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Antioxidative response of Indian mustard subjected to drought stress

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Abstract

The growth and yield of plants are severely affected by different environmental stresses. Water deficit imposed by drought brings about severe growth retardation and yield loss of crops. Therefore, in order to evaluate the response of antioxidant defense system of Indian mustard varieties to drought stress, a field experiment was conducted at Oilseeds Research Area, CCS HAU, Hisar, by taking two varieties *i.e.* RH 0406 (drought-tolerant) and RH 0749 (drought-sensitive). A different response of drought stress was observed on enzymatic and non-enzymatic components of antioxidant system in leaves of drought-tolerant and drought-sensitive varieties of Indian mustard (*Brassica juncea*). Drought stress was created by withholding the irrigation after sowing. The antioxidative enzymes *viz*. superoxide dismutase (SOD), peroxidase (POX), ascorbate peroxidase (APX) and glutathione reductase (GR) showed higher basal activities in leaves of drought-tolerant variety (RH 0406) as compared to drought-sensitive variety (RH 0749). Plants under drought stress indicated a significant increase in antioxidative enzyme but this increase was more pronounced in drought-tolerant variety *i.e.* RH 0406 as compared to RH 0749, a drought sensitive variety. The content of ascorbic acid, carotenoids, total glutathione and proline content were also recorded higher in tolerant variety than in sensitive variety under drought stress. It can be inferred that leaves of drought-tolerant variety (RH 0406) had greater capacity to perform reaction of antioxidative pathways under drought stress to control drought-induced oxidative stress.

Key words: *Antioxidative enzymes, drought stress, glutathione, Indian mustard, proline*

Introduction

Environmental stresses affect every aspect of physiological and biochemical processes in plants which results in stomatal closure, decrease in rate of transpiration and pigment content. Drought is one of the major environmental stress which affects crop growth and yield (Hasanuzzaman *et al*., 2012). Plants experience drought stress due to high rate of transpiration or due to low supply of water to roots. The adverse effects of drought stress on plant biomass production are due to inhibition in cell expansion, alteration in plant metabolism and reduction in the activities of different metabolic enzymes (Ashraf *et al*., 2013).

Drought stress enhances the production of reactive oxygen species (ROS) such as superoxide radical, hydrogen peroxide, hydroxyl radical and singlet oxygen. The production of ROS has harmful effects on different plant physiological and metabolic processes such as photosynthesis and antioxidative defense system leading to lipid peroxidation, chlorophyll degradation, ion leakage and biomolecules deterioration (Hossain *et al*., 2013). For the protection of deleterious effect of ROS, plants possess an antioxidative system consisting of enzymatic and non-enzymatic components. Enzymatic system includes superoxide dismutase (SOD), peroxidase (POX), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR); non-enzymatic system includes water soluble molecules (ascorbate and glutathione) and lipid soluble antioxidants such as carotenoids etc. (Sekman *et al*., 2007). Although plants with high levels of antioxidants, either constitutive or induced, osmotic adjustment, osmoprotection and scavenging defense system have been reported to possess greater resistance to oxidative damage and most important physiochemical/ biochemical basis responsible for drought tolerance (Omprakash *et al*., 2017).

The induction and regulation of the antioxidative system are necessary to obtain substantial tolerance against oxidative stress and detoxification of ROS might be a strategy for tolerance against various abiotic stresses (Hasanuzzaman *et al*., 2012). Improving yield under drought stress is a major goal of plant breeding. However, scanty information is available on the effect of drought

stress on antioxidant system in Indian mustard. The present work therefore, was undertaken to study the drought-induced changes in oxidative stress and antioxidative system in Indian mustard.

Materials and Methods

The present investigation was carried out by growing two varieties of Indian mustard *viz*. RH 0406 (drought-tolerant) and RH 0749 (drought-sensitive) under field conditions at Oilseeds Research Area, CCS HAU, Hisar, India. Drought stress was created by withholding irrigation after sowing and leaf samples were collected from control and stressed plants.

