Annals of Plant and Soil Research 22(1): 60-65 (2020)

Influence of Salicylic Acid on non-enzymatic antioxidative synthesis in Indian mustard (*Brassica juncea* L.) cultivars under stress conditions

RAJANI CHAUHAN[1]AND DHEERA SANADHYA*

School of Life Sciences, Jaipur National University, Jaipur, Rajasthan, India (302025)

Received: August, 2019; Revised accepted: October, 2019

ABSTRACT

In the present work the possible roles of non-enzymatic antioxidants (TGSH, TPC, GST and phenol) in SA-mediated protection against osmotic stresses were investigated. The research work was conducted in the Plant tissue culture laboratory, Department of Life Sciences, Jaipur National University, Jaipur (Rajasthan) in 2017. Drought situations were imposed under in-vitro conditions to observe two sets of 7-d-old seedling, Various concentrations of PEG 6000 like 5, 10 and 15 % PEG and for salinity three potential levels of NaCl (50, 100 and 150 mM and distilled water as control were used. On the other hand, second set of seedlings were also supplemented with same stress conditions along with the application of SA (8 µm).Treatment with osmotic stress increased the PC levels in roots of B. juncea, but only slight changes were observed in the leaves. Long-term exposure to stresses decreased the phytochelatin synthase (PCS) activity in the roots and led to an increase in PCS and glutathione reductase (GR) activities in B. juncea leaves. The phenolic content decreased consistently with imposed stress in both cultivars. Treatment with osmotic stress increased the all non-enzymatic antioxidant levels in the leaves of B. juncea cv. in comparison to root. SA application protected antioxidant system to reduce oxidative damage. This protection was not directly connected with the altered regulation of PCs. Tolerant mustard variety showed less oxidative damage compared to susceptible variety under stress conditions.

Key words: Phytochelatins, Salicylic acid, *B. juncea*, total glutathione, glutathione-s-transferees, phenol, Drought, Salinity

INTRODUCTION

Among the oil seed crops, Indian mustard (*Brassica juncea* (L.)Czern & Coss.)is one of the most important crop, due to its edible oil production. Decreased yield of Indian mustard due to drought and salinity stresses has been reported by many researches (Khan *et al.*, 2014). Both drought and salinity alters the osmotic homeostasis in plants. Many toxic symptoms may result if the osmotic concentration exceeds a critical level. These symptoms include the inhibition of growth and photosynthesis, activation or inhibition of enzymes, and disturbances in water and ion metabolism. It is well demonstrated that, antioxidant systems play an important role in protection against various stress; however, antioxidant capacity may not be sufficient to minimize the harmful effects of oxidative injury. In addition to general stress responses, plants synthesize special complex-forming agents called phytochelatins (PCs),which are produced in the cytosol and play a special role in the detoxification of toxic osmotic stress.They have the structure [(γ-Glu– Cys)n – Gly], where n is the number of replications of (γ-Glu – Cys) units, generally in the range of 2–11. PCs are synthesised by phytochelatin synthase (PCS) from glutathione (GSH) by transferring ag-Glu-Cys moiety from a donor to an acceptor molecule. However, Glutathione (GSH; γ-glutamyl-cysteinyl-glycine) is a small intracellular thiol molecule which is considered as a strong non-enzymatic antioxidant. It regulates multiple metabolic functions; for example, it protects membranes by maintaining the reduced state of both α-tocopherol and zeaxanthin, it prevents the oxidative denaturation of proteins under stress conditions by protecting their thiol groups, and it serves as a substrate for both glutathione peroxidase and glutathione *S*-transferase (Hasanuzzaman *et al.*, 2017).Glutathione-S-transferases (GSTs) are ubiquitous enzymes encoded by a large family of genes, which play an important role in cellular detoxification to a wide variety of endobiotic and xenobiotic substrates by conjugating the tripeptide glutathione.

