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Comparative Analysis of Defence Response of Soybean by Seed Soaking in Gibberellic Acid to Salinity

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ABSTRACT

The present study was conducted to determine the effects of gibberellic acid (GA_3) presoaking of soybean (*Glycine max* L.) seeds on the biomass yield, regulation of reactive oxygen species and some antioxidant enzyme activities in radicle, hypocotyl and cotyledons under salinity. Within this scope, the seeds were subjected to priming with 150 ppm GA_3 for 6 h at room temperature. Then, 150 mM NaCl was added in a group and the control was not primed with GA_3. The results clearly revealed that salt stress caused a slight induction of biomass yield of radicle and hypocotyl, photosynthetic pigments in cotyledon, reactive oxygen species content in all organs. Moreover, GA_3 pretreatment alleviated salt-induced oxidative damage by maintaining superoxide dismutase, ascorbate peroxidase and glutathione reductase enzyme activities in soybean plants. This study firstly elucidates the effects of GA_3 priming on the comparative response mechanisms to salinity with different organs of soybean plants.

Key words: Gibberellic acid, Reactive oxygen species, Salinity, Soybean.

INTRODUCTION

Salinity is a major factor limiting crop productivity. Specifically, over seven percentage of the world's total land and approximately twenty percentage of irrigated land is affected by high salinity (Munns and Gilliham, 2015). In the soil, high salinity deposition leads to osmotic and ion effects, which further caused oxidative stress in plants. The production of reactive oxygen species (ROS) due to salinity denatures the proteins, enzymes, bio-membranes and nucleic acids (Gomez *et al.,* 2004). To cope with oxidative stress, plants have developed an antioxidant defense system, comprising of antioxidant enzymes like superoxide dismutase (SOD), ascorbate peroxidase (APX), peroxidase (POX), catalase (CAT) and glutathione reductase (GR). Salinity regulates these enzymes depending on the plant species, age, degree of stress induced damage and exposure time to stress. Most studies proved that tolerant plant genotypes usually have a better antioxidant defense system under salt stress conditions. Beside this, plants also adapt various biochemical and molecular strategies such as compartmentalization into vacuoles, maintained uptake of ions and accumulating solutes and induced phytohormone levels.

Phytohormones are molecules produced in very low concentrations but able to regulate all cellular processes in plants. Abiotic stress conditions caused to the production of signaling molecules that induce several metabolites including phytohormones like gibberellic acid (GA), abscisic acid (ABA), ethylene (ETH), auxin (IAA), cytokinin (CTK), brassinosteroid (BR) for stress tolerance. Their role in salinity stress is important in modulating physiological responses that lead to the adaptation of plants to stress conditions. Additionally, these hormones have significant effects on plant antioxidant enzyme activities to resist the effects of salinity.

Gibberellins (GAs) are a class of phytohormones that control plant growth process including seed germination, leaf expansion, flower initiation, fruit development (Li *et al.,* 2010). However, recent investigations determined that GAs are used to be a regulator to increase stress tolerance in plants. Presoaking treatment or exogenous spraying of GAs in some crops has improved seed performance under salinity (Leite *et al.,* 2003; Saeidi-Sar *et al.,* 2013; Younesi and Moradi, 2014; Sofy *et al.,* 2016; Jiao *et al.,* 2019; Miladinov *et al.,* 2019).

Soybean [*Glycine max* (L.) Merr.] is an important member of the family of Leguminosae, because of its high-quality protein and oil for livestock and human. Although some reports have been determined the positive effects of gibberellic acid on soybean plants there is no data available about the effects of presoaking GA_3 with seeds on the ROS regulation and antioxidant enzymes in cotyledon, radicle and hypocotyl. This is the first report of seed priming of soybean with GA_3 and the change in antioxidant defense system and ROS maintenance in organs.

