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Full Length Article

Role of Medium Supplementation of Ethylene Biosynthesis Inhibitors in Improving Grain Yield and Quality in Rice

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Abstract

Soil salinity is a noxious factor that declines the growth and yield of crop plants. Recently increased production of ethylene has been regarded as one of the reasons for enhanced ion toxicity. The inhibition of ethylene synthesis can be used to reduce the ethylene biosynthesis and improve salinity tolerance. Silver (Ag) and cobalt (Co) ions are inhibitors of ethylene action and biosynthesis (EIs), and can be used to reduce ethylene biosynthesis and improve plant salt tolerance. In this pot experiment, screened levels of Ag (15 µM) and Co (25 µM) were applied to salinity (120 mM NaCl) grown salt resistant (Shaheen Basmati) and salt susceptible (Super Basmati) rice varieties to monitor possible improvement in the grain yield components, grain quality and grain nutritional status. Results revealed that both the varieties suffered a substantial reduction in the number of panicles per plant, panicle length, number of grains per panicle, 1000 grain weight, grain yield per plant and harvest index by salinity while medium supplementation of Ag was effective in reversing the salinity effect of salinity on all these attributes. The applied salt or EIs treatments did not affect the grain starch and grain proteins contents but indicated prominent differences in the grain vitamins including ascorbate, niacin and riboflavin, which were highly increased in salinity stressed sensitive Basmati rice. The contents of Na^+ and Cl^- were much increased in grains of salinity stressed rice but were enormously decreased under the medium supply of EIs. Furthermore, EIs enhanced the grain nitrate-N, phosphate-P, K^+ and Ca^{2+} contents, although Ag was more effective than Co. In conclusion, that suppression of ethylene action is of greater significance than its biosynthesis. So the medium supplementation of both the EIs could be useful in fetching better grain yield of plants grown in the marginally saline areas. © 2018 Friends Science Publishers

Keywords: Ethylene inhibition; Grain yield; Vitamins; Grain nutrients; Salinity; Basmati rice

Introduction

Salinity leads to a sizeable decline in the productivity of crop plants. Sodium (Na^+) and chloride (Cl^-) ions are among the predominant ions in salt affected soils, which reduce plant growth. Kumar *et al*. (2009) reported that salt resistant rice cultivars generated larger biomass than the sensitive ones when irrigated with saline water. The roots of rice plants readily absorbed Na^+ due to its small size, which is distributed in all plant organs to upset osmotic stress and cause imbalanced nutrition (Siringam *et al*., 2011). Accumulation of tissue Na^+ in lesser amounts may explain the basis of salt tolerance in the resistant plants (Ghosh *et al*., 2016). Reducing stress-induced ethylene by any means may encounter the adverse effect of salinity and crop plants can display better yield.

Ethylene, also called acetylene, has simpler structure as compared to other plant hormones. Being gaseous in nature, it can penetrate intracellularly and effectively effects physiological mechanisms (Arteca and Arteca, 2008). Popularly known as stress hormone, the production of ethylene is enhanced under all type of stresses including salinity, drought, heat, flooding metal toxicity etc. (Lin *et al*., 2009). Once accumulated in greater concentrations it induces a range of signaling mechanisms and modulates gene expression (Müller and Munné-Bosch, 2015). Few of the signaling mechanisms triggered by ethylene are light dependent while other involve the crosstalk with other hormones (Murphy, 2015).

In plants methionine is used as a precursor for the synthesis of ethylene. After conversion of methionine into S-adenosylmethionine (SAM), the SAM is enzymatically converted into 1-aminocyclopropanecarboxylic acid (ACC). The enzyme involved is SAM synthase. In the next step the ACC is produced with the activity of enzyme ACC synthetase, while ACC oxidase converts ACC to ethylene. Once synthesized, the ethylene performs its multifarious actions. This implies that blocking the pathway of synthesis at any of these steps or stopping the action of ethylene could be of high significance in combating the

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abiotic stress effects. Among the known compounds used to inhibit or minimize the cell and tissue synthesis of ethylene are 2-aminoethoxyvinyl glycine (AVG), silver (Ag^+) ions and 1-methylcyclopropene (MCP).Of these, Ag^+ and MCP are the inhibitors of ethylene receptors, which act during signaling (Schaller and Binder, 2017). In addition, cobalt (Co^{2+}) inhibits the activity of enzyme ACC oxidase, which is an ultimate step in the ethylene biosynthesis (Locke *et al*., 2000).

