Exogenous application of gibberellic acid and silicon to promote salinity tolerance in pea (Pisum sativum L.) through Na\(^+\) exclusion

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**Article Info**

**Abstract**

Salinity is a worldwide problem limiting the plant growth and risking food security. This study was conducted to examine exogenous application of silicon (Si), gibberellic acid (GA\(_3\)) upon the ion transport, growth, yield, and antioxidant enzymes activities of pea plant in saline conditions. Two pea varieties Meteor-FSD and Samrina Zard were pre-treated with GA\(_3\) (10\(^{-4}\) M) for 12 h. Plants were allowed to grow with or without silicon in washed silica sand. Ten days old seedlings were shifted in pots with 10 kg soil. Twenty-five days old plants were exposed to 0 and 5 dS m\(^{-1}\) sodium stress. Results showed that exogenous application of GA\(_3\) + Si was the best treatment for increasing plant biomass and yield in the presence and absence of NaCl. Furthermore, application of Si or GA\(_3\) enhanced chlorophyll content in the leaves, thereby increasing the net assimilation rate of pea varieties under NaCl stress by increasing the antioxidant enzyme activity. Treatment of Si alone or in combination with GA\(_3\) significantly reduced Na\(^+\) movement in both pea varieties. Results showed that Si has more prominent role than GA\(_3\) alone to build-up high plant biomass, yield, soluble protein content and reduction of Na\(^+\) transport. Samrina Zard variety showed higher yield, shoot and root dry weight as compared to Meteor-FSD variety in presence and absence of salt. It was concluded that Si can be used as a nutrient for pea under saline or non-saline conditions. Moreover, application of GA\(_3\) has a potential role for increasing salinity tolerance, mostly in sensitive pea varieties.

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**1. Introduction**

Saline soils in dry and semi-arid areas of the globe are increasing rapidly because of various climatic changes and insufficient irrigation water (Ding et al. 2020). Soil salinity is key constraints in sustainable agriculture that significantly decline plant production worldwide (Zhang et al. 2013; Shahid et al. 2018). World population is increasing rapidly and estimated to be 9.8 billion at the end of 2050 and 11.2 billion in 2100 (McNabb 2019). Salt prone area of the world is 1125 million hectares; out of these 76 million hectares converted to saline and sodic conditions through human being activities (Akram et al. 2020). Salinity stress in plants was partly managed by techniques of plant breeding, development of transgenic plants. Soil reclamation from salinity can be done with management practices like mulching, deep tillage, and incorporation of organic matter. However, production of salt resilient varieties is a lengthy process due to complicated nature of multigenic control in salt tolerance. Production of salt tolerant through plant breeding, biotechnological and genetic transformation in some crops showed unsuccessful results (Maribel et al. 2000). Consequently, exogenous application of various biochemicals and plant growth regulators emerged as alternative options to mitigate salinity stress in cheaper price and low risk (Gurmani et al. 2018; Iqbal and Ashraf 2013).

Salt tolerance in plants is associated with Na\(^+/K^+\) discrimination which usually controlled by Na\(^+\) transportation (Chakraborty et al. 2004).
Various physiological, biochemical attributes as well as plant growth and production declined due to higher external Cl− and Na+ transport in plant tissues (Magda et al. 2021). Salt stress declines leaf expansion, photosynthetic pigments, and diminishes plant growth because of water stress produced through osmotic stress and toxic level of Na+ uptake in plant tissues (Ashraf and Harris 2013). Higher external Na+ and Cl− level damages process of electron transport and enhance creation of reactive oxygen species (ROS). High ROS level has deleterious effect on membrane, chloroplast, lipid, protein, and nucleic acid. In avoiding deleterious effects of salinity, plants have established various important antioxidant resistance techniques that control ROS mechanism (Al-Huqail et al. 2018).

Salt affected soils can potentially be reclaimed through rigorous procedures of physical, chemical, and biological reclamation methods; however, such methods are costly and time consuming. While development of salt tolerance in plants through exogenous application of phytohormones is a cheap and quick alternate solution (Zhang and Shi, 2013). In avoiding adverse effects of salinity stress on plants, seed pretreatment is an inexpensive and shotgun approach. Though, efficiency of diverse seed pre-treatment agents depends on different abiotic stresses and plant species (Gurmani et al. 2018; Iqbal and Ashraf 2013). According to reports, plant growth regulators have successfully minimized the harmful effects of salt stress on the growth of various plant species (Nimir, Lu et al. 2015; Gurmani et al. 2018). Giberellin acid (GA3) plays a vital role in leaf expansion, seed growth, stem elongation and plant yield and growth (Tsegay and Andargie 2018; Khan et al. 2002; Iqbal and Ashraf 2013). Exogenously application of GA3 had no reliable influence on gaseous exchange characteristics in some crop species such as wheat (Ashraf et al. 2000). Seed treatment with GA3 enhances salinity tolerance through maintaining superoxide dismutase (SOD), catalase (CAT) and peroxidase dismutase (POD) activity (Ahmed et al. 2021). Exogenous application of GA3 has neutralized toxic effects of salt stress by increasing relative water content, electrolyte leakage, chlorophyll content and plant growth in mustard crop (Ahmad et al. 2012).

