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Exogenous salicylic acid and thiourea ameliorate salt stress in wheat by enhancing photosynthetic attributes and antioxidant defense

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ABSTRACT

Salinity stress is a worldwide problem, constraining global crop production seriously. Recent global climate change has made this situation more serious. Exogenous application of potential protectants such as salicylic acid (SA) and thiourea (TU) can be an important approach to alleviate the adverse effects of salinity stress on plants. Therefore, a hydroponic experiment was conducted to investigate the ameliorating effect of SA and TU on salt stress in a wheat genotype namely BARI Gom-30 considering the growth traits, photosynthetic parameters and antioxidant enzyme activities. The experiment was laid out by following randomized complete block design (RCBD) with three replications and five treatments *viz.,* control, salt, salt + SA, salt + TU, and salt + SA + TU. Salinity stress caused significant reduction of different growth traits such as plant height, shoot and root length, fresh weight and dry weight of root and shoot in wheat genotype. The leaf water status, different photosynthetic parameters and intracellular proline contents were severely shortened in the leaves of salt stressed plants. However, exogenous application of SA or TU on salt stressed wheat plants showed a significant increase in growth traits by up regulating the levels of chlorophyll content, photosynthetic pigments and proline contents in comparison to salt treated plants alone. The higher Na^+/K^+ was noted in the leaves of stressed seedlings and therefore, the wheat plants suffered more oxidative damage due to the higher production of H_2O_2 and MDA under salinity stress. Besides these, the activities of antioxidant enzymes namely CAT, POD and APX were slightly enhanced due to the imposition of salt on wheat seedlings compared to control treated plants. In contrast, SA or TU mediated ameliorating effect of salt stress maintained the lower Na^+/K^+ as well as the minimum production of H_2O_2 and MDA in BARI Gom-30. This is might be due to the higher increment of CAT, POD and APX enzyme activities in the stressed wheat genotypes supplemented with SA or TU reflecting the positive role of SA or TU against salt-induced oxidative stress in BARI Gom-30. Individual foliar application of SA and TU was found to be more effective in improving salt stress tolerance in BARI Gom-30 than that of the combined application of SA and TU.

Introduction

Wheat is one of the most important cereal crops having global production of >700 million tones, and provides 20% of the daily protein requirements, and calories for 4.5 billion people globally (Arzani and Ashraf, 2017). Wheat *(Triticum aestivum L.)* is also considered as the second largest cereal crop next to rice in respect to production in Bangladesh (Hossain *et al.,* 2013). During the year 2010-2011, 1.25 million metric tons of wheat was produced from 0.64 million hectares of land with an average yield of 1.96 mt/ha in Bangladesh (BBS, 2018). However, salt stress in the saline prone coastal belt of Bangladesh hit hard the productivity of wheat to a great extent (Hossain *et al.,* 2013). Soil salinity is an intense issue to oblige crop production because of antagonistic environmental change in the shoreline zones, particularly in the low-lying developing nations around the world (Nicholls *et al.,* 2007). Retardation in crop production occurs, resulting a 30-50% crop loss as the coastal areas of Bangladesh encompass approximately 30% of the cultivable lands, wherein, 53% of the coastal areas are affected by varying degrees of salinity (Khatun *et al.,* 2019). Salt stress caused to happen through a combined effect of osmotic and ion toxicity (primary effect), and oxidative stress (secondary effect) (Nounjan *et al.,* 2012). Wheat varieties usually categorized as moderately salt tolerant crop undergoing a threshold

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level of 6-7 dS m^{-1} (Sajid *et al.,* 2017). Lower germination rate, photosynthesis, transpiration, and higher accumulation of Na^+ and Cl^- ions can be mentioned as some drawbacks of excess salinity which disturbs the normal metabolic processes of wheat plants (Hasanuzzaman *et al.,* 2017). Soluble salts, Na^+ in particular deposits on plants ultimately limiting the growth and development by disturbing both physiological and biochemical processes namely osmotic adjustment, nutrient homeostasis, bio-molecules synthesis, photosynthesis, enzymes activity and water balance accompanying ionic and osmotic stress because of hyper-ionic and osmotic stresses (Mathur *et al.,* 2019). While in a salinity stress condition, biosynthesis of compatible solutes like proline increases which protects cells against hyperosmotic stress. The high concentration of proline under salt stress is able to balance the concentration of salts outside the cell consequently counteract the high concentrations of Na^+ and Cl^- in the vacuole (Türkan and Demiral, 2009). It also offers a wide range of protective roles including stabilizer for cellular structure and reduction of damage to the photosynthetic apparatus. Salt stress often exhibits oxidative damage that can be determined in terms of the accumulation of malondialdehyde (MDA) and H_2O_2 (Aghaleh *et al.,* 2009). However, the deterioration of salt-affected plants resulted in the production of significant level of reactive oxygen species (ROS) like hydrogen peroxide (H_2O_2), superoxide ($O\cdot^{-2}$), the hydroxyl radical (OH·) and singlet oxygen ($1O^{\cdot 2}$), as one of the earliest responses of plant cells to salt stresses, results biological damage, oxidative stress, redox imbalance and metabolic changes (Halliwell, 2016; Mathur *et al.,* 2019).