Corresponding author: email dheerasanadhya@yahoo.com
[1]Department of Plant Breeding and Genetics, R.B. (P.G.) College, Agriculture Campus, Mudi Crossing Agra, (U.P.), India (282007)

In the past few decades, there are so many strategies such as plant breeding, genetic engineering, plant growth regulators (PGRs) etc. used to combat drought and salinity stresses, exogenous application of plant growth regulators has received considerable attention.There are so many plant growth regulators have been reported to combat the deleterious effects of osmotic stress (Chauhan *et al.*, 2018; Shahnwaz *et al.*, 2017). Salicylic acid (SA) is one of them which play a key role in the signal transduction pathways of various stress responses (Shahnawaz *et al.*, 2017). Exogenously SA is applied to stressed plants, either through seed priming, adding to the nutrient solution; irrigating or foliar spraying was addressed to induce major abiotic stress tolerance mechanisms (Chauhan *et al.*, 2019). However, the influence of exogenous SA treatment in the reduction of drought, salinity and also their combined stress is still in its infancy. Hence, the current study was undertaken to find out the possible role of TGSH, TPC, GST and phenol in the SA mediated protection against drought and salinitystressesof *B. juncea* cv. "PUSA-AGRANI (Tolerant variety) and CS-52 (Susceptible variety)" under *in-vitro* conditions.

MATERIALS AND METHOD

The experiments were conducted in the Plant tissue culture laboratory, Department of life science, Jaipur National University, Jaipur (Rajasthan) in 2017. Various concentrations of PEG 6000 were used like (0), 5, 10 and 15 % PEG, Three potential levels of NaCl (50, 100 and 150 mM and distilled water as control were used.Two sets of seedlings were subjected to two different varieties of *B. juncea*. Another set of seedlings of both varieties were treated with 8 µM SA simultaneously. In order to assess the response of the two varieties of *B. juncea* (tolerant PUSA-AGRANI and another is susceptible CS-52) under different concentration of PEG and NaCl. Seeds of both varieties were pretreated with antifungal agent and surface sterilized with 0.1% $HgCl_2$ prior to germination in a hydroponic system for 48 h in darkness. For further growth, seedlings were germinated under control condition, i.e., (25 ± 2ºC, 70 % relative humidity (HR) and 16 hour photoperiod). Each replicate was inspected intensively and at the last day (7th day) seedlings were harvested for extraction of TGSH, TPC and GST. Total Glutathione (TGSH) was extracted and assessed according to the method reported by Anderson, (1985). Total Phytochelatins (TPC) were extracted and assayed according to the method suggested by Pagliari *et al.* (2005).

Total PC = (Tot. vol. / sample Vol.) × OD412nm / 13600 = 100 × OD412nm / 13600

Glutathione-s-Transferase (GST) (EC 2.5.1.18)activity was assayed as per method suggested by Ezaki *et al.* (2004) with some modifications.The method of Marinova*et al.* (2005) was used for the extraction and quantification of total soluble phenolic contents.

RESULTS AND DISCUSSION

Total Glutathione (TGSH) and Total Phytochelatins (TPC)

The concentration of TGSH and TPC in leaves and roots significantly differed depending upon both cultivars and stress treatments. With an increase in the concentrations levels of each stress, there was an increase in the amount of TGSH and TPC in root and leaves of both cultivars (Table 1), The amount of TGSH in cultivar CS-52 (Susceptible variety) was higher at all concentrations of each stress in comparison to PUSA-AGRANI. With the addition of SA, there was a remarkable increase in TGSH and TPC content in both varieties as compared to untreated plants at all concentrations of both the stresses. These investigations were consistent with findings of Alam *et al.* (2013) and Nahar *et al.* (2013). Naturally, GSH is oxidized to GSSG when it is participated in ROS scavenging process that results in reduced GSH content (Hasanuzzaman *et al.*, 2012). Glutathione is a low molecular weight thiol tripeptide (γ-glutamyl-cysteinyl-glycine) abundantly found in almost all cellular compartments. GSH scavenges H_2O_2, $^1O_2$, $OH^{\cdot}$ and $O_2^{\cdot-}$ and protects the different biomolecules by forming adducts (Glutathiolated) or by reducing them in presence of ROS or organic free radicals and generating GSSG as a byproduct. GSH also plays a vital role in generating AsA to yield GSSG. The GSSG thus generated is converted back to GSH, either by de novo synthesis or enzymatically by GR.