For the extraction of ascorbate and glutathione (reduced and oxidized), 1 g of leaves from control and stressed plants were ground in 5 ml of chilled 0.8 N $HClO_4$ (Jimenez *et al*., 1997), and centrifuged at 10,000 x g for 25 min. The clear supernatant was used for the estimation of these metabolites. Ascorbic acid content was estimated according to the method of Roe (1964) which is based on the reduction of 2, 6-dichlorophenol indophenol by ascorbic acid. Method of Smith (1985) was employed for determining the level of total glutathione. The proline content was determined by using method of Bates *et al.*, 1973. The method of wellburn, 1994 was employed for the estimation of carotenoids.

Extraction medium for SOD, CAT, APX and GR consisted of 0.1 M phosphate buffer (pH 7.5) containing 5 % (*w/v*) polyvinyl polypyrrolidone (PVPP), 1 mM EDTA, and 10 mM â-mercaptoethanol. For POX, the extraction was done in 0.01 M phosphate buffer (pH 7.5) containing 3% (*w/v*) PVPP. The homogenate was prepared by grinding 1 g of tissue in 5 ml of ice cold extraction medium in pre-cooled mortar and pestle. The homogenate thus prepared, was centrifuged at 10,000 rpm for 15 min at 4^0C. The supernatant was carefully decanted and used as the crude enzyme preparation. All the estimations were carried out in three replicates.

Superoxide dismutase (SOD) activity was determined by quantifying the ability of the enzyme to inhibit light induced conversion of nitroblue tetrazolium (NBT) to formazan (Nishikimi *et al*., 1972). One enzyme unit was defined as the amount of enzyme which could cause 50 per cent inhibition of the photochemical reaction. The activity of catalase was estimated according to the procedure described by Aebi (1984). One unit of enzyme activity corresponded to one µmole of H_2O_2 consumed during the reaction. Peroxidase activity was assayed at 37^oC as described by Shannon *et al*. (1966). The POX activity was defined as 1.0 µmole of H_2O_2 utilized per min. Method of Nakano and Asada (1981) was employed to assay APX and decrease in absorbance due to oxidation of ascorbic acid at 290 nm was recorded. One enzyme unit was defined as amount of enzyme required to oxidize 1 ìmole of ascorbic acid per min. Glutathione reductase was determined by the method of Halliwell and Foyer, 1978. Oxidation of NADPH by GR was monitored at 340 nm and the rate (µmoles min^{-1}) was calculated using the extinction coefficient of 6.12 mM^{-1} cm^{-1}.

Results and Discussion

Among the abiotic stresses drought stress is the most severe environmental stress which affects crop growth and yield. The effect of drought depends on the time of its occurrence, duration and its intensity. Under stress conditions, reactive oxygen species (ROS) accumulates in leaves resulting in the oxidation of cellular components *i.e* proteins, chlorophyll and lipids. To cope with oxidative stress, plants are equipped with an effective antioxidant defence system comprising of antioxidant enzymes like superoxide dismutase (SOD), peroxidase (POD), ascorbate peroxidase (APX), catalase (CAT) and other enzymes of ascorbate-glutathione pathway. Results presented in figure 1A, demonstrate the activity of SOD that catalyses the oxidation of superoxide radical to H_2O_2 which had higher constitutive level (3.32 units/g fresh wt) in tolerant cv. RH 0406 as compared to that in sensitive cv. RH 0749 (2.86 units/ g fresh wt). Imposition of drought stress resulted in 60.8 % and 31.1 % increase in SOD activity in tolerant and sensitive variety, respectively. Results of the present investigation corroborate the previous report (Dawood and Sadak, 2014) that SOD activity increased in response to drought stress. The salt-tolerant cultivars of *B. juncea* and *Najas graminia* have also been reported to possess higher basal levels of SOD than the sensitive cultivars (Bor *et al*., 2003). Higher basal level of SOD has been proposed to be the first line of defense, and it signifies the possible involvement of this enzyme in drought tolerance.