Although at high concentration, ROS causes damage to biomolecules and at low/moderate concentration, it serves the purpose of second messenger in intracellular signaling cascades that arbitrates several responses in plant cells (Das and Roychoudhury, 2014). These toxic molecules (such as ROS) efficiently distract normal metabolism through attacking the proteins, DNA, and membrane lipids in plant cell structures (Talaat and Shawky, 2014b). ROS accumulation during stress greatly depends on the balance between ROS production and ROS scavenging (Mittler *et al.,* 2004). In order to scavenge or detoxify the over-generated ROS, plants have developed a complex oxidative defense system comprising of both enzymatic and non-enzymatic antioxidants (Noctor and Foyer, 1998). These enzymes are enzymatic antioxidant includes superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), dehydroascorbate reductase (DHAR), and glutathione reductase (GR) (Yan *et al.,* 2018). These enzymes slink H_2O_2 with different mechanisms under stress conditions, and therefore, plants activate these enzymatic antioxidant systems to avoid excessive ROS accumulation when in a stress condition (Hassan *et al.,* 2017).

Induction of stress tolerance methods in plants, regarded as a vital and still receives considerable attention. The various approaches in has been undertaken for the development of stress-tolerant plants for instance, vitro selection, and the use of growth regulators (Baninasab and Ghobadi, 2011). In addition, priming plants with various growth regulators, including hormones, before or at the onset of salinity, have turned out to be a promising technique in case of minimizing salt toxicity and maximizing plant productivity (Akram *et al.,* 2017; Shahbaz *et al.,* 2017). Therefore, the exogenous use of stress alleviating chemicals such as salicylic acid (SA) and thiourea (TU) could be undergone (Wahid *et al.*, 2007). SA is an important signaling molecule, which is involved in local and systemic acquired resistance (SAR) induced by a wide range of abiotic stresses (Gao *et al.,* 2015). SA takes part in salt stress acclimation enhancement in plants by ion exclusion and/or compartmentalization, osmoregulation, reduction in membrane lipid peroxidation, synthesis of protein kinase, and regulation of antioxidative system (Yadu *et al.,* 2017).

SA increases the activity of anti-oxidant enzymes such as superoxide dismutase (Singh and Usha, 2003; Yang *et al.,* 2004). In addition, exogenous application of SA was reported to have influences on a wide range of physiological processes in salt stress, including plant growth and development, photosynthetic rate, stomatal conductance, and transpiration (Bastam *et al.,* 2012). Moreover, SA can be found further involved in the functioning of different anti-stress mechanisms in plants under osmotic stress, for example, by increasing the accumulation of sugars (Khodary, 2004) and activating of protein kinase (Mikolajczyk *et al.,* 2000). Thiourea is also an important growth regulator and is thought to be involved in improving stress tolerance in crops (Kumawat *et al.,* 2004). TU application modulates key physiological events and mechanisms, including photosynthesis, nitrogen metabolism, proline metabolism, antioxidant defense systems, and plant water relations during different plant developmental stages (Wakchaure *et al.,* 2018; Kaya *et al.,* 2019). Even though conventional wheat varieties in Bangladesh being high yielding yet to keep pace with population growth as these varieties proved not suitable for the cultivation in saline prone coastal belt of Bangladesh. In these circumstances, to meet the demand of an increasing population as well as to secure future food security, finding effective technologies addressing the reduction of wheat productivity under salt stress bears significant importance for the nation's food security (Hossain *et al.,* 2013). Therefore, the present research aims to examine the positive role of SA and TU on mitigation of salt stress for the improvement of growth and development of a wheat genotype namely BARI Gom-30 and to reveal the physiological mechanisms of SA and TU for salt tolerance in wheat.

Materials and Methods

Plant materials and treatments

A wheat cultivar BARI Gom-30 (*Triticum aestivum* L.) was used as plant materials in this study. The hydroponic pot experiment was performed with two factorial arrangement of Salicylic Acid (SA) and Thiourea (TU) and salt treatments using a randomized complete block design (RCBD) with three replications. The salinity factor (NaCl) comprised of one level (150mM), and the SA and TU were applied at 150mM and 15mM levels, respectively. Therefore, the treatment combinations were as follows: 'C' 0 mM NaCl + 0 mM SA + 0mM TU (control); 'S' 150 mM NaCl + 0 mM SA/0mM TU; 'S + SA' 150 mM NaCl + 10 mM SA; 'S + TU' 150 mM NaCl + 15 mM TU; 'S + SA + TU' 150 mM NaCl + 10 mM SA+ 15 mM TU.

Plant cultures and treatment induction

A homogenous lot of wheat seeds were separately surface sterilized by using 5% sodium hypochlorite + 2% Tween-20 for 25 min. Subsequently, presoaking of the seeds were done in dH_2O for 24 h and kept in a petri-dish with moistened filter paper for 3 days at room temperature (30°C) to induce germination and 20 seeds were placed per petri-dish. Evenly germinated seeds were placed in a Styrofoam seedling float (28 cm × 32 cm × 1.25cm) containing 60 (2×30) hole having nylon net at the bottom and fitted in a pot with 4L water capacity. Wheat seedlings were grown hydroponically using nutrient solution (Modified Cooper's nutrient solution, Cooper, 1996). The control (T0) plants were grown on the nutrient solutions only with distilled water (dH20). After 7 days of seedling establishment in the pot, the salt treatment (T1, 150 mM NaCl) were applied in six steps employing 25mM in each pot in each time at an interval of 7 days (total six spray). The first and last salt stress imposition being on day 10th and day 42th, respectively. Simultaneously, the exogenous application of SA (T2, 10mM) and TU (T3, 15mM) were employed to the leaf surface of salt treated plants 20 days after seedling establishment (i.e. 10 days after stress imposition) individually to ensure the maximal penetration of externally applied compounds into the leaf tissues. The nutrient solutions were renewed at an interval of 15 days through the experimental period.