In plants growth under saline conditions, the roots are the first organ to encounter the excess of ions dissolved in the soil solution. So to induce salinity tolerance by producing profound changes in the plant root may be of immense significance in promoting stress tolerance (Khan *et al*., 2016). Salinity significantly reduced the tillering, panicle length, number of grains per panicle, 1000 grain weight, and grain yield at maturity in rice (Mahmood *et al*., 2009). A decrease in tillering capacity might be due to the toxic effect of salinity on plant growth, while development of greater number of tillers may be a mechanism of salt tolerance concerned with the dilution of ions in rice (Aslam *et al*., 1989).

Rice is a staple food for around 70% of the world population. It is a submergence tolerant crop but shows sensitivity to salinity stress with salt tolerance threshold of 3.0 dS/m (Venkateswarlu *et al*., 1972; Hoang *et al*., 2016). Such attributes make rice a model species for studying the salinity response and using the inhibitors of ethylene biosynthesis. No comprehensive studies are previously available on the use of ethylene inhibitors in improved salinity tolerance in plants. It is predicted that application of ethylene inhibitors may improve rice yield and grain quality under salinity stress. The objectives of this study were to study the comparative effect of Co and Ag ions respectively as potential inhibitor of ethylene production and action and their resultant effects on grain yield and kernel quality under salt stress.

Materials and Methods

Plant Materials and Selection of Treatments

The experiments were conducted on two Basmati rice (*Oryza sativa* L.) varieties, viz., Super Basmati and Shaheen Basmati. Variety Super Basmati exhibited 77.3% yield loss while var. Shaheen Basmati indicated 52.6% yield loss when both were tested at 7 dS/m salinity level in the natural field conditions (Shabbir *et al.*, 2001). The seed of both the varieties were obtained from Rice Research Institute (RRI), Kala Shah Kaku, District Sheikhupura, Pakistan. Experiments were conducted in the net house facility in the Department of Botany, University of Agriculture, Faisalabad, Pakistan. Screening trials were done to select a most suitable level out of a range of levels of each of the silver ions (Ag^+; medium supplemented as $AgNO_3$) and cobalt ions (Co^{2+}; medium supplemented as $CoCl_2$) as inhibitors of ethylene (EIs) biosynthesis yielded 15 µM Ag^+ and 25 µM Co^{2+} as optimum levels (Kabir, 2017), which were used in this study.

Treatment Application

Nursery of the Basmati rice varieties (Shaheen Basmati and Super Basmati) was raised in small beds. Five seedlings were transplanted in each pot containing 30 kg soil. The age of the nursery plants at the time of transplantation was 20 days, and the plants of comparable size from both the varieties were transplanted. The soil was kept puddled throughout the experimental period. The plants of both the varieties were normally grown up to anthesis stage. At anthesis stage, the plants were treated with 120 mM NaCl (99% pure). This salt level was developed in the pots in three days (~40 mM) based on the full saturation percentage of soil. On the last day of salt addition, supplementation of optimized levels of EIs (15 µM Ag^+ and 25 µM Co^{2+}) was done by dissolving in water. A control set with salt stress but with or without Ag^+ and Co^{2+} treatments was run. The physico-chemical properties of the soil used in this study, determined using authentic methods (Moodie *et al.*, 1959; Black, 1965) were: clayey, with light brown in color, pH 7.91, Ece 2.31 dS/m; SAR 14.32; organic matter 0.91%; N 0.59%; P, K and Ca+Mg 7.48, 86.32 and 35.31 mg/kg, respectively. The plants were harvested when the panicles were mature.