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MATERIALS AND METHODS

Seeds of soybean cv. SA88 were obtained from a commercial provider (Agarova, Adana, TR). This study was conducted in 2019 at the Department of Biology Laboratory, Sinop University to determine the effect of GA_3 applied on soybean seeds before germination. The seeds of the selected cultivar were sterilized in 5% sodium hypochlorite for 10 min and washed with distilled water. Then, the seeds were subjected to priming with 150 ppm GA_3 for 6 h at room temperature. Non primed seeds were used as the control group. 50 seeds were placed in petri dishes with double-layer filter paper in the presence of 150 mM NaCl in darkness. These concentrations of GA_3 (50, 100, 150 ppm) and NaCl (50, 100, 150 mM NaCl) and duration (6h, 12h, 24h) were determined with previous experiments (data not shown). Germinated seeds were checked up for 10 days. Radicle, hypocotyl and cotyledons were harvested and stored at -80°C. The experiment consisted of 3 replicates.

Biomass analysis was examined after exposure to salt for 10 days; fresh and dry weight (FW and DW) were measured. Samples were dried in the oven at 70°C for 48 hours for dry weight (DW) calculations, (Bohm, 1979). The chlorophylls and carotenoids content of samples was measured by the method specified by Lichtenthaler and Wellburn (1983).

The level of lipid peroxidation in samples was determined in terms of the malondialdehyde (MDA) content according to the method specified by Madhava Rao and Sresty (2000). The MDA content, an end product of lipid peroxidation, was determined by using the thiobarbituric acid reaction. The MDA concentration was calculated from the absorbance at 532 nm and measurements were corrected for nonspecific turbidity by subtracting the absorbance at 600 nm. An extinction coefficient of 155 mM^{-1} cm^{-1} was used to determine the MDA concentration.

The H_2O_2 content was determined according to Velikova *et al*. (2000). Fresh samples (0.1 g) were homogenized in 5ml of 0.1% trichloroacetic acid (TCA) and centrifuged at 12.000 rpm for 15 minutes.

For protein and enzyme extractions, 0.5 g of fresh samples were homogenized in 1.5 ml of 50 mM sodium phosphate buffer (pH 7.8). Samples were centrifuged at 14.000 × g for 30 min and supernatants were used for the determination of protein content and enzyme activities. The total soluble protein contents of the enzyme extracts were determined according to Bradford (1976) using bovine serum albumin as a standard. Superoxide dismutase (SOD; EC 1.15.1.1) activity was assayed based on its ability to inhibit the photochemical reduction of nitrotetrazolium blue chloride (NBT) at 560 nm (Beauchamp and Fridovich, 1973). Ascorbate peroxidase (APX; EC 1.11.1.11) activity was measured according to Nakano and Asada (1981). Glutathione reductase (GR; EC 1.6.4.2) activity was determined according to Foyer and Halliwell (1976). All operations were performed at 4°C.

Statistical analysis

The experiment was conducted in a completely randomized design and measurements were performed with 6 replicates (n = 6). Statistical variance analysis of the data was performed using ANOVA and differences among treatments were compared using Turkey's posthoc analysis with least significant differences at the 5% level.

RESULTS AND DISCUSSION

Table 1 reveals that (FW) of radicle and hypocotyl under salinity was reduced by 62.22% and 64.23% as compared to controls respectively. It has been known that salinity reduced biomass yield seriously in plants and many papers have determined this. In the results, the pretreatment of GA_3 alleviated this value by 29.41% and 2.58 fold compared with salt stress alone. Similar to these results, Maggio *et al*. (2010) reported that GA_3 induced growth in tomato plants under salinity. This could be explained by the roles of GA_3 in cell division, elongation. However, the FW of cotyledon was not affected by salt stress in this experiment. These results also agree with the reports of Ruffino *et al.* (2010) who determined the less damage of salt stress on cotyledons of quinoa plants. Considering the DW of soybean organs, the highest reduction was in the radicle by 83.33% while it was 60% in the hypocotyl. In accordance with these findings, Bai *et al*. (2019) determined that there was a slight reduction in DW values in soybean with salinity. Similar to the fresh weight results, DW of cotyledons also was not changed with stress. This finding indicated that cotyledons have different tolerance capacity comparing with the hypocotyl. Nevertheless, the pretreatment of GA_3 increased this value by 4 fold in radicle and 50% in the hypocotyl. In recent years,

Table 1: The effects of pretreatment of gibberellic acid on fresh and dry weight on radicle, hypocotyl and cotyledon of soybean (*Glycine max* L.) seedlings under salt stress.