Determination of morphological parameters

The plant height was measured on the 42th day of planting. Shoot length (SL) and root length (RL) was measured from shoot initiation point to highest shoot tip by using centimeter (cm) scale. Percentages of the live leaves (LL %) were determined by dividing the number of live leaves by total number of leaves multiplied by hundred. The fresh weight of shoot (SFW) and root (RFW) were taken carefully by using an electric balance immediately after collecting the sample. Afterwards, plant samples (Root + Shoot) were separately stored in a brown envelop (20x10 cm) and oven-dried at 60ºC for 72 hr. and then dry weight of root and shoot were also measured by an electric balance.

Determination of photosynthetic pigments

Chlorophyll content (Chl a, Chl b, Total Chlorophyll content) and carotenoids were determined by randomly collected 42 days old leaf sample. An amount of 50 mg fresh leaf samples were taken into a small vial containing 10 ml of 80% acetone and was covered by aluminium foil and preserved in the dark for 7-10 days. The absorbance was measured at 663 nm for Chl a, 645 nm for Chl b and 663 nm wave length for total chlorophyll by using a spectrophotometer (Shimadzu UV-2550, Kyoto, Japan). Afterwards, the concentrations of Chl a, Chl b and total chlorophyll were calculated using the following formula: Chl a: 12.7(A663) – 2.69(A645); Chl b: 22.9(A645) – 4.68(A663) and total chlorophyll: 20.2(A645) + 8.02(A663) and expressed as µg g-1 fresh weight (F.W.) of leaf.

Measurement of leaf water related traits

Leaf water related traits *viz.,* relative water content (RWC), relative water loss (RWL), and excised leaf water retention (ELWR) were determined according to Mostofa and Fujita (2013). In case of RWC measurement, leaf samples were collected after 42 days of planting and then weighed (FW) and immersed in dH_2O in a petridish for 4 hr. Subsequently, excess water was removed with a paper towel and turgid weight (TW) was immediately determined. After 48-hr oven-drying at 70 °C, dry weight (DW) was recorded, and leaf RWC was calculated according to the following formula: RWC (%) = FW – DW /TW – DW× 100. For the measurement of RWL, fresh weight (FW) of leaves were recorded and kept at 30ºC for 4 hours and reweighed (WW4h) and finally, oven dried at 72°C for 24 h to attain dry weight (DW). The RWL was measured according to the following formula: RWL (%) = [(FM – WW4h)/ (FW – DW)] × 100. For the measurement of ELWR, fresh weight (FW) of leaves were recorded and kept at 30ºC for 4 hours and reweighed (WW4h). Finally, ELWR was then calculated using the following formula: ELWR (%) = [1– (FW – WW4h)/ FW] ×100.

Determination of Na^{+}/K^{+}

After harvesting, shoot samples were oven dried at 60°C for 3 days and the finely powdered plant material was digested with HNO_3: $HClO_4$ (2:1v/v) mixture at 220°C for 1.5 - 2 hours according to the method of Tahjib-Ul-Arif *et al.* (2018). Na^+ and K^+ contents were quantified by flame photometry (Jencon PFP 7, JENCONS-PLS, UK) following to Brown and Lilleland (1946).

Determination of Proline content

Proline content was measured from the leaf tissues according to the procedure of Bates *et al.* (1973) with some modifications as described by Rasel *et al.* (2019).

Determination of H_2O_2 and MDA Content

H_2O_2 was determined from leaf tissues (Velikova *et al.* 2000) using an extinction coefficient of 0.28 μM^{-1} cm^{-1}, and with values expressed as µmol g^{-1} FW. The lipid peroxidation product, MDA, was estimated in the fresh maize leaves harvested on the 85th day of plantation. MDA was extracted and measured after reaction with thiobarbituric acid reactant substances using the protocol as described by Rasel *et al.* (2019).

Measurement of antioxidant enzyme activity

The activities of three antioxidant enzymes *viz.,* catalase (CAT), ascorbate peroxidase (APX), and peroxidase (POD) were determined in extracts from the shoot tissues. To extract the enzymes, fresh leaf samples (0.05 g) were homogenized with 1mL of 50 mM K-phosphate buffer (pH 8.0), using pre-chilled mortars and pestles. The homogenates were centrifuged at 11,500×g for 10 min and the resultant supernatants were collected to analyze enzyme activities. All procedures were performed at 0-4°C and all of the spectrophotometric assays were performed using a UV-VIS spectrophotometer (Shimadzu, UV-1201, Tokyo, Japan).

CAT (EC: 1.11.1.6) activity was assayed following the method of Aebi (1984) by monitoring the decrease in absorbance at 240 nm. The specific activity was determined using an extinction coefficient of 39.4 M^{-1} cm^{-1} and expressed as mmol min^{-1} g^{-1} FW.

APX (EC: 1.11.1.11) activity was measured by monitoring the decrease in absorbance at 290 nm as AsA was oxidized, according to the method of Hoque *et al.* (2007a, b). The specific activity was measured using the extinction coefficient 2.8 mM^{-1} cm^{-1} and expressed as µmol min^{-1} g^{-1} FW.

