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ORIGINAL ARTICLE

Improvement of Salt Tolerance in *Trigonella foenum-graecum* L. var. PEB by Plant Growth Regulators

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The crop yield is reduced under saline conditions and this hampers agricultural productivity. The incorporation of plant growth regulators (PGRs) during presoaking treatments in many crops has improved seed performance under saline conditions. In order to study the ameliorative effect of plant growth regulators, experiments were conducted to study the variation in organic constituents in the leaves of *Trigonella foenum-graecum* L. var.PEB, where the seeds were primed with different plant growth regulators and grown under NaCl salinity. After a pre-soaking treatment of six hours in 20 mg L^{-1} solutions of gibberllic acid (GA_3), 6-furfuryladenine (Kinetin) and benzyl adenine (BA), the seeds were allowed to germinate and grow for forty-five days under saline conditions. On the analysis of mature leaves, it was observed that chlorophyll a and b, total chlorophyll and protein showed an increase in PGR-treated plants compared to the untreated set. The accumulation of the stress metabolite such as proline and sugars, which increase under saline conditions, showed a significant decrease in the plants pretreated with PGRs.

Key words: Benzyle adenine, Gibberellic acid, Kinetin, Plant growth regulators, Salinity

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Key words: Benzyle adenine, Gibberellic acid, Kinetin, Plant growth regulators, Salinity

Salinity exists naturally in arid and semiarid regions of the world. The incorporation of plant growth regulators (PGRs) during presoaking treatments in many crops has improved seed performance under saline conditions (Akbari *et al*., 2007; Gurmani *et al*., 2009). It is also possible that under highly saline conditions, naturally present hormones are suppressed and seed soaking with plant growth regulators helps to ameliorate the adverse effects of salinity by supplying hormones for normal growth (Afzal *et al.*, 2005). Phytohormones are known to influence a number of physiological processes, including enzyme activation (Bera *et al*., 2006). According to Jamil and Rha (2007), one of the most effective ways to overcome the problems of salinity is the use of plant growth regulators. Chauhan *et al*. (2009) suggested that plant growth regulators help in overcoming the harmful effects of salinity on growth by changing the endogenous growth regulators which affect plant water balance.

In order to study the ameliorative effect of plant growth regulators, experiments were conducted to study the variation in organic constituents in the leaves of *Trigonella foenum-graecum* L. Var. PEB, a leafy vegetable. The seeds were primed with different plant growth regulators and grown under NaCl salinity.

MATERIALS AND METHODS

Seeds of *Trigonella foenum-graecum* L. were obtained from Indian Agricultural Research Institute, Pusa, New Delhi. After a pre-soaking treatment of six hours in 20 mg L^{-1} solutions of gibberellic acid (GA_3), 6-furfuryladenine (Kinetin) and benzyl adenine (BA), seeds were sown in earthen pots (20 cm diameter, 29 cm height). The pots were irrigated using tap water till the phase of germination. A pot served as control where the seeds were irrigated with tap water. Remaining pots were irrigated with NaCl solutions. In order to acclimatize the plants to NaCl, the concentrations were raised after every three-four days in a stepwise manner starting from 5 mM till a final concentration of 60 mM NaCl. The plants were grown for forty-five days. Mature leaves of these plants were used to estimate organic constituents like chlorophyll a, chlorophyll b, total chlorophyll, reducing sugars, non-reducing sugars, proteins, proline, β-carotene, thiamine, riboflavin and ascorbic acid using standard protocols of Arnon (1949), Miller (1959), Lowry *et al*. (1951), Bates *et al*. (1973), AOAC (1980), Strohecker and Henning (1966) and Birch *et al*. (1933) respectively.

F test was used to test for the statistical significance and Student's *t*-test was use to compare the treatment means.

RESULTS AND DISCUSSION

Chlorophyll:

In *Trigonella foenum-graecum* leaves, a decrease in chlorophyll a as well as chlorophyll b was observed with a significant reduction of 30 percent in total chlorophyll content at 60 mM NaCl concentration as compared to control. Mane *et al*. (2010) related the decrease in chlorophyll content under NaCl salinity to the disruption in cellular functions and membrane deterioration. Disrupted photosynthetic electron transport chain or the instability of the pigment protein complex with increased activity of chlorophyllase may also be the reason for decrease in chlorophyll content under saline conditions (Mane *et al*., 2010). Presoaking *Trigonella foenum-graecum* seeds in GA_3, has improved chlorophyll a, chlorophyll b and total chlorophyll contents in leaves as compared to unprimed seeds grown at 60 mM NaCl concentration (Table 1). Similarly, Kinetin pretreatment resulted in an increase in chlorophyll a, chlorophyll b and total chlorophyll content. An ameliorative effect of BA was also found in *Trigonella foenum-graecum*, as chlorophyll a, chlorophyll b and total chlorophyll contents were observed to increase by about 21, 27 and 21 percent respectively. Zeid (2011) also reported an alleviation of adverse effects of salinity on chlorophyll content in barley with GA_3 treatment.