Silicon is an essential plant nutrient that is present at highest concentration in earth’s crust (Kaushik and Saini, 2019). Silicon in the form of silica (SiO2) reacts with the constituent of cell wall and gives strength to plant leaves and stem. Exogenous application of Si strengthens plant cell wall through increasing suberization, silicification and lignification of (Currie and Perry, 2007). Additionally, high stress tolerance capability in plant cell develops due to bio-silicification within cell apoplast lead to the improvement of amorphous silica blockade (Guerriero et al. 2016). Silicon augmented salt tolerance by increasing plant growth and production in different plant species such as rice, maize, and Acacia gerrardii Benth (Abdel Latef and Tran, 2016; Al-Huqail et al. 2019; Gurmani et al. 2013). Silicon alleviates stress by enhancing plant growth, chlorophyll content, photosynthetic activity, and K+: Na+ ratio (Garg and Bhandari, 2016). Exogenous application of Si enhances transpiration and increases production of important polyamines under saline conditions and hence gives improved salt tolerance (Yin et al. 2016).

Pea (Pisum sativum L.) is a one of leading profitable vegetable in the world including Pakistan and grown in winter. Pea has high nutritional value having proteins (21 – 32%) and starch (37 – 49%) content, water soluble fibers, vitamins, minerals, and phytochemicals (Dahl et al. 2012). Peas are utilized as fresh peas, edible podded pea, freeze and dried peas. The growth and production of peas get declined due to salinity stress as it is a salt sensitive plant (Shahid et al. 2011). Several studies reported that the exogenously use of GA3 and Si can improve the salt tolerance (Iqbal and Ashraf 2013; Al-Huqail et al. 2019; Shekari et al. 2015). However, the mechanism of salt tolerance in pea plant via seed pretreatment with GA3 and soil application of Si is not fully known yet. Salt stress changes stability of hormone within plant. Consequently, induction of GA3 through seed pretreatment is a possible mechanism for hormonal homeostasis under salt stress. Addition of silicon to the soil can regulate physiological processes such as transpiration rate, stomatal conductivity, and relative water content by increasing the activity of peroxidase (POD), superoxide dismutase (SOD) and catalase (CAT). Silicon plays a significant role in the positive regulation of the antioxidant defense system (Shekari et al. 2015). Thus, purpose of this study was to determine that how seed pre-treatment through application of GA3 and Si in soil separately and in combination could improve growth and yield of two different varieties of peas. Furthermore, determine the effect of seed pretreatment with GA3 and application of Si treatments on ions selectivity, photosynthesis, and antioxidant enzymes activities in both pea varieties.

2. Materials and methods

2.1. Plant material and growth conditions

This study included seed priming with GA3 and silicate for the improvement of salt tolerance in two pea varieties (Samrina Zard and Meteor-FSD). Seeds of pea varieties were acquired from Horticulture Research Institute, National Agricultural Research Center, Islamabad, Pakistan. There were two salt levels (0 and 5 dS m−1 NaCl), four treatments such as control (distilled water treated seeds), GA3 seed pretreatment, soil application of silicon, and GA3 seed pretreatment + soil applied silicon. The development requirements were light with an intensity of 500–700 μmol m−2 s−1 for 12 h. Temperature ranging from 18 °C to 21 °C (daytime) to 8 °C to 10 °C (night) was observed during the study. Giberellin acid solution was prepared at the concentration of 10−4 M and used for seed pretreatment. Seeds were kept in solution for 12 h at 21 °C. Total 350 seeds of every pea variety were pretreated with aerated solution of GA3 or distilled water for 12 h at 21 °C. Two concentrations of NaCl (0 and 5 dS m−1) were selected to examine the effects of GA3 and Si on some biochemical, physiological, and productivity characteristics of pea varieties in the soil pots. Silicon is used as calcium silicate fertilizer, with a silicon content of 14.02%. The study was conducted in a completely randomized three-factor study design, with four replicates in 10 kg soil pots. Factors were pea varieties (Samrina Zard and Meteor-FSD), dual levels of salt (0 and 5 dS m−1 NaCl), four treatments (distilled water treated plants (control), GA3, Si, and GA3 + Si). Soil with 10 kg weight was collected from topsoil layer (0–15 cm depth), thoroughly mixed and filled in pots. Eight seeds after pretreatment were sown in polyethylene pots. The electrical conductivity of the soil used in study was 0.36 dS m−1; pH was 7.6; texture was sandy loam; CaCO3 (equivalent) 4.5%; Na+ 1.20 m Eq. L−1; available phosphorus was 2.4 mg kg−1; organic matter 0.82%; extractable potassium was 70 mg kg−1 and AB-DTPA extractable nitrate-N was 1.2 mg kg−1. Salinization was made by the inclusion of NaCl on soil weight basis in three equal splits in three regular days. The concentration of salt was 0 g L−1 and 3.5 g L−1 of NaCl per pot. Basic fertilizer was applied before planting consisting of 150 mg N kg−1 soil as urea was introduced in 3 parts, adding 200 mg K kg−1 as potassium sulfate and 40 mg P kg−1 as CaH2PO4·2H2O Four plants were harvested from each pot for analysis of various biochemical and physiological parameters after 25 days of salinization. According to the visual observation of soil moisture, plants were irrigated regularly with distilled water. Only two pots planted plants were grown to maturity. Plants were harvested at 125 days after seeding and 4–5 pickings at 6–10-day intervals were
added to determine the total amount of seed. Number of pods plant\(^{-1}\) and 1000 seed weight was noted.

