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Some Growth Promoting Essential Mineral Elements Alleviates The Salinity Effect on Nitrate Reductase and Hill Reaction Activities in Cotton (*Gossypium hirsutum*) cv. "CIM 496"

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Abstract: The effect of salinity was observed on the activity of nitrate reductase and hill reaction activity in the leaves of Cotton (*Gossypium hirsutum L.*) cv. "CIM 496" grown in large size plastic pot culture, irrigated with sea salt concentrations of 0.0%(EC iw: 0.6 dS/m), 0.4%(EC iw: 6.2 dS/m) and 0.8%(EC iw: 10.8 dS/m) and subjected to foliar application with non-spray (control), water spray, KNO_3 (500 ppm), KCl (500 ppm), Urea(1000 ppm), Fe-EDTA (5ppm), $MnCl_2$ (5ppm) and MoO_3 (5ppm) alone and their mixture. Foliar spray of the mixture showed better result as compared to that of above mentioned individual mineral. Conclusion made on the comparative performance of nitrate reductase and hill reaction enzymes under above number of treatments in the leaves of cotton growing under various sea salt concentrations is given below: Nitrate Reductase Activity (NRA): Nonspray< water spray< KCl< Mo< KNO_3< Urea< KNO_3 + Mo< KNO_3 +Fe + Mn + Mo; Hill Reaction Activity (HRA): Nonspray< water spray< *KNO3<Fe<Mn<* KNO_3 *+ Fe<* KNO_3 *+Mn <*KNO_3 *+Fe + Mn + Mo*.

Keywords: Salinity, Gossypium, EC iw (Electrical Conductivity of Irrigation Water), Foliar Spray, Nitrate Reductase Activity, Hill Reaction Activity

1. Introduction

Excessive soil salinity can result from natural processes, or from crop irrigation with saline water under poor drainage conditions. This effect is mostly reported in semi-arid to arid regions where it inhibits the growth and yields of crop plants [1, 2, 3]. The physiology of plant responses to salinity and their relation to salinity resistance have been much researched and frequently reviewed in recent years e.g. [4, 5, 6, 7, 8, 9].The ability of plants to tolerate salt is determined by multiple biochemical pathways that facilitate retention and/or acquisition of water, protect chloroplast functions, and maintain ion homeostasis. Essential pathways in this connection are referred those which lead to synthesis of osmotically active metabolites, specific proteins, and certain free radical scavenging enzymes [10]. Therefore, foliar application of essential mineral elements is an alternative approach to cope with saline rooting medium. In this respect [11, 12, 13] stated that foliar fertilization of both macro and micronutrient is practiced whenever, nutrients uptake through the root system is restricted due to salt stress. Foliar spray did not only increase the crop yields but also reduce the quantities of fertilizer applied through soil. Islam et al., [14] used 0.1% KNO_3 as foliar spray on jute plant leaves and obtained good results whereas, 250 ppm of KNO_3 produce promising results in *Lagenaria siceraria* documented by [15]. Jabeen & Ahmad [16] studied the response of cotton grown at high salinity supplemented with foliar application of KCl (500 ppm) and NH_4NO_3 (500 ppm) alone and in mixture offset the inhibitory effect due to salinity. Nitrate reductase (NR) contains molybdenum, it is apparent that in higher plants it plays important role in facilitating the assimilation of nitrate. In *Prosopis alba* [17] found only highest NaCl concentration affected the nitrate content and nitrate reductase activity in leaves and roots when treated 0, 300 and 600 $mmol^{-1}$NaCl. Nitrate reductase activity (NRA) is considered as the main limiting step in nitrogen assimilation in most plants [18, 19]. The activity of nitrate reductase depends of various factors, including the level of nitrate supply, the plant species, the plant age, and has important consequences for mineral nutrition and carbon economy of plants. In general, when the external nitrate supply is low, a high proportion of nitrate is reduced in the roots. Chaves et al., [20] Reported that photosynthesis is a key factor in the determination of plant development. The decrease in crop performance detected in various plant groups exposed to salinity is linked to the decline in photosynthesis. Reduction in the photosynthetic capability of various plant species by salt stress has been documented in a number of reports [21, 22, 23]. Foliar supply of manganese (Mn) in suitable quantity may result an increase in hill reaction activity and growth rate of cells in barley under saline rhizosphere [24]. Manganese (Mn) has been known for many years as an essential unique metallo-enzyme required for oxygenic photosynthesis in all plants and cyanobacteria in the water oxidizing process [25]. The function of Mn at the cellular level of plant is to bind firmly to lamellae of chloroplast, possibly to the outer surface of thylakoid membranes, affecting the chloroplast structure and photosynthesis [26].The present study was undertaken to see the effect of foliar application of some sodium antagonistic essential minerals on plants raised with sea salt water irrigation at sandy loam soil. The aim was to investigate whether it will inhibit toxic effect of excessive sodium and improve physiological parameters like nitrate reductase and Hill reaction activity.