POD (EC: 1.11.1.6) activity was determined by following the method of Nakano and Asada (1981) with some modifications. The activity of POD was calculated from the increase in absorbance per minute when the extinction coefficient of H_2O_2 was 26.6 mM^{-1} cm^{-1}.

Statistical analysis

Data were analyzed by Minitab 17 by a one way analysis of variance and significant differences between mean values with standard errors were presented indicated by different alphabetical letters in the same column at the $p < 0.05$ level using the least significant difference (LSD) test.

Results

Growth traits response of wheat genotypes under NaCl, SA and TU treatments

The wheat seedlings grown in saline condition showed several symptoms of salt injury such as yellowing and drying of leaves, reduction in root and shoot growth, reduced stem thickness and in many cases dying of seedlings were also observed, however, the exogenous application of signaling molecules like SA and TU significantly improved the growth characteristics under salt stress conditions in BARI Gom-30 (Table 1).

Root length and shoot length

The exposure of plants to salt stress resulted in a considerable decrease in the root and shoot length in wheat genotype (Table 1). In the present investigation, 150 mM NaCl application to wheat plants reduced shoot and root length by 14.8% and 42.6% in BARI Gom-30 respectively compared to the control treated plants (Table 1). However, the supplementation of salt stressed plant with SA or TU enhanced the shoot length and root length by ameliorating the salt toxicity effect in salt stressed wheat seedlings. In the combination of NaCl + SA, shoot length increased by 106.5% and root length by 108.0% respectively compared to the salt treated plants only. In the similar way, foliar spray of TU also amending the stress impact on shoot length and root length by increasing 91.8% and 63.6% in comparison to the salt treated plants only (Table 1).

Table 1. The exogenous effects of SA and TU on growth characteristics of wheat plants under non-salinized and salinized conditions

Genotype	Treatments	SL	RL	SFW	RFW	SDW	RDW
BARI Gom-30	Control	43.09^{a}	33.25^{a}	3.19^{a}	1.59^{a}	0.36^{a}	0.14^{b}
	S	24.97^{d}	25.22^{b}	1.36^{d}	1.0^{c}	0.32^{b}	0.09^{d}
	S+SA	32.84^{b}	33.60^{a}	1.45^{c}	1.34^{b}	0.22^{c}	0.11^{c}
	S+TU	29.16^{c}	22.40^{c}	1.64^{b}	0.94^{d}	0.19^{c}	0.07^{d}
	S+SA+TU	20.87^{e}	17.67^{d}	0.38^{e}	0.25^{e}	0.32^{b}	0.21^{a}

Here, S denotes Salts, SA denotes Salicylic acid and TU denotes Thiourea; *SL* Shoot length (cm), *RL* Root length (cm), *SFW* Shoot fresh weight (g), *RFW* Root fresh weight (g), *SDW* Shoot dry weight (g), and *RDW* Root dry weight (g); Data are presented with mean values indicated by different alphabetical letters in the same column at the $p < 0.05$ level using the least significant difference (LSD) test.

Root and shoot biomass

The root and shoot biomass in wheat was markedly changed under the treatments of salt and priming molecules like SA and TU. Salt treatment declined shoot fresh and dry weight by 39.5% and 87.6% compared to the control condition plant whereas root fresh and dry weight were reduced by 65.1% and 62.9% under salt stress (Table 1). However, the foliar application of SA or TU to salinized wheat plants caused a significant increase in fresh and dry weight of shoot and root, though the values were still significantly lower than those of the control plants. In the present study, the application of protectant such as SA enhanced the shoot fresh and dry weight by 104.6% and 69.1% respectively and similarly, root fresh and dry weight also increased by 125.2% and 127.4% with the foliar spray of TU on stressed plant in compared to the NaCl treated plant only. Similarly, the supplementation of salt stressed wheat seedlings with TU also experienced the improvement of shoot fresh weight and dry weight by 30% and 25% in BARI Gom-30 under salt stress conditions in compared to the salt treated plant only (Table 1). The combined application of SA and TU on the salt stressed plants also didn't make any significant improvement of morphological parameters in wheat genotype under salt stress conditions (Table 1).

Photosynthetic pigments

The exposure of wheat plants to 150 mM NaCl for 42 days severely affected different photosynthetic pigments such as total chlorophyll content, chlorophyll a, chlorophyll b, and carotenoid contents (Table 1). In contrast, a significant improvement in chlorophyll contents was recorded when SA or TU was applied on wheat seedlings under salt stress condition. In the present study, application of 150 mM NaCl treatment led the moderate reduction of total chlorophyll content by 53.04% in the leaves of BARI Gom-30 correspondingly in comparison with the non-salt treated plants (Table 2). However, the alleviation effect of SA or TU in the salt treated plants caused the great increment of total chlorophyll content by 128.8% and 149.3% compared to NaCl-alone treatment. The wheat seedlings subjected the salt stress also caused the severe curbing of chl a and chl b content in wheat plants by 63.0% and 46.6%, respectively. The alleviation effect of TU on salt stressed plants was considered best in comparison with SA treatment alone. When the wheat plants were treated with TU in the presence of NaCl caused the increment of chl a and chl b by 163.1% and 127.3% in BARI Gom 30 compared with salt treated plant only. Similar response was also exhibited in case of carotenoid concentrations in wheat cultivar where salt treatment decreased the carotenoid content by 90.3% in comparison with control treated plants. Nevertheless, the combination of NaCl+ SA and NaCl+ TU also enhanced the carotenoid content under salt stress condition by 272.5% and 366.8% in BARI Gom-30 as compared with only NaCl treated plants condition (Table 2).

