High temperature induced changes in antioxidative enzymes in *Brassica juncea* (L) Czern&Coss

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ABSTRACT

The present investigations were undertaken to study the changes in antioxidative enzymes under heat stress and its revival in *B. juncea* genotypes. Five days old seedlings of thermo tolerant genotype namely BPR-542-6 and thermo susceptible genotype namely NPJ-119 of *B. juncea* were subjected to high temperature ($45.0\pm0.5^{O}C$) stress. These were analyzed for antioxidative enzymes under control, stressed and revived conditions. The activities of Superoxide dismutase (SOD), Peroxidase (POX) , Catalase (CAT), Ascorbate peroxidase (APX) and Glutathione reductase (GR)] increased under high temperature stress but increase was found significantly higher in tolerant genotype. The basal level of all antioxidative enzymes except CAT was found more in tolerant genotype. On revival, SOD and CAT started decreasing but activity of POX and GR still continued increasing in both the genotypes however, APX enzyme exhibited differential behaviour which increased in tolerant genotype but decreased in susceptible genotype.

Key words: Superoxide dismutase - Peroxidase - Catalase - Ascorbate peroxidase - Glutathione reductase - Heat stress.

INTRODUCTION

Abiotic stresses such as drought, salinity, extreme temperature and chemical toxicity are the serious threats to agriculture and significantly diminish the plant productivity. These 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 ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^{\cdot}$) 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 (Davis, 1987).Plants have developed the scavenging mechanism of ROS categorized as enzymatic and nonenzymatic defense system. When ROS increases, chain reactions start in which superoxide dismutase (SOD), a metalloenzyme catalyses the dismutation of $O_2^{\cdot-}$ radical to molecular O_2 and H_2O_2. The H_2O_2 is then detoxified either by catalase/peroxidase or in ascorbate glutathione cycle which involves oxidation and reduction of ascorbate and glutathione through ascorbate peroxidase (APX) and glutathione reductase (GR) action (Noctor and Foyer, 1998). 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. The equilibrium between the production and scavenging of ROS may be perturbed under adverse abiotic stresses thereby results into reduction in crop yield.

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. Active oxygen species produced under temperature stress brings about heat induced oxidative stress which causes denaturation of functional and structural proteins and activates cell signalling pathway and cellular responses such as production of stress proteins, upregulation of antioxidants and accumulation of compatible solutes. *Brassica* is an important oilseed crop of winter season and its early sowing implies many important advantages.. But high temperature prevailing at the sowing time imposes severe limitation on early germination pattern and subsequent seedling establishment and yield of *Brassica juncea*. Keeping in view the burning problem of global warming which may result into yield loss, the present investigation was proposed to understand the response of tolerant and susceptible genotypes of *B. juncea* to high temperature stress and

its revival with main emphasis to study the role of antioxidative enzymes in imparting tolerance to *Brassica* crop.

MATERIALS AND METHODS

Raising of seedlings

Seeds of thermotolerant (BPR-542-6) and thermosusceptible (NPJ-119) genotype of *Brassica juncea* 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). Seedlings were allowed to grow at optimum temperature (25±0.5°C), relative humidity (70%) and 16 hrs light, 8hrs dark cycle for five days. Five days old seedlings were exposed to threshold high temperature (45±0.5°C) and 30% relative humidity continuously. After subjecting the *B. juncea* seedlings to heat stress, the seedlings were revived by placing the trays at 25±0.5°C for 24 hrs. The stressed and revived seedlings were taken for further studies.

Enzyme extraction and Assays

Seedlings of B*rassica juncea* genotypes were taken and washed with cold distilled water and wiped to dry with several folds of filter paper. All the steps of extraction were carried out at 0 - 4°C. The tissue was macerated in chilled pestle and mortar in the presence of 3 ml of 0.1 M phosphate buffer (pH 7.5) containing 5% (w/v) polyvinylpyrrolidone(PVP),1mM EDTA and 10mM β-mercaptoethanol. The homogenate was centrifuged at 10,000 x g for 20 min at 4°C. The supernatant was carefully decanted and used as the crude enzyme preparation for determining the activity of superoxide dismutase (SOD), peroxidase (POX), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR) .Superoxide dismutase was assayed by measuring its ability to inhibit the photochemical reduction of nitro blue tetrazolium (NBT) adopting the method of Beauchamp and Fridovich (1971). One enzyme unit was defined as the amount of enzyme that inhibits the nitro blue tetrazolium photoreduction by 50%.The peroxidase enzyme was assayed by adopting the method of Shannon et al. (1966) . One unit of peroxidase represents 1.0 O.D. change in one minute.Catalase activity was measured according to the method of Sinha (1972) and one enzyme unit was defined as the amount of enzyme which catalyzed the oxidation of 1 μmole H_2O_2 per minute under assay conditions**.** The activity of ascorbate peroxidase enzyme was assayed by the method of Nakano and Asada (1981) following the oxidation of ascorbic acid. The decrease in absorbance at 290 nm was recorded spectrophotometrically which corresponded to oxidation of ascorbic acid. One enzyme unit was defined as 1 μmole of ascorbic acid oxidized per min at 290 nm. Glutathione reductase was assayed by using the method of Halliwell and Foyer (1978). One enzyme unit was defined as 100 μmole of NADPH oxidized per minute.

RESULTS

The results presented in Table-1 demonstrate the activities of antioxidative enzymes i,e. Superoxide dismutase (SOD), Peroxidase (POX), Catalase (CAT), Ascorbate peroxidase (APX) and Glutathione reductase (GR) in control, stressed and revived seedlings of thermotolerant and thermosusceptible genotypes of *B.juncea*.