2.2. Determination of biochemical and physiological parameters of plant

2.2.1. Photosynthesis

Photosynthesis was determined after 25 days (10 days after salinization) of sowing. For this purpose, a portable photosynthesis system (LI-6400, Li-cor Inc., Lincoln, NE) having a chamber (6 cm\(^2\), photo flux density of 500 \(\mu\)mol m\(^{-2}\) s\(^{-1}\)) was used. Completely expanded, and recently developed pea leaf was placed in chamber of a portable photosynthesis system. Transpiration rate (E), photosynthetic rate (PN) and stomatal conductance (gs) data was measured.

2.2.2. Chlorophyll, Na\(^{+}\), and K\(^{+}\) concentration

A fully extended, freshly produced young leaves were collected and immersed in 15 mL ethanol (80\%) in a glass vial. Subsequently, covered and incubated for 10 min at 85 \(^\circ\)C in a water bath. Extract was utilized to assess Na\(^{+}\), K\(^{+}\), and chlorophyll after the tubes were placed were chilled in the shade at 25 \(^\circ\)C. A spectrophotometer (T80 + UV/VIS China) was used to test the samples collected out of the same extract in A666. Chlorophyll activity was calculated, according to Arnon (1949).

Further, leaf tissue was re-extracted at 4 \(^\circ\)C for 24 h by adding acetic acid to determine Na\(^{+}\) and K\(^{+}\) concentration (Yeo and Flower, 1983). Then, concentration of K\(^{+}\) and Na\(^{+}\) in extract was determined by using flame photometer (PFP7 Jenway). An oven is used to dry remaining leaf material and weighed for dry weight. Leaf dry weight was used to calculate the concentrations of Na\(^{+}\), K\(^{+}\), and chlorophyll.

2.2.3. Electrolyte leakage

The EC meter was used to calculate electrolyte loss based on the permeability of leaf cell membranes (Lutts et al. 1996). Pea leaves were immediately sliced into 1 cm\(^2\) bits, separated in 10 mL vials with deionized water and washed 3 times. At 25 \(^\circ\)C, samples were incubated in a shaker at the rate of 100 round per minutes for 24 h and electrical conductivity of solution (EC\(_1\)) was recorded. At 95 \(^\circ\)C, sample was placed in a water bath for 15 min and measured electrical conductivity (EC\(_2\)) after cooled to 25 \(^\circ\)C. By evaluating the ratio of EC\(_1\) to EC\(_2\), electrolyte leakage was determined and expressed in percentage.

2.2.4. Proline content

The proline content was measured by homogenizing fresh leaves in 10 mL of 3\% aqueous solution of sulfosalicylic acid. The filtrate was mixed with 2 mL of acid-ninhydrin and 2 mL of glacial acetic acid and placed in a water bath at 100 \(^\circ\)C for 1 h. This one was split by 4 mL toluene and measured with a spectrophotometer (T80 + UV/VIS China) at 520 nm (Bates et al., 1973).

2.2.5. Determination of SOD, POD, and CAT

Fresh plant sample (1 g) was added to sodium phosphate buffer. Subsequently, sample was loaded in centrifuge machine at 13000 rpm for twenty minutes. Sample was incubated at 4 \(^\circ\)C for the determination of enzymatic activity. Method of Giannopolitis and Ries (1977) was used to determined superoxide dismutase (SOD) activity from leaf samples. One unit of SOD activity was defined as the quantity of enzyme that could hold a 50\% decrease in tetrazolium blue when measured at A650 nm. The superoxide dismutase activity was checked by arbitrary units mg\(^{-1}\) of fresh weight.

Fresh leaf sample (0.25 g) was used to examine peroxidase activity (POD) according to Pundir et al. (1999). Sample was treated with sodium phosphate buffer in an ice bath. Subsequently, mixture was loaded in centrifuge machine and run at 10,000 rpm for twenty minutes at 4 \(^\circ\)C. Same sample was used to determine activity of catalase and peroxidase. The analyzed mixture was treated in incubator at 40 \(^\circ\)C for five minutes. Therefore, hydrogen peroxide (1 mM L\(^{-1}\)) was recorded at 520 nm followed by incubation and absorbance. The amount of hydrogen peroxide utilized during incubation was determined from standard curve. Activity of enzyme was indicated as amount of hydrogen peroxide utilized min\(^{-1}\) mg\(^{-1}\) protein. Supernatant was used for catalase determination. 50 mM L-1 H2O2, 50 mM L-1 sodium phosphate buffer, and 50 mL of enzyme extract were employed in the reaction (Aebi 1984). The effectiveness of catalase was assessed via examining the decrease in reflectance at 240 nm because of the hydrogen peroxide used.

2.3. Statistical analysis

Statistical analysis on various parameters were evaluated by applying Statistics 8.1 software. Duncan's Multiple Range Test was performed on three-way ANOVA to examine the variation among treatments (\(p < 0.05\)). Microsoft Excel 2019 was used to calculate treatments mean and standard errors.