	FW (g)			DW (g)		
Treatments	Radicle	Hypocotyl	Cotyledon	Radicle	Hypocotyl	Cotyledon
C	0.045 ± 0.002^{a}	0.26 ± 0.01^{a}	0.347 ± 0.002^{a}	0.006 ± 0.01^{a}	0.02 ± 0.03^{a}	0.088 ± 0.02^{a}
GA_3	0.043 ± 0.002^{a}	0.24 ± 0.03^{a}	0.366 ± 0.003^{a}	0.003 ± 0.01^{b}	0.014 ± 0.02^{a}	0.087 ± 0.01^{a}
S	0.017 ± 0.001^{b}	0.093 ± 0.04^{b}	0.308 ± 0.02^{a}	0.001 ± 0.03^{c}	0.008 ± 0.03^{b}	0.090 ± 0.04^{a}
GA_3 + S	0.022 ± 0.003^{c}	0.24 ± 0.08^{a}	0.361 ± 0.03^{a}	0.004 ± 0.02^{b}	0.012 ± 0.01^{c}	0.090 ± 0.02^{a}

Control (C), Gibberellic acid (GA_3), Salt stress (S), Gibberellic acid + Salt stress (GA_3+S). Columns with different letters represent significantly different ($P < 0.05$) values of soybean (*Glycine max* L.) seedlings under salt stress.

Chauhan *et al.* (2019) determined that GA_3 treated seeds of oat cultivars showed a slight increase in biomass yield. From these results, it could also be said that GA_3 had a positive effect on soybean organs under salinity.

The pigment content of cotyledons was showed in Table 2. In our results, salinity inhibited photosynthetic pigments in cotyledons significant. This fact had already been proved by a researcher that salt stress had negative effects on photosynthetic mechanisms in soybean (Khan, 2018). This is also an agreement with the results of MDA and H_2O_2 contents in cotyledons (Table 3). However, treatment with GA_3 on soybean seeds improved photosynthetic capacity in cotyledons. It could be suggested that this hormone might play a role in increasing organic matter and maintain water balance in cotyledons. In parallel with this result, Al Mahmud *et al.* (2019) said that exogenous GA_3 induced chlorophyll content in wheat plants under drought conditions.

In the results, MDA content was increased with salt stress (150 mM NaCl) by 11.75%, 32.53%, 48.65% in different soybean organs (cotyledon, radicle and hypocotyl) but GA_3 pretreatment reduced this levels under stress condition and the highest reduction (40.36%) was determined in hypocotyl (Table 3). It can be concluded that GA_3 lead to an improvement in hypocotyl rather than cotyledon and radicle of soybean.

The present study revealed that salt stress-induced SOD enzyme activity in each part of soybean plants. This result was the agreement with the increased MDA contents with salinity (Table 3). Fig 1 reveals that SOD enzyme activities were increased by 5.95 fold, 42.85% and 3.08 fold with salt treatment as compared to control groups in cotyledon, radicle and hypocotyl but decreased significantly by 64.7%, 50% and 59.25% with GA_3 and salt application according to salt stress alone. In accordance with our results, Amor *et al.* (2019) reported that SOD enzyme activity was induced with salinity in *Cakile maritima*. In the present study, GA_3 pretreated plants have decreased SOD activity according to NaCl treated ones. It is suggested that GA_3 may play a role as an antioxidant instead of SOD enzyme. These results also agree with the reports of Chakrabarti and Mukharji (2003) and Ahmad (2010) who reported that there was a decreased activity of SOD enzyme with GA_3 treatment in *Vigna radiata* and mustard plants respectively. In contrast, Sofy (2016) determined that GA_3 primed soybean seeds had a higher SOD activity according to controls. This paradox can be explained with the differences in concentration of GA_3 and NaCl with different cultivars of soybean.

