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# Effect of heat stress on enzymatic and non-enzymatic antioxidants in *Brassica rapa*

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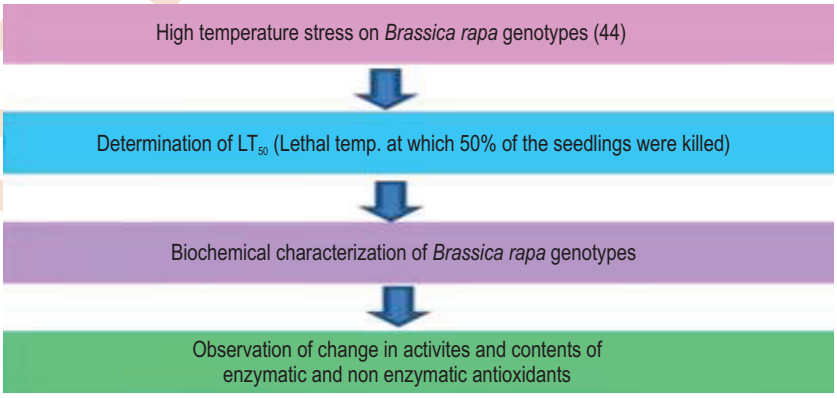
## Abstract

**Aim :** Heat stress due to increase in global temperature is posing a serious threat to the agricultural sector in many parts of the world. The present investigation was, therefore, undertaken to study the mechanism of thermos-tolerance in four-day-old seedlings of *Brassica rapa* (44 genotypes) on the basis of various enzymatic and non-enzymatic antioxidants. The information gathered through the present investigation can pave way for imparting tolerance to *Brassica* genotypes by altering enzyme activities through genetic engineering interventions.

**Methodology :** A total of 44 genotypes were evaluated for survival percentage, electrolyte leakage and chlorophyll content under heat stress conditions. Seedlings were characterized by membrane lipid peroxidation and antioxidants viz. peroxidase and catalase activities, proline and glutathione. Heat stress conditions were created by exposing four-day-old seedlings to 45ºC for 4.5 hr. Out of 44 genotypes, four genotypes (JMT-04-03, TL-2035, TL-98-01 and PBT-37) were thermos-tolerant. Tolerant genotypes registered survival greater than 65%, moderately tolerant between 35-65% and susceptible less than 35%.

**Results :** Among various parameters studied, under heat stress, a significant increase in electrolyte leakage, lipid peroxidation, peroxidase activity, glutathione and proline content was observed in comparison to control seedlings, whereas a decline in CAT activity and chlorophyll content was recorded.




**Interpretation :** Biochemical changes observed in the activities and contents of various parameters studied could be linked with enhanced tolerance to heat stress damage in *Brassica rapa* which could further be used as a marker for screening against heat stress.

**Key words:** Antioxidants, *Brassica rapa,* Heat stress, Lipid Peroxidation, Reactive oxygen species

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## Introduction

Out of various abiotic stresses, high temperature due to global warming is the second most important stress which can strike crop at any time and cause an array of morpho-anatomical, physiological and biochemical changes in plants which impose many limitations on plant growth and development (Kaur *et al.,* 2009; Jukanti *et al.,* 2017). High temperature at sowing time reduces germination, emergence and survival of seedling, resulting in loss of productivity (Wilson *et al.,* 2014). High temperature is further associated with oxidative stress in plants through overproduction of reactive oxygen species such as superoxide radicals ($O_2$-), hydroxyl (OH-), singlet oxygen and hydrogen peroxide ($H_2O_2$) (Krishnamurthy and Rathinasabapathi, 2013; Harsh *et al.,* 2016). The overproduced ROS under stress conditions react directly with lipids, proteins and nucleic acids, causing lipid peroxidation mediated membrane injury, protein degradation, enzyme inactivation, pigment bleaching and disruption of DNA structure (Ershova *et al.,* 2011; Sharma *et al.,* 2012). Estimation of lipid peroxidation is a direct index of oxidative stress induced damage due to temperature stress (Pradhan *et al.,* 2013). To counteract the deleterious effects of over-produced ROS, plants have developed scavenging mechanisms categorized as enzymatic and non-enzymatic defence system (Rani *et al.,* 2016).

