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RESPONSE OF ANTIOXIDATIVE SYSTEM OF *BRASSICA JUNCEA* (L.) CZERN. TO TERMINAL HEAT STRESS

NARENDER MOHAN[1], NISHA KUMARI[1]*, MINAKSHI JATTAN, RAM AVTAR, BABITA RANI[1] AND SUSHIL[2]

Oilseed Section, Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar-125 004, India

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Abstract

A differential response to terminal heat stress was observed on enzymatic and non-enzymatic components of antioxidant system of two thermo-tolerant genotypes *viz*., RGN-368 & RH-1566 and two thermo-sensitive genotypes *viz*., RH-1134 & RH-0749 in leaves of Indian mustard. The antioxidative enzymes *viz*., superoxide dismutase, peroxidase, catalase, ascorbate peroxidase and glutathione reductase showed higher activity in leaves of two thermo-tolerant genotypes as compared to thermo-sensitive. Terminal heat stress resulted in concomitant increase in non-enzymatic components like carotenoids, ascorbic acid and proline in all the genotypes but maximum increase was observed in thermo-tolerant genotypes. A remarkable accumulation was observed in oxidative stress indicators i.e., malondialdehyde, hydrogen peroxide and electrolyte leakage in all the genotypes, whereas, significant increase was observed in thermo-sensitive genotypes as compared to thermo-tolerant. It is inferred that leaves of thermo-tolerant genotypes tend to attain greater capacity to perform reaction of antioxidative pathway under the condition of terminal heat stress to combat thermo-induced oxidative stress.

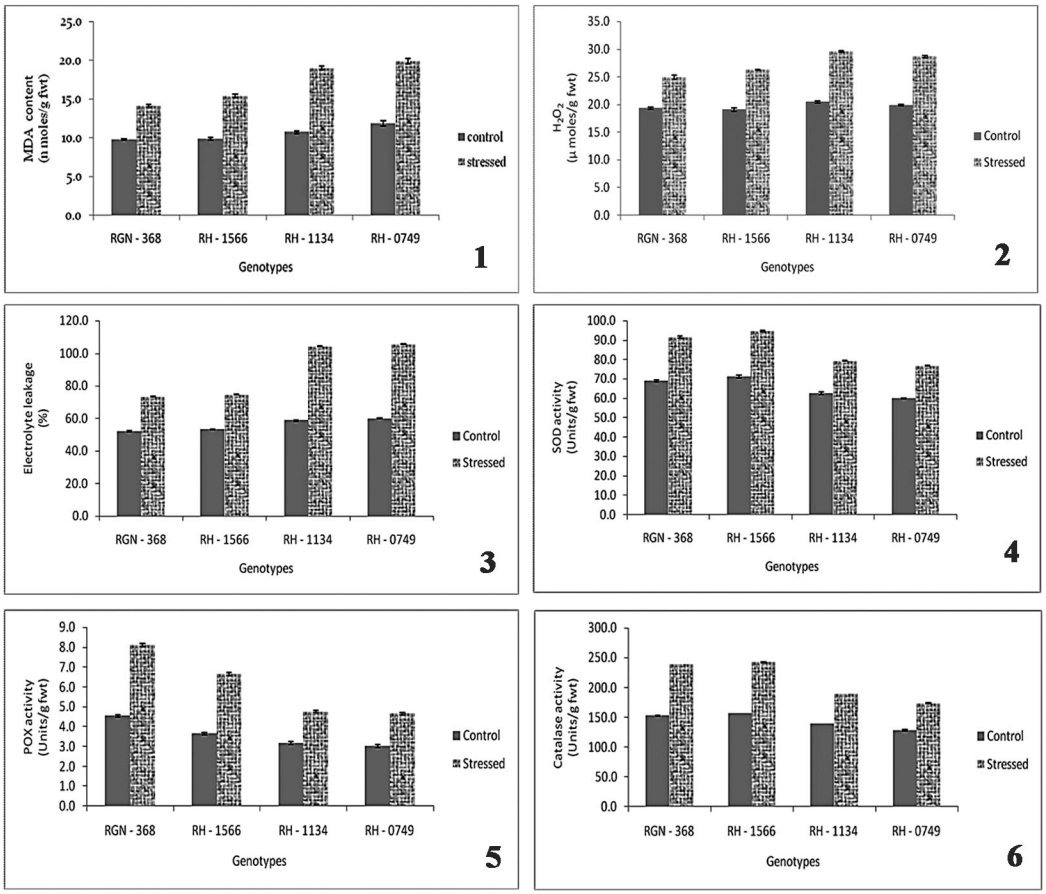
Indian mustard (*Brassica juncea* (L.) Czern. & Coss. belonging to Brassicaceae is the second largest oilseed crop in India after soybean. It accounts for nearly 30% of the total oilseeds and 27% to edible oil pool of the country. High temperature, specially terminal heat stress in late sown crop, is the second most important stress next to drought. It has devastating effects on plant growth and metabolism (Asthir 2015). Variability in temperature affects the grain and seed yield of annual crops. Generally plants respond to heat stress through developmental, biochemical and physiological changes and the type of observed responses depends on several factors such as stress intensity, stress duration and genotype (Moradshahi *et al.* 2004). This stress leads to generation of reactive oxygen species (ROS) and further tissue dehydration.