It is evident from the above mentioned data that in the present investigation, GA_3 treatment was observed to be most effective as compared to Kinetin and BA with respect to chlorophyll contents in the selected leafy vegetable. Similar observations of mitigative effects of PGRs on chlorophyll contents in plants grown under saline environment have been reported by several workers. Jat and

Sharma (2006), while working on the influence of different PGRs, on wheat seedlings recorded an increase in chlorophyll content with GA_3 and indole acetic acid (IAA) in salt tolerant cultivar Raj-3777 and salt sensitive cultivar Lok-1 respectively. In *Brassica campestris*, application of ascorbic acid through presoaking treatment was found to be effective as chlorophyll b and total chlorophyll pigments were observed to increase under saline conditions (Khan *et al*., 2010). Azzedine *et al*. (2011) recorded an improvement of salt tolerance in durum wheat by exogenous application of ascorbic acid. They observed a significant increase in photosynthetic pigments like chlorophyll and carotenoid with ascorbic acid application. Zeid (2011) reported that the adverse effects of salinity on chlorophyll content in barley were reversed with glycine betaine, proline and GA_3 treatments. According to him, this enhancement may be attributed to some extent to improved water status of plant cells and membrane stability.

Sugars:

In case of *Trigonella foenum-graecum* leaves, a significant increase in reducing and non-reducing sugars was observed at a concentration of 60 mM NaCl as compared to control plants (Table 2). According to Heidari and Mesri (2008), compatible solutes such as carbohydrates accumulate under salinity to regulate osmotic adjustments by providing suitable conditions to continue water and nutrient uptake by the plant, which may be true even in the present study. In the leaves of PGR treated *Trigonella foenum-graecum* plants, reducing sugars were observed to decrease by about 46, 39 and 40 percent with GA_3, Kinetin and BA treatments respectively, as compared to unprimed seeds grown under 60 mM NaCl concentration (Table 2). Non-reducing sugars from leaves were also found to decrease with PGR treatment (Table 2).

Protein:

In *Trigonella foenum-graecum* leaves, a reduction of about 42 percent was observed in proteins at 60 mM NaCl treatment as compared to control (Table 2). However, in PGR treated *Trigonella foenum-graecum* plants, leaf proteins were found to increase by about 75 percent with Kinetin, followed by BA (64 percent) and GA_3 (53 percent) treatments as compared to untreated seeds grown at 60mM NaCl concentration (Table 2). Similarly Kinetin, BA and GA_3 were found to be effective in alleviating the effect of NaCl salinity in *Spinacia oleracea* (Ratnakar and Rai, 2013). Kinetin was also recorded to be beneficial when used for pretreating the seeds of *Hordeum vulgare* as the protein contents were found to increase under saline environment (Sarwat and El-Sherif, 2007). An increase in seed protein contents in mustard due to foliar application of GA_3 under saline conditions has also been recorded by Shah (2007). Bagdi and Afria (2008) also observed that presoaking the seeds of wheat with BA, helped the plants by increasing protein contents under saline conditions. Jat and Sharma (2006) while working with wheat reported ameliorative effects of indole acetic acid on protein contents when seedlings were grown under saline environment.

Proline:

When *Trigonella foenum-graecum* plants were grown under saline environment, leaf proline was found to increase by about 90 percent as compared to control (Table 2). However, PGR treated plants of *Trigonella foenum-graecum* exhibited significant reduction in leaf proline content as compared to untreated seeds grown under 60 mM NaCl concentration, maximum reduction being observed with GA_3, followed by Kinetin and BA, although, the contents were found to be slightly higher than that of control plants (Table 2).

In the present investigation, proline contents were observed to increase in the selected leafy vegetable at 60 mM NaCl concentration. According to Harinasut *et al*. (2000), proline is one of the compatible organic solute that is used by plants as an osmoprotectant. Seed soaking with all the three PGRs resulted in a decrease in proline content as compared to unprimed seeds grown under 60 mM NaCl salinity (Table 2).