Similar to the results of MDA, salinity also induced H_2O_2 levels by 22.1%, 15.48% and 10.88%, while it was decreased by GA_3 before the application. This amelioration was the highest in radicle by 24.8% (Table 3). Otherwise, there was no change in APX enzyme activity in cotyledon and radicle with salinity, while it was increased remarkably by 3.33 fold in hypocotyl (Fig 2). In contrast, GA_3 treatment induced this activity in cotyledon and radicle but decreased by 33.33%

Table 2: The effects of pretreatment of gibberellic acid on chlorophyll (CHL) and carotenoids content on cotyledons of soybean (*Glycine max* L.) seedlings under salt stress.

Treatment	CHL (mg g^{-1} FW)	Carotenoids (mg g^{-1} FW)
C	39.34 ±1.26^{a}	4.93±0.48 ^{a}
GA_3	38.26±1.22^{a}	8.92±1.20^{b}
S	8.60±2.30^{b}	1.75±0.21^{c}
GA_3 + S	43.18±1.35^{c}	3.70±0.021^{a}

Control (C), Gibberellic acid (GA_3), Salt stress (S), Gibberellic acid + Salt stress (GA_3+S). Columns with different letters represent significantly different ($P < 0.05$) values of soybean (*Glycine max* L.) seedlings under salt stress.

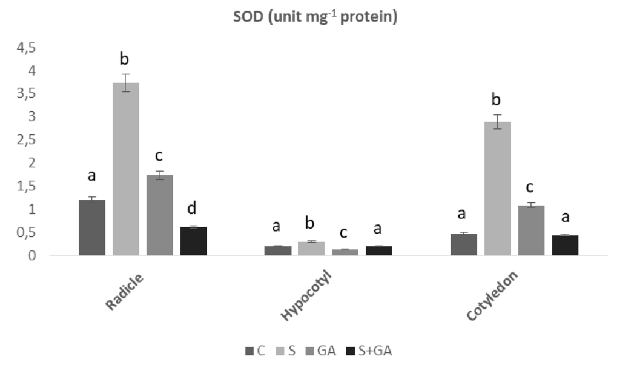

Fig 1: The effects of pretreatment of gibberellic acid on SOD (superoxide dismutase) enzyme activity of soybean (*Glycine max* L.) seedlings under salt stress.

Table 3: The effects of pretreatment of gibberellic acid on hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) content on radicle, hypocotyl and cotyledon of soybean (*Glycine max* L.) seedlings under salt stress.

	H_2O_2 (µmol / g^{-1} FW)			MDA (nmol / g^{-1} FW)		
Treatments	Radicle	Hypocotyl	Cotyledon	Radicle	Hypocotyl	Cotyledon
C	73.6 ±1.2^{a}	57.53 ±0.53^{a}	17.6±0.87^{a}	12.6±0.9^{a}	14.9±0.51^{a}	18.3±0.4^{a}
GA_3	74.1 ±1.5^{a}	59.00 ±0.67^{a}	17.4 ±0.54^{a}	13±0.4^{a}	14.8±0.64^{a}	19.1±0.9^{a}
S	85 ±1.9^{b}	66.01 ±0.47^{b}	21.5 ±0.91^{b}	16.7±0.3^{b}	22.15±0.81^{b}	22.35±0.7^{b}
GA_3 + S	63.9±1.3^{c}	59.72±0.42^{a}	18.9±0.37^{a}	13.06±0.6^{a}	15.21±0.46^{a}	18.8±0.5^{a}

Control (C), Gibberellic acid (GA_3), Salt stress (S), Gibberellic acid + Salt stress (GA_3+S). Columns with different letters represent significantly different ($P < 0.05$) values of soybean (*Glycine max* L.) seedlings under salt stress.