The activity of SOD increased due to heat stress in tolerant and susceptible genotypes.The increase was significantly higher in tolerant genotypes i.e. BPR-542-6 (106.57%) as compared to susceptible genotypes *viz.* NPJ-119 (100.08%) .The basal level of SOD was also found more in thermotolerant genotype On revival, the activity decreased significantly as compared to stressed seedlings but remained still higher than the control. Almeselmani et al. (2006) also reported an increase in SOD activity under high temperature stress in wheat genotypes.

The peroxidase(POX) activity increased in all the genotypes under high temperature stress and the activity also remained higher than the control during the recovery period. Basal level of POX activity was found more in BPR-542-6 (11.79 units g^{-1} fresh weight) than in NPJ-119 (6.02 units g^{-1} fresh weight). The POX activity was found significantly higher in tolerant genotypes under stressed condition i.e. 14.64 units g^{-1} fresh weight (BPR-542-6)

The significant increase in catalase activity under heat stress in both the genotypes was observed but increase was found more in tolerant genotypes i.e. 41.32% (BPR-542-6) when

compared with the susceptible genotypes i.e. 17.63% (NPJ-119). The basal level of CAT was found more in susceptible genotype NPJ-119 (342.40 units g^{-1} fresh weight) as compared to tolerant genotype BPR-542-6 (163.53 units g^{-1} fresh weight). After relieving the stress, CAT activity decreased significantly but remained higher than the control in all genotypes.Increase in CAT and POX activity under heat stress was also observed by Kaur et al. (2009) in *B.juncea* species.

Table 1. Effect of high temperature treatment (45±0.5°C) on the activities (units/g fresh weight) of antioxidative enzymes [Superoxide dismutase (SOD) Peroxidase (POX) Catalase (CAT) Ascorbate peroxidase (APX) Glutathione reductase (GR)] in thermotolerant and thermosusceptible genotypes of *B.juncea* seedlings.

Enzymes	Genotypes					
	BPR-542-6 (Tolerant)			NPJ-119 (Susceptible)		
	Control	Stressed	Revived	Control	Stressed	Revived
SOD	4.18	8.63	7.51	3.46	6.92	5.76
POX	11.79	14.64	15.23	6.02	9.40	11.78
CAT	163.53	231.10	193.81	342.40	402.77	385.65
APX	1.60	2.12	2.44	0.98	1.61	1.39
GR	35.90	54.70	63.80	24.90	33.30	36.60

CD at 5%

SOD : G* = 0.21 T** = 0.19 GxT = 0.37
* Genotype

POX : G = 0.21 T = 0.18 GxT = 0.36
** Treatment

CAT : G = 13.26 T = 11.48 GxT = 22.96

APX : G = 0.07 T = 0.06 GxT = 0.12

GR : G = 1.42 T = 1.23 GxT = 2.45

Data on APX activity showed that under heat stress, both the thermotolerant and thermosusceptible genotypes exhibited an increase in APX activity but the level of APX activity was more in tolerant genotype under stressed condition. The basal activity of APX was also found higher in thermotolerant genotypes i.e. BPR-542-6 (1.60 units g^{-1} fresh weight) when compared to theremosusceptible genotypes i.e. NPJ-119 (0.98 units g^{-1} fresh weight). But response of APX activity during recovery period was different in tolerant and susceptible genotypes, the activity continued to increase in thermotolerant genotypes but decreased in susceptible genotypes. Almeselamni et al. (2006) also found an increase in APX activity under heat stress in wheat genotypes and it was found more in tolerant genotype.

The response of GR activity under stress and revival is similar to the activity of POX in both the genotypes. GR activity increased in all the genotypes under stressed condition but the increase was significantly higher in tolerant genotype i.e. BPR-542-6 (52.36%) as compared to susceptible genotype where the increase was33.73% in NPJ-119 . The GR activity continued to increase under revival condition in both the genotypes.Locatto et al. (2009) also observed an increase in GR activity when the cell suspension of tobacco was exposed to 55°C. However initial increase and then decrease in GR activity was observed by Ma et al. (2008) in apple leaves after increasing the duration of high temperature stress.

DISCUSSION

The activities of all the antioxidative enzymes viz. SOD, POX, CAT, APX and GR increased significantly after subjecting the seedlings of thermotolerant & thermosusceptible genotypes of

B.juncea to heat stress.The increase in enzyme activities explain that ROS are overproduced due to heat stress which have resulted into upregulation of enzyme activities The basal level of all the antioxidative enzymes except CAT was found more in tolernt genotypes. On revival, the activities of SOD and CAT decreased but the activities of POX and GR increased in both the genotypes when compared to stressed seedlings. APX showed differential behaviour on revival,it continued increasing in tolerant genotype and started decreasing in susceptible genotype.

Higher basal level of antioxidative enzymes and signifcant increase in their activities during stress in tolerant genotype may be responsible for imparting tolerance to *Brassica* genotypes. Moreover increase in APX throughout stressed and revived condition in tolerant genotype may explain that glutathione- ascorbate cycle is more efficiently operating in tolerant genotype thereby imparting more tolerance to the seedlings.

CONCLUSION

The differential response of these genotypes to heat stress as a result of variation in the activities of their antioxidative enzymes suggests that by manipulation of the enzyme activities through genetic engineering may imprt tolerance to the genotypes.

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