Table 1: Effect of SA application on induced changes in total glutathione (TGSH) in leaves and roots of *B. juncea* cultivars at 7th day after sowing (mean ± SE of three replicates) under stress conditions

Genotypes	Stress	Concentration	Total Glutathion in leaves (nM g^{-1})			Total Glutathion in roots (nM g^{-1})		
			0Hrs	48Hrs	72Hrs	0Hrs	48Hrs	72Hrs
PUSA-AG. (Tolerance)	Drought	Control	7.26±0.015	23.2±0.020	38.2±0.021	3.6±0.043	6.3±0.015	14.2±0.017
		5% PEG	8.37±0.009	25.3±0.015	39.7±0.025	1.84±0.020	3.2±0.015	8.2±0.015
		10 % PEG	13.2±0.015	29.3±0.035	42.8±0.020	4.64±0.043	8.1±0.020	15.2±0.025
		15% PEG	15.3±0.010	34.3±0.038	47.4±0.021	6.7±0.015	12.6±0.010	21.2±0.010
		Control (SA)	16.2±0.021	34.8±0.038	50.3±0.040	11.9±0.030	17.2±0.044	28.3±0.006
		5% PEG (SA)	19.3±0.025	38.4±0.020	53.3±0.035	5.7±0.015	10.2±0.010	21.2±0.012
		10 % PEG (SA)	24.2±0.025	41.3±0.031	57.3±0.025	9.31±0.020	16.2±0.026	31.1±0.010
		15% PEG (SA)	31.2±0.025	47.3±0.038	61.1±0.072	22.2±0.025	23.2±0.006	39.8±0.032
CS-52 (Susceptible)	Drought	Control	8.34±0.036	25.5±0.020	34.3±0.035	2.1±0.023	5.0±0.031	11.2±0.015
		5% PEG	8.7±0.015	25.9±0.021	35.2±0.145	1.6±0.015	3.1±0.026	9.2±0.038
		10 % PEG	14.7±0.012	30.3±0.057	41.2±0.035	3.7±0.015	6.3±0.023	14.2±0.015
		15% PEG	16.2±0.04	36.2±0.020	44.2±0.045	5.8±0.043	12.2±0.021	21.2±0.017
		Control (SA)	17.2±0.032	37.8±0.045	48.2±0.060	10.2±0.040	21.2±0.021	29.6±0.050
		5% PEG (SA)	21.2±0.045	42.3±0.015	56.3±0.040	5.1±0.025	10.2±0.012	16.3±0.021
		10 % PEG (SA)	29.3±0.031	48.4±0.030	59.8±0.021	6.2±0.04	13.8±0.032	24.8±0.010
		15% PEG (SA)	36.2±0.015	51.5±0.517	67.3±0.036	16.2±0.026	23.3±0.067	41.2±0.012
PUSA-AG	Salinity	Control	9.86±0.032	14.5±0.040	19.8±0.021	7.6±0.037	17.8±0.015	21.6±0.021
		50Mm	8.37±0.017	12.13±0.029	17.9±0.038	9.3±0.015	19.6±0.025	27.9±0.044
		100mM	10.2±0.035	19.3±0.015	23.1±0.025	11.6±0.032	22.3±0.015	32.1±0.015
		150mM	12.2±0.015	20.6±0.036	29.0±0.042	16.6±0.02	29.6±0.098	39.7±0.006
		Control (SA)	12.6±0.021	21.7±0.020	30.2±0.032	15.7±0.01	28.8±0.036	38.2±0.042
		50mM (SA)	17.2±0.015	28.6±0.020	37.3±0.015	17.2±0.02	32.6±0.047	42.8±0.036
		100mM (SA)	21.3±0.031	32.8±0.010	41.3±0.015	23.5±0.02	39.8±0.035	48.2±0.044
		150mM (SA)	26.3±0.010	39.2±0.015	48.3±0.015	29.6±0.025	42.7±0.010	52.7±0.020
CS-52	Salinity	Control	10.2±0.015	15.3±0.015	19.9±0.035	6.2±0.03	16.8±0.032	20.1±0.021
		50mM	9.3±0.031	12.2±0.020	18.7±0.023	8.2±0.015	19.3±0.021	27.6±0.031
		100mM	12.4±0.03	19.2±0.025	28.3±0.017	10.2±0.03	21.8±0.026	31.8±0.010
		150mM	29.3±0.015	32.2±0.015	31.3±0.015	16.0±0.030	29.1±0.015	40.2±0.010
		Control (SA)	11.3±0.040	18.2±0.020	36.2±0.015	13.8±0.026	25.2±0.006	35.2±0.017
		50mM (SA)	11.8±0.010	19.3±0.025	42.3±0.020	16.8±0.035	31.2±0.012	40.1±0.025
		100mM (SA)	13.3±0.04	22.3±0.012	53.3±0.025	21.8±0.036	37.8±0.015	47.2±0.032
		150mM (SA)	31.3±0.015	43.2±0.010	61.3±0.020	31.8±0.01	46.7±0.010	53.2±0.021