3. Results

3.1. Shoot and root dry weight

Salinity stress significantly declined shoot dry weight in both varieties. However, exogenous application of GA\(_3\), and Si significantly increased shoot dry weight under salt stress and without salt stress in Meteor-FSD and Samrina Zard pea varieties. Soil application of Si significantly augmented shoot dry weight in both pea varieties under salt stress and without salt stress conditions. Soil application of Si showed higher shoot dry weight as compared to sole application of GA\(_3\). Application of GA\(_3\) + Si showed high plant shoot dry weight under both salt levels. Overall, Samrina Zard showed highest shoot dry weight as compared to Meteor-FSD. Average shoot dry weight was reduced in application of 50 mM NaCl over control by 23\% in Meteor-FSD and 17\% in Samrina Zard (Table 1).

Root dry weight was declined non significantly in both varieties due to salinity stress. Root dry weight was increased significantly by exogenous application of GA\(_3\) + Si in both pea varieties under both salinity levels. Seed pretreated with GA\(_3\), and soil application of Si significantly ameliorate root dry weight of both pea varieties under salt stress and without salt stress conditions in both varieties. In NaCl treated plants, mean root dry weight was declined 17\% in Meteor-FSD and 25\% in Samrina Zard, respectively (Table 1).

3.2. Ion concentrations

The sodium ions concentration was significantly enhanced with the application of salt in Meteor-FSD and Samrina Zard. The Na\(^{+}\) concentration of Meteor-FSD was significantly greater than Samrina Zard. Exogenous application of GA\(_3\) + Si significantly decline Na\(^{+}\) transportation under saline conditions in both varieties. However, K\(^{+}\) concentration decreased significantly in both varieties respectively from non-saline to saline conditions. Exogenous application of Si significantly ameliorated Na\(^{+}\) transportation from non-saline to saline condition of both varieties and similarly K\(^{+}\) concentration reduced significantly in both varieties respectively from non-saline to saline conditions. There was significant augmentation of Na\(^{+}\) transport in Meteor-FSD and Samrina Zard from non-saline to saline condition while K\(^{+}\) transport was declined signifi-
Effect of seed pretreatment with gibberellic acid and silicate on Na+, K+ concentrations and K+:Na+ ratio of two pea varieties viz. Meteor-FSD and Samrina Zard at saline and non-saline conditions.

3.3. Electrolyte leakage, proline, and chlorophyll contents

A salt substrate significantly reduced chlorophyll content of Meteor-FSD and Samrina Zard; however, combined application of GA3 and Si non-significantly improved chlorophyll content without salt treatment and significant increase with salt stress. Proline content was increased (non-significantly) in both pea varieties under saline soil conditions as compared to non-saline condition; even though in Meteor-FSD combined treatment of GA3 and Si declined the proline contents under salinity stress. While in Samrina Zard proline showed ameliorative effect under salinity. Both pea varieties Meteor-FSD and Samrina Zard improved proline content (non-significantly) by applying GA3 and Si treatments individually. But reverse results were acquired with combined treatment of GA3 and Si (Table 3).

The percentage of electrolyte leakage was increased in both pea varieties under salt stress environment. Compared to control, there was decline effect of GA3 and Si treatments on Meteor-FSD under normal and saline condition. Exogenous treatment of GA3 and Si treatment reduced the electrolyte leakage percentage in Samrina Zard under both saline and non-saline environments (Table 3).

3.4. Influence of anti-oxidative enzymes

Salt stress levels significantly ameliorated SOD activities in both pea varieties. Meteor-FSD showed significantly higher SOD activity than Samrina Zard under salt stress levels by applying GA3 and Si individually. While non-significant ameliorative results were shown under normal environment (non-saline). There was significant augmentation of SOD activity in both pea varieties from non-saline to saline conditions when GA3 and Si were applied exogenously. POD activity was accelerated in both pea varieties under salt stress conditions. In Meteor-FSD, there was significant variation in SOD and POD activity under saline and non-saline conditions. While there was non-significant difference of SOD and POD activity under normal soil condition and significant difference under saline condition in Samrina Zard. POD activity was reduced non-significantly with combined treatment of GA3 and Si under non-saline condition but enhanced non-significantly under saline condition in tolerate variety (Samrina Zard). As compared with control treatment, exogenously applied GA3 and Si showed more ameliorative effect than combined treatment of GA3 + Si in Meteor-FSD under normal

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**Table 1**

| Varieties      | Treatments | Shoot dry weight (g) | Root dry weight (g) |
|----------------|------------|----------------------|---------------------|
|                |            | 0 dS m⁻¹       | 5 dS m⁻¹       | 0 dS m⁻¹       | 5 dS m⁻¹       |
|----------------|------------|----------------------|---------------------|
| Meteor FSD     | Control    | 1.32 ± 0.036       | 0.90 ± 0.12       | 1.02 ± 0.044   | 0.74 ± 0.124   |
|                | GA3        | 1.44 ± 0.049       | 1.13 ± 0.039      | 1.16 ± 0.033   | 0.98 ± 0.034   |
|                | Si         | 1.57 ± 0.039       | 1.22 ± 0.029      | 1.28 ± 0.048   | 1.12 ± 0.038   |
|                | GA3 + Si   | 1.73 ± 0.046       | 1.42 ± 0.069      | 1.38 ± 0.032   | 1.22 ± 0.020   |
| Samrina Zard   | Control    | 1.60 ± 0.058       | 1.45 ± 0.078      | 0.94 ± 0.046   | 0.53 ± 0.038   |
|                | GA3        | 1.80 ± 0.058       | 1.56 ± 0.100      | 1.11 ± 0.017   | 0.79 ± 0.047   |
|                | Si         | 1.90 ± 0.029       | 1.62 ± 0.063      | 1.18 ± 0.036   | 0.94 ± 0.039   |
|                | GA3 + Si   | 2.10 ± 0.058       | 1.83 ± 0.004      | 1.27 ± 0.032   | 1.09 ± 0.028   |

GA3 (10⁻⁴ M) was applied during seed treatment for 12 h; while the silicate was applied as soil application. Twenty-five days old seedlings were supplied with 0 and 5 dS m⁻¹ NaCl. Shoot and root dry weight was calculated 25 days after the completion of Salt. LSD (0.05) was applied on both Salt level. Means followed by similar letters were not significantly different at p < 0.05 on both varieties. GA3, gibberellic acid; Si, silicon; (n = 4).