Leaf water parameters

Different leaf water related parameters *viz.,* RWC, RWL, and ELWR were estimated to investigate the alleviating role of SA and TU on salt stressed wheat seedlings. A significant reduction of RWC and ELWR approximately by 21.1% and 120.4% was reported in BARI Gom-30 due to salt stress imposition. With the treatment of NaCl +SA to salt stressed plant led the increment of RWC and ELWR by 63.2% and 158.7%, respectively in comparison with that of salt treated condition (Table 3). Correspondingly, exogenous application of TU also showed an increment of RWC and ELWR by 71.8% and 139.0% compared to that in the salt stressed condition (Table 3). The RWL content showed the similar pattern like RWC and ELWR under saline conditions by decreasing 74.8% in BARI Gom-30, however, the supplementations of salt stressed plants with SA or TU significantly enhanced the RWL by 101.7% and 176.7% in comparison to the NaCl treated plant condition only (Table 3).

Table 2. The exogenous effects of SA and TU on photosynthetic parameters of wheat plants under non-salinized and salinized conditions

Genotypes	Treatments	Chl a	Chl b	Total Chl content	Carotenoid
BARI Gom-30	Control	4.07^b	2.78^a	6.85^a	0.41^d
	S	2.73^d	1.37^d	4.10^d	0.38^d
	S+SA	3.97^c	1.49^c	5.45^c	1.02^c
	S+TU	4.52^a	1.77^b	6.29^b	1.38^a
	S+SA+TU	2.86^d	1.29^e	4.16^d	1.17^b

Here, *S* denotes Salts, *SA* denotes Salicylic acid, *TU* denotes Thiourea Data are presented with mean values indicated by different alphabetical letters in the same column at the p< 0.05 level using the least significant difference (LSD) test.

Table 3. The exogenous effects of SA and TU on leaf water related parameters of wheat plants under non-salinized and salinized conditions

Genotype	Treatments	RWC (%)	RWL (%)	ELWR (%)
BARI Gom-30	Control	69.67^d	0.45^b	0.26^d
	S	63.27^e	0.34^c	0.32^c
	S+SA	80.03^b	0.34^c	0.51^a
	S+TU	85.50^a	0.60^a	0.44^b
	S+SA+TU	79.03^c	0.59^a	0.36^c

Here, *S* denotes Salts, *SA* denotes Salicylic acid, *TU* denotes Thiourea *RWC (%)* Relative water content (%), *RWL (%)*, Relative water loss (%), *ELWR (%)* Excised leaf water retention (%); Data are presented with mean values indicated by different alphabetical letters in the same column at the p< 0.05 level using the least significant difference (LSD) test.

Ion concentrations

The Na^+/K^+ content in the leaves of wheat genotype increased under salt stressed condition in comparison to the control treated plants (Fig. 1A). In control treatment, the Na+/K+ ratio was 2.21% and salt stress led to the increment of 20.6% in BARI Gom-30 in the level of control condition. However, exogenous application of SA and TU to the plant successfully mitigate the toxic effect of salt stress in the wheat seedlings. The individual foliar spray of SA and TU on salt stressed plant demonstrated the reduction of Na^+/K^+ by 8.4% and 14.8% respectively in stressed wheat seedlings in comparison with the salt treated (150 mM NaCl) condition only. Similarly, the combined application of SA+TU on wheat seedlings also reduced the Na^+/K^+ by 12.54% in the level of stressed plant only (Fig. 1A).

Proline contents

In the present study, proline level increased in a salt stress condition (150 mM NaCl) by 130.4% as compared with that of control. However, the accumulation of proline was further increased in the cultivars BARI Gom-30 (8.50%) with the application of priming molecule SA under salt stress conditions compared with the salt treated plant only (Fig. 1B). However, the foliar spray of TU and combined treatment of SA+TU showed the reduction of proline content by 12.2% and 4.29% respectively in BARI Gom-30 as compared with the 150mM NaCl condition plant only (Fig. 1B).

H_2O_2 and MDA contents

The result of the study reflected that salt stress enhanced the production of H_2O_2 causing substantial rise in MDA content in wheat seedlings, which however declined with the application of exogenous protectant under saline conditions (Fig. 1C, 1D). The accumulation of H_2O_2 and MDA were enhanced by 5.90% and 77.1%, respectively in BARI Gom-30 under 150 mM NaCl salt treatment in comparison to that of untreated plants. The application of SA and TU showed the reduction of H_2O_2 and MDA contents and the maximum reduction of H_2O_2 by 9.66%, and MDA by 36.2% were found in BARI Gom-30 with the application of NaCl +SA treatment as compared with that of the salt treated condition only. Similarly, decreasing trend in H_2O_2 and MDA content by 13.0% and 47.11%, respectively were also observed in BARI Gom-30 with the combination of NaCl +SA+TU in the level of salt treated plant only (Fig. 1C, 1D).

