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# Exogenous application of Plant Growth Regulators (PGRs) improves the nutritional status of Maize grown under salinity stress in response to Etiolation and De-etiolation conditions

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## Abstract

Plant growth and developmental processes are affected by both absences of light and salinity stress. Light and salinity affect the plant's essential nutrients by affecting the mineral uptake through the rooting system. A pot experiment was conducted under the ambient condition of Turbat, Balochistan, to explore etiolation and de-etiolation response of maize hybrid (SP-17S23) to salinity stress under exogenous application of plant growth regulators (PGRs). Maize seedlings in three sets, i.e., non-etiolated, etiolated, de-etiolated, subjected to salinity stress (120 mM NaCl) after 15 days of seed germination. After a week, the seedlings were sprayed with optimized levels of PGRs, including thiourea (10 mM), salicylic acid (250 µM), and Kinetin (3 µM). Salinity stress affected maize growth by affecting the nutritional status. Salinity negatively affects plant's nutrient contents such as (Nitrate, K, Mg). However, the foliar supplementation of PGRs significantly enhanced the nutrient content under salinity. Concludingly, the data recommend that foliar application of PGRs plays a key role in hampering the impacts of salinity by improving plant's nutrients contents, thereby improving the growth of plants.

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## Introduction

Light is one of the major abiotic factors which play a vital role in the life of the organism. Light influences various plant attributes like development, germination, diurnal rhythms, flowering, etc. (Nemhauser and Chory 2002; Svriz *et al.* 2014; Tiryaki and Kaplan 2019). A plant completes its life in two ways in the absence of light (etiolation) and the presence of light (de-etiolation). In the absence of light (etiolation response), plant growth and development processes get hindered. However, it promotes stem elongation but very weak seedlings as they lack chloroplast. However, shifting the seedlings back to light from etiolated conditions (de-etiolated response) promotes successful and healthy plant growth. Various genes that consist of information help the seedling in proper shifting from etiolation (dark) to de-etiolation (light), which involves the synthesis of chloroplast, formation of different pigments and photosystem assemblage at thylakoid membranes. The function of these genes includes encoding chlorophyll a/b and binding proteins. When plants are transferred from etiolation to de-etiolation enzymes become active, and stem and leaf length become reduced (Garcia and Gil, 2002; Campel and Reece, 2004).

Salinity is the major limiting environmental factor that limits plant production worldwide. It negatively affects both the growth and yield attributes of plants (Ghassemi *et al.* 1995). With the increase in salinity level, a reduction in growth and productivity occurs (Flowers *et al.,* 2010, Akram and Ashraf, 2011). Salinity decreases growth by affecting various nutrient contents and morphological attributes of Maize (Zia. 2011). Due to salinity, a high level of Na and Cl accumulate in tissues of plants which is toxic for plants; when $Na^+$ and $Cl^-$ are accumulated in the cytoplasm, enzyme activities and the photosynthesis process become hindered, and K gets replaced by Na (Kant *et al.* 2006).

Plant growth regulators (PGRs) help successful plant growth under stressful environments (Brien *et al.* 1985). The growth of etiolated seedlings can be controlled by PGRs, and many studies showed that PGRs are essential and control the transition between the etiolated and de- etiolated processes (Vandenbussche *et al.* 2005). Phytohormones are natural and regulate the physiological process of plants, such as transduction of signals, water uptake and respiration, transport of ions, gas exchanges and regulation of proteinase inhibitor genes (Mok and Mok 1994; Riefler *et al.*, 2006).

The exogenous application of PGRs is effective in enhancing crop growth and productivity under optimal and sub-optimal conditions (Binenbaum *et al.*, 2018). Bio-regulatory hormones have the potential to provide reasonable and easy solutions to ensure sustainable agriculture in salt-affected lands (Srivastava *et al.*, 2009). The reported number of natural and synthetic plant growth regulators (PGRs) molecules can be used to improve stress tolerance in plants and increase germination under various environmental cues (Wahid *et al.*, 2007; Wahid *et al.*, 2008).