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**Table 2**

| Varieties      | Treatments | Na⁺ (mg g⁻¹ dry weight) | K⁺ (mg g⁻¹ dry weight) | K⁺: Na⁺ (ratio) |
|----------------|------------|-------------------------|------------------------|----------------|
|                |            | 0 dS m⁻¹   | 5 dS m⁻¹   | 0 dS m⁻¹ | 5 dS m⁻¹ | 0 dS m⁻¹ | 5 dS m⁻¹ |
|----------------|------------|------------|------------|----------|----------|----------|----------|
| Meteor FSD     | Control    | 8.85 ± 0.058 | 18.80 ± 0.21 | 2.10 | 1.83 | 1.27 | 1.13 |
|                | GA3        | 5.58 ± 0.097 | 13.47 ± 0.12 | 2.56 | 2.24 | 1.77 | 1.52 |
|                | Si         | 5.40 ± 0.097 | 12.37 ± 0.15 | 2.70 | 2.40 | 2.03 | 1.83 |
|                | GA3 + Si   | 5.24 ± 0.097 | 12.57 ± 0.12 | 2.93 | 2.63 | 2.15 | 1.89 |
| Samrina Zard   | Control    | 4.98 ± 0.12 | 16.37 ± 0.15 | 3.01 | 2.71 | 2.07 | 1.81 |
|                | GA3        | 3.60 ± 0.097 | 12.07 ± 0.15 | 3.10 | 2.80 | 2.01 | 1.75 |
|                | Si         | 3.63 ± 0.15  | 11.88 ± 0.12 | 3.31 | 3.01 | 2.17 | 1.93 |
|                | GA3 + Si   | 3.41 ± 0.12  | 11.47 ± 0.15 | 3.35 | 3.05 | 2.12 | 1.87 |

GA3 (10⁻⁴ M) was applied during seed treatment for 12 h; while the silicate was applied as soil application. Twenty-five days old seedlings were treated with 0 and 5 dS m⁻¹ NaCl ions were measured from 25-days old plants (10-days after Salination). LSD (0.05) was applied on both Salt level. Means followed by similar letters were not significantly different at p < 0.05 on both varieties. GA3, gibberellic acid; Si, silicon; (n = 4).
Effect of seed pretreatment with gibberellic acid and silicate on chlorophyll, proline content and electrolyte leakage in two pea varieties viz. Meteor-FSD and Samrina Zard at saline and non-saline conditions.

| Varieties | Treatments | Chlorophyll Content (mg g⁻¹ D. wt.) | Proline (µg g⁻¹) | Electrolyte Leakage (%) |
|-----------|------------|------------------------------------|-----------------|-------------------------|
|           |            | 0 dS m⁻¹ 5 dS m⁻¹                  | 0 dS m⁻¹ 5 dS m⁻¹ | 0 dS m⁻¹ 5 dS m⁻¹       |
| Meteor-FSD| Control    | 14.27 ± 0.54a                      | 10.57 ± 0.82ab   | 1.97 ± 0.44bcd          | 2.17 ± 0.08bc         |
|           | GA₃        | 14.23 ± 0.50a                      | 12.80 ± 0.64bc   | 2.97 ± 0.35ab           | 3.73 ± 0.58a          |
|           | Si         | 14.23 ± 0.59a                      | 11.17 ± 0.84bcd  | 2.93 ± 0.44ab           | 3.97 ± 0.67b          |
|           | GA₃ + Si   | 15.07 ± 0.81ab                     | 13.20 ± 0.82ab   | 1.40 ± 0.15bc           | 1.30 ± 0.53cd         |
|           | Control    | 11.27 ± 0.56cde                    | 7.56 ± 0.70d    | 1.00 ± 0.32bc           | 0.77 ± 0.41i          |
|           | GA₃        | 10.80 ± 0.76cde                    | 8.84 ± 2.70ef   | 1.24 ± 0.26cd           | 1.40 ± 0.49f          |
|           | Si         | 10.80 ± 0.76cde                    | 9.20 ± 1.22de   | 1.20 ± 0.21cd           | 1.43 ± 0.61f          |
|           | GA₃ + Si   | 12.97 ± 0.37bde                    | 10.50 ± 0.38e   | 0.73 ± 0.13d            | 0.75 ± 0.15f          |

GA₂ (10⁻⁴ M) was applied during seed treatment for 12 h; while the silicate was applied as soil application. Twenty-five days old seedlings were treated with 0 and 5 dS m⁻¹ NaCl. Antioxidant activities were measured from 25-days old plants (10-days after Salination). LSD (0.05) was applied on both Salt level. Means followed by similar letters were not significantly different at p < 0.05 on both varieties. GA₃; gibberellic acid; Si; silicon (n = 4).

