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Research Article

EFFECT OF IODINE ON NON-ENZYMATIC ANTIOXIDANT LEVELS OF *GLYCINE MAX* L. GROWN UNDER HEAVY METALAND HEAT STRESS

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ABSTRACT

Objective: The present research work was designed response of iodine on non-enzymatic antioxidant levels grown under heavy metal and heat stress in soybean.

Methods: Soybean seeds exposed to different concentrations, three of which were applied in increasing doses of IO_3– (20, 40, and 80 µM as KIO_3^-) with heavy metal and heat stresses were subjected to analysis of non-enzymatic biochemical parameters including ascorbic acid, proline, glutathione using the standard protocols.

Results: The application of IO_3^- can improve the levels of non-enzymatic antioxidant levels to severe cadmium chloride and heat stress in soybean seeds (*Glycine max* L). Our study recommends that IO_3^- could be considered a possible beneficial element to counteract the harmful effects of cadmium chloride and heat stress.

Conclusion: Our study recommends that IO_3^- could be considered as a possible beneficial element to counteract the harmful effects of cadmium chloride and heat stress.

Keywords: *Glycine max* L., Heavy metal stress, Heat stress, Ascorbic acid, Proline, Glutathione, Iodine.

INTRODUCTION

Heavy metal and heat stresses are important threat limitations to plant growth and sustainable agriculture worldwide. Abiotic stresses are often interrelated, either individually or in combination, they cause morphological, physiological, biochemical, and molecular changes that adversely affect plant growth and productivity and ultimately yield. Heat, drought, cold, and salinity are the major abiotic stresses that induce severe cellular damage in plant species including crop plants [1]. Currently, it is estimated that 250-300 thousand hectares of agricultural land is lost every year to heavy metal contamination. Heavy metal concentration has increased in soil, surface water, and posed potential threat to terrestrial and aquatic biota [2]. Cadmium (Cd^{2+}) is a strongly phytotoxic heavy metal that inhibits plant growth and leads to plant death as studied in a number of plants. The antioxidative response due to oxidative stress induced by Cd^{2+} varies in different plants and is dependent on the concentration of Cd^{2+}. Interestingly, Cd^{2+} hyperaccumulator plants show strong tolerance to oxidative stress [3].

Heat stress during key reproductive events, including pollination and seed development, has been demonstrated to be a major factor limiting soybean seed yield [4,5]. Significant, complex interactions of temperature, drought, and increased carbon dioxide are expected for future climatic conditions, but predictions on the impact and severity of these stresses on soybean yield are inconsistent [6,4].

Iodine is vital to human health, and iodine biofortification programs help improve the human intake through plant consumption. Iodine is applied in two forms; one as potassium iodide (KI) and another as KIO_3. Research has shown the successful use of KI as a chemical desiccant first for the screening of drought tolerant upland rice varieties at the reproductive stage [7] and second in simulating the effect of terminal drought by chemical desiccants during grain filling [8].

The application of iodine in the form of iodate influences the antioxidant capacity of a number of plants such as lettuce and tomato [9,10]. Our principle goal was to study the effect of application of iodine in the form of potassium iodate on the antioxidant system of *Glycine max* L. seeds.

METHODS

Plant materials and growth conditions

The experiment was conducted the rainy season in 2013 field of the Sharda University, Department of Biotechnology, Greater Noida, India. Seeds of soybean were procured from the Indian Agriculture Research Institute, Pusa, New Delhi. Seeds were sown in pots carrying soil and cow dung manure in the ratio 3:1. 10 days old seedlings having four-five true leaves were displaced and preincubated in a growth chamber at 35°C/33°C (day/night), and 12/12 h (light/dark) photoperiod with light supplied at an intensity of 72 µmol $m^{-2}s^{-1}$ for 3 days. Three of which were applied in increasing doses of IO_3^- (20, 40, and 80 µM as KIO_3^-) [11]. Two controls were kept. One was supplied with heat and another was administered only Hoagland Nutrient solution. Iodine was supplied in Hoagland Nutrient Solution for 1 month (alternate days). The young plants were harvested after 15 days of the last treatment. The seeds were collected and wrapped in an aluminum foil and stored at −20°C for further study.

Seed growth and germination

Seeds after harvesting were measured for their size and mass. Standard blotter technique was followed for seed germination percentage of soybean. For germination percentage, pure soaked seeds of each sample were spread over a wet filter paper in a Petri plate. The entire setup was placed in dark for 72 h. Seeds were observed for germination at an interval of 4 h till 3 days.