Antioxidant enzymatic activities

Activities of different antioxidant enzymes such as CAT, APX and POD were measured in the leaves of wheat seedlings under salt stress with the supplementation effect of SA and TU (Fig. 1E, 1F, 1G). The great enhancement of catalase activity by 117.3% was observed in BARI Gom-30 due to the imposition of NaCl treatments as compared to the control treated plant. While the application of SA and TU reduced harmful effect of salinity stress through further enhancing of CAT activities in the salt-treated plants. Foliar applications of SA increased the CAT activity by 2.81% whereas TU increased the CAT activity by 12.1% under salinity stress condition which indicating a high value of CAT to detoxify the ROS content (Fig. 1E). In contrast, the APX activity was reduced by 47.8% under salt stress in BARI Gom-30 correspondingly. However, the individual exogenous application of protectants to wheat plant showed maximum enhancement of the APX activity by 53.9% in NaCl+TU treatment combinations and by 89.9% in NaCl+SA treatment combinations in wheat plants in comparison to the salt treated seedlings (Fig. 1F). The study exposed that salt stress led the significant increment of POD activity in wheat cultivars by 20.13% as compared with untreated conditions (Fig. 1G). However, the highest activity of POD was recorded when the salt stressed wheat plants were supplemented with SA. In this case, the foliar spray of SA on stressed plants exhibited the increment of POD activity by 3.23% in BARI Gom-30 in comparison to that of the salt treated plant only (Fig. 1G). The combined foliar application of SA+TU on salt stressed wheat plants didn't enhance the enzymatic activities of CAT and POD in the leaves of wheat seedlings.

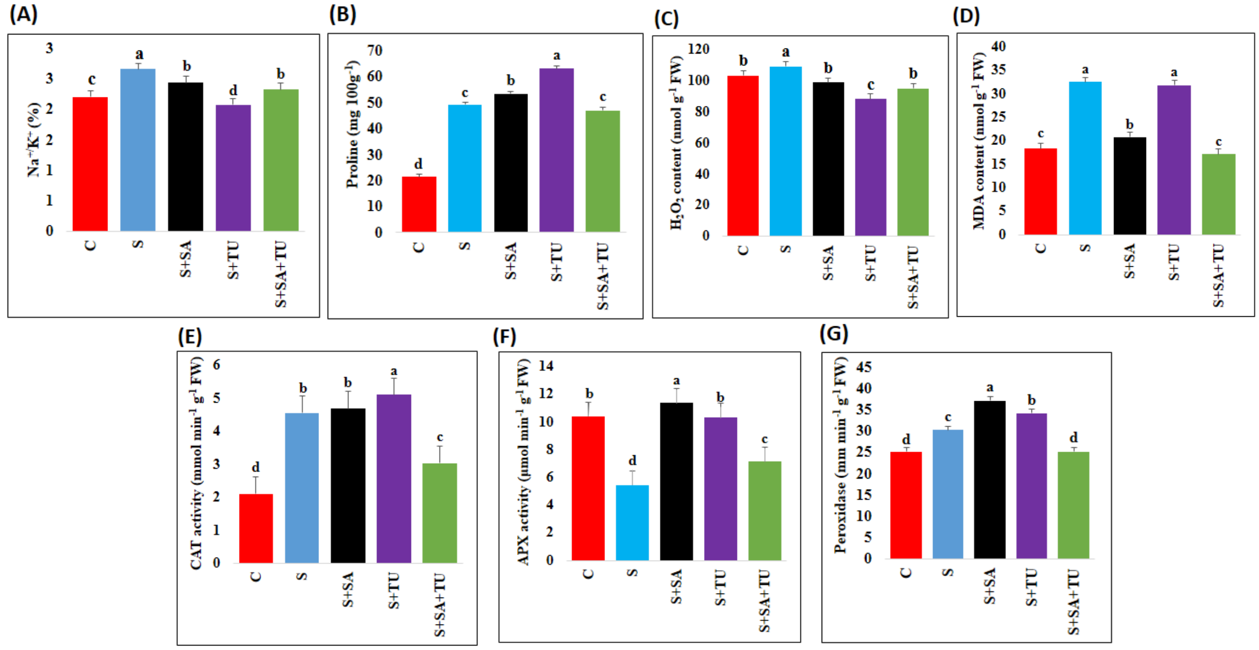

Fig. 1. Effects of exogenous SA and TU on A. Na^+/K^+, B. proline content, C. Hydrogen peroxide (H_2O_2), D. Malondialdehyde (MDA) content, E. Catalase (CAT) activity, F. Ascorbate peroxidase (APX) activity and G. Peroxidase (POD) activity in the leaves of salt stressed wheat leaves . Data are presented with mean values indicated by different alphabetical letters in the same column at the $p< 0.05$ level using the least significant difference (LSD) test.

Discussion

Salinity is one of the most significant natural factors far and wide restricting the yield of plants to a great extent (Hossain *et al.,* 2015; Khatun *et al.,* 2019). Numerous methodologies, including exogenous use of plant growth regulators, have indicated promising impacts in the mitigation of the detrimental effects of salinity on various crop plants, such as tobacco, wheat, rice, and soybean (Akram *et al.,* 2017). Prior to evaluate the alleviation of SA and TU on salt stress in wheat plant, several morphological and biochemical parameters were taken into account in this study.