3.5. Growth and physiological responses of pea varieties

3.5.1. Photosynthesis characteristics

The photosynthetic rate (PN) was found to be significantly reduced in the both pea varieties exposed to salinity; the photosynthetic rate (PN) of Meteor-FSD was significantly greater than Samrina Zard. Exogenously combined application of GA₃ and Si augment the photosynthetic rate of two pea varieties under normal and saline soil conditions. Statistically there was no significant difference in PN observed by applying GA₃ and Si individually on both pea varieties under the saline and normal soil conditions. As compared with control, combined application of GA₃ and Si ameliorate the photosynthetic rate PN in both pea varieties under normal and salt stress levels. (Fig. 1).

The salt treatment considerably lowered the stomatal conductance (gs) in the leaves of both pea cultivars. Individual use of GA₃ and Si showed statistically non-significance increase in Meteor-FSD while significantly augment in Samrina Zard under saline and non-saline salt levels. Combined treatment of GA₃ and Si enhanced stomatal conductance (gs) non-significantly in sensitive variety (Meteor-FSD) under both salt levels. While significantly enhanced in tolerant variety (Samrina Zard) under non-saline condition and non-significantly under saline condition (Fig. 2).

A marked decrease was observed in transpiration rate (E) of both pea varieties owing to salt stress. Compared with control transpiration rate (E) was increased non-significantly due to individually GA₃ and Si treatments in Meteor-FSD under normal and salt stress conditions while diminished non-significantly in Samrina Zard under salt stress while ameliorated significantly without salt stress (Fig. 3).

3.6. Yield attributes

Despite salt stress reduced the number of seeds per pod in both pea varieties, treatment with GA₃ and Si was effective in improving this trait regardless of the varieties under normal and salt stress conditions. However, compared with control, Si treatment enhanced the number of seeds under saline conditions. Individually applied GA₃ enhanced the number of seeds per pod in Meteor-FSD under non-saline condition as compared to Si application. While reverse results were acquired in salt stress levels. But in Samrina Zard, exogenously applied Si showed throughout higher results than GA₃ under both Salt levels. Significantly maximum number of seeds per pods were achieved in Samrina Zard than Meteor-FSD under both salinity levels. It was found that the seed weight of each pod was reduced due to the salinity in both pea varieties. Under Salt stress, Si treated plants showed positive results than GA₃ in both pea varieties. There was significant augmentation in number of plants per pod due to Si and GA₃ application.

| Varieties | Treatment | SOD (unit mg⁻¹) | POD (unit mg⁻¹) | CAT (unit mg⁻¹) |
|-----------|-----------|----------------|----------------|----------------|
|           |           | 0 dS m⁻¹ 5 dS m⁻¹ | 0 dS m⁻¹ 5 dS m⁻¹ | 0 dS m⁻¹ 5 dS m⁻¹ |
| Meteor-FSD| Control   | 12.53 ± 0.57abc | 15.73 ± 1.17bc | 6.90 ± 1.00cd  | 10.37 ± 1.21b    |
|           | GA₃       | 13.63 ± 0.73cd | 19.37 ± 1.15a  | 8.60 ± 0.40bc  | 13.17 ± 0.97a    |
|           | Si        | 13.70 ± 0.82a  | 19.47 ± 1.21a  | 8.67 ± 0.48a   | 13.13 ± 0.98a    |
|           | GA₃ + Si  | 14.37 ± 1.34a  | 20.93 ± 1.67a  | 7.23 ± 0.53cd  | 10.57 ± 0.41a    |
|           | Control   | 8.50 ± 0.75ef  | 10.70 ± 0.69ef | 6.17 ± 0.50d   | 7.50 ± 0.52cd    |
|           | GA₃       | 9.17 ± 1.02d   | 14.73 ± 1.15b  | 6.87 ± 0.83cd  | 10.60 ± 0.59b    |
|           | Si        | 9.20 ± 1.04c   | 14.90 ± 1.12bc | 6.90 ± 0.83cd  | 10.50 ± 0.53a    |
|           | GA₃ + Si  | 9.77 ± 0.83cd  | 16.57 ± 0.86b  | 5.43 ± 0.28d   | 8.60 ± 1.10bc    |

GA₂ (10⁻⁴ M) was applied during seed treatment for 12 h; while the silicate was applied as soil application. Twenty-five days old seedlings were treated with 0 and 5 dS m⁻¹ NaCl. Antioxidant activities were measured from 25-days old plants (10-days after Salination). LSD (0.05) was applied on both Salt level. Means followed by similar letters were not significantly different at p < 0.05 on both varieties. GA₃; gibberellic acid; Si; silicon (n = 4).
tion in both pea varieties under both salt levels. Individually applied Si treatment significantly increased number of pods per plant in both pea varieties as compared to control and GA3 application. Average reduction in number of pods per plant was 17%, 10% in Meteor-FSD and Samrina Zard respectively, while combined effect of GA3 and Si showed augmentation in both pea varieties under both salinity levels (Table 5).