Grain Yield Components

At maturity, the data on yield components like number of tillers per plant, number of panicles per plant, panicle length, number of grains per panicle, 1000 grain weight per plant, grain yield per plant and straw yield per plant were determined by taking measurements. The harvest index (HI) was calculated according to the following formula:

$$\text{HI (\%)} = (\text{grain yield}/\text{straw yield}) \times 100$$

Grain Quality Determination

To determine the grain quality parameters, the grain were dried up to 12% moisture and milled for determination of grain quality attributes. The grains were determined for starch, proteins, ascorbic acid, riboflavin, thiamine, niacin contents. The grain **s**tarch content was estimated by the iodine blue test of Gilbert and Spragg (1964). For this 75 mg of rice grain powder boiled in 50 mL of distilled water and filtered after cooling. To 3 mL of the filtrate, 40 mL of distilled water, 1.0 mL of iodine reagent and 1.0 mL concentrated HCl were added and the volume was made up to 50 mL. The transmission was read at 600 nm using a spectrophotometer (Shimadzu, Japan) and values of starch calculated by comparing with a standard curve. Total grain proteins were determined using Bradford (1976) method. Dye stock (BioRad, USA) was added to the samples in equal amount, vortexed, incubated at room temperature for 30 min and absorbance noted at 595 nm. The amount of proteins were estimated from a standard curve prepared from a range of BSA standards.

To estimate ascorbic acid by the method described by Mukherjee and Choudhuri (1983), the seed powder (0.25 g) was extracted in 10 mL solution of 6% TCA. To 4 mL extract, 2 mL of 2% dinitrophenyl hydrazine solution was added followed by one drop of 10% thiourea solution. The reaction mixture was heated in a water bath for 20 min, cooled and 5 mL of 80% H_2SO_4 (v/v) added. The absorbance was taken at 530 nm. To determine riboflavin with the method of Okwu and Josiah (2006), 0.5 g fat free sample was extracted in 10 mL of 50% ethanol for 1 h and filtered. To 1 mL of the filtrate, 1 mL of 5% potassium permanganate and 1 mL of 30% H_2O_2 was added followed by heating in a water bath at 50ºC for 30 min. On cooling, 0.2 mL of 40% sodium sulfate was added and diluted the reaction mixture up to 5 mL. After 5 min, the absorbance of the colored complex was measured at 510 nm. Niacin contents were measured according to the method of Okwu and Josiah (2006). Fat free sample (0.5 g) was mixed with 5 mL of 1 N H_2SO_4 with shaking. Then three drops of NH_3 solution were mixed and filtered. Filtrate (1 mL) was taken and mixed with 0.5 mL potassium cyanide solution and 0.5 mL of 0.02 N H_2SO_4. The absorbance was noted at 470 nm. Thiamine was determined with the method of Hassan and Azeez (2005). To 2 mL of 1 M HCl, 1.4 mL of 0.4% sodium nitrite solution was added and cooled in ice bath for 5 min. Then added 4 mL of 2% urea solution shaken and allowed to stand for 3 min. Then 5 mL of 0.05 M sulfanilic acid was added; shaken well and then added 2 mL of sample extract and 5 mL of 30% NaOH. The absorbance of reddish-brown colored complex was noted at 490 nm. Actual quantities of all vitamins in the unknown samples were determined after comparing with respective standard curves prepared from a range of standard of each vitamin.

Grain Nutritional Status

The contents of Na^+, K^+ and Ca^{2+} in grain were determined by digesting 0.2 g of grain powder in 2.5 mL of mixture of concentrated nitric acid and perchloric acid (2:1 ratio) at 250ºC on a heating block till the samples became clear; diluted up to 50 mL with distilled water. Ca^{2+}, Na^+ and K^+ were estimated by using flame photometer (Sherwood model, 410 UK). Standard curves were constructed by running the different grade series (10, 20, 30, 40 and 50 ppm) of Ca^{2+}, Na^+ and K^+, and actual quantities were computed by comparing the values from the flame photometer with the standard curve. The nitrate-N was determined by using the method of Kowalenko and Lowe (1973) using chromotropic acid (CTA) solution spectrophotometrically. For soluble PO_4^{3-} determination, the molybdate-vanadate method was used as described by Yoshida *et al.* (1976). For the determination of Cl^-, dried ground plant material (0.5 g) was extracted in distilled water at 70ºC for 8 h, filtered and made the volume to 50 mL with distilled water. The Cl^- concentration was determined with Chloride Analyzer (Model 926, Sherwood Scientific Ltd., Cambridge) and expressed as mg/g dry weight.

Statistical Analysis

The layout of experiments was completely randomized factorial with three replicates. Analyses of variance were computed using COSTAT computer package (CoHort Software, 2003, Monterey, California). The least significance difference between the mean values was calculated. The Duncan's New Multiple Range test (DMRT) at 5% level of probability was used to find out the differences among the mean values (Steel *et al.*, 1996). Letters have been applied to the data points in the figures, where the overall interaction of all the factors was significant ($P<0.05$).