The species *Brassica rapa* includes various vegetable and oil crops. Production of these crops is usually impaired by heat stress. *B. rapa* is known to be more sensitive to heat stress as compared to *B. juncea* and *B. napus* (Angadi *et al.,* 2000). The optimum temperature required for germination and seed development in *Brassica species* is 25-33ºC (Wilson *et al.,* 2014). Previous studies have reported yield loss due to floral sterility and impaired seed filling as the crop experience terminal high temperature stress at grain filling stage (33-35ºC) (Young *et al.,* 2004). Improved thermo-tolerance in plants has been observed due to the synthesis of isoprene or glycinebetaine, production of antioxidant enzymes and reduction in α-linolenic acid concentration (Wahid *et al.,* 2007, Wilson *et al.,* 2014). Survival of the plant during high temperature stress through antioxidant defence system is the urgent need of the time. Research on the effect of high temperature stress on *B. rapa* species is limited in comparison to other *Brassica* species. Hence, keeping in view the situation leading to crop yield loss, the present research was designed with the objective to understand the effect of high temperature stress in *B. rapa* genotypes and to evaluate the changes in biochemical and antioxidative defence mechanisms.

## Materials and Methods

The seeds of *B. rapa* (44 genotypes) were procured from Oilseeds Section, Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana. Seeds were surface sterilized with 0.1% $HgCl_2$ solution and then washed under running tap water followed by distilled water. To analyze heat stress tolerance, 20 seeds of each genotype were germinated in a glass jar (500 ml capacity) in duplicate. The seeds were grown for four days in an incubator under 16 hr light and 8 hr dark period at 25±2ºC. Out of two jar sets, one set was used as control and the second set was given heat shock treatment at $LT_{50}$ *i.e.,* 45ºC for 4.5 hr, after 4 days of germination. $LT_{50}$ is the lethal temperature at which 50% seedlings of TL-15 (released check variety of *B. rapa*) were killed. Stress tolerance was estimated on the basis of number of seedlings survived 24 hr after heat shock treatment (Dat *et al.,*1998).

Genotypes were subdivided into categories on the basis of persent survival, i.e., tolerant (having survival greater than 65%), moderately tolerant (having survival within the range of 35-65%) and susceptible (having survival less than 35%). Cell membrane stability (electrolyte leakage), chlorophyll content, lipid peroxidation, peroxidase activity, catalase activity, glutathione content and proline content were measured as described in our previous study (Wilson *et al.,* 2014).

**Statistical analyses :** The experiments were performed in a completely randomised design (CRD). Paired t-test was performed to analyse the effect of heat stress on seedlings (control vs heat stressed). An alpha level of 0.05 was adopted throughout to reduce Type I statistical errors.

## Results and Discussion

A set of forty-four *B. rapa* genotypes were evaluated under heat stress along with the control for thermo-tolerance (45°C) in terms of survival percentage. On the basis of survival percentage, four genotypes (JMT-04-03, TL-2035, TL-98-01 and PBT-37) were found to be highly tolerant *i.e.,* high rate of survival at 45 ºC for 4.5 hrs; twenty two were moderately tolerant and eighteen were susceptible (Fig.1a). Tolerant genotypes had 3.6 fold higher percent survival (74.41%) than susceptible genotypes (20.79%), and survival (%) was 50.82 *i.e.,* 2.5 fold higher than susceptible genotypes in case of moderately tolerant ones.

Electrolyte leakage data, an indicator of cell membrane stability during heat stress (Wilson *et al.,* 2014), supported the survival data as it increased significantly ($p<0.001$) under heat stress in seedlings of all the three categories of genotypes as compared to their respective controls (Fig.1b). In response to heat stress, tolerant genotypes registered 48.26% of electrolyte leakage, which was two-fold higher than control. In moderately tolerant and susceptible genotypes, it was 58.83% and 64.50% which was 2.5 and 3.15 fold higher than their respective controls. The increase in electrolyte leakage during heat stress indicated that heat stress caused cell membrane damage in seedlings, though damage being higher (1.5 fold) in susceptible genotypes than the resistant ones. These results are in confirmation with the previous reports of Al-Jebory (2013) in wheat, and Wilson *et al.* (2014) in *B. juncea*.