β-carotene:

An increase of about 6 percent as compared to control was recorded in leaf β-carotene content in case of *Trigonella foenum-graecum* plants when grown under saline conditions (Table 3). Pisal and Lele (2005) observed increased production of β-carotene in cells with increasing NaCl in the growth medium. They suggested that, β-carotene is a secondary metabolite and these molecules are produced by the cells under stress for protection. It is evident from table 3 that, leaf β-carotene content was observed to decrease with $GA_{3,}$ BA and Kinetin treatments as compared to unprimed seeds grown under 60 mM NaCl salinity. However, β-carotene content remained little above the level of control plants.

Thiamine:

In *Trigonella foenum-graecum*, an increase of about 17 percent was observed in leaf thiamine content at 60 mM NaCl salinity (Table 3). An increase in total thiamine content has been observed in *Arabidopsis thaliana* seedlings subjected to saline conditions by Tunc-Ozdemir *et al*. (2009). Their study showed that salt stress induced accumulation of thiamine, was at least in part due to the increased expression of thiamine biosynthesis genes. They have also observed that thiamine accumulation in the plant resulted in enhanced tolerance to oxidative stress. PGR treatment in the present study resulted in a decrease in thiamine content under saline environment. Although, thiamine contents were increased by about 5 percent with $GA_{3,}$ and Kinetin treatment as compared to control, they were found to be restored to the level of control plants, with application of BA (Table 3).

Tunc-Ozdemir *et al*. (2009) reported that exogenous application of thiamine protects the plants during oxidative stress. They are of the opinion that, thiamine deficiency may lead to increased sensitivity of plants to stress.

Riboflavin:

In the present investigation, reduction of about 19 percent in riboflavin content at 60 mM NaCl concentration was observed in the leaves of *Trigonella foenum-graecum* plants as compared to control plants (Table 3). However, leaf riboflavin content was found to increase with GA_3 and Kinetin treatment, as compared to unprimed seeds grown under 60 mM NaCl salinity. BA treatment resulted in an increase of almost 5 percent as compared to plants grown only under saline environment. Although, riboflavin content were found to increase with all the three PGR treatment as compared to unprimed seeds grown at 60 mM NaCl salinity, the value of riboflavin remained lower than control plants (Table 3).

Abdel-Rahman *et al*. (2005) reported that riboflavin treatments alleviated the inhibitory effect of NaCl and enhanced growth and pigment content in *Chlorella vulgaris* and *Chlorococcum humicola* as compared to when grown under only saline treatment. An increase in carbohydrate and total proteins in salinised cells of both algae treated with riboflavin was also recorded as compared to those subjected to saline environment. Free proline and total free amino acids were observed to reduce in riboflavin treated algae. According to Abdel-Rahman *et al*. (2005), incorporation of free amino acids into protein was enhanced by riboflavin treatment in both the algae. An increase in carbohydrate content with foliar application of riboflavin to enhance resistance of *Hibiscus sabdariffa* to salinity stress has been reported (Azooz, 2009). According to Azooz (2009), such an accumulation of carbohydrates might be attributed to increased leaf area, which leads to increased photosynthetic activity and plant productivity.

On the basis of above findings, it can be said that riboflavin helps plants to cope with the adverse environmental conditions. In the present study, increased riboflavin contents in plants pretreated with PGRs, compared to plants grown only under saline environment may help plants to cope under saline environment.

Ascorbic acid:

In the present investigation, in *Trigonella foenum-graecum* leaves, reduction in ascorbic acid content was observed at 60 mM NaCl concentration as compared to control (Table 3). Decreased ascorbic acid content has also been reported in wheat under the influence of salinity (Seth *et al*., 2007). Emam and Helal (2008) observed that salinity is capable of inducing significant decrease in ascorbic acid content in *Linum usitatissimum* plants. Similarly, a decrease in ascorbic acid content has also been recorded in wheat seedlings under NaCl salinity by Mandhania *et al*. (2010).

Increase in ascorbic acid when wheat seeds were presoaked with gibberellic acid and salicylic acid under saline conditions has been reported (Seth *et al*., 2007). Azooz and Al-Fredan (2009) recorded an increased content of endogenous ascorbic acid under saline conditions, when plants were treated with exogenous ascorbic acid in *Vicia faba*. According to them, ascorbic acid plays an inductive role in overcoming the detrimental effects of seawater salinity. Khan *et al*. (2010) also reported reduced uptake of sodium in ascorbic acid treated *Brassica*, when grown under saline conditions. According to them, ascorbic acid can mitigate the harmful effects of salinity when applied as a seed soaking agent.