The data on peroxidase and catalase activity (Fig 1B and 1C), H_2O_2 scavenging enzymes, showed an enhancement in leaves of both varieties when exposed to drought stress. In the present study, high POX and CAT activity under drought-stress in both the varieties suggests an active involvement of these enzymes in H_2O_2 detoxification. This enhancement was more pronounced (88.14 % & 50.13 %, respectively) in RH 0406 as compared to RH 0749 (57.0 % & 21.0 %, respectively). The results on the effect of drought stress on peroxidase and catalase are in agreement with those of Dawood and Sadak (2014) who reported a significant increase in peroxidase and catalase activitiy due to drought stress in *B. napus*.

Further, under drought stress, the activities of CAT and POX were increased and involved in elimination of H_2O_2 from stressed cells and presumed to limit cellular damage and enhance the plants oxidative capacity to defend stress (Nojavan and Khorshidi, 2006). High activity of CAT indicated drought tolerance in some of the canola cultivars (Omidi, 2010).

Ascorbate peroxidase is another H_2O_2 scavenging enzyme using ascorbate as electron donor and it has very high affinity for H_2O_2 than POX and CAT. Drought stress resulted in increase in APX activity in both the varieties (Fig 1D). This increase was more in case of tolerant variety (69.3 %) as compared to the sensitive one (41.0 %). Increase in APX activity under drought stress in the present study is in agreement with the findings of Dawood and Sadak (2014) who reported the enhancement in APX activity due to drought stress in *B. napus*. High activity of APX indicated tolerance against drought in some of the canola cultivars (Omidi, 2010). Stress-induced increase in APX activity could impart tolerance by detoxifying H_2O_2 generated upon exposure of plants to stress conditions. Glutathione reductase (GR) is one of the enzymes involved in scavenging of active oxygen species by maintaining ascorbate pool in reduced form. In the present study, the activity of GR (Fig. 1E) also increased under drought stress condition. This increase was more in RH 0406 (42.5 %) as compared to RH 0749 (29.9 %). Increased GR activity under drought stress has also been reported in *B. napus* (Dawood and Sadak, 2014) and in some of the canola cultivars (Omidi, 2010). The elevated levels of GR activity perhaps could increase the ratio of NADP/NADPH, thereby ensuring the availability of NADP to accept electrons from the photosynthetic electron transport chain. Increase in the GR activity in plants results in the accumulation of glutathione (GSH) levels and ultimately confers tolerance to plants. The APX and GR are the key enzymes of the ascorbate glutathione cycle; the cycle may be a potential mechanism of mustard adaptation to drought stress.

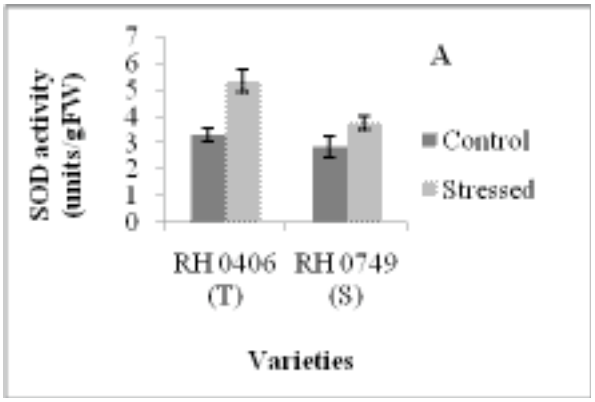
Among the non-enzymatic antioxidants, the contents of ascorbic acid, total glutathione and proline were monitored. Results presented in Table 1 reveal that all these non-enzymatic antioxidants content in leaves of both *B. juncea* varieties increased under stress condition, the basal level as well as the magnitude of increment was higher in tolerant variety (RH 0406) than in sensitive (RH 0749). The increase in ascorbate content was 24 % in RH 0406 and 20.6 % in RH 0749. The high basal level of glutathione content was observed in RH 0749 (22.4 µg/g FW) but the percent increase was more in RH 0406 (52.9 %) under stress condition. Ascorbic acid and glutathione are important ROS scavenging metabolites. Ascorbate plays an important role in imparting protection against ROS, as it acts as an electron donor for ascorbate peroxidase. Results of present study are in agreement with those of Rani *et al*. (2012) who observed more ascorbate content in heat-tolerant than in heat-sensitive cultivars of *B. juncea*. Glutathione, a low molecular weight antioxidant, is a powerful regulator of major cell functions. Reduced glutathione can react directly with free radicals, hence preventing inactivation of enzymes due to oxidation of essential thiol groups (Wang *et al*., 1991). Similar results were also obtained by Wilson *et al*. (2014) who observed more glutathione content in heat-tolerant than in heat-sensitive cultivars of *B. juncea*.