There was significant increase observed in thousand seed weight of both pea varieties under saline and non-saline conditions due to exogenous treatment of GA3 and Si. Exogeneous application of GA3, Si, and GA3 + Si significantly enhanced thousand seed weight 4.54%, 9.09% and 11.82% respectively in Meteor-FSD under non-saline conditions. Similarly, thousand seed was significantly enhanced 10.53%, 18.42% and 11.58% due to exogenous application of GA3, Si, and GA3 + Si in Meteor-FSD under saline conditions. In Samrina Zard, thousand seed weight was increased significantly under normal soil conditions due to exogenous treatment of GA3 and Si and their combinations. Thousand seed weight was also
enhanced significantly due to exogenous application of GA$_3$ and Si under saline conditions in Samrina Zard pea variety. Exogenous application of GA$_3$, Si, and GA$_3$ + Si significantly enhanced thousand seed weight 3.50%, 6.23% and 8.95% respectively in Samrina Zard under non-saline conditions. Similarly, thousand seed was significantly enhanced 7.39%, 8.70% and 13.04% due to exogenous application of GA$_3$, Si, and GA$_3$ + Si in Samrina Zard under saline conditions. Results showed that exogenous application of GA$_3$, Si, and GA$_3$ + Si significantly improved thousand seed weight of both pea varieties under both salt levels.

### 4. Discussion

The current study showed that salt stress has a negative influence on the growth characteristics of peas. In the present study, the development of peas was declined by salinity stress. The tentative seed pretreatment with GA$_3$ and Si enhanced the salinity tolerance of Samrina Zard pea variety and reduced in the Meteor-FSD. Exogenous application of GA$_3$ and proline ameliorate the growth and yield of pea plants (Abbas et al. 2018). When growing under a stress salt stress condition, the optimal concentration of certain plant regulators can boost the germination, development, and production of various crops (Iqbal & Ashraf 2013). It was reported that different concentrations of GA$_3$ are required for plant growth at different stages of the life cycle (Iftikhar et al. 2020). This study showed that exogenously applied GA$_3$ and Si significantly ameliorated shoot and root dry weight of both pea varieties under both salt levels. All treatments tested with GA$_3$ statistically enhanced growth parameters, i.e., fresh, and dry weights of leaves, number of leaves per plant (Enas Safaa Azab, 2018). Plant growth and development is known to be regulated by various exogenous and endogenous factors, including the growth regulators (Taiz & Zeiger, 2010). It has also been observed that exogenous application of GA$_3$ promote growth in Catharanthus roseus (Srivasvasta & Srivasvasta, 2007) and Nigella sativa (Shah & Ahmad 2006). The effect of GA$_3$ on plant biomass has been mentioned by several workers (El-Shraiy & Hegazi, 2009). Application of Si declined Na$^+$ transport in both pea varieties, regardless of the variety, Si treatment was found to be more efficient than GA$_3$ treatment and enhanced dry plant biomass and lower salt damage. The results showed that silicon treatment enhanced K$^+$ concentration in both varieties. As the K$^+$ concentration in both varieties increased, treatment with GA$_3$ reduced Na$^+$ concentrations in the shoots and roots and improved K$^+$ concentrations in the roots of wheat crop (Iqbal & Ashraf 2013).

#### Table 5

Effect of seed pretreatment with gibberellic acid and silicate on yield of pea varieties viz. Meteor-FSD and Samrina Zard at saline and non-saline conditions.

| Varieties         | Treatment      | Number of Seeds Pod$^{-1}$ | 1000 seed Weight (g pod$^{-1}$) | Number of Pods Plant$^{-1}$ |
|-------------------|----------------|-----------------------------|---------------------------------|-------------------------------|
|                   |                | 0 ds m$^{-1}$ | 5 ds m$^{-1}$ | 0 ds m$^{-1}$ | 5 ds m$^{-1}$ | 0 ds m$^{-1}$ | 5 ds m$^{-1}$ | 0 ds m$^{-1}$ | 5 ds m$^{-1}$ |
| Meteor-FSD        | Control        | 6.27 ± 0.09$^{G}$ | 4.03 ± 0.12$^{I}$ | 220 ± 1.15$^{L}$ | 190 ± 1.73$^{L}$ | 14.50 ± 0.35$^{I}$ | 11.60 ± 0.15$^{K}$ |
|                   | GA$_3$         | 7.57 ± 0.18$^{DE}$ | 5.60 ± 0.17$^{H}$ | 230 ± 2.65$^{H}$ | 210 ± 1.56$^{H}$ | 16 ± 0.15$^{G}$ | 13.50 ± 0.05$^{L}$ |
|                   | Si             | 7.32 ± 0.07$^{E}$ | 6.23 ± 0.20$^{G}$ | 240 ± 1.56$^{G}$ | 225 ± 0.20$^{G}$ | 16.50 ± 0.15$^{C}$ | 13.60 ± 0.15$^{I}$ |
|                   | GA$_3$ + Si    | 8.17 ± 0.15$^{C}$ | 7.00 ± 0.06$^{F}$ | 246 ± 1.53$^{F}$ | 212 ± 1.56$^{F}$ | 17.40 ± 0.15$^{DE}$ | 14.50 ± 0.10$^{I}$ |
| Samrina Zard      | Control        | 7.43 ± 0.28$^{C}$ | 6.12 ± 0.01$^{G}$ | 257 ± 1.53$^{G}$ | 230 ± 1.53$^{G}$ | 17 ± 0.58$^{D}$  | 15.50 ± 0.35$^{I}$ |
|                   | GA$_3$         | 8.57 ± 0.13$^{B}$ | 6.82 ± 0.14$^{E}$ | 266 ± 1.16$^{E}$ | 247 ± 1.15$^{E}$ | 19.20 ± 0.06$^{B}$ | 17.20 ± 0.26$^{E}$ |
|                   | Si             | 8.80 ± 0.10$^{B}$ | 7.54 ± 0.11$^{DE}$ | 273 ± 0.58$^{D}$ | 250 ± 2.64$^{D}$ | 20.47 ± 0.55$^{B}$ | 18 ± 0.15$^{DE}$ |
|                   | GA$_3$ + Si    | 10 ± 0.15$^{A}$  | 8.80 ± 0.15$^{B}$ | 280 ± 1.73$^{A}$ | 260 ± 1.15$^{B}$ | 21.50 ± 0.87$^{A}$ | 20 ± 0.15$^{BC}$ |