Therefore, higher GSH/GSSG is considered as supportive for improved abiotic stress tolerances including drought, salinity and their combination also because GSH/GSSG ratio has vital roles in maintaining cellular redox balance and in transduction of stress signals (Gill and Tuteja, 2010; Hasanuzzaman *et al.*, 2012).The value of phytochelatins increased with increased stress levels (Table 2). Increased PCs concentration with more difference against stress in root and shoot in both varieties was observed; Phytochelatins increased in leaves of PUSA-AGRANI and CS-52. SA treated seedlings showed more increase in phytochelatin concentration in shoot and root of tolerant variety as compared to susceptible variety under drought condition. However, plant synthesized the low molecular weight peptide called phytochelatins, which are polymers of GSH. PCs syntheses have been shown to be activated by a broad range of abiotic stresses in particular drought, salinity and some-times our together (Das and Roychoudhury, 2014).

Table 2: Effect of SA application on induced changes in total Phytochelatin (TPC) include data in leaves and roots of *B. juncea* cultivars at 7th day after sowing (mean ± SE of three replicates) under stress conditions

Genotypes	Stress	Concentration	Total phytochelatins in leaves(nM g^{-1})			Total phytochelatins in roots(nM g^{-1})		
			0Hrs	48Hrs	72Hrs	0Hrs	48Hrs	72Hrs
PUSA-AG. (Tolerance)	Drought	Control	14.7±0.021	27.9±0.025	32.1±0.021	11.8±0.010	13.4±0.036	15.3±0.017
		5% PEG	15.8±0.015	28.6±0.032	37.0±0.020	16.1±0.015	18.7±0.015	21.8±0.015
		10 % PEG	20.3±0.021	33.3±0.015	42.6±0.026	19.2±0.025	22.6±0.021	25.8±0.025
		15% PEG	29.6±0.021	37.8±0.010	47.3±0.057	22.6±0.006	26.2±0.021	29.8±0.032
		Control (SA)	19.0±0.036	31.5±0.026	41.2±0.021	14.8±0.015	17.8±0.006	21.8±0.015
		5% PEG (SA)	18.1±0.031	22.9±0.010	26.8±0.015	18.7±0.010	21.7±0.010	26.3±0.010
		10 % PEG (SA)	28.3±0.015	36.2±0.017	45.7±0.021	21.4±0.025	25.5±0.012	29.8±0.012
		15% PEG (SA)	31.7±0.015	41.3±0.010	49.3±0.020	25.8±0.015	28.7±0.015	31.2±0.015
CS-52 (Susceptible)	Drought	Control	13.2±0.015	26.8±0.020	31.6±0.006	10.6±0.021	12.7±0.010	14.7±0.015
		5% PEG	14.8±0.021	28.3±0.030	35.7±0.010	15.4±0.010	17.2±0.025	21.6±0.023
		10 % PEG	19.2±0.035	29.3±0.025	36.7±0.010	18.7±0.023	21.5±0.031	26.5±0.023
		15% PEG	27.4±0.010	35.8±0.030	46.8±0.031	21.6±0.021	25.6±0.053	30.8±0.015
		Control (SA)	18.7±0.010	24.8±0.031	29.7±0.125	13.7±0.026	15.6±0.012	18.2±0.006
		5% PEG (SA)	17.2±0.021	22.7±0.015	26.4±0.035	17.6±0.030	19.8±0.010	23.4±0.031
		10 % PEG (SA)	29.8±0.015	39.7±0.044	45.8±0.006	20.5±0.036	24.8±0.012	27.8±0.015
		15% PEG (SA)	32.9±0.025	43.7±0.026	53.7±0.025	24.5±0.042	27.5±0.026	30.2±0.010