Plants being sessile are unable to escape the stress as such, but try to maintain cellular equilibrium by mechanisms of avoidance and tolerance which includes early maturation, alteration of membrane lipids composition, expression of stress proteins and other enzymatic and non-enzymatic mechanisms to scavenge the rapidly evolving ROS (Esfandiari *et al.* 2007). So the objective of this study was to evaluate the response of thermo-tolerant and thermo-sensitive genotypes of *B. juncea* to terminal heat stress in terms of antioxidants.

The four genotypes of *B. juncea viz.,* RGN-368, RH-1566, RH-1134 and RH-0749 were sown in research area of Oilseeds Section, Department of Genetics and Plant Breeding in RBD on two dates of sowing i.e. October 13 (timely) and November 15 (late) in three replications. Leaves of *B. juncea* genotypes after 90 days of sowing were used for further studies.

*Author for correspondence: <nishaahlawat211@gmail.com>. [1]Department of Biochemistry, CCS Haryana Agricultural University, Hisar-125 004, India. [2]Department of Chemistry, CCS Haryana Agricultural University, Hisar-125 004, India.

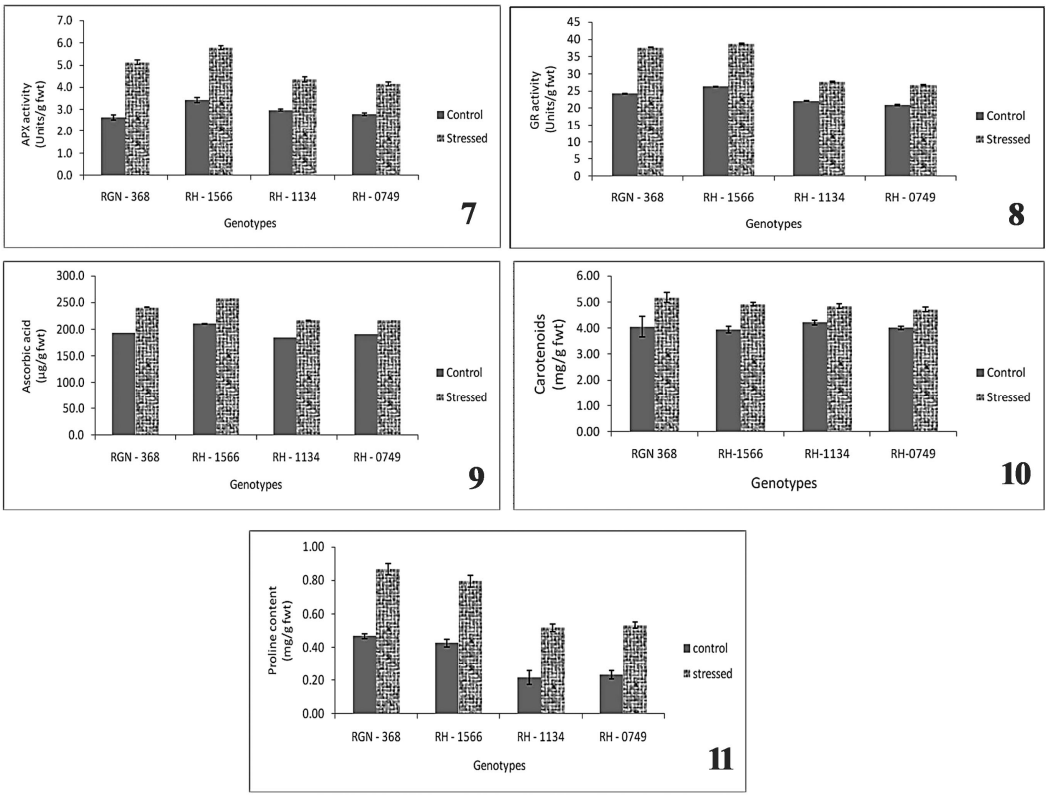
The MDA was estimated according to the method of Heath and Packer (1968). The H_2O_2 was estimated by the method of Sinha (1972). The relative intactness of plasma membrane was measured as the leakage percentage of electrolytes, as described by Gong *et al.* (1998). The SOD activity was determined by quantifying the ability of the enzyme to inhibit the photochemical reduction of nitro blue tetrazolium (NBT) to formazan (Beauchamp and Fridovich 1971). One unit of SOD activity is the amount of enzyme which causes 50 per cent inhibition of the photochemical reaction. One unit of POX activity is equivalent to µmole of H_2O_2 oxidized per minute (Shannon *et al.* 1966). One unit of catalase activity is the amount of enzyme required to consume one µmole H_2O_2 per minute under assay conditions (Sinha 1972). One unit of APX activity is the amount of enzyme utilized to oxidize one µmole of ascorbic acid per minute (Nakano and Asada 1981). One unit of glutathione reductase is defined as 100 µmole of NADPH oxidized per minute (Halliwell and Foyer 1978).