GA$_3$ (10$^{-4}$ M) was applied during seed treatment for 12 h; while the silicate was applied as soil application. Twenty-five days old seedlings were treated with 0 and 5 dS m$^{-1}$ NaCl. Si was supplied during imbibition for 12 h; silicate was applied throughout the irrigation water, LSD (0.05) was applied on both Salt level. Means followed by similar letters were not significantly different at p < 0.05 on both varieties. GA$_3$: gibberellic acid, Si: silicon (n = 4).
At high external NaCl concentration, internal K⁺ level reduced due to the competition between Na⁺ and K⁺. Several studies have been reported the positive effects of GA₃ seed treatment and soil application of silicon on plant biomass and physiological responses (Fahad et al. 2015).

Due to the increased in electrolyte leakage of the two pea varieties, salt stress damaged membrane permeability. The membrane permeability and chlorophyll content were enhanced with the use of Si and GA₃ treatments under salinity stress. The reduction of photosynthesis and chlorophyll content caused by salinity was neutralized by using Si and GA₃. The amelioration in plant biomass increased nutrient absorption, improved photosynthesis, and increased the translocation of photosynthesis of Capsicum annum (Miniraj and Shammugavelu 1987).

Under salt conditions, silicon treatment was more effective than GA₃ in reducing proline accumulation. Proline content in the leaves of the both pea varieties increased significantly, while the proline content of Samrina Zard was lower than that of Meteor-FSD under salinity stress. Against saline conditions, cultivars with a higher photosynthetic efficiency and a higher K⁺/Na⁺ ratio function well and exhibit osmotic modification with decreasing proline levels. Hence, the potential salt tolerance of Samrina Zard enhanced solutes accretion in the leaf tissues under salinity stress. It is well known that proline plays a role in the adaptation stress within the cell (Gilbert et al. 1998).

In this study, Si and GA₃ treatments improved the activity levels of superoxide dismutase and catalase under saline soil conditions, which showed that the defense mechanism to reduce oxidative damage was improved. Salinization of wheat shoot (Meneguzzo et al. 1999; Sairam and Srivastava, 2002) and pea (Hernandez et al. 1999) was declined by enhancing the activity of antioxidant enzymes. Similar increases of SOD enzyme activity were observed in cotton varieties (Meloni et al. 2003), in Cassia angustifolia plants (Agarwal & Pandey, 2004) and Beta maritima and Beta vulgaris (Bor et al. 2003) exposed to salt stress. The salt content inhibited the growth and yield characteristics of pea varieties. The silicon treatment under saline and non-saline soil conditions has a positive effect on fruit yield characteristics. The fruits treated with GA₃ maintained a higher fruit weight (Rokaya et al. 2016). Exogenously applied GA₃ was found effective in increasing the yield, pulp weight, Juice content, seed weight in Populus ciliata (Lal and Das, 2017). It was reported that the number of fruits and fruit weight were significantly improved in tomato due to the GA₃ and a highest yield was obtained due to use of GA₃ at 80 ppm (Singh et al. 2018).

5. Conclusion

GA₃ increased plant growth and reduced Na⁺ transport in the sensitive pea variety Samrina Zard. In conjunction with silicate, GA₃ elevated the salinity tolerance of both varieties and reduced Na⁺ transport and increased stomatal conductance, K⁺ transport and shoot biomass under salt stress. It suggests that GA₃ has a regulatory role by influencing Na⁺ uptake and transport to produce salt tolerance in peas. POD activity enhanced by combined application of GA₃ and Si in tolerant pea variety (Meteor-FSD) while opposite results were showed in Samrina Zard which is salt sensitive pea variety. GA₃ ameliorate CAT activity more efficiently in Samrina Zard than Meteor-FSD under both salt levels. It was found that silicon efficiently enhanced plant development and growth, salinity tolerance and productivity of Samrina Zard and Meteor-FSD. Seed treatment with GA₃ increased the plant’s antioxidant activity, dry biomass, chlorophyll contents, and productivity of salt-susceptible varieties. Based on results, soil application of Si may be recommended to ameliorate the adverse effect of salinity in pea varieties.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Authors are thankful to Dr Naushnerwan Nobel Nawab, Senior Scientific Officer, Vegetable Program, Horticultural Research Institute, National Agricultural Research Center, Islamabad, Pakistan for providing the seeds of pea varieties and fruitful technical discussion.

Funding

The publication of the present work is supported by the Shaanxi Innovation Capacity Support Project (2018QJ5218) and the National Natural Science Foundation of China (51809224), Top Young Talents of Shaanxi Special Support Program.

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