PUSA-AG	Salinity	Control	2.8±0.026	4.7±0.015	6.8±0.010	13.6±0.026	15.3±0.006	19.7±0.015
		50mM	5.4±0.032	8.6±0.038	11.1±0.015	15.0±0.026	17.3±0.012	21.8±0.021
		100mM	8.5±0.031	10.8±0.020	13.8±0.017	18.0±0.031	20.3±0.006	26.7±0.015
		150mM	11.4±0.035	14.7±0.031	16.3±0.012	22.3±0.023	25.3±0.012	29.8±0.026
		Control (SA)	1.2±0.015	2.6±0.021	3.8±0.040	10.2±0.010	13.2±0.010	16.2±0.015
		50mM (SA)	2.7±0.036	4.7±0.012	6.1±0.026	11.6±0.015	15.8±0.021	20.2±0.012
		100mM (SA)	5.9±0.583	8.7±0.020	10.8±0.021	13.6±0.032	18.3±0.015	24.9±0.020
		150mM (SA)	8.5±0.026	10.6±0.010	12.8±0.015	16.3±0.015	21.3±0.012	28.7±0.025
CS-52	Salinity	Control	2.8±0.590	4.3±0.006	6.2±0.064	12.8±0.010	16.3±0.017	21.8±0.015
		50mM	4.8±0.021	7.3±0.015	10.7±0.015	14.8±0.026	19.2±0.017	26.7±0.020
		100mM	7.7±0.015	9.2±0.015	13.7±0.035	17.3±0.012	23.8±0.020	29.7±0.010
		150mM	10.8±0.015	13.2±0.020	17.6±0.010	19.6±0.012	26.8±0.025	30.6±0.025
		Control (SA)	1.1±0.030	2.7±0.032	4.2±0.021	9.5±0.026	13.6±0.026	16.8±0.010
		50mM (SA)	2.6±0.025	4.8±0.015	6.8±0.010	10.2±0.006	15.8±0.040	20.8±0.015
		100mM (SA)	4.6±0.021	7.7±0.020	9.6±0.015	12.7±0.017	18.4±0.012	25.8±0.021
		150mM (SA)	6.5±0.032	9.7±0.067	11.3±0.015	14.3±0.010	22.8±0.020	27.7±0.023

Glutathione-s-Transferase (GST)

The results (Table 3) elucidated that the activity of GST significantly increased in the root and shoot of both cultivars in response to all concentrations of each stress. Comparably, more amount of GST was found in shoot and root of cultivar PUSA-AGRANI than that cultivar CS-52 under all stress conditions.The value of GST increased with increased stress levels. Increased GST concentration with more difference against stress in root and shoot in both varieties was observed; GST level increased in leaves of CS-52 and PUSA-AGRANI. SA treated seedlings showed more increase in GST concentration in shoot and root of susceptible variety as compared to tolerant variety under drought condition. The similar results were observed by Nahar *et al.* (2015) in mung bean under water deficit conditions. In response to increased ROS and membrane injury, Non enzymatic antioxidant activated in oxidative damage such as, Glutathione, Phenolic, Ascorbic acid, Phytochelatins and Glutathione-s-transferees (GST) etc. Such non-enzymatic antioxidants neutralized the reactive oxygen species. GSH provided protection against oxidative stress by reduction of ascorbate via ascorbate-glutathione cycle. Glutathione and Phenolic content significantly decreased and Phytochelatins and GST enhanced under stress conditions. SA application supported antioxidant system to reduce oxidative damage. Tolerant mustard variety showed less oxidative damage compared to susceptible variety.