Non-enzymatic antioxidant assay

Determination of ascorbic acid (AsA)

AsA content was measured [12] 0.1 g seed homogenized in 10 ml of 0.4% oxalic acid and then centrifuged at 8000 rpm at 4°C for 15 minutes 500 µl of supernatant was taken in a tube and 7 ml of

2,6- dichloroindophenol dye solution was added to the same tube. The absorbance was taken at 518 nm in a spectrophotometer. The results were expressed as mmol total AsA g^{-1} fresh weight (FW).

Determination of proline

Proline content was determined based on the method of Bates *et al.* [13]; 0.1 g of seed was homogenized with 2 ml of 3% aqueous sulfosalicylic acid and centrifuged at 10,000 rpm for 10 minutes, 1 ml of supernatant were mixed with 1 ml of glacial acetic acid and 1 ml of acid ninhydrin for 1 h at 100°C. The developed color was extracted in 2 ml toluene and measured at 520 nm.

Determination of glutathione (GSH)

Total GSH was determined by the GSH recycling method of Anderson [14]. Fresh seed (0.1 g) was homogenized in 2 ml of 5% sulphosalicylic acid at 4°C. The homogenate was centrifuged at 10,000 rpm for 10 minutes. To a 0.5 ml of supernatant, 0.6 ml of reaction buffer (0.1 M Na-phosphate, pH 7, 1mM EDTA) and 50 µl of 3 mM 5, 5-dithiobis-2-nitrobenzoic acid were added and read at 412 nm after 5 minutes.

Statistical analysis

All the experiments were performed in triplicate. Values in the tables indicate mean values ± SD. Differences among treatments were analyzed by two-way analysis of variance with multiple observations, taking $p<0.05$ as significant according to Fisher's multiple range test.

RESULTS AND DISCUSSION

The major challenge imposed by heavy metal stress on plants is reduction in growth and, therefore, major loss in yield. Biomass reduction is one of the major parameters of agricultural indices for defining heavy metal stress tolerance. Here, in our experiment, the maximum tolerance of *G. max* seeds was studied in the pots containing Hoagland Nutrient Solution with 100 mM of $CdCl_2$ treated with 80 µM of IO_3^-.

Non-enzymatic antioxidants such as AsA, proline, and GSH constitute an important plant defense system against environmental stresses [15,16]. They are found abundantly in all cell compartments. AsA and GSH are the substrates of Halliwell-Asada pathway and work to keep the levels of H_2O_2 under control. Our present findings show an increase in the activity of both AsA and GSH. The maximum concentration of AsA was reached in 80 µM of IO_3^- perhaps to balance the O_2 accumulation with this dosage. GSH reached its peak value at 20 µM IO_3^- exhibiting an increase of 21% with respect to 100 mM treatment of $CdCl_2$. Proline has been shown to play an important role in recovering from environmental stresses in plants, and its accumulation might be induced as a result of reactive oxygen species (ROS). The mechanism by which proline reduces oxidative damage include physical quenching of singlet oxygen and chemical reaction with hydroxyl radicals [17,18]. Proline, an important osmolyte, was also measured, and it showed an increase of 16% in 100 mM $CdCl_2$ treatment against control. Among the combination dosages, it reached its peak value in 20 µM IO_3^- $CdCl_2$ treatment (Table 1) (Fig. 1).

The major results of exposure of heavy metal stress to plants are reduction in biomass and hence yield. Under stress conditions, transfer of electrons leads to the free radical generation. These electrons damage DNA, lipids, and proteins.

In the case of heat stress, non-enzymatic antioxidants, AsA, and GSH are the substrates of Halliwell-Asada pathway and work to keep the levels of H_2O_2 under control. Our present findings show an increase in the activity of AsA against control, similar to the activity of GSH which also increased against control. The maximum concentration of AsA was reached in 80 µM of IO_3^- perhaps to balance the O_2 accumulation with this dosage. GSH reached its peak value at 80 µM IO_3^- exhibiting an increase of 45% with respect to heat treatment. Proline, an osmolyte, has been shown to play an important role in recovering from environmental stresses in plants and its accumulation is induced as a result of ROS. Proline, an important osmolyte, was also measured, and it showed an increase of 72% in heat treatment against control. Among the combination dosages, it reached its peak value in 40 µM IO_3^- heat treatment (Table 2) (Fig. 2). Our findings are in agreement of the findings of Leyva *et al.* [17], where they showed the beneficial effects of low concentration of iodine (>40 µM) in lettuce plant under 100 mM NaCl stress.