Chlorophyll content (Fig.1c) decreased significantly ($p<0.05$) in heat stressed seedlings as compared to control in all the test material, suggesting damage to photosynthetic apparatus (Karim *et al.,* 1997). The decrease in chlorophyll

content was at par in all the three categories. Similar to the present study, earlier Chaitanya *et al.* (2001) and Jiang and Huang (2001), reported decline in chlorophyll content under drought, heat or combined stress conditions in mulberry and Turfgrass, respectively. The unstressed seedlings of all the categories showed non-significant variation. In some cases, unchanged chlorophyll level has also been reported during drought stress (Hayat *et al.,* 2009).

Control seedlings did not show any significant variation with respect to lipid peroxidation. Under heat stress, lipid peroxidation increased significantly ($p<0.05$) in all the categories, except tolerant genotype where increase observed was non-significant. Lipid peroxidation was maximum in susceptible genotypes (10.78 µm MDA $g^{-1}$ f.wt.) followed by moderately tolerant genotypes (8.06 µm MDA $g^{-1}$ f.wt.) and tolerant genotypes (7.67 µm MDA $g^{-1}$ f.wt.) (Fig. 1d). Maximum lipid peroxidation in susceptible genotypes could be due to disturbance in balance between the production and scavenging of ROS (Bowler *et al.,* 1992). The present results are supported by the studies of Jiang and Huang (2001) in turfgrass and Wilson *et al.* (2014) in *B. juncea.* High temperature affects membrane linked processes due to alteration in membrane fluidity and permeability (Larkindale and Knight, 2002) which inturn is due to lipid peroxidation. The hydroperoxides, thus, formed decompose into oxy, peroxy radicals including malondialdehyde (Rani *et al.,* 2016).

Higher increase in lipid peroxidation in susceptible genotypes leads to more disruption of membrane integrity (Almeselmani *et al.,* 2006). Low cell membrane integrity is a repercussion of lipid peroxidation of membrane lipids caused by ROS generated during stress. ROS generated during heat stress reacts with unsaturated lipids in membranes and cause lipid peroxidation leading to MDA accumulation (Liu and Huang, 2000). MDA, a product of peroxidation of unsaturated fatty acids in phospholipids, is responsible for cell membrane damage (DaCosta and Huang, 2007).

Since lipid peroxidation was estimated in seedlings, it becomes incumbent to estimate the antioxidant status of the seedlings under heat stress. The different antioxidants studied were: peroxidase (POD), catalase (CAT), proline and glutathione (GSH). A significant ($p<0.01$) increase in POD activity was registered in all the categories in response to heat stress. The average values for POD action observed was highest in tolerant genotypes (460.0 ΔE $minute^{-1}$ $g^{-1}$ f.wt. of tissue, followed by moderately tolerant (328.13 ΔE $minute^{-1}$ $g^{-1}$ f.wt. of tissue) and susceptible ones (302.50 ΔE $minute^{-1}$ $g^{-1}$ f.wt. of tissue). In comparison to controls, percent increase was 45.45%, 40.70% and 30.95% in tolerant genotypes, moderately tolerant and susceptible genotypes, respectively (Fig.1e). No significant difference in POD activity was recorded in case of control seedlings. The results presented are in agreement with the previous repotrs of Chakraborty and Tongden (2005) and Rani *et al.* (2016) on *Cicer arietinum* and *B. juncea,* respectively. The increase in POD activity could be associated with chlorophyll degradation during heat stress and was induced probably due to increased levels of peroxide radicals as a result of increased lipid peroxidation (Chakraborty and Tongden, 2005).

Catalase activity decreased significantly ($p<0.001$) in all the three classes under heat stress. The seedlings of susceptible and moderately tolerant categories showed a higher decrease in CAT activity as compared to tolerant one. The decrease was 55.25% in susceptible seedlings, 48.68% in moderately tolerant genotypes and 11.85% in tolerant genotypes, respectively (Fig.1f). The results obtained in the present investigation are in accordance with those observed by Jiang and Huang (2001), who also reported decrease in CAT activity upon heat stress in turfgrass, respectively. The results are also in consonance with the results of He and Huang (2010) in Kentucky bluegrass. Dat *et al.* (1998) reported decrease in CAT activity by 9.6% following heat stress. However, in the present study decrease ranged from 10 to 35%. The CAT activity in unstressed seedlings of all categories showed non-significant difference but activity after heat stress varied significantly among seedlings of three categories. Catalase is a peroxisomal enzyme and helps in scavenging of $H_2O_2$. The decrease in CAT activity could be due to its non-robust nature and sensitivity to heat stress (Fadzillah *et al.,* 1996), and thus would not be playing role in scavenging $H_2O_2$ generated during heat stress (Liu and Huang, 2000). In different studies, catalase activity varied under heat stress suggesting that its activity may vary from species to species (Kaur *et al.,* 2009).