Results

Yield and Yield Components

As regards number of tiller per plant, salt levels, EIs and vars. showed significant ($P<0.05$) while only EIs × salt interaction was significant ($P<0.001$). Applied salinity declined the number of tillers by 24 and 30% in vars. Shaheen Basmati and Super Basmati, respectively. With the medium supply of Ag an increase in number of tillers was 2 and 7% under control condition while it was 23 and 38% under salinity stress. Likewise, treatment with Co, an increase in this character was 0 and 4% in control plants but the same increase was 63 and 28% under salinity stress in vars. Shaheen Basmati and Super Basmati, respectively (Table 1). The number of panicles per plant indicated significant ($P<0.05$) difference in EIs and salt levels but no difference in Var was noted. Among the various interactions, EIs × salt interaction was significant ($P<0.001$) only, while there was a significant ($P<0.05$) overall interaction of these factors. Applied salinity declined the number of panicles by 36 and 61% in vars. Shaheen Basmati and Super Basmati, respectively. With the medium supply of Ag an increase in this attribute was 29 and 36% under non-saline condition, while it was 156 and 412% under salinity stress. With the medium supply of Co, increase in this character was 2 and 16% under non-saline but 111 and 271% under salinity stress (Table 1). Panicle length data revealed significant ($P<0.05$) difference in salt levels and EIs and vars. with a significant ($P<0.05$) overall interaction of these factors. Applied salinity declined this character in both the varieties by 22 and 46% in vars. Shaheen Basmati and Super Basmati. With medium supply of Ag an increase in panicle length was 29 and 12% under non-saline control while it was 52 and 100% under salinity stress. Treatment with Co increased this character by 16 and

Table 1: Changes in yield components and grain yield attributes of differentially salt resistant rice varieties by medium supplementation of selected levels of inhibitors of ethylene biosynthesis under salt stress

Basmati rice varieties	NaCl level (mM)	EIs levels	No. of tiller/plant	No. of panicles/plant	Panicle length (cm)	No. of grains/ panicle	1000 grain weight (g)	Grain yield (g) per plant	Straw yield (g/plant)	Harvest index (%)
Shaheen	Control	N.A.	15.33±1.53	14.00±1.73d	12.67±0.58d	69.67±4.51d	17.04±0.97e	11.67±0.76d	36.06±2.72	32.37±0.86c
		15 µM Ag	15.67±115	18.00±1.73bc	16.33±1.15a	87.67±5.86b	23.25±1.99ab	16.55±1.15b	44.15±3.84	37.52±0.69a
		25 µM Co	15.33±1.53	14.33±1.45d	14.67±0.58bc	84.00±5.57b	18.50±2.31d	13.85±0.60c	38.13±2.57	36.45±3.16ab
	120	N.A.	11.67±0.58	9.00±1.15e	9.67±0.58e	46.00±4.58e	12.55±1.00f	8.73±0.62e	34.08±1.98	25.68±2.35d
		15 µM Ag	14.33±1.15	23.00±1.73b	14.67±0.58bc	85.67±7.51b	23.79±2.32ab	13.56±0.54c	37.02±3.64	36.77±2.17a
		25 µM Co	19.00±2.00	19.00±2.03c	14.33±0.58bc	78.67±6.43c	21.29±1.16bc	13.25±1.03c	37.29±1.30	35.56±2.98b
Super	Control	N.A.	15.33±1.15	14.67±2.02d	13.67±0.58cd	73.33±4.51cd	18.41±1.82d	12.79±0.86c	38.98±2.79	32.83±0.83c
		15 µM Ag	16.33±0.58	20.00±1.52bc	15.33±0.58b	98.33±4.93a	24.25±1.86a	18.77±1.03d	48.15±2.30	38.98±1.23a
		25 µM Co	16.00±1.00	19.00±1.73c	14.33±0.58bc	93.67±5.86ab	20.70±0.74c	16.43±1.16ab	44.46±4.35	37.10±3.27a
	120	N.A.	10.67±0.58	5.66±2.03f	7.33±0.58f	30.33±2.52f	8.96±0.38g	7.33±0.71f	31.49±2.52	23.31±2.00a
		15 µM Ag	14.67±0.58	19.00±1.52a	14.67±0.58bc	88.33±4.51b	25.23±1.88a	13.51±0.47c	36.32±1.54	37.22±1.16a
		25 µM Co	13.67±0.58	21.00±1.73b	14.00±1.00c	73.67±5.13cd	23.16±1.99b	12.39±0.96d	34.05±2.42	36.39±0.64ab

N.A., EIs not applied; Means sharing same letter(s) differ non-significantly (P>0.05)

5% in control plants but the same was 48 and 91% in salinity stressed plants (Table 1).