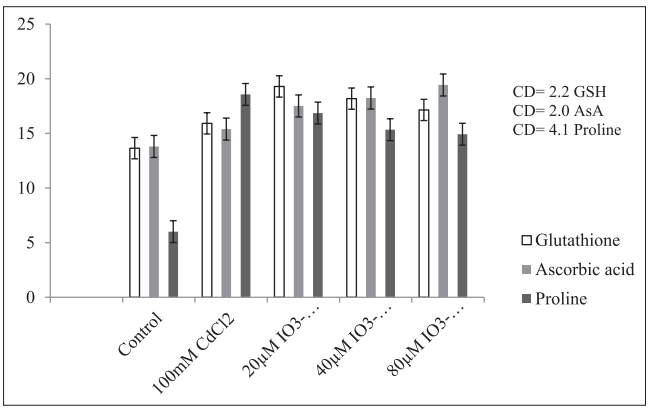

Fig. 1: Effect of IO_3^- application on (A) glutathione (GSH), (B) ascorbic acid (AsA), and (C) proline activities in seeds of *Glycine max* L. plants under heavy metal stress. Data represent mean value (n = 5) for one control and three treatment each of three replicate. (CD = coefficient of determination)

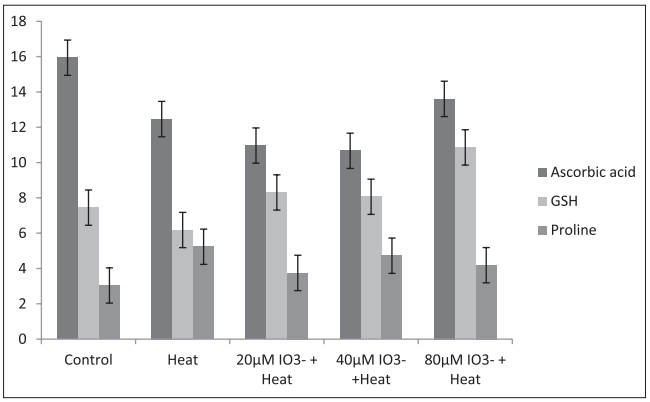

Fig. 2: Effect of IO_3^- application on (A) AsA (B) GSH and (C) proline activities in seeds of *Glycine max* L plants under heat metal stress. Data represent ± SE of value for one control and three treatment each of three replicate

Table 1: The effect of iodate supplementation on AsA, GSH, and proline concentration in $CdCl_2$-stressed *Glycine max*-L

Treatments		AsA (mmol. g^{-1}FW)	GSH (mmol. G^{-1} FW)	Proline (µg g–1 FW)
IO_3^- (µM)	$CdCl_2$ (mM)			
0	0	13.804±0.437	13.646±0.553	16.006±0.802
0	100	15.392±0.198	15.917±0.241	18.566±0.0665
20	100	17.511±0.395	19.294±0.202	16.856±0.460
40	100	18.237±0.179	18.180±0.150	15.336±0.589
80	100	19.428±0.094	17.143±0.110	14.923±0.683
Mean±SE		0.332	0.363	0.592
CD		2.018	2.210	3.599

SE: Standard error, CD: Coefficient of determination, GSH: Glutathione, FW: Fresh weight, AsA: Ascorbic acid

Table 2: The effect of iodate supplementation on AsA, GSH, and Proline concentration in Heat stressed *Glycine max*-L seeds

Treatments		AsA (mmol. g^{-1}FW)	GSH (mmol. g^{-1} FW)	Proline (µg g^{-1} FW)
IO_3^- (µM)	Heat			
0	0	15.94±0.312	7.448±0.814	3.039±0.178
0	Heat	12.464±0.357	6.178±0.075	5.231±0.288
20	Heat	10.963±0.520	8.309±0.814	3.747±0.221
40	Heat	10.666±1.056	8.062±0.334	4.722±0.284
80	Heat	13.606±0.168	10.859±0.043	4.187±0.190
Mean±SE		0.672	0.587	0.223
CD		4.087	3.569	1.356

SE: Standard error, CD: Coefficient of determination, GSH: Glutathione, AsA: Ascorbic acid

Dai *et al*.,[19] have shown that iodine when supplied in the form of IO_3^- has positive effects on the biomass of plants used for their edible leaves. Our results are almost in agreement with Dai's findings showing that supply of iodine in the form of IO_3^- increased the antioxidant response of *G. max* seed proteins.

Dai *et al*. have shown that iodine when supplied in the form of IO_3^- has positive effects on the biomass of plants used for their edible leaves.

CONCLUSION

IO_3^- also played a significant role in increasing the concentration of AsA and GSH. Our study strongly recommends that use of low concentration of IO_3^- (20 µM) improves the response of *G. max* L. under 100 mM $CdCl_2$ stress. Further research is needed in this respect to prove the beneficial effects of exogenous application of iodine in eliminating or suppressing the harmful effects of other stresses.

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