Table 3 depicts significantly higher amounts of leaf ascorbic acid when plants were pretreated with PGRs and grown under saline environment, as compared to untreated plants grown under NaCl treatment, thus indicating an ameliorative effect of PGRs.

Table 1: Effect of seed priming with PGRs on chlorophyll content from the leaves of *Trigonella foenum-graecum* L. var. PEB grown under saline (60 mM NaCl) environment

NaCl Concentration/ PGR Treatment	Chlorophyll a (mg/ 100g)*	Chlorophyll b (mg/ 100g) *	Total Chlorophyll (mg/ 100g) *
0 mM (Control)	54.337 ± 1.227	42.964 ± 1.191	97.272 ± 2.556
60 mM	39.488 ± 1.102	28.159 ± 1.248	67.912 ± 2.064
60 mM + GA_3	51.752 ± 0.309 #	36.522 ± 1.256 #	88.249 ± 1.564 #
60 mM + Kinetin	43.144 ± 0.216 #	30.930 ± 0.742	74.054 ± 0.958 #
60 mM + BA	48.028 ± 0.309 #	35.905 ± 2.554 #	82.613 ± 1.563 #

Results are the mean of three determinants. *One-way ANOVA was carried out and F ratio was significant at 5% level of significance. # Significant at $p < 0.05$ (*t*-test was carried out to test whether there is significant difference between control and individual salt concentration).

Table 2: Effect of seed priming with PGRs on reducing sugar, non-reducing sugar, protein and proline content from the leaves of *Trigonella foenum-graecum* L. var. PEB grown under saline (60 mM NaCl) environment

NaCl Concentration/PGR Treatment	Reducing Sugars (mg/ 100g) *	Non-Reducing Sugars (g/ 100g) *	Protein (g/100g FW) •	Proline (mg/100g FW) *
0 mM (Control)	516.6 ± 24.608	3.630 ± 0.148	3.200 ± 0.327	1.11 ± 0.124
60 mM	970.0 ± 36.285	4.763 ± 0.122	1.860 ± 0.134	2.12 ± 0.127
60 mM + GA_3	521.6 ± 26.562 #	3.605 ± 0.120 #	2.860 ± 0.175	1.27 ± 0.073#
60 mM + Kinetin	583.3 ± 30.641 #	3.816 ± 0.107 #	3.260 ± 0.099	1.33 ± 0.090#
60 mM + BA	573.3 ± 51.370 #	3.906 ± 0.192 #	3.060 ± 0.215	1.48 ± 0.090#

Results are the mean of three determinants.
*One-way ANOVA was carried out and F ratio was significant at 5% level of significance.
Significant at $p < 0.05$ (*t*-test was carried out to test whether there is significant difference between control and individual salt concentration).
• No significant difference was observed in the group, so data is not analyzed further for pair-wise comparisons among the treatments.

Table 3. Effect of seed priming with PGRs on β-carotene, thiamine, riboflavin and ascorbic acid content from the leaves of *Trigonella foenum-graecum* grown under saline (60 mM NaCl) environment

NaCl Concentration /PGR Treatment	β-carotene (mg/100g FW) *	Thiamine (mg/100g DW) *	Riboflavin (mg/100g DW) *	Ascorbic acid (mg/100g FW) •
0 mM (Control)	2.416± 0.028	0.017 ± 0.0003	0.046 ± 0.0006	32.400±6.547
60 mM	2.574± 0.040	0.020 ± 0.0003	0.037 ± 0.0004	23.612±1.965
60 mM + GA_3	2.502± 0.035	0.018 ± 0.0004	0.042 ± 0.0007#	28.930±1.640
60 mM + Kinetin	2.453± 0.044 #	0.018 ± 0.0004	0.043 ± 0.0005#	28.000±2.557
60 mM + BA	2.473± 0.052	0.017 ± 0.0005	0.039 ± 0.0006	25.000±0.000

Results are the mean of three determinants.
*One-way ANOVA was carried out and F ratio was significant at 5% level of significance.
Significant at $p < 0.05$ (*t*-test was carried out to test whether there is significant difference between control and individual salt concentration).
• No significant difference was observed in the group, so data is not analyzed further for pair-wise comparisons among the treatments.

CONCLUSION

On the basis of present findings, it is clear that all the three plant growth regulators are found to mitigate the adverse effects of NaCl salinity on *Trigonella foenum-graecum* L. var. PEB. Of the three plant growth regulators used in the present study GA_3 was found to be most effective.

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