Maize is an economically important crop; its productivity is increasing every year. (Farooq *et al.* 2015). Maize is a $C_4$ plant and has a good photosynthetic process than $C_3$ plants (Ashrafuzaman *et al.*, 2002). Maize is nutritive, a valuable plant that contains 72% starch, 10% protein, 8.5% fiber, 4.8% oil, 3% sugar, 1.7% ash and 5% vitamin A and B (Ahsan *et al.*, 2007). For maize growth, soil should be fertile and pH 6.5 -7.5 (Wang *et al.* 2008). During the salt stress, seed germination becomes delayed, and the germination rate becomes reduced because Maize is sensitive to salt (Ashraf and Foolad 2005). Saline soil retard maize growth and productivity (Ouda *et al.* 2008). Many researchers observed that the early seedling growth of Maize is the sensitive period (Shalhevet, 1995). The present study was performed to explore the possible bio-regulatory role of foliar spray of optimized levels of selected PGRs including salicylic acid (SA), thiourea (TU) and Kinetin (KIN) in the etiolated and de-etiolated maize seedlings under salinity stress based on an array of nutritional attributes.

## Materials and methods

*Experimental layout*

A pot experiment was conducted under ambient environmental conditions of Turbat, Balochistan, Pakistan to explore etiolation and de-etiolation response of maize hybrid (SP-17S23) to salinity stress under exogenous application of plant growth regulators (PGRs). Pots of 32 cm diameter were filled with 10 kg of soil. Ten seeds were sown per pot at 1-inch depth. The design was a completely randomized design (CRD) with 3 replicates. Two complete sets, i.e., etiolation and de-etiolation were generated in comparison to the control (Non-etiolated). Etiolated pots were covered with a black sheet. After 15 days of seed germination, NaCl (120mM) was applied as soil medium supplementation in comparison to non-stressed plants (no salinity/control). After 1 week of salinity stress, plants were foliar supplemented with optimized levels of plant growth regulators, thiourea (10 mM), salicylic acid (250 µM), and Kinetin (3 µM). After 1 week of PGRs treatment, plants were harvested (i.e., etiolated and de-etiolated and non-etiolated sets) and oven-dried at 75°C.

*Nutrients analysis*

For nutrient analysis, plant samples (both shoot and root) were oven-dried at 75°C. Root and shoot about 0.1gm were weighed and digested in 5mL nitric acid placed on the hot plate and the sample was diluted with distilled water up to 50mL.

*Nitrate-N*

Nitrate was analyzed by the method developed by Kowalenko & Lowe (1973). To 3 mL of extract 7 mL of chromotropic acid (CTA) was added and vortexed. After 20 minutes, the absorbance of colored complex was taken at 430 nm using distilled water as blank.

*Potassium and Magnesium*

Potassium and Magnesium were determined using atomic absorption spectrophotometer.

*Statistical analysis*

The data was statistically analyzed using "STATISTIX 8.1". The graphs and mean, standard deviation were calculated using "MS EXCEL".

## Results

*Shoot Nitrate-N*

The data obtained for shoot nitrate showed statistically significant results, with TU Spray under salinity stress at de-etiolation conditions showing the highest nitrate content. In contrast, the lowest concentration of nitrate was recorded in Kin foliar spray at control conditions under etiolation (Fig. 1).