2. Materials and Methods

Since broader leaf plants provide greater surface for retention of minerals given through foliar spray, commercially important *Gossypium hirsutum* cv. "CIM 496" cotton) included in family Malvaceae was selected for present investigation. The seeds were obtained from Agriculture University, Faisalabad, Pakistan. An experiment was conducted in large size plastic pots during autumn: April - December 2006. A set of 180 large size plastic pots containing approximately 18 Kg of sandy loam soil each having basal outlet for drainage were used in this experiment. Cow dung manure with pH 7.9 was added in the soil at 9:1 ratio (w/w) whereas NPK (Nitrogen, Phosphorus and Potassium) fertilizer concentrations were followed by [27, 28]. The seeds were delinted with concentrated H_2SO_4 for one minute to remove the fiber and immediately washed with running distilled water and then surface sterilized with 0.1 % (w/v) $HgCl_2$ for five minute. Composition of foliar supply medium was based on potassium (K^+) as macro element and some essential trace elements which being bivalent and trivalent show antagonism with monovalent sodium (Na^+) were given in concentration with potassium (K^+), which appeared to be growth promoting for plants raised under saline water irrigation. Experiment was divided into XII sets i:e I-Non-spray, II-Foliar spray with water, III-Foliar spray with Fe-EDTA (5 ppm), IV-Foliar spray with $MnCl_2$, (5 ppm), V-Foliar spray with MoO_3 (5 ppm), VI-Foliar spray with KNO_3 (500 ppm), VII-Foliar spray with KCl (500 ppm), VIII-Foliar spray with Urea (1000 ppm) IX-Foliar spray with KNO_3 (500 ppm)+ Fe-EDTA (5 ppm), X-Foliar spray with KNO_3 (500 ppm)+ $MnCl_2$ (5 ppm), XI-Foliar spray with KNO_3 (500 ppm)+MoO_3 (5 ppm) and XII-Foliar spray with KNO_3 (500 ppm)+ Fe-EDTA (5 ppm)+$MnCl_2$ (5 ppm)+MoO_3 (5 ppm). A Randomized Complete Block Design (RCBD) with five replications was used. Out of a total 180 pot 15 were used in each foliar spray set, comprising of three different irrigation treatment i: e 0.0% sea salt irrigation water (EC iw: 0.6 dS/m), 0.4% sea salt irrigation water (EC iw: 6.2 dS/m) and 0.8% seasalt irrigation water (EC iw: 10.8 dS/m) given to five pots under each treatment. Liquid transparent soap solution with concentration of 100 ppm was used as a surfactant for each treatment. Spray solution of 500 mL was applied to each plant with fine mist sprayer whereas water spray set was sprayed with only water. The pH values of spray solution was in range of 3.0 – 5.5 which are optimal ranges for the maximum uptake of mineral nutrients as reported by[29].The plants were sprayed constantly till the leaves were entirely showery and the solution ran off the leaves. Control (0.0% sea salt irrigation water) treatment plants were irrigated with 3L of tap water and plants under saline treatments were irrigated with same volume of water with their respective sea salt concentrations ensuring proper leaching. Insecticides were used to control pest management during the growth season according to directions. Fresh cotton leaves sample were collected for NRA by the method [30] from 3rd and 4th node at grand period of growth and then central portion of leaves were cut into discs of 1.0 cm in diameter avoiding the main vein. The NRA spectrophotometric assay method was based on incubation of fresh leaf tissue (300 mg) in 10.0 mL of a buffer mixture comprising 25 mMol /L potassium phosphate buffer (pH 7.2), 25 mMol /L KNO_3 and 1%(w/v) n-propanol . In order to induce anaerobic conditions in the incubation medium, the system was subjected to vacuum infiltration three times to remove air from the tissue. The buffer medium was maintained in the dark at 30^{o}C for 60 minutes. The nitrite produced by action of the NR enzyme was determined by drawing 0.5 mL aliquot of the incubation medium, and treating this sample with 0.5mL of 10 g/L sulfanilamide in 3 mol/L HCl and 0.5 mL of 0.2 g/L N-(1-naphtyl)-ethylenediamine dihydrochloride. After 20 minutes, the solution was diluted to 4.5 mL with deionized water, and the absorbance was noted at 540 nm. Hill reaction activity was conducted with Ferricyanide Dye [1, 2, 3] as an electron acceptor by using the method [31]. 300 mg of fresh leaves samples were deveined and grind with 5 ml of isolating medium consisting of 10% (w /v) polyethylene glycol and 0.1 M potassium phosphate buffer adjusted to a pH of 7.6. The homogenate was squeezed through about eight layers of clean cheese cloth. Chloroplast extracts were centrifuged at 8000g for 3 min. After two washes with 10 ml of the isolating medium, the chloroplasts were stored for short periods in the isolation medium at 1 ^{0}C. The measurement of the Hill reaction is accomplished as follows: chloroplast aliquot is mixed with 0.05 M phosphate buffer with pH 7.0, 0.4 M sucrose, 0.01 M KCl mixture medium, and 0.1 ml of