Glutathione content was significantly higher in control seedlings of tolerant genotypes in comparison to other two categories. After heat stress, remarkable significant increase ($p<0.001$) was observed in GSH content (Fig.1g) in seedlings of all the three categories of genotypes. Maximum GSH content was recorded in tolerant genotypes (0.674 nmoles $g^{-1}$ f.wt.), followed by moderately tolerant (0.404 nmoles $g^{-1}$ f.wt.) and susceptible genotypes (0.293nmoles $g^{-1}$ f.wt.), respectively. Higher GSH content could be responsible for alleviating the oxidative stress due to heat shock as GSH could have detoxified free radicals, and thus act as an antioxidant in heterotrophic as well as in phototrophic tissues (Wise and Naylor, 1987). $H_2O_2$ accumulated during heat stress is quenched by GSH, therefore higher glutathione content could plays a defensive role against heat stress generated oxidative stress (Noctor and Foyer, 1998). The increased GSH levels not only protect against free radicals but also switch the whole panoply of stress resistance response (Noctor and Foyer, 1998; Kumar and chattopadhyay, 2018).

Proline plays a protective role in various stresses (Wahid and Ghazanfer, 2006). In case of control seedlings of all three categories, variation in proline content was non-significant. But heat shock treatment caused significant ($p<0.001$) increase in proline content, ~ 1.5 fold in tolerant and moderately tolerant categories and 1.4 fold in susceptible ones as compared to control. The maximum proline content was found in tolerant genotypes (0.136 mg $g^{-1}$ f.wt tissue), followed by moderately