Number of grains per panicle revealed that significant (P<0.01) difference over treatments. Among interactions, a significant (P<0.05) overall interaction was noticed. Salt stress declined the number of grains per panicle by 34 and 59% in vars. Shaheen Basmati and Super Basmati. By treatment with Ag, an increase in number of grains per panicle was found to be 26 and 34% under control condition while it was 86 and 191% under salinity stress. With Co treatment an increase in this character was 21 and 28% under non-saline control plants but the same was 71 and 143% under salinity stress (Table 1). Data for 1000 grains weight revealed that salt levels and EIs showed (P<0.05) with a significant (P<0.01) overall interactions for all these factors. Applied salinity reduced 1000 grain weight by 26 and 51% in vars. Shaheen Basmati and Super Basmati, respectively. With medium supply of Ag, 1000 grain weight was increased 36 and 32% under control condition, which was 90 and 182% under salt stress. Likewise, treatment with Co increased this character was 9 and 12% under condition but the same was 70 and 159% under salinity stress (Table 1).

Grain yield data revealed significant (P<0.01) differences in the salt levels, EIs and vars. with significant (P<0.05) overall interaction of these factors. Soil salinity declined this attribute by 25 and 43% in vars. Shaheen Basmati and Super Basmati, respectively. With the medium supply of Ag, grain yield per plant increased by 42 and 47% under control condition while it was 55 and 84% under salinity stress. Likewise, with the medium supply of Co, the increase in this character was 19 and 28% under control condition but the same was 52 and 69% under salinity stress (Table 1). Straw yield data revealed significant (P<0.01) differences in the salt levels and EIs but vars. while overall interaction of all these factors was non-significant (P>0.05). Salinity stress declined the straw yield by 5 and 19% in vars. Shaheen Basmati and Super Basmati, respectively. With the medium supply of Agan increase in the straw yield was noted as 22 and 24% under control condition but by 9 and 15% under salt stress. With the medium supply of Co, an increase in this character was 6 and 14% in control plants but the same was 9 and 8% under salinity stress in vars. Shaheen Basmati and Super Basmati (Table 1). Data regarding harvest index (HI) showed significant (P<0.01) difference for salt levels and EIs but not for vars. An overall interaction for all of these three factors was significant (P<0.05). Applied salinity declined HI in both of the varieties by 21 and 29% in vars. Shaheen Basmati and Super Basmati, respectively. With the Ag supply, HI was increased by 16 and 19% under non-saline control condition while by 43 and 60% under salinity stress. Co treatment, increased this character was 13% in control plants but the same was 38 and 56% in salt treated plants (Table 1).

Grain Quality Attributes

Statistical analysis for grain starch data indicated significant (P<0.01) differences in the salt levels and EIs while vars. Showed no significant (P>0.05) difference but interactions of these factors were non-significant (P>0.05). Salt stress decreased grain starch contents by 9 and 14% in vars. Shaheen Basmati and Super Basmati, but medium supply of Ag increased this attribute under control condition by 5 and 3% while such an increase in salinity treated plants was 12 and 16% in vars. Shaheen Basmati and Super Basmati, respectively. With the medium supply of Co, an increase in grain starch content was 2% under control condition while it was 5 and 12% in salinity treated plants of the respective varieties (Fig. 1). Grain protein content presented a significant (P<0.01) differences for salt levels and EIs in all three growing media. Salt stress decreased the grain protein content by 25 and 35% in vars. Shaheen Basmati and Super Basmati. With Ag treatment, under control condition, an increase in grain protein content was 16 and 13% in vars. Shaheen Basmati and Super Basmati while such an increase in salinity treated plants was 36 and 56%. With Co treatment grain proteins enhanced by 6 and 10% under