Phenol

The concentration of phenol decreased consistently with imposed stress. The phenolic content was considerably higher in PUSA-AGRANI genotype in comparison to CS-52.The results are in confirmation with Ali and Abbas,

Table 3: Effect of SA application on induced changes in glutathione-s-tranferees (GST) weight in leaves and roots of *B. juncea* cultivars at 7th day after sowing (mean ± SE of three replicates) under stress conditions

Genotypes	Stress	Concentration	Glutathione-s-transferees in leaves (micro mole /g^{-1} min^{-1})			Glutathione-s-transferees in roots (micro mole /g^{-1} min^{-1})		
			0Hrs	48Hrs	72Hrs	0Hrs	48Hrs	72Hrs
PUSA-AG. (Tolerance)	Drought	Control	8.23±0.068	24.3±0.012	39.3±0.020	4.7±0.085	8.3±0.040	16.2±0.021
		5% PEG	9.4±0.023	26.3±0.020	40.7±0.021	2.8±0.015	5.3±0.030	8.2±0.040
		10 % PEG	13.3±0.023	30.3±0.010	43.8±0.015	5.7±0.012	9.2±0.030	16.3±0.015
		15% PEG	17.3±0.012	35.4±0.015	48.3±0.021	7.8±0.015	13.7±0.006	23.4±0.026
		Control (SA)	17.2±0.015	35.8±0.025	51.2±0.023	12.8±0.059	18.5±0.035	29.3±0.006
		5% PEG (SA)	20.2±0.021	39.4±0.010	54.2±0.012	6.7±0.032	11.3±0.021	23.4±0.012
		10 % PEG (SA)	25.3±0.025	42.2±0.031	58.2±0.036	10.3±0.020	17.3±0.035	34.1±0.025
		15% PEG (SA)	34.3±0.012	48.2±0.035	63.1±0.030	23.2±0.021	24.2±0.026	40.6±0.025
CS-52 (Susceptible)	Drought	Control	7.3±0.026	26.5±0.010	35.2±0.010	3.2±0.036	6.5±0.012	12.7±0.031
		5% PEG	9.8±0.026	28.7±0.038	36.2±0.017	2.7±0.010	4.1±0.021	10.2±0.015
		10 % PEG	15.7±0.010	31.3±0.010	21.2±0.025	4.7±0.026	7.4±0.021	15.3±0.032
		15% PEG	18.2±0.021	37.3±0.015	45.3±0.015	6.6±0.042	13.4±0.015	23.3±0.012
		Control (SA)	18.2±0.025	38.8±0.032	49.3±28.5	11.3±0.017	24.2±0.025	30.6±0.006
		5% PEG (SA)	22.2±0.026	43.4±0.010	57.3±0.021	6.1±0.032	11.2±0.026	17.2±0.020
		10 % PEG (SA)	28.8±0.010	39.4±0.015	60.8±0.031	7.2±0.040	14.8±0.010	25.8±0.031
		15% PEG (SA)	37.4±0.015	53.2±0.042	68.3±0.085	17.6±0.025	24.3±0.015	44.2±0.017
PUSA-AG	Salinity	Control	9.9±0.010	15.5±0.006	21.8±0.015	8.7±0.044	18.7±0.113	22.7±0.006
		50mM	9.3±0.006	15.1±0.025	18.9±0.035	10.3±0.023	20.6±0.015	28.7±0.023
		100mM	11.2±0.006	20.3±0.021	25.1±0.017	12.7±0.021	23.3±0.015	33.2±0.012
		150mM	13.4±0.010	22.7±0.035	30.0±0.035	17.7±0.010	30.7±0.035	40.6±0.010
		Control (SA)	13.7±0.017	22.8±0.015	32.2±0.015	16.7±0.012	27.7±0.023	41.2±0.010
		50mM (SA)	18.3±0.010	29.2±0.015	38.2±0.040	18.4±0.038	33.7±0.040	43.8±0.031
		100mM (SA)	22.6±0.032	34.8±0.032	42.2±0.040	24.5±0.021	40.8±0.025	49.4±0.023
		150mM (SA)	27.5±0.031	40.2±0.015	50.3±0.020	30.7±0.010	43.7±0.020	55.6±0.021
CS-52	Salinity	Control	11.3±0.015	16.5±0.026	20.9±0.012	7.2±0.020	17.9±0.012	23.1±0.026
		50mM	9.5±0.023	13.6±0.015	19.7±0.006	9.3±0.015	20.3±0.015	27.6±0.015
		100mM	13.3±0.015	20.2±0.017	30.6±0.021	11.2±0.035	22.8±0.021	39.8±0.015
		150mM	19.3±0.015	33.7±0.017	32.2±0.020	17.6±0.000	30.2±0.038	42.4±0.026
		Control (SA)	12.3±0.015	20.2±0.044	37.3±0.017	14.7±0.021	26.4±0.021	38.2±0.035
		50mM (SA)	12.8±0.012	20.3±0.012	43.3±0.025	17.7±0.029	33.4±0.021	41.3±0.026
		100mM (SA)	15.2±0.026	24.2±0.040	46.9±6.3	22.8±0.035	39.7±0.017	48.2±0.040
		150mM (SA)	37.3±0.021	47.1±0.036	64.2±0.026	33.8±0.015	50.4±0.032	54.4±0.025