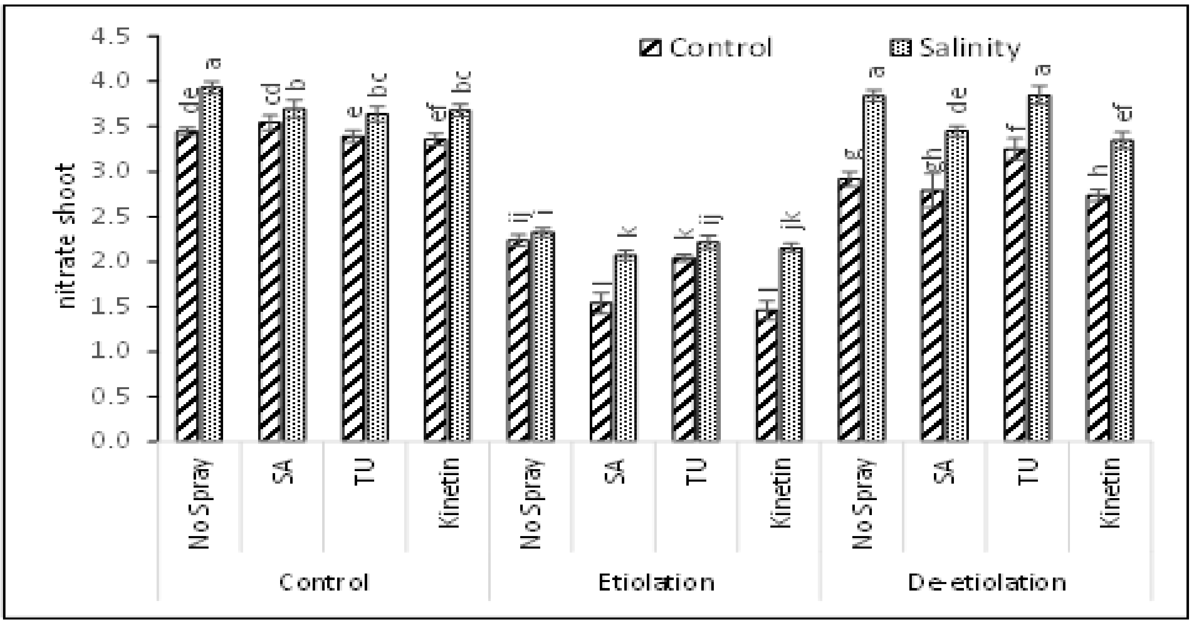


**Fig. 1.** Changes in Shoot nitrate content of Maize as affected under salinity stress in response to etiolation and de-etiolation. Plants were foliarly supplemented with different PGRs.

In non-etiolated seedlings, under salinity with no foliar treatment, increased nitrate content while a spray of Kin under control conditions had minimum nitrate content. The order of improvement for shoot nitrate contents in control was observed as No spray S > SA S > Kin S > TU S > SA Con > No spray control> TU Con > Kin Con.

In Etiolated seedlings, the results indicated that maximum nitrate content was noted under salinity with no foliar spray, and minimum was noticed under Kin control. However, shoot nitrate content under etiolation was observed as: No spray S > No spray control> TU S > Kin S > SA S > TU Con > SA Con > Kin Con.

Considering de-etiolated seedlings, TU under salinity condition had maximum nitrate content in shoot and minimum was noticed with Kin spray under control condition. In de-etiolation condition the trend was observed as: TU S> No spray S > SA S > Kin S > TU Con > No spray control> SA Con > Kin Con.

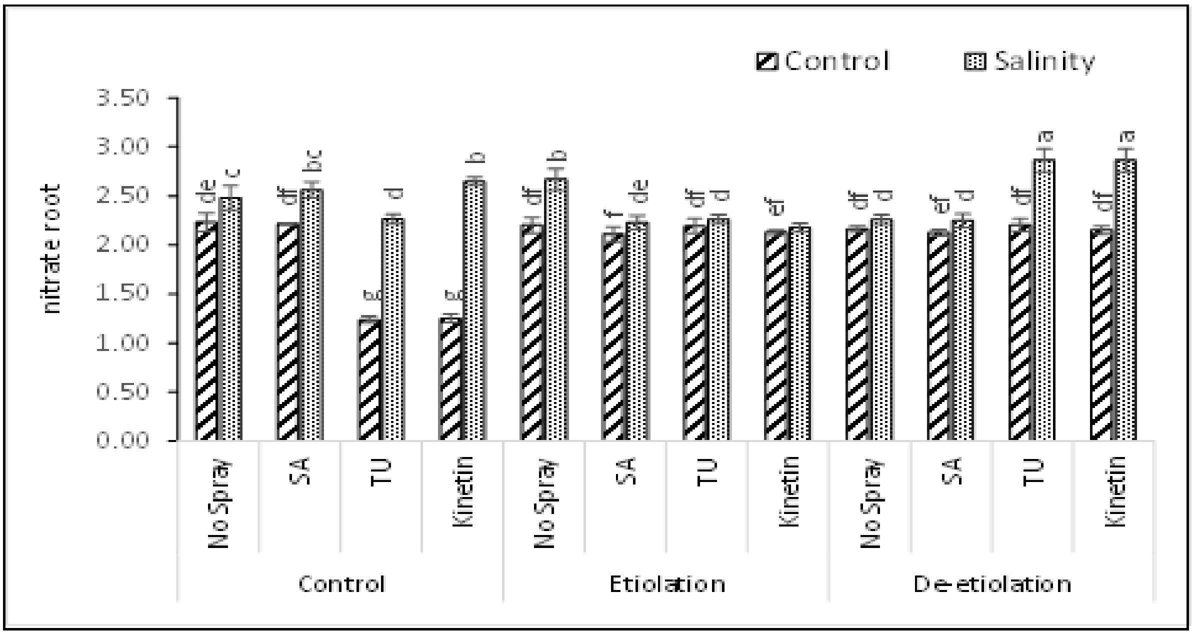


**Fig. 2.** Changes in root nitrate content of Maize as effected under salinity stress in response to etiolation and de-etiolation. Plants were foliarly supplemented with different PGRs.