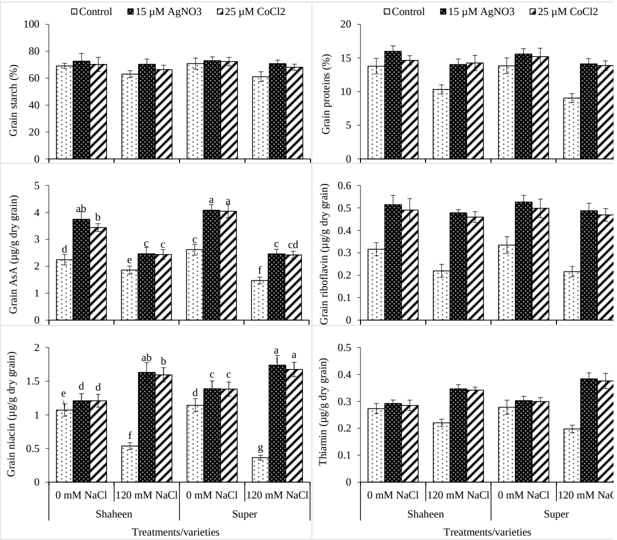

Fig. 1: Changes in grain quality attributes of differentially salt resistant rice varieties by medium supplementation of selected levels of inhibitors of ethylene biosynthesis under salt stress. The data points in a figure subset differ non-significantly (P>0.05)

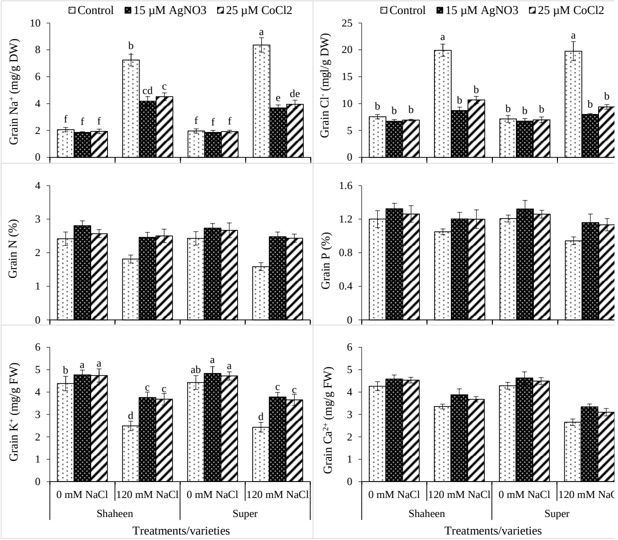

Fig. 2: Changes in grain nutritional status of differentially salt resistant rice varieties by medium supplementation of selected levels of inhibitors of ethylene biosynthesis under salt stress. The data points in a figure subset differ non-significantly (P>0.05)

control, while it was 38 and 53% in salinity treated plants (Fig. 1).

The grain AsA content showed significant ($P<0.01$) difference in salt levels, EIs and vars. Various interactions and overall interaction of all factors were significance ($P<0.01$). Salinity stress decrease the grain AsA content by 17 and 44% in vars. Shaheen Basmati and Super Basmati. Medium supply of Ag increased this attribute in control plants by 67 and 56% while this increase in salinity treated plants was 33 and 67% in vars. Shaheen Basmati and Super Basmati, respectively. Medium supply of Co, increased grain AsA content by 53 and 54% under control while it was 31 and 64% in salinity treated plants in vars. Shaheen Basmati and Super Basmati, respectively (Fig. 1). Data for riboflavin content indicated significant ($P<0.01$) differences in the salt levels and EIs, while overall interactions of all these factors were non-significant ($P>0.05$). Soil salinity decreased in the grain riboflavin content by 30 and 35% in vars. Shaheen Basmati and Super Basmati. Medium supply of Ag increased riboflavin contents under control condition by 63 and 57% in vars. Shaheen Basmati and Super Basmati while such an increase in salinity treated plants was 118 and 126%. Treatment with Co increased grain riboflavin content by 56 and 49% under control while by 109 and 117% in salinity treated plants (Fig. 1). Grain niacin indicated significant ($P<0.01$) differences in EIs, salt levels and vars, with a significant ($P<0.01$) overall interaction of all these factors. Salt stress decreased the grain niacin content by 50 and 68% in vars. Shaheen Basmati and Super Basmati, respectively. Medium supply of Ag increased this character by 13 and 22% in vars. Shaheen Basmati and Super Basmati under control condition, while by 204 and 378% salinity treated plants. Under Co treatment grain niacin increased by 13 and 21% under control while by 197 and 360% in salinity treated plants (Fig. 1). Thiamine content indicated significant ($P<0.01$) differences in salt levels and EIs, while overall interactions of these factors was non-significant ($P>0.05$). The salinity stress decreased the grain thiamine content by 19 and 29% in vars. Shaheen Basmati and Super Basmati. Medium supply of 15 µM Ag increased this attribute under control condition by 7 and 9% while such an increase in salinity treated plants was 57 and 94% in vars. Shaheen Basmati and Super Basmati. Under medium supplyof Co, an increase in the grain thiamine content was 4 and 8% in control plants while 55 and 90% in salinity treated plants (Fig. 1).