0.01 M potassium ferricyanide is added. The optical density of the mixture is recorded at 625 nm. The test tube or cuvette is illuminated, and then 0.1 ml of 0.2 N sodium citrate and 0.1 ml of 0.01 N ferric chloride in 0.1 N acetic acid are added, followed by 0.1 ml of 0.1 M orthophenanthroline in absolute alcohol and allowing four minutes for complete color development, the optical density is compared to that of a control cuvette which had not been illuminated, but is otherwise identical. All the data were statistically analyzed by computer program Costat 3.03 and SPSS version 11[32]. Mean separation of data was carried out using Duncan Multiple Range Test [33].

3. Results and Discussion

3.1. Activity of Nitrate Reductase (NRA)

In present study in *Gossypium hirsutum* it is observed that leaf nitrate reductase activity increased at 0.4% sea salt irrigation water (EC iw: 6.2 dS/m) irrespective of any foliar treatment. The inhibitory effect was found at 0.8% sea salt irrigation water (EC iw: 10.8 dS/m) irrespective of any foliar treatment (*table 1*). Foliar spray of KNO_3 with three microelement (Fe, Mn, Mo) mixture proved to be best spray medium, while the foliar spray of KNO_3 with (Mo) was second best medium, foliar spray of alone, Urea, KNO_3, Mo and KCl was found at third, fourth, fifth and sixth order respectively. Hence the spray of mineral elements overcomes the toxic effect of root zone salinity of sea salt irrigation. Conclusion made on the comparative performance of this enzyme under above number of treatments in the leaves of cotton growing under various regimes of sea salt irrigation water is shown as follows: *Nonspray< water spray< KCl< Mo<* KNO_3*< Urea<* KNO_3 *+ Mo<* KNO_3 *+Fe + Mn + Mo.* In tanner grass (*Brachiaria radicans* Napper) [34] reported that nitrate reductase activity was enhanced when fertilized with NaNO3 as comparison with NH_4Cl and Urea while the application of molybdenum in the absence of external nitrogen improved NRA without affecting leaf and stalk growth. Whereas in the presence of nitrogen, application of the molybdenum levels limited leaf NRA and plant development. Highest levels of NRA was found by [35] in the leaf of *Verbascum* L. species in comparison with root and stem, significant differences were found between the samples collected from different altitudes. The NRA was found highest in *Verbascum olympicim Bioss*, and lowest in *Verbascum lagurus Fisch* and *Mey*. In *Azospirillium brasilense* was reported [36] the application of molybdenum leads to an increased accumulation of potassium ions up to 2 folds compared to the respective molybdenum untreated plants under drought condition. Activity of nitrate reductase in leaf is reported to be dependent on the source of nitrogen [37] and continuous supply of nitrates through the xylem [38, 39]. Interactions between the different nitrogen sources and molybdenum have been worked out on NRA by [40].

Table 1. *Effect of foliar spray of mineral nutrients and sea salt irrigation water of different salinity levels on nitrate reductase activity (NRA) (µmole* NO_2*/g f.w.t/min) in Gossypium hirsutum.*

Foliar Spray Treatment / Sea salt irrigation water Treatment (%)	0.0% (EC: 0.6 dS/m)	0.4% (EC: 6.2 dS/m)	0.8% (EC: 10.8 dS/m)
Non-Spray	15.93 a	16.39 a	14.26 b
Foliar spray with water	17.04 a	17.4 a	16.67 b
Foliar spray with KNO3 (500 ppm)	18.52 a	18.89 a	17.19 a
Foliar spray with Urea(1000 ppm)	20.65 a	20.83 a	19.01 b
Foliar spray with MoO3 (5 ppm)	18.16 b	17.87 a	18.00 b
Foliar spray with KCl (500 ppm)	17.59 a	17.71 a	16.83 b
Foliar spray with KNO3 (500 ppm) + MoO3 (5 ppm)	21.57 b	23.31 a	20.93 a
Foliar spray with KNO3 (500 ppm) + Fe-EDTA (5 ppm)+ MnCl2 (5 ppm)+ MoO3 (5 ppm)	24.07 b	26.94 a	23.15 b

Mean followed by different letters in the same column differ significantly at 95% probability level according to New Duncan's Multiple Range Test.