Figs 1-6: 1. Effect of terminal heat stress on MDA content in Indian mustard. CD at 5%: Genotype = 0.5 treatment = 0.353 genotype × treatment = 0.707. 2. Effect of terminal heat stress on H_2O_2 content in Indian mustard. CD at 5%: Genotype = 0.448 treatment = 0.317 genotype × treatment = 0.634. 3. Effect of terminal heat stress on electrolyte leakage in Indian mustard. CD at 5 %: Genotype = 0.594 treatment = 0.42 genotype × treatment = 0.84. 4. Effect of terminal heat stress on SOD activity in Indian mustard. CD at 5%: Genotype = 0.888 treatment = 0.628 genotype × treatment = 1.256. 5. Effect of terminal heat stress on POX activity in Indian mustard. CD at 5 %: Genotype = 0.13 treatment = 0.092 genotype × treatment = 0.184. 6. Effect of terminal heat stress on CAT activity in Indian mustard. CD at 5 %: Genotype = 1.178 treatment = 0.833 genotype × treatment = 1.666.

Ascorbic acid content was estimated by the method of Roe (1964). The carotenoids extracted in the DMSO were estimated by the method of Hiscox and Israelstam (1979). For proline estimation, standard method of Bates *et al*. (1973) was used. The data were analyzed statistically using CRD at 5% level of significance using OPSTAT software (CCS HAU, Hisar).

Malondialdehyde, H_2O_2 and electrolyte leakage are considered as indicators of degree of oxidative damage. Effect of THS on the MDA, H_2O_2 content and electrolyte leakage showed a significant increase in all genotypes under stress condition (Figs 1 - 3). However, this increment for MDA, H_2O_2 content and electrolyte leakage was more in thermo-sensitive genotypes i.e. RH-1134 (77.16, 44.71 and 77.65%, respectively) and RH-0749 (67.88, 44.36 and 76.09%, respectively). These results are in agreement with those reported by Kumar *et al*. (2013), Rani *et al*. (2016) and Kavita and Pandey (2017) in Indian mustard under heat stress.



Figs 7-11: 7. Effect of terminal heat stress on APX activity in Indian mustard. CD at 5%: Genotype = 0.199 treatment = 0.141 genotype × treatment = 0.282. 8. Effect of THS on GR activity in Indian mustard. CD at 5%: Genotype = 0.303 treatment = 0.214 genotype × treatment = 0.428. 9. Effect of terminal heat stress on ascorbic acid in Indian mustard. CD at 5%: Genotype = 0.715 treatment = 0.506 genotype × treatment = 1.011. 10. Effect of terminal heat stress on carotenoids in Indian mustard. CD at 5%: Genotype = N.S treatment = 0.257 genotype × treatment = N.S. 11. Effect of terminal heat stress on proline content in Indian mustard. CD at 5%: Genotype = 0.058 treatment = 0.041 genotype × treatment = 0.082.

The activity of antioxidative enzymes i.e. SOD, POX, CAT, APX and GR increased significantly in all the genotypes under late sown condition. However, the maximum per cent increase for all these enzymes was observed in thermo-tolerant genotypes as compared to thermo-

sensitive (Figs 4 - 8). Higher activity of these enzymes under THS in tolerant genotypes might be responsible for providing tolerance (Kumar *et al*. 2013, Rani *et al*. 2016).

All the genotypes showed significant increase in ascorbic acid, carotenoid and proline content with a higher increase seen in thermo-tolerant genotypes as compared to thermo-sensitive (Figs 9-11). This higher increase in thermo-tolerant genotypes might be an adaptive advantage to cope with terminal heat stress. These results are in conformity with Kumar *et al*. (2013), Rani *et al*. (2016) and Kumar *et al*. (2018) in Indian mustard under heat stress.

From the results it may be concluded that under terminal heat stress significant increase in antioxidative enzymes and metabolites in thermo-tolerant genotypes might be responsible for imparting tolerance. It may be suggested that the sensitive genotypes can be accorded by exploiting the activity of antioxidative enzymes through genetic engineering.

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