*Root Nitrate-N*

The results obtained for root nitrate showed statistically significant results, with maximum nitrate content in TU and Kin foliar treatment under salinity of de-etiolation condition, while the lowest root nitrate content was noted under TU control conditions (Fig. 2).

In non-etiolated seedlings, under salinity with Kin foliar spray had maximum nitrate content, while a spray of TU under control conditions had the least nitrate content. The order observed for root nitrate accumulation under control was: Kin S > SA S > No spray S > TU S > No spray control> SA Con > Kin Con > TU Con.

However, in etiolated seedlings, data indicated that maximum nitrate content was noted under salinity with no spray, and minimum nitrate concentration was noticed with foliar spray of SA under control conditions. The trend observed in etiolation seedlings was: No spray S > TU S > SA S > No spray control> TU Con > Kin S > Kin Con > SA Con.

While in de-etiolated seedlings, a spray of TU and Kin foliar treatment under salinity conditions increased the nitrate content in the root, and the minimum amount of nitrate content was observed in SA spray under control conditions. The order of changes observed as Kin S=TU S > No spray S > SA S> TU Con > No spray control> Kin Con > SA Con.

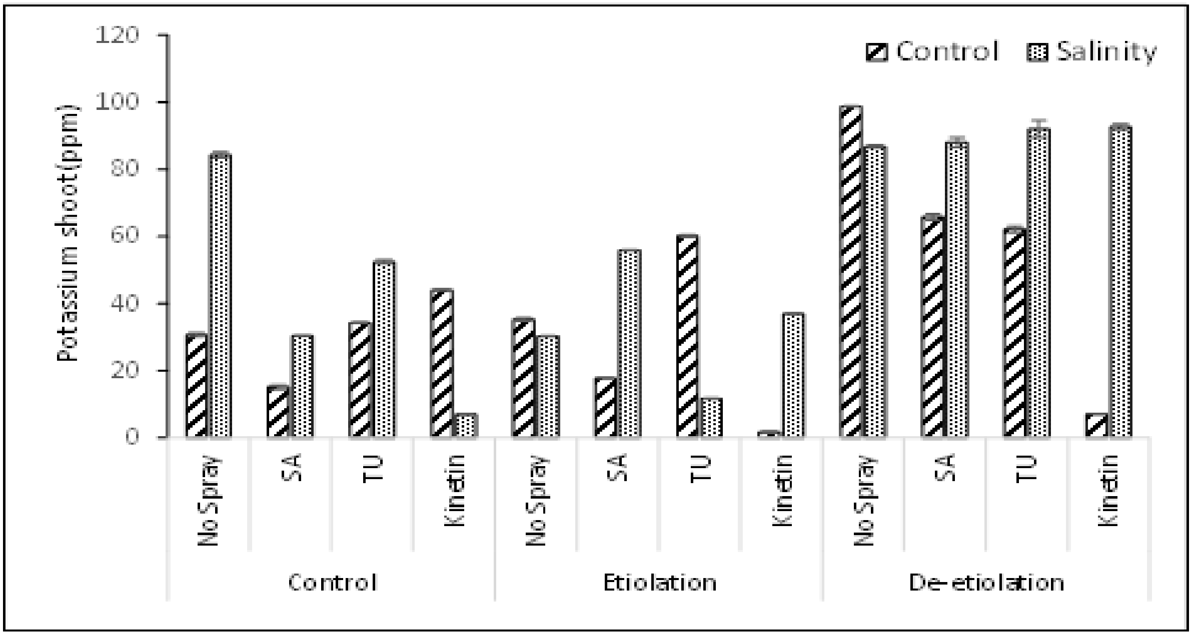


**Fig. 3.** Changes in shoot potassium content of Maize as affected under salinity stress in response to etiolation and de-etiolation. Plants were foliar supplemented with different PGRs.

*Shoot Potassium ($K^+$)*

Data recorded for potassium content in shoot showed that no foliar spray under control of de-etiolation had the maximum $K^+$ content, while the lowest potassium content was noted with the foliar application of Kin under control of etiolation (Fig. 3).

In non-etiolated seedlings, no foliar spray under salinity had maximum potassium content and foliar treatment of Kin under salt stress had the minimum $K^+$ content. The trend observed was: No spray S > TU S > Kin Con > TU Con > No spray control> SA S > SA Con > Kin S.