3.2. Hill Reaction Activity (HRA)

Hill reaction activity determine by the reduction of potassium ferricyanide increased at 0.4% sea salt irrigation water (EC iw: 6.2 dS/m) but the inhibitory effect was found at 0.8% sea salt irrigation water (EC iw: 10.8 dS/m) irrespective of any foliar treatment (Table 2) in present investigation. Foliar spray of KNO_3 with three microelement (Fe, Mn, Mo) mixture occupies 1st position in improving this reaction, while the spray of KNO_3 with (Mn), KNO_3 with (Fe) were found second and third best medium, foliar spray of alone, Mn, Fe and KNO_3, was found at fourth, fifth and sixth order. Conclusion made on the comparative performance of this enzyme under above number of treatments in the leaves of cotton growing under various dilutions of sea salt water irrigation is given as follows: *Nonspray< water spray< KNO3<Fe<Mn<* KNO_3 *+ Fe<* KNO_3 *+Mn <*KNO_3 *+Fe + Mn + Mo.* Similar result of inhibitory effect of salinity on Hill reaction activity were also documented by others [41, 42, 43, 44]. In sunflower plants [45] exogenous supply of micronutrients i.e., manganese and boron alone and their mixture partially offset the inhibitory effect of salt stress and improved the Hill reaction activity irrespective to their growth under non saline or saline conditions. In *Oryza sativa* L. cv. Safari [46] found similar results when treated with varying Mn concentrations between 0.125 and 32 mg/L in nutrient solutions. The plant shoots and the thylakoid membranes showed an overall increase in Mn content and the photosynthetic electron transport rates coupled to PSII and PSI also showed a significant increase up to 8 mg/L Mn treatment. Krogmann & Jagendorf [47] reported that ferricyanide reduction is seen to be proportional to the concentration of chlorophyll added, and only 12 % less than the dye reduction achieved under similar conditions.

Ferricyanide reduction is also found to be directly proportional to the duration of exposure to saturating light. The method appears to be usable in measuring the reduction of ferricyanide by the Hill reaction, at low oxidant concentrations, over short periods of time. The substitution of quinones for ferricyanide proved impractical due to complicating nonenzymatic reactions between the quinones and ferric chloride. In cotton [48] reported, actively reduction of ferricyanide when assay for Hill reaction activity was increased 133% with 10% (w/v) polyethylene glycol when replaced by 0.5 M sucrose at pH 7.6.

Table 2. *Effect of foliar spray of mineral nutrients and sea salt irrigation water of different salinity levels on Hill reaction activity (HRA) / gram fresh weight (µmole ferricyanide/mg chlorophyll/hr) in Gossypium hirsutum.*

Foliar Spray Treatment/ Sea salt irrigation water Treatment (%)	0.0% (EC: 0.6 dS/m)	0.4% (EC: 6.2 dS/m)	0.8% (EC: 10.8 dS/m)
Non-Spray	0.029 [a]	0.037 [b]	0.028 [a]
Foliar Spray with water	0.048 [a]	0.056 [a]	0.041 [c]
Foliar spray with KNO_3 (500 ppm)	0.060 [b]	0.066 [a]	0.058 [c]
Foliar spray with $MnCl_2$ (5 ppm)	0.059 [a]	0.065 [b]	0.059 [a]
Foliar spray with Fe-EDTA (5 ppm)	0.057 [a]	0.065 [b]	0.059 [a]
Foliar spray with KNO_3 (500 ppm) + $MnCl_2$ (5 ppm)	0.063 [a]	0.068 [b]	0.065 [a]
Foliar spray with KNO_3 (500 ppm) + Fe-EDTA (5 ppm)	0.062 [b]	0.067 [a]	0.061
Foliar spray with KNO_3 (500 ppm) + Fe-EDTA (5 ppm)+ $MnCl_2$ (5 ppm)+ MoO_3 (5 ppm)	0.086 [b]	0.088 [a]	0.075 [c]

Mean followed by different letters in the same column differ significantly at 95% probability level according to New Duncan's Multiple Range Test.

4. Conclusions

Our results have highlighted the usefulness of foliar spray practice in plant growth regulation. One of the more significant findings to emerge from this study is that foliar application of macro and micro mineral elements can improve plant physiological efficiency, including nitrate reductase and Hill reaction activities under saline environmental conditions. In present investigation the critical values of salts in rooting medium beyond which retardation occurs at both NRA and HRA enzymatic activities was significantly extended due to foliar supply of above mentioned essential minerals alone or in mixture of two or three salts, which could provide incentive to grow salt tolerant crop through foliar application of essential sodium antagonistic mineral elements at a bit higher levels of salinities.

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