The downshift in growth of many plants may be attributed to the effect of salt stress on various physiological processes such as photosynthesis, ion homeostasis and antioxidant enzyme activities (Talaat, 2019). In many cases, the negative effects of salinity predicated to have increased Na^+ and Cl^- ions in different plants; hence, these ions produce the critical conditions for plant survival by intercepting different plant mechanisms. In the present study, salt stress (150 mM NaCl) deemed a considerable holdback shoot and root length, root and shoot biomass in wheat seedlings (Table 1). This growth inhibitory effect of salinity could be characterized to the high salt concentration-mediated disturbance of several photosynthesis, nutrient homeostasis, accumulation of compatible solutes, and activities of antioxidant enzymes (Tariq *et al.,* 2011). However, plants provided with SA and TU, exerts a lessening in the extent of growth suppression, and these treated plants had greater growth parameters, such as fresh and dry weights of shoot and root, length of root and shoot in comparison to that of untreated salt stressed plants. SA-induced increase in growth traits could be due to the SA-enhanced increase in the net photosynthetic rate under salt stress (Li *et al.,* 2014). In addition, an expansion in growth parameters of salt influenced plants in response of SA may be identified with the defensive job of SA on membranes that may build the resistance of plants to salt stress (Aftab *et al.,* 2010). Furthermore, Liu *et al.* (2014) reported that an SA-mediated increase in growth parameters under salt stress could be due to the SA-induced antioxidant functioning and metabolic activities in plants. Besides, the exogenous use of TU additionally advances roots development which has been recognized as a significant characteristic in giving salinity tolerance in plants (Perveen *et al.,* 2015). The present study outcomes were supported by Arfan *et al.* (2007), who concluded that exogenous foliar application of SA ameliorated the hostile effects of salt stress on barley and wheat growth traits, respectively.

Photosynthetic pigments, for example, chlorophyll a and b were major components of photosystems driving the mechanism of photosynthesis and hence growth as far as biomass production (Noreen *et al.,* 2011). Salt stress causes deterioration in the structure of chloroplast e.g., thylakoid membranes and plastids due to direct Na+ toxicity and cellular oxidative damage (Mittler, 2002). Accumulation of NaCl has been accounted for unfavorably influence chlorophyll biosynthesis and photosynthetic process (especially photosystem-II) in plants (Jiang *et al.,* 2017). In the current study, chlorophyll content and the related photosynthetic content in wheat plant leaves subjected to saline condition considerably declined (Table 2). However, the application of SA and TU fundamentally improved the leaf chlorophyll contents and photosynthetic pigments in the salt stressed wheat seedlings. These findings are similar to those of Ghai *et al.,* (2002) who showed a considerable improvement in chlorophyll content. Therefore, SA mediated improvement in photosynthesis, partly due to increasing chlorophyll synthesis, is important for improving plant salt tolerance. SA might promotes the activity of enzymes related to chlorophyll biosynthesis or might calm the impedance of the photosynthetic framework, subsequently decreasing chlorophyll degradation (Ma *et al.,* 2017).

The maintenance of plant water status or osmoregulation is regarded as a vital physiological process for maintaining optimal plant growth under stress conditions (Taiz and Zeiger, 2006). RWC is a useful measure of the physiological water status of plants (González and González-Vilar, 2006). Salt stress led leaf electrolyte leakage in plants (Parida and Das, 2005) of different crops, however SA treatments reduce the electrolyte leakage in lettuce plants affected by salt stress (Khalifa et al. 2016). In the present study, various leaf water related parameters like RWC, RWL and ELWR were severely diminished when plant confronted to salt stress (Table 3). This decrease might be occurred due to reduced water uptake (Parvin *et al.,* 2019) and/or its harmful effect on cell wall structure (Abdelaal *et al.,* 2018). Excess accumulation of Na^+ in the plants causes direct toxic effects to the cell membrane causing leakage of electrolytes and adversely affecting metabolic activities in cytosol. It leads to depress physiological and biochemical processes and an overproduction of ROS, which turn out to premature leaf senescence and loss of photosynthetic efficiency that, in turn, reduce carbon assimilation and ultimately, the growth (Sedigheh *et al.,* 2011). In contrast, when the salt stressed wheat seedlings were supplemented with priming molecules such as SA or TU demonstrated the improvement of water status in plants (Table 3). These results were in accordance with Tari *et al.* (2002) who found the positive role of SA to increase RWC and water potential tolerance of plants to salt stress. The elevating effects of these treatments on RWC could be due to the increase in osmo regulators, as well as to osmotic adjustment in plant cells (Gholami Zali, *et al.,* 2018; Hafez *et al.,* 2019). The exogenous SA or TU applications could enhance membrane deterioration in plants exposed to salt stress, thus indicating SA that facilitated the maintenance of membrane functions (Gunes *et al.,* 2007). In addition, SA increases ABA, which ultimately helps to maintain better water balance in the plants

(Sakhabutdinova *et al.,* 2003). In accordance with present results, less electrolyte also has been previously reported in the laves of salt stressed plants due to the foliar spray of SA and TU (Yildirim *et al.,* 2008). Ionic imbalances, specific ion effects and nutrient deficiency symptoms in plants have found to be prominent in plants grown in salinity prone areas provoking excess Na^+ and Cl^-accumulation (Siringam *et al.,* 2009). The results of the present observation demonstrated the enhancement of Na^+/K^+ in salt stressed wheat genotype although mitigating effects were noted when SA or TU were sprayed on salt treated wheat seedling leaves (Figure 1A). The exogenous SA or TU application expanded the K^+ content and diminished Na^+ aggregation in the shoot of Arabidopsis under salt stress condition (Jayakannan *et al.,* 2013). Khan *et al.* (2010) also found that the application of SA enhanced salinity tolerance of mungbean by reducing Na^+ and increasing K^+ content.