In etiolated seedlings, TU spray under control showed the highest potassium content while Kin without salinity had the least potassium content in maize seedlings observing the trend as: TU Con > SA S > Kin S > No spray control> No spray S> SA Con > TU S > Kin Con.

However, De-Etiolated seedlings indicated maximum potassium content under control with no spray and minimum was noticed with the foliar treatment of Kin under control. Shoot potassium content in de-etiolation condition observed as: No spray control> TU S > Kin S > SA S > No spray S > SA Con >TU Con > Kin Con.

*Root Potassium ($K^+$)*

Obtained results regarding root potassium revealed that maximum root potassium content was observed in TU spray control of de-etiolated seedlings, while minimum root potassium contents were observed under the foliar spray of TU of salt-treated de-etiolated seedlings (Fig. 4).

In non-etiolated seedlings, maximum $K^+$ content was observed under TU foliar treatment control and minimum content was noted with foliar supplementation of Kin under control. The order of changes recorded as: TU Con >KIN S > Con S >SA S > TU S> SA Con >No spray control> KIN Con.

In etiolated conditions, maximum potassium content in root was observed with the application of Kin under control and minimum was recorded with no foliar spray under salt stress. Following trend was observed: KIN Con > KIN S > TU S > SA Con > Con S > TU Con > SA S > No spray Con.

In de-etiolated seedlings, the root potassium increased in TU spray under control, and a minimum was observed with foliar application of SA under control. Under De-etiolation, the order of changes observed as follows: TU Con > KIN Con > No spray control> SA S > No spray S> TU S > KIN S > SA Con.

*Shoot Magnesium ($Mg^{+2}$)*

Results related to shooting magnesium revealed that the highest amount of magnesium was accumulated under the foliar spray of Kin control of etiolation condition while low concentration was recorded in TU foliar treatment under salinity stress of etiolation conditions (Fig. 5).Considering non-etiolated seedlings, it was observed that the highest content of magnesium accumulation was noted in salinity conditions with the application of Kin spray, while the lowest content was observed in salinity stress under SA spray. Trend observed for shoot magnesium was: KIN S >Con S > TU S >KIN Con > No spray control> TU Con > SA Con > SA S.

While in etiolated seedlings, maximum magnesium content in the shoot was observed with the application of Kin under control and minimum was with TU foliar treatment under salt stress. The order of improvement observed as: KIN Con > TU Con > SA Con > SA S > No spray control> Con S > KIN S > TU S.