(2003)in barleyand Chaparzadeh and Behboud, (2015) in radish under saline water. In contrast, Pandey and Chikara, (2014) they reported significant increase in the concentration of phenol in response to drought. Also, SA treatment increased phenolic compounds of *Panax ginseng* (Bhardwaz *et al.*, 2015). Further it is also suggested by Bhardwaz *et al.* (2015) that increased phenylalanine ammonia-lyase (PAL) activity could be a response to the cellular damage provoked by higher osmotic concentrations. So, enhancement of PAL activity could be related to the implication of enzyme in the plant response to stresses. It is observed in present work that phenolic compounds, as antioxidants, can reduce the toxic effects of stress and thus prevent physiological damages of plants; however, this is critically dependent on the salt sensitivity of plants. Induced accumulation of phenolic compounds can control the production of H_2O_2, so, these compounds may play an important role in the oxidative stress tolerance of plants (Lu *et al.*, 2007). SA is considered to be a plant signaling molecule that plays a key role in the plant growth, development and defense responses. SA, probably, can induce particular enzymes of the secondary metabolism to produce defense compounds such as phenolic compounds.

SA application improved drought and salinity stress tolerance by reducing the highly reactive oxygen species and enhancing of antioxidative enzymes in Indian mustard. Alleviated PCs, the low activity of TGSH in the roots of SA-treated indian mustard plants indicated a lower level of oxidative stress. Ultimately, these results suggest that the exogenous application of SA assisted the plants to become more tolerant to drought and salinity stress-induced oxidative damage by enhancing their antioxidant defense and non-enzymatic antioxidant system.

REFERENCES

Alam, M.N, Bristi, N.J. and Rafiquzzaman, M. (2013) Review on *in vivo* and in vitro methods evaluation of antioxidant activity. *Saudi Pharmaceutical Journal* 21(2): 143-152.

Ali, R. M. and Abbas, H.M. (2003) Response of salt stressed barley seedlings to phenylurea. *Plant Soil and Environment* 49(4): 158-162.

Anderson, M.E. (1985) Determination of glutathione and glutathione disulphide.*Methods in Enzymology* 13: 548-555.