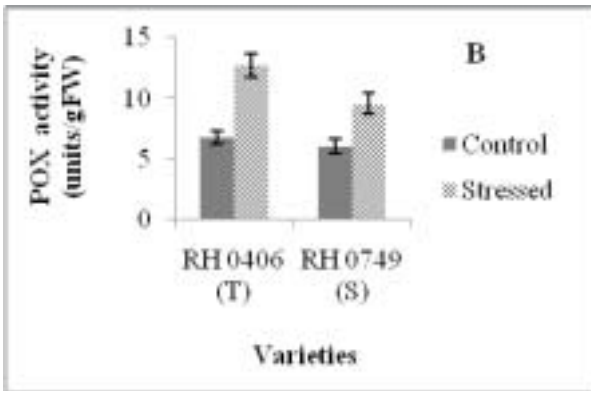
Drought stress resulted in increase in proline content in both the varieties. This increase was 29.4 % in tolerant variety as compared to 17.6 % in sensitive variety. Proline is reported to act as a protective osmolyte, membrane stabilizer and reactive oxygen species scavenger. The accumulation of proline, which is synthesized from the amino acids glutamate, is an enzyme-regulated process. The enzymes involved in its biosynthesis are reported to be elevated under drought stress, whereas those involved in its degradation are inhibited (Sumithra and Reddy, 2004). Therefore, the level of proline in the plants exposed to drought stress was higher than those of the control. It may be due to the fact that BRs activates the enzymes of

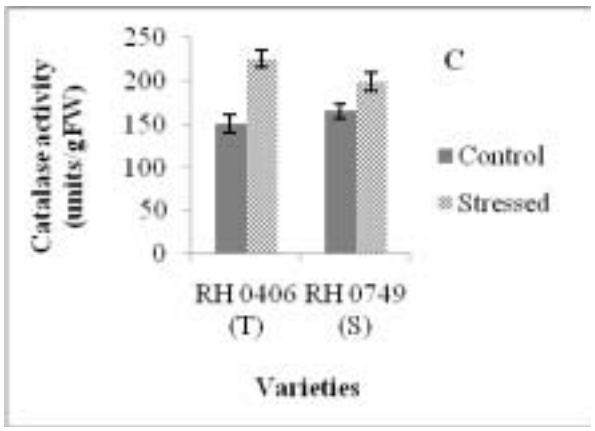
Table 1: Effect of drought stress on different antioxidative parameters in Indian mustard

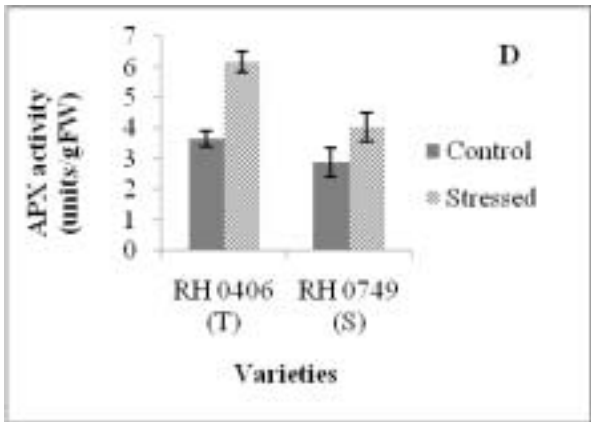
Antioxidative parameters	Varieties			
	RH 0406 (T)		RH 0749 (S)	
	Control	Stressed	Control	Stressed
Ascorbate content (µg/gFW)	237.2	294.1 (+24.0)	220.5	265.9 (+20.6)
Carotenoids (mg/gFW)	2.5	3.0 (+20.2)	2.6	2.8 (+7.4)
Total glutathione (µg/gFW)	22.4	31.2 (+39.2)	19.4	29.7 (+52.9)
Proline (mg/gFW)	4.7	6.1 (+29.4)	4.1	4.8 (+17.6)