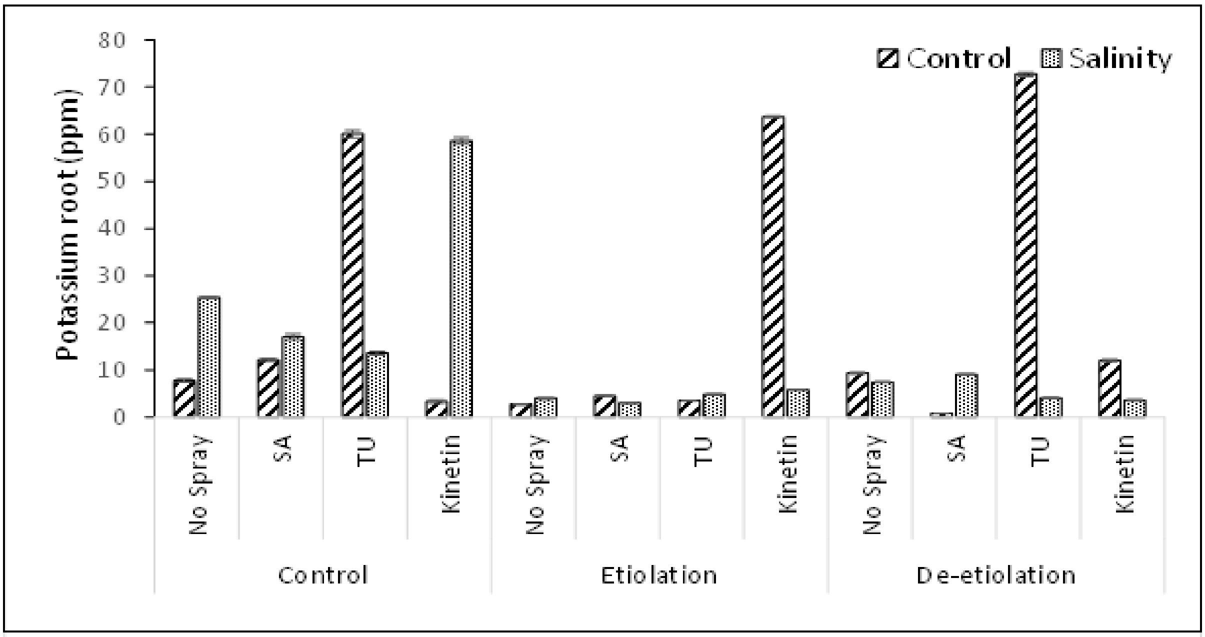


**Fig. 4.** Changes in root potassium content of Maize as affected under salinity stress in response to etiolation and de-etiolation. Plants were foliar supplemented with different PGRs.

In de-etiolated seedlings, the highest amount of shoot magnesium was noticed with no spray under salinity and the lowest was observed with the application of Kin spray under salt stress. The order of changes in shoot magnesium content under de-etiolation observed as: No spray S > SA S > SA Con > TU S > Control of C > TU Con > KIN Con > KIN S.

*Root Magnesium ($Mg^{+2}$)*

Data regarding root magnesium revealed that foliar spray of TU under salinity stress of non-etiolated condition had maximum content while the least content of magnesium was observed under the foliar spray of SA under salinity stress of non-etiolated condition (Fig. 6).

In non-etiolated seedlings, the highest root magnesium content was noted with TU foliar treatment under salinity stress, lowest was noted with the application of SA spray under salt stress. The trend observed for root magnesium was: TU S > Con S > SA Con > No spray control> KIN S > TU Con > KIN Con > SA S.

In etiolated conditions, maximum magnesium content in root was observed with no spray under control conditions and the minimum was with the foliar treatment of TU under salinity. The order of changes observed in etiolation for root magnesium was recorded as: Control of C > SA S > SA Con > TU C > Con S > KIN Con > KIN S > TU S.

In de-etiolated seedlings, the highest root magnesium was observed without spray under salt stress and minimum content was observed with no spray with control conditions. The order of changes observed in De-etiolation for root magnesium was recorded as: Con S > KIN S > SA S > SA Con >TU C > TU S > KIN C > Control of C.

## Discussion

Salinity stress is reported to cause various physiological and biochemical changes in crops by reducing the growth potential and yield, particularly because of osmotic imbalance and ionic toxicity (Munns and Tester, 2008; Lin *et al.*, 2017), micro and macronutrients deficiencies (Niste *et al.*, 2014). Nutrients are important for physiological processes such as enzyme activation, production of macromolecules, osmotic homeostasis and stomatal regulation (Fageria and Moreira, 2011; Shahid *et al.*, 2020). Ionic toxicity due to Na+ and Cl- decrease the uptake of other nutrients and causes metabolic imbalance (Munns, 2002). During salt stress, the germination of seeds gets delayed because maize plant is sensitive to salinity stress (Ashraf and Foolad 2005). Saline soil is reported to delay the growth and productivity of Maize (Ouda *et al.* 2008). However, the present research showed that the foliar supplementation of PGRs proved very effective by ameliorating salinity and etiolation impacts on maize seedlings and the PGRs improved tolerance potential of plants w.r.t improved nutrient status under salinity stress with the consideration of light variations remain to be the key concern for the development of healthy and vigor seedling.