Proline is the most regular compatible solute prevailing in a wide variety of plants. It may acts as a non-toxic osmotic solute preferentially stabilizing the structure of macromolecules and organelles (Sumithra *et al.,* 2006). The proline content, typically viewed as associated to stress resistance mechanisms, is one of the most frequently reported modifications induced by salt stress in plants (Misra and Saxena, 2009). In the current study, considerable proline accumulated in BARI Gom-30 under salt stress (Figure 1B). Increased proline in the stressed plants may be an adaptation to balance the energy for growth and survival and thus help the plant to survive at stress, as also observed in spinach (Kuzuoglu-Ozturk et al. 2012) and in *Phyllanthus amarus* (Jaleel *et al*., 2009). While the plants supplemented with SA exhibited further increment of intracellular proline content in the leaves of wheat plant compared to salt treated plant (Figure 1D). This usually happens because proline might contribute to osmotic adjustment at the cellular level when plant face stress, thus protect membrane integrity and consequently, mediate the damage induced by salt. The results of the present study was in line with Sharma et al. (2019) who observed that the protective effects of SA on salt stress-induced oxidative damage in cotton leaves might be related with its regulation roles in organic solute like proline.

Reactive oxygen species reportedly a central component of plant's adaptive responses to biotic and abiotic stresses. Production of ROS is intimately related with the stressful condition which leads to the membrane retardation and electrolyte leakage (Kumar *et al.,* 2015). Salt stress caused significant damage on plant growth and development is partial due to the generation of reactive oxygen species (ROS), such as hydrogen peroxide (H_2O^2) and superoxide ($O.^{-2}$) (Asada, 2006). Studies usually consider the malonaldehyde (MDA) content as an important indicator to reflect the level of lipid peroxidation due to the production of ROS (Li *et al.,* 2014). In our exploration work, salt stress altogether improved the MDA and H_2O_2 content in wheat genotype in contrast with the control condition which implies a significant level of oxidative harm in the lipid membrane because of salinity stress in the wheat seedlings (Figure 1C, 1D). On the contrary, the production of MDA as well as H_2O_2 was severely reduced when the SA or TU were exogenously applied on salt stressed wheat seedlings (Figure 1C, 1D). This was because of the prevention of membrane damage and induction of antioxidant responses by SA or TU, which protects the plant from oxidative damage (Khalifa *et al.,* 2016). SA reduced ROS by increasing the activity of APX, CAT, and POD and as a result, oxidative damage to the membranes occurs. The findings of our study is analogous to another researcher who suggested that SA acts as a natural signal molecule activating the response system of plant through mediating the peroxidatic reaction (Klessig and Malamy, 1994).

Plants can by using Enzymes such as Ascorbate peroxidase (APX), catalase (CAT) and peroxidase (POD) can come into action protecting plant tissues from the toxic effects of salt-accumulated ROS (Ahmed *et al.,* 2010). CAT converts H_2O_2 to water in the peroxisomes (Fridovich, 1989), and prevents oxidative damage. APX is regarded as detoxifying system in plant cells in the ascorbate glutathione cycle, in which, ascorbate peroxidase (APX) enzymes play a key role catalyzing the conversion of H_2O_2 into H_2O, using ascorbate as a specific electron donor (Hong *et al.,* 2013). POD is one of the few important antioxidant enzymes with the ability to fix oxidative damage brought about by ROS (Jini and Joseph, 2017). The effect of exogenous SA on reducing the accumulation of O^{2-} and on increasing POD activity have been well documented in cotton and *Pisum sativum* L. (Yadu *et al.,* 2017). In the present analysis, the higher increment of different antioxidant enzymes viz., CAT, APX or POD were focused in wheat genotype namely BARI Gom-30 due to the supplementation of stressed plant with SA or TU in comparison to the control treated plant (Figure 1E, 1F, 1G). The antioxidant defenses highly appeared salt stressed wheat seedlings to provide crucial protection against oxidative degradation in cellular membranes and organelles in plants grown under salt stress (Hossain *et al.,* 2015). The results of our study coincides with results of several researchers (Pirasteh-Anosheh and Emam 2018) who discovered a conspicuous positive role of exogenous SA in oxidative protection through the enhancement of antioxidant capacity that might intensify the photosynthetic process and reduce the toxic effects of salinity on maize plants.

Conclusion

The exposure of wheat seedlings to salt stress caused the severe reduction of growth parameters as well as photosynthetic pigments and leaf water status. The imposition of salt treatment lead the higher Na^+/K^+, H_20_2 and MDA content in stressed wheat genotypes. In addition, the activities of different antioxidants were somewhat enhanced in the leaves of wheat seedlings under salt stress. On the other hand, the foliar spray of SA and TU greatly ameliorated the salt induced toxic effect and led the increment of growth parameters by the enhancement of photosynthetic attributes and by the prevention of electrolytic leakage in the leaves. Besides, SA and TU mediated effect lowered the Na^+/K^+ and protect the plant from oxidative damage by the maintenance of lower H_2O_2 and MDA content and by the up regulation of antioxidant enzymatic activities in salt stressed wheat seedlings.

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