Grain Nutritional Status

The grain Na^+ data revealed that salt levels and EIs were significantly ($P<0.01$) different in all the three growing media but Var showed significant ($P<0.01$) difference with a significant ($P<0.01$) overall interactions. The salt stress increased the grain Na^+ content by 252 and 326% in vars. Shaheen Basmati and Super Basmati, respectively. The application of Ag decreased the amount of this ion by 10 and 5% in vars. Shaheen Basmati and Super Basmati under control condition while such an decrease in salinity treated plants was 42 and 56%. Under the supplementation of Co, a decrease in grain Na^+ content was 6 and 2% under control but 38 and 53% in salinity treated plants (Fig. 2). Variance analysis of data for grain Cl^- content revealed that salt levels and EIs were significantly ($P<0.05$) different. Among various interactions, EIs × salt, vars.× salt and vars × EIs and overall interaction of these factors was significant ($P<0.05$). Applied salt stress increased the grain Cl^- content by 163 and 176% in vars. Shaheen Basmati and Super Basmati. The medium supply of Ag decreased the grain Cl^- under control condition by 11 and 6% while such a decrease in salinity treated plants was 56 and 60%in vars. Shaheen Basmati and Super Basmati. Under the medium supply of Co a decrease in grain Cl^- content was 8 and 2% under control condition, while it was 46 and 52% in salinity treated plants (Fig. 2).

Grain nitrate-N data indicated significant ($P<0.01$) differences in the salt levels and EIs while vars. showed no significant ($P>0.05$) difference. Among the various interactions, EIs × salt interaction showed significance ($P<0.01$) while other interactions and overall interactions of all of these factors were non-significant ($P>0.05$). Applied salinity decreased grain nitrate-N content by 25and 35% in vars. Shaheen Basmati and Super Basmati, respectively. The medium supply of Ag increased this attribute under control condition by 16 and 13%, while a comparative increase in salinity treated plants was 36 and 56% in vars. Shaheen Basmati and Super Basmati. Under the medium supply of Co, an increase in grain nitrate-N content was 6 and 10% under control while 38 and 53% under salt stress (Fig. 2). Grain phosphate-P content showed significant ($P<0.01$) differences in the salt levels and EIs while vars. showed no significant ($P>0.05$) difference. Salinity stress decreased the grain phosphate-P by 13 and 22% in vars. Shaheen Basmati and Super Basmati, respectively. medium supply of Ag increased grain phosphate-P under control condition by 10 and 9% while an increase in salinity treated plants was 15 and 23% in the respective vars. With Co treatment, an increase in grain phosphate-P content was 5 and 4% under control condition while it was 14 and 20% in salinity treated plants of both the varieties (Fig. 2).

Statistical analysis of grain K^+ data showed that salt levels, EIs and vars. were significantly ($P<0.01$) different. EIs × salt interaction was significant ($P<0.01$) while all other interactions were non-significant ($P>0.05$). The grain K^+ content decreased by 43 and 45% in vars. Shaheen Basmati and Super Basmati, respectively under salinity stress. The medium supply of Ag increased this attribute under control condition by 9% both in the respective varieties while such an increase in salinity treated plants was 51 and 56%. With Co treatment an increase in grain K^+ content was 8 and 7% under control while 48 and 50% in salinity treated plants of both the varieties (Fig. 2). Statistical data analysis of grain Ca^{2+} indicated significant ($P<0.01$) differences in the salt levels, vars. and EIs. Among the interactions, vars. × salt and EIs × salt interactions were significant ($P<0.01$) while rest of the interactions were non-significant ($P>0.05$). Applied salinity decreased the grain Ca^{2+}content by 21 and 38% in vars. Shaheen Basmati and Super Basmati under salinity. Medium supply of Ag increased this attribute under control condition by 8% in both vars. Shaheen Basmati and Super Basmati while increased by 16 and 26% under salt stress. Under the medium supply of Co, increase in grain Ca^{2+}content was 6 and 5% under control while it was 10 and 17% in salinity treated plants (Fig. 2).