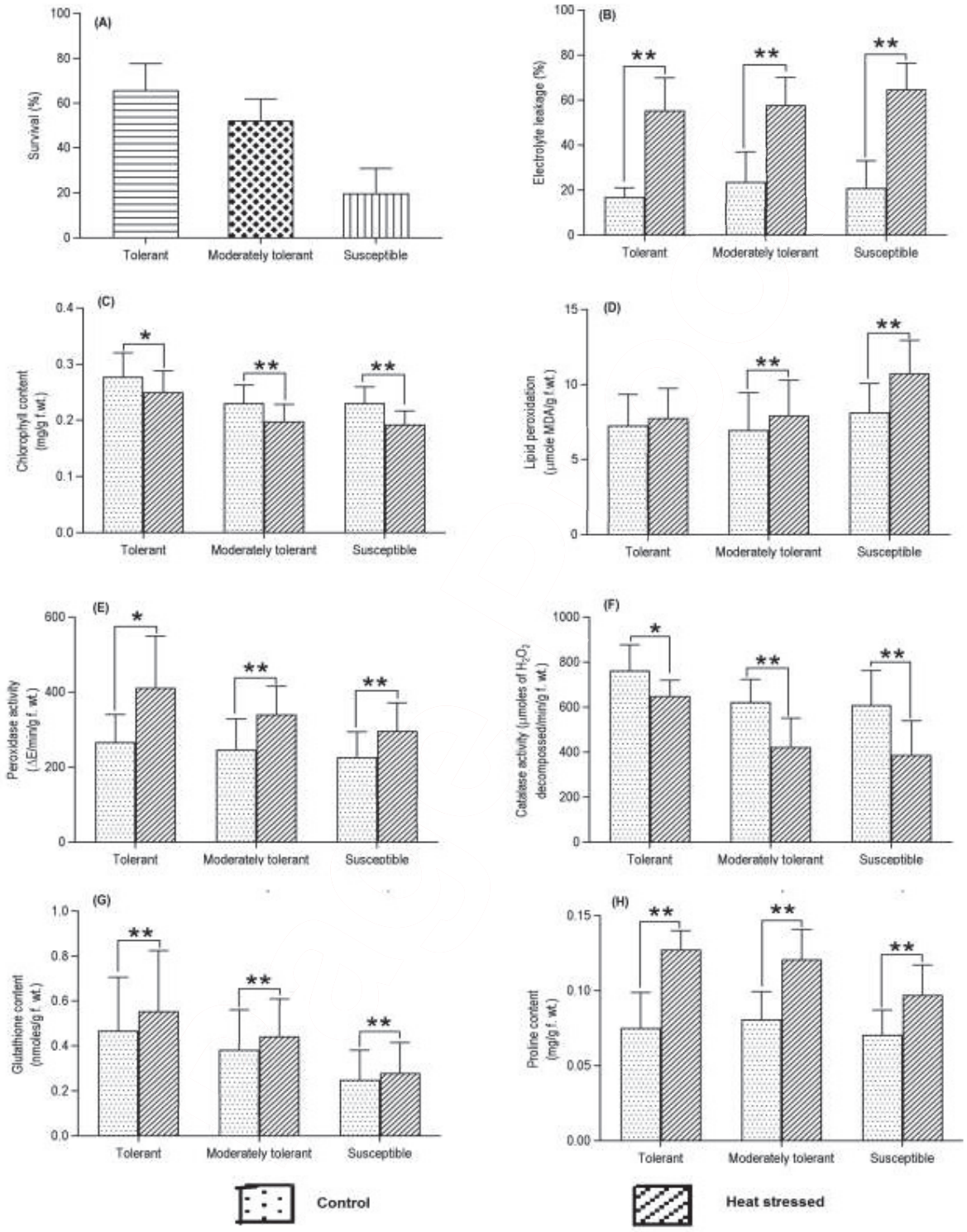


**Fig. 1 :** Effect of heat stress on (A) percent survival, (B) electrolyte leakage, (C) chlorophyll content, (D) lipid peroxidation, (E) peroxidase activity, (F) catalase activity, (G) glutathione content and (H) proline content of four-day-old *B. rapa* seedlings. Bars and error bars represent mean and standard deviation respectively. Asterisk (*) and (**) brackets represent significant difference between control and heat stressed seedlings at $P<0.05$ and $p<0.01$ level of significance respectively.

tolerant (0.117 mg $g^{-1}$ f.wt. tissue) and susceptible genotypes (0.100 mg $g^{-1}$ f.wt tissue) (Fig.1h). Similar increase in proline content was also reported by Pradhan *et al.* (2013) in *Brassica* spp. Proline is an osmolyte that accumulates during stress conditions (Schafleitner *et al.,* 2007) preventing water loss from the cell. (Gupta *et al.,* 2013). Higher proline content in tolerant genotypes is responsible for higher cell membrane stability (Verbruggen and Hermans, 2008). The accumulation of proline, particularly proline residues in protein, provides additional protection against oxidative stress (Ashraf and Foolad, 2007;Harsh *et al.,* 2016) which can be attributed to its cellular redox potential under heat (Wahid *et al.,* 2007) and other environmental stresses like drought and salinity (Ashraf and Foolad, 2007). Increased proline content during heat stress results due to from stimulation of proline synthesis from glutamate (Hayat *et al.,* 2009) by loss of feed-back inhibition, decrease in proline oxidation or due to decreased incorporation into proteins (Handa *et al.,* 1986).

The tolerant genotypes with higher survival percentage showed significantly lower ($p<0.01$) lipid peroxidation than susceptible genotypes, indicating tolerance towards heat induced damage. Biochemically, this heat stress tolerance could be due to higher induction of both enzymatic and non-enzymatic antioxidants (Yildiztugay *et al.*, 2017). The tolerant group showed significantly ($p<0.05$) higher increase in peroxidase activity in comparison to susceptible group. Likewise, tolerant group showed significantly higher increase in proline ($p<0.05$) and GSH content ($p<0.01$) in comparison to susceptible group. The antioxidant defence system has been considered as part of heat stress adaptation and the strength of this system is related to tolerance to heat stress (Wahid *et al.,* 2007; Zandalinas *et al*., 2017). To conclude, out of forty-four genotypes studied, only four were found to be heat tolerant. The information gathered through present investigation can pave way for imparting tolerance to genotypes via altering enzyme activities through genetic engineering.

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