotolerance, Photosynthesis, Seedling mortality, Yield attributes**

(An abstract of the dissertation submitted to the CCS Haryana Agricultural University in partial fulfillment of the requirements for the degree of M.Sc.).

The present investigations were undertaken to study the role of epibrassinolide (EBR) on seed germination, seedling growth, 50% seedling mortality, relative water content, membrane stability, free proline content, antioxidative enzymes and metabolites at seedling stage, while gaseous exchange and yield attributes at terminal stage in field grown *Brassica juncea* plants.

When seedlings were grown at 25°C, 30°C or 35°C, it was observed that 25°C is optimum temperature for germination and seedling growth. The germination, speed of germination and seedling growth of *Brassica juncea* in general reduced when temperature was increased from 25°C to 30°C and 35°C. Soaking seeds in EBR (10^{-6} M, 10^{-8} M or 10^{-10} M) for 2 hrs in general reduced germination, seedling growth and delayed seedling mortality. Higher the concentration of EBR used, more was the reduction in germination and seedling growth.

Acclimation at sublethal temperature (32°C) and EBR pretreatments (10^{-8} M and 10^{-10} M) for 24 hrs prior to high temperature stress (45°C) were found to be effective in imparting thermo protection at seedling stage, which is the crucial stage of plant establishment. These pretreatments helped seedling to recover from heat stress by improved relative water content, reduced per cent injury and increased free proline content. Increase in enzymatic activity of catalase, peroxidase, and also reduction in MDA, H_2O_2 content conferred thermotolerance.

At flower cessation stage, compared to normal sown, in late sown crop the rate of photosynthesis and seed yield decreased significantly whereas, rate of transpiration and stomatal conductance increased. Rate of photosynthesis, transpiration and stomatal conductance increased in plants sprayed with EBR at flower cessation stage. Lower concentration of EBR (10^{-10} M) was more effective to bring consecutive improvement in gaseous exchange. EBR spray (10^{-10} M) increased the seed yield (13.6%) and oil content (4.23%) as compared to control. Lower concentration of EBR (10^{-10} M) in general was more effective in imparting thermotolerance in *B. juncea.*

MAJOR ADVISOR **DEGREE HOLDER**

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CURRICULUM VITAE

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(b)	Date of Birth	:	22-09-1986
(c)	Place of Birth	:	Daund (Maharashtra)
(d)	Mother's Name	:	Sht. Walke Kusum Bapu
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(j) **Academic Qualification**

Degree	Univ./Board	Year of Passing	Percentage of marks	Subjects
M.Sc. (Plant Physiology)	CCS HAU, Hisar	2010	80.30%	Major : Plant Physiology Minor : Biochemistry
B.Sc. (Agriculture)	M. P. K. V. Rahuri (Maharashtra)	2008	84.70%	Agriculture
Senior Secondary	Kolhapur board (Maharashtra)	2004	89.33%	Biology, Crop Science, Chemistry, Physics
Matriculation	Pune board (Maharashtra)	2002	81.20%	Maths, Social Sciences, Science

(k) Co-Curricular Activities :

- Completed two year NSS program with social service camp of 10 days.
- Participated in Inter-collegiate "Volleyball Tournament" (2006-07) and won runner prize.

(l) Medals/Honours received :

- Awarded ICAR merit cum scholarship during B.Sc. (Agriculture) degree programme.
- Awarded ICAR's Junior Research Fellowship during M.Sc. (Plant Physiology) degree programme.
- Selected in Student Council as convenor of debate, elocution and cultural committee (2006-07).

(m) List of Publications : –

Walke Mahadev Bapu

UNDERTAKING OF THE COPY RIGHT

I, **Walke Mahadev Bapu**, Admission No. **2008BS115M**, undertake that I give copyright to the CCS HAU, Hisar of my thesis entitled "**Studies on epibrassinolide induced amelioration of high temperature stress in *Brassica juncea* (L.) Czern & Coss**."

I also undertake that patent, if any, arising out of the research work conducted during the programme shall be filed by me only with due permission of the competent authority of CCSHAU, Hisar.

Walke Mahadev Bapu