Discussion

The ultimate impact of changes produced by different treatments is on the final yield and yield components (Alam *et al*., 2015). The data for number of tillers, panicles length and number of grains and weight of 1000 grains, straw weight and harvest index revealed that applied salinity substantially reduced all the yield attributes in both the rice varieties but the adverse effects of salinity were remarkably higher on Super Basmati (a known salt sensitive variety) while salt tolerant variety Shaheen Basmati fairly well under salinity stress irrespective of the growing media used (Table 1). The medium supplementation of both the EIs quite effectively minimized the adverse effect of salinity on the yield and yield contributory parameters. The most important of these was 1000 grains weight and grain yield which were much improved by EIs treatment. Straw yield on the hand was not much affected, while the changes in grain yield directly influenced the harvest index which was considered as very important (Fageria *et al*., 2011; Amanullah and Inamullah, 2016). The improved 1000 grain weight is assignable to the fact that EIs improved the overall plant growth leading to better assimilate partitioning to the developing grain. On the other hand, improved grain yield can be assigned to various reasons which may include greater partitioning of assimilates to developing grain in the panicles. Thus greater number of panicles and number of grains as observed here under EIs in salinity grown plants (Table 1) are of greater significance. These data suggested that the inhibition of ethylene synthesis is a likely factor that plays a key role in the final yield exhibited by both the rice varieties. This substantiated the view that ethylene is a growth inhibitor and salinity has a marked influence on the enhanced production of ethylene (Kim *et al*., 2012; Ma *et al*., 2014).

The grain quality is important as far as the grain marketing and its value in the food consumption is considered (Wilson, 1990). The greater the contents of starch, proteins, vitamin and minerals, the most likely will be the grain priced (Thalang, 2016). Among the various grain quality characteristics, starch, proteins, ascorbic acid, riboflavin, niacin, and thiamine (Fig. 1) are of great significance (Mahmood *et al*., 2009). Results of this study showed that applied salinity although adversely affected the grain quality attributes of both the vars., treatment with EIs nullified the adverse effect of salinity on all these parameters thereby resulting in better grain yield and quality. In this study, root zone salinity enhanced the contents of Na^+ and Cl^- in the grain (Fig. 2), which was the results of partitioning of both the ions from leaves to developing grain (Wu *et al*., 2016). As an antagonistic effect, there was a concomitant reduction in the contents of nitrate-N, phosphate-P, K and Ca and other biochemical constituents of making the grain more qualitative (Fig. 2). This notion is supported by presence of strong interactions of different factors. Taking into account the nutrient contents of grain, it is quite evident that both the EIs reduced the partitioning of Na^+ and Cl^- to grain whilst improving the status of beneficial nutrients. As regard, the comparative efficiency of both the EIs is concerned; Ag was more effective than Co in positively influencing the grain yield and quality attributes especially under saline conditions. The improved grain quality attributes are important in view of the consumption of the grains for food and other purposes (Wilson, 1990; Thalang, 2016).

The above findings revealed that treatment with both the EIs was highly effective in improving grain yield and quality, especially the improved vitamins contents, under salinity stress. Of the two EIs, Ag was more effective. Since Ag is an inhibitor of ethylene receptor (Schaller and Binder, 2017), and was observed to be more effective than Co, it is conceivable that stopping the ethylene action in the salinity grown plants is of greater importance.

Conclusion

The adverse effect of salinity observed on the grain yield components and grain quality attributes can be effectively lessened by treating with EIs. The major effects of EIs were on the alleviation of salinity damage twined with improved development of panicles, number of grains per panicle, greater 1000 grain weight, harvest index, and improved contents of vitamins (especially ascorbic acid and niacin) and essential nutrients including K and Ca. The data from provoke the implementation of these findings for their field used of EIs. Of the two EIs, the field use of Ag is likely to pay rich dividends when applied in in the marginally to moderately saline fields.

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