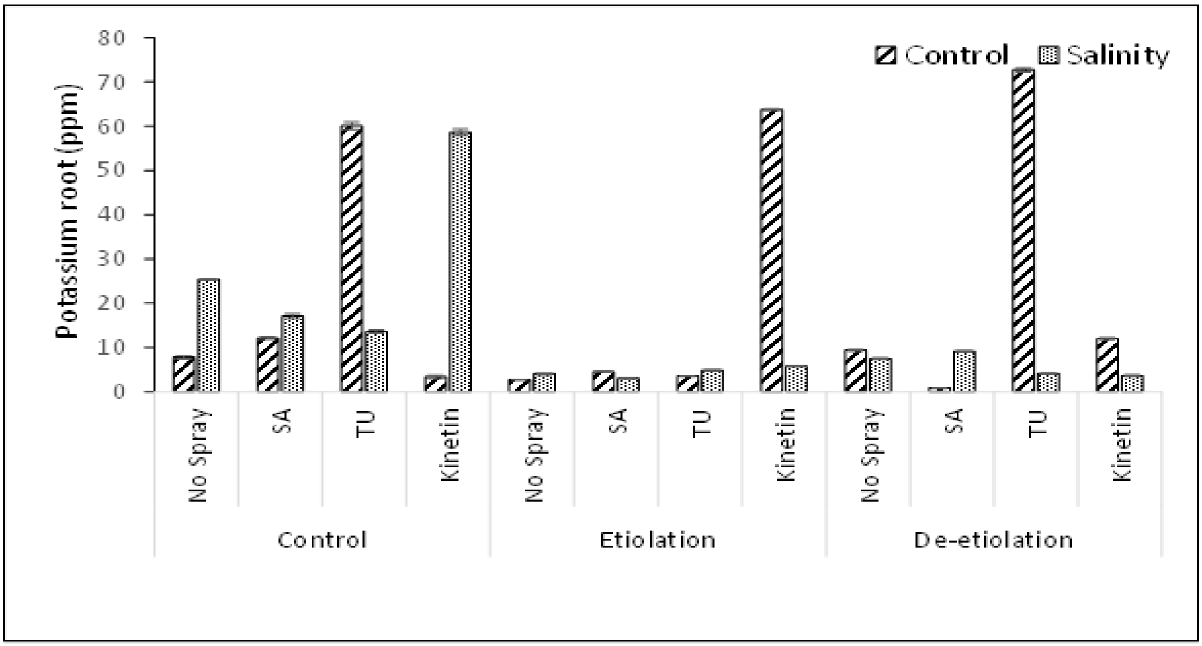


**Fig. 5.** Changes in shoot magnesium content of Maize as affected under salinity stress in response to etiolation and de-etiolation. Plants were foliar supplemented with different PGRs.

Exogenous application of growth regulators (PGRs) can enhance plant growth and antioxidant capacity and improve plant tolerance under various abiotic stress, and selective application of plant growth regulators is effective in accumulating the nitrate content under stress (Khan *et al.*, 2021). While in the present study, results that in the shoot of Maize, the nitrate content increased due to the foliar supplementation of TU under salinity at de-etiolation condition, while the lowest nitrate content was observed by the foliar supplementation of Kin under control of etiolation condition. Further, the results obtained for root nitrate showed that the maximum nitrate was observed in TU and Kin foliar treatments under salinity of de-etiolation conditions, while the lowest root nitrate content was observed in TU spray under control condition in maize seedlings. Potassium is among the essential nutrient for plant growth and development (Taiz and Zeiger, 2009). It plays important role in plants, like maintaining the osmotic pressure inside the vacuole that help in cell turgidity and activation of many enzymatic reactions (Maathuis and Amtmann, 1999).