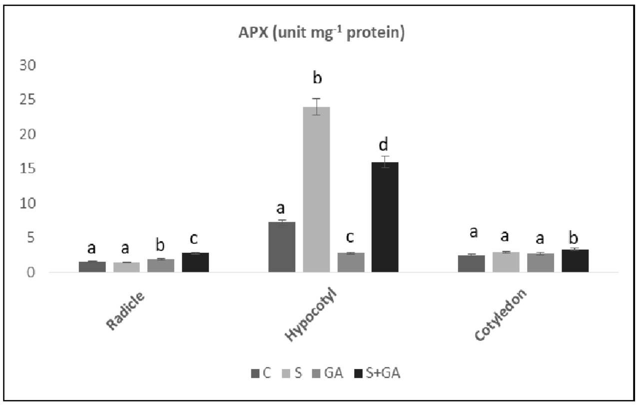

Fig 2: The effects of pretreatment of gibberellic acid on APX (ascorbate peroxidase) enzyme activity of soybean (*Glycine max* L.) seedlings under salt stress.

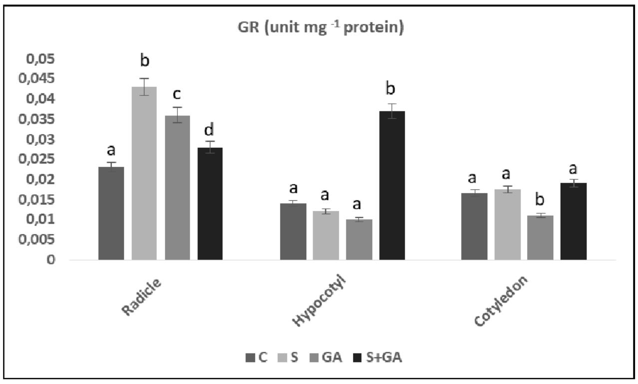

Fig 3: The effects of pretreatment of gibberellic acid on GR (glutathione reductase) enzyme activity of soybean (*Glycine max* L.) seedlings under salt stress.

in hypocotyl as compared to salt stress alone (Fig 4). Similarly, Xie *et al.* (2008) and Verma and Mishra (2005) determined that salt stress did not affect the APX enzyme activity in *Brassica juncea* and *Gossypium hirsutum* plants. Nevertheless, there was a significant increase in APX activity as it determined by Weisany *et al.* (2012) in soybean plants. However, in the present study, GA_3 treated plants under salinity showed an increased APX activity in cotyledon and radicle according to salt stress alone. This result is also agreement with the reports of Younesi and Moradi (2014) in *Medicago sativa.* Also, it was determined that the GA_3 application reduced APX enzyme activity in hypocotyl while it was induced with salt stress. This result showed that GA_3 plays antagonist action with salt stress in soybean plants to increase salt tolerance.

Salt stress-induced GR enzyme activity by 86.95% in radicle but did not affect in cotyledon and hypocotyl. However, GA_3 pretreatment under stress-induced this activity in cotyledon (11.76%) and hypocotyl (3.08 fold), while decreased in radicle according to salt stress groups (Fig 3). These results also agree with the reports of the Yassin *et al.* (2019) and Srivastava *et al.* (2015) in bread wheat and *Sesavium portulacastrum*. Otherwise, GA_3 increased GR activity under salinity in cotyledon and hypocotyl, although it was decreased in radicle. Following the evaluation of the all results, the role of GA_3 as an antioxidant (by reducing ROS level) and signal molecule (by inducing antioxidant enzymes activities) in soybean plants under salinity is observed explicitly.

CONCLUSION

Although GA_3 has been known as a growth regulator for plants they also involved in alleviating salt stress-induced damage. Totally, in the present study, to treat soybean seeds with GA promoted response mechanisms to salinity in soybean plants. It was showed that GA_3 protects soybean plants from salinity and induced biomass yield and photosynthetic capacity significantly as well as maintains the antioxidant enzyme system and lead to reduce oxidative damage. Collectively, this is the report of GA_3 which determined that might behave as a protector in different organs of soybean plants.

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