Figures in parentheses are % increase (+)/decrease (-) over control









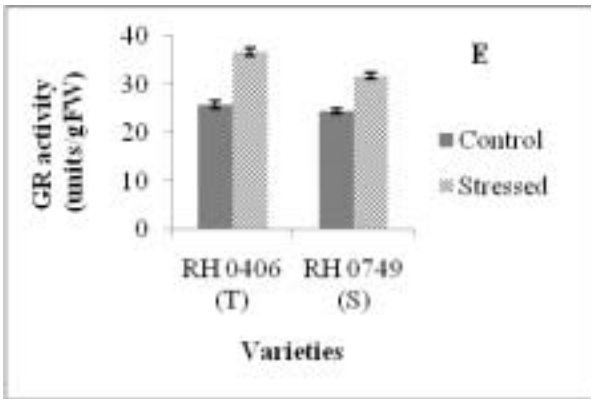


Fig. 1: Effect of drought stress on (A) SOD (B) POX (C) CAT (D) APX and (E) GR activities in Indian mustard varieties (Values are mean ± S.D)

proline biosynthesis, which caused an additive effect on the proline content. The accumulation of proline in the plants exposed to temperature stress (Wilson *et al*., 2014) and salt stress (Ali *et al*., 2007) has also been reported earlier. Carotenes form a key part of the plant antioxidant defense system. Carotenoids, despite their capacity to scavenge singlet oxygen and lipid peroxy radicals, as well as to inhibit lipid peroxidation and superoxide generation under dehydrative forces (Deltoro *et al.*,1998). Table 1 depicts that the percent increase in carotenoids was 20.24 % in tolerant variety while it was 7.42 % in sensitive variety. The increase in carotenoid content in heat-tolerant than in heat-sensitive cultivars of *B. juncea* was also reported by Rani *et al*. (2012).

The differential response of these varieties to drought 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 impart tolerance. The tolerant variety also showed increased levels of antioxidant enzymes and metabolites and thus, registered lesser oxidative damage. These techniques may also be used in pre-breeding programmes for screening of genotypes against drought tolerance and identification for future use in crossing programmes for developing Indian mustard varieties tolerant to the drought stress.

References

Aebi H. 1984. Catalase *in vitro. Methods in Enzymol* **105**: 121-126.

Ali B, Hayat S, Ahmad A. 2007. 28-Homobrassinolide ameliorates the saline stress in chickpea (*Cicer arietinum* L). *Environ Exp Bot* **59**: 217–223.

Ashraf M, Shahbaz M and Ali Q. 2013. Drought- induced modulation in growth and mineral nutrients in canola (*Brassica napus* L.). *Pak J Bot* **45**: 93-98.

Bates LS, Waldren RP and Teare ID. 1973. Rapid determination of free proline for water stress studies. *Plant Soil* **39**: 205-207.

Bor M, Ozdemir F and Turkan I. 2003. The effect of salt stress on lipid peroxidation and antioxidants in leaves of sugar beet *Beta vulgaris* (L.) and wild beet *Beta maritima* (L.). *Plant Sci* **164**: 74-77.

Dawood MG and Sadak M. 2014. Physiological role of glycine betaine in alleviating the deleterious effects of drought stress on canola plants (*B. napus* L.). *Middle East J Agric Res* **3**: 943-954.