Bhardwaj, A.R. Joshi, G, Kukreja, B, Malik, V, Arora, P, Pandey, R, Shukla, R.N, Bankar, K,G, Agarwal, S,K, Goel, S, Jagannath. A, Kumar, A. and Agarwal, M. (2015) Global insights into high temperature and drought stress regulated genes by RNA-Seq in economically important oilseed crop *Brassica juncea. BMC Plant Biology* 15(9): 1-15.

Chaparzadeh, N. and Hosseinzad-Behboud, E. (2015) Evidence for enhancement of salinity induced oxidative damages by salicylic acid in radish (*Raphanus sativus* L.). *Journal of Plant Physiology and Breeding* 5(1): 23-33.

Chauhan R, Sanadhya, D. and Kumar A. (2019) Effects of salicylic acid on growth and germination parameters in *in-vitro* raised Indian mustard (*Brassica juncea* L.) genotypes under water stress *Annals of Plant and Soil Research* 21(3): 275-279.

Chauhan, R. and Chaudhary, A. (2018) Recent Breakthroughs in the Study of Salicylic Acid Biosynthesis under Changing Environment: A Review, *International Journal for Research in Applied Science & Engineering Technology (IJRASET)* 6(2): 1107-1122.

Das, K. and Roy Choudhury A. (2014) Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants. *Frontiers in Environmental Science* 2: Article 53 1-13.

Ezaki, B, Suzuki, M. Motoda, H, Kawamura, M. Nakashima, S. and Matsumoto H. (2004) Mechanism of gene expression of Arabidopsis glutathione S-transferase, AtGST1, and AtGST11 in response to aluminum stress.*Plant Physiology* 134: 1672-1682.

Gill, S.S. and Tuteja, N. (2010) Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiology and Biochemistry* 48(12): 909-930.

Hasanuzzaman, M, Hossain, M.A. Da Silva, JAT, and Fujita, M. (2012). Plant response and tolerance to abiotic oxidative stress: antioxidant defense is a key factor. In *Crop stress and its management: Perspectives and Strategies* (Springer, Berlin) pp 261-316.

Hasanuzzaman, M. Nahar, K.Anee, T.I. and Fujita,M. (2017) Glutathione in plants: biosynthesis and physiological role in environmental stress tolerance.*Physiology and Molecular Biology of Plants* 23(2): 249–268.

Khan, M.I.R, Asgher, M. and Khan, N.A. (2014) Alleviation of salt-induced photosynthesis and growth inhibition by salicylic acid involves glycinebetaine and ethylene in mungbean (*Vigna radiata* L.). *Plant Physiology and Biochemistry* 80: 67-74.

Lu, Z, Liu, D. and Liu, S. (2007) Two rice cytosolic ascorbate peroxidases differentially improve salt tolerance in transgenic Arabidopsis. *Plant Cell Reports*, 26: 1909-1917.

Marinova, D, Ribarova, F. and Atanassova, M. (2005) Total phenolics and total flavonoids in Bulgarian fruits and vegetables.*Journal of the University Chemical Technology Metallurgy*40: 255-260.

Nahar, K, Hasanuzzaman, M, Alam, M.M. and Fujita, M. (2015) Exogenous glutathione confers high temperature stress tolerance in mung bean (*Vigna radiata* L.) by modulating antioxidant defense and methyl glyoxal detoxification system. *Environmental and Experimental Botany*, 112: 44-54.

Nahar, K, Kyndt, T, Hause, B, Höfte, M. and Gheysen, G. (2013) Brassinosteroids suppress rice defense against root-knot nematodes through antagonism with the jasmonate pathway. *Molecular Plant-Microbe Interactions* 26(1): 106-115.

Pandey, M. and Chikara, S.K. (2014) In vitro Regeneration and effect of abiotic stress on physiology and bio chemical content of *Stevia rebaudiana* 'Bertoni'.*Journal of Plant Science and Research* 1(3): 1-9.

Shahnawaz, M.D. Chauhan, R. and Sanadhya, D. (2017) Impact of aluminum toxicity on physiological aspects of barley (*Hordeum vulgare* L.) cultivars and its alleviation through ascorbic acid and salicylic acid seed priming, *International Journal of Current Microbiology and Applied Science*6 (5): 875-891.