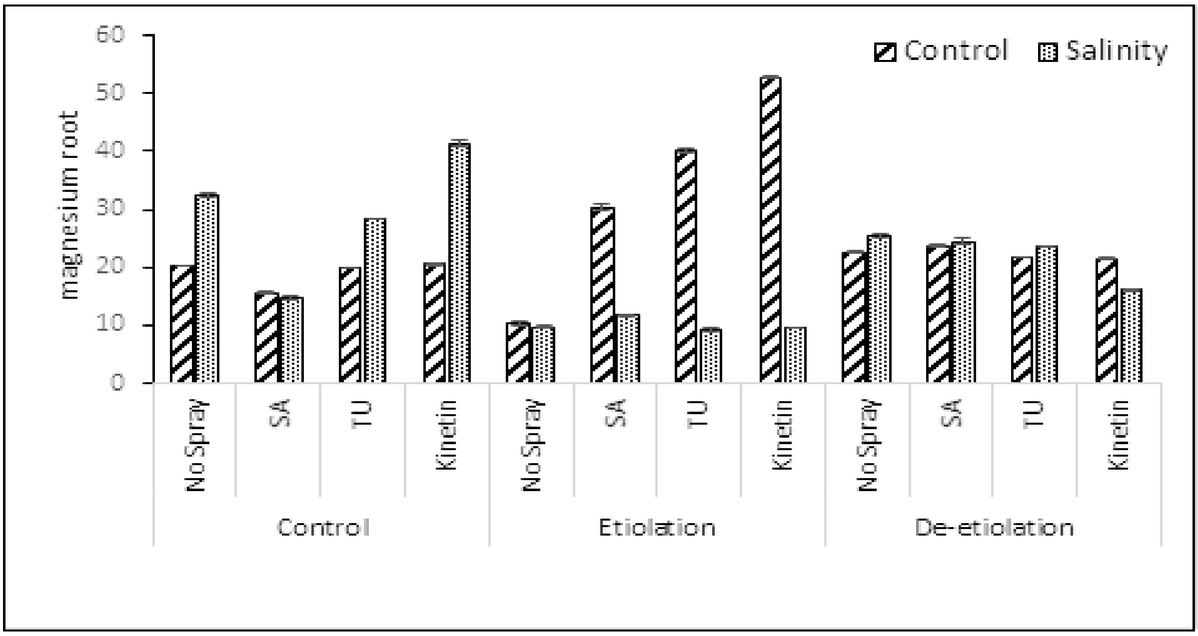


**Fig. 6.** Changes in root magnesium content of Maize as affected under salinity stress in response to etiolation and de-etiolation. Plants were foliar supplemented with different PGRs.

The deficiency of $K^+$ causes a reduction in chlorophyll contents and carbon fixation and reduces photosynthesis activity (Zhao *et al.*, 2001). Salinity also reduces the $K^+$ contents. The reduction in $K^+$ concentration was due to the presence of unnecessary $Na^+$ in the growth medium and $Na^+$ content have a negative effect on $K^+$ uptake in the plant (Sarwar and Ashraf, 2003), which hampers plant growth (Shannon, 1979). Therefore, salinity is reported to hinder the concentration of $K^+$ content (Dasgan *et al.*, 2002; Akram *et al.*, 2010). Application of mannitol or thiourea was found to be effective in enhancing the leaf K, $Ca^{+2}$ and P content (Kaya, 2013). Application of Ascorbic acid (AsA), Kinetin (Kin) and thiourea (TU) were found to be the most effective treatment in enhancing the K content during etiolation and de-etiolation (Batool *et al.*, 2020). The data of present research revealed that the highest potassium content in the shoot was recorded without foliar spray treatment under control of de-etiolation, while the lowest potassium content was noted with foliar supplementation of Kin under control of etiolation condition in maize seedlings. Furthermore, the obtained results revealed that maximum root potassium content was reported in TU spray under control of de etiolation condition, while minimum root potassium contents were observed with no salt stress in de-etiolation condition. Many researchers revealed that magnesium is the most effective nutrient in reducing the toxic effects of salinity. Inside the cell, Mg regulates the cation-anion balance and pH. It has a vital role in being the central atom of chlorophyll (Walker and Weinstein, 1991). It is essential for ATP synthesis (Lin and Nobel, 1971) and plays an important role in hampering the toxic impacts of salinity, cadmium toxicity, etc. (Kashem and Kawai, 2007). SA strongly inhibits $Na^+$ and $Cl^-$ accumulation by enhancing Mg, N, and Cu content in

Maize (Gunes and Cicek 2007). $Mg^{+2}$ is a key factor in the molecules of chlorophyll and a highly mobile nutrient by having a connection with nitrogen and potassium, thus, having a great potentiality in the dry matter that is portioning from the sink to the source. While the exogenous application of foliar spray improves the concentration of various nutrients by helping plants for better growth and development under stressful environments (Senbayram *et al.* 2016; Ashraf *et al.* 2008). The present study documents that in the shoot, maximum amount of magnesium was accumulated with foliar spray of Kin under control of etiolation condition while low concentration was recorded in TU foliar treatment under salinity stress of etiolation conditions. Hence, in root maize seedlings, it was observed that the spray of TU treatments under salinity enhanced the magnesium contents. The foliar supplementation of Kin and TU in roots and shoots of maize plants showed the greatest effect on magnesium accumulation in overall conditions because the exogenous application of foliar spray was very helpful in enhancing the magnesium contents.

The mineral nutrients have essential roles in plant growth and development. Any deficiency or excess of essential nutrients leads to the development of respective symptoms in different parts of the affected plants (Batool *et al.*, 2020). According to this research, it was noted that the changes in tissue nutrient contents (Potassium, Magnesium, and Nitrate) with foliar supplementation of PGRs gets improved, thereby hampering the negative impacts of salinity stress under non-etiolated/control, etiolation and de-etiolation conditions.

**Conclusion**

In conclusion, the results of this study recommend the application of PGRs, i.e., SA, TU and Kin, in regulating the salt stress tolerance by improving nutrient contents in maize seedlings under etiolation and de-etiolation conditions. Thus, exogenous supplementation of PGRs induced salt tolerance in Maize by improving growth with maintaining essential nutrients and hampering the impacts of salinity both in the shoot and root of maize seedlings.

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