Deltoro VI, Calatayud A, Gimeno C, Abadoaa A and Barreno E. 1998. Changes in chlorophyll a fuorescence, photosynthetic CO_2 assimilation and xanthophyll cycle interconversions during dehydration in desiccation-tolerant and intolerant liverworts, *Planta* **207**: 224–228.

Halliwell B and Foyer CH. 1978. Properties and physiological functions of a glutathione reductase purified from spinach leaves by affinity chromatography. *Planta* **139**: 9-17.

Hasanuzzaman M, Hossain MA, da Silva JAT, Fujita M. 2012. Plant responses and tolerance to abiotic oxidative stress: Antioxidant defense is a key factor. In: Bandi V, Shanker AK, Shanker C, Mandapaka M (eds.) *Crop stress and its management: perspectives and strategies*. Berlin: Springer. p. 261–316.

Hossain MA, Mostofa MG and Fujita M. 2013. Cross protection by cold-shock to salinity and drought stress-induced oxidative stress in mustard (*Brassica campestris* L.) seedlings. *Molecular Plant Breeding* **4**: 50-70.

Jimenez A, Hernandez JA, Del Rio LA and Sevilla F. 1997. Evidence for presence of ascorbate- glutathione cyclein mitochondria and peroxisomes of pea leaves. *Plant Physiol* **114**: 275-284.

Nakano Y and Asada K. 1981. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant Cell Physiol* **2**: 867-880.

Nishikimi, M, Rao NA and Yagi K. 1972. The occurrence of superoxide anion in the reaction of reduced phenazine metosulphate and molecular oxygen. *Biochem Biophy Res Comm* **48**: 849-854.

Nojavan AM and Khorshidi M. 2006. An investigation of vanillin imposed oxidative stress in corn (*Zea mays* L.) and the activities of antioxidative enzymes, *Pak J Biol Sci* **9**: 34-38.

Omidi H. 2010. Changes of proline content and activity of antioxidative enzymes in two canola genotype under drought stress. *Am J Plant Physiol* **5**: 338-349.

Omprakash, R. Gobu, Prashant Bisen, Murlimanohar Baghel and Kumar Nishant Chourasia 2017. Resistance/Tolerance mechanism under water deficit (Drought) condition in plants. *Int J Curr Microbiol App Sci* **6**: 66-78.

Rani B, Jain V, Chhabra ML, Dhawan K, Kumari N and Yadav P. 2012. Oxidative stress and antioxidative system in *B. juncea* (L.) under high temperature stress. *Ann Biol* **28**:110-115.

Roe JH. 1964. Chemical determination of ascorbic, dehydroascorbic and diketogluconic acids. In: D Glick (ed.) *Methods in Biochemical analysis*, Interscience, New York, **1**: 115-139.

Sekmen AH, Turkan I and Takio S. 2007. Differential responses of antioxidative enzymes and lipid peroxidation to salt stress in salt-tolerant *Plantago maritima* and salt-sensitive Plantago media. *Physiol Plant* **131**: 399-411.

Shannon LM, Key E and Law JY. 1966. Peroxidase isoenzymes from horse reddish roots: isolation and phys physical properties. *J Biol Chem* **241**: 2166-2172.

Smith IK. 1985. Stimulation of glutathione synthesis in photorespiring plants by catalase inhibitors. *Plant Physiol* **79**: 1044-1047.

Sumithra K and Reddy AR. 2004. Changes in proline metabolism of cowpea seedlings under water deficit. *J Plant Biol* **31**: 201–204

Wang SY, Jiac HJ and Faust M. 1991. Changes in ascorbate, glutathione and related enzyme activities during thiadia zuren-induced bud break of apple. *Physiol Plant* **82**: 231-236.

Wellbrun AR. 1994. The spectral determination of cholorphyll a and b as well as total carotenoids, using various solvents with spectrophotometer of different resolution. *J. Plant Physiol* **144**: 307-313.

Wilson RA, Sangha MK, Banga SS, Atwal AK and Gupta S. 2014. Heat stress tolerance in relation to oxidative stress and antioxidants in *B. juncea*. *J Env Biol* **35**: 383-387.