Annals of Agricultural Science (2014) **59**(1), 89–94

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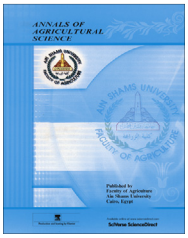

ORIGINAL ARTICLE

A comparison study on the effect of some growth regulators on the nutrients content of maize plant under salinity conditions

Rama T. Rashad *, **Rashad A. Hussien**

Soils, Water and Environment Research Institute, Agricultural Research Center, Giza, Egypt

Received 11 November 2013; accepted 31 March 2014
Available online 6 August 2014

KEYWORDS

Gibberellic acid GA3;
Salicylic acid SA;
Silicon;
Salinity resistance;
Plant growth regulators PGR;
Chlorophyll

Abstract A comparison study between the foliar application effects of the gibberellic acid (GA3), salicylic acid (SA) and silicon on the nutritional content of the maize plant leaves (*Zea mays* L. CV.) has been carried out through a pot experiment using an irrigation saline water. Chlorophyll, macro- and micro-nutrients contents of the plant leaves were estimated for the untreated and the treated plants by a 100 mg L^{-1} solution of GA3, SA or Si. GA3 was found to be the most effective for resisting the severe salinity effects on the leaves' chlorophyll followed by the Si then the SA. In almost the same order, the Fe, Zn and Si toxicity due to the salinity effects on the leaves could be reduced. Cu and Mn deficiency might be controlled but to a limited extent by SA then by GA3. Silicon ions may compete for the Na^+ ions and hence reduce their absorption by the maize plants.

Introduction

Crops grown in the arid and semi-arid regions are often exposed to adverse environmental factors such as high soil salinity. The reduction in the plant growth in the saline environments may be due to either water relations or the toxic effects of Na^+ and Cl^- ions on the metabolism. Na^+ influx into the root cells elevates the cytoplasm Na^+ concentration and causes toxicity symptoms.

When plants are subjected to stress conditions, highly reactive oxygen species ROS (cytotoxic species) are produced. In the absence of any protective mechanism, excessive amounts of ROS can seriously disrupt the normal metabolism through oxidative damage to lipids, protein and nucleic acids. They can enhance membrane lipid per-oxidation, electrolyte leakage, damage chloroplast; inhibit photochemical reactions, decrease photosynthesis and loss of cell membrane integrity. The balance between ROS generation and scavenging may be disrupted by salt stress and high light or UV exposure. Plants have a number of antioxidant enzymes protecting themselves that used as indicators for the salinity stress (Chen et al., 2011).

In the field, the solid phase of the soil system, the concentration and composition of the solutes in the soil solution and its pH control the concentrations and activity of the

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* Corresponding author. Tel.: +20 01062856224.
E-mail address: rtalat2005@yahoo.com (R.T. Rashad).

Peer review under responsibility of Faculty of Agriculture, Ain-Shams University.

http://dx.doi.org/10.1016/j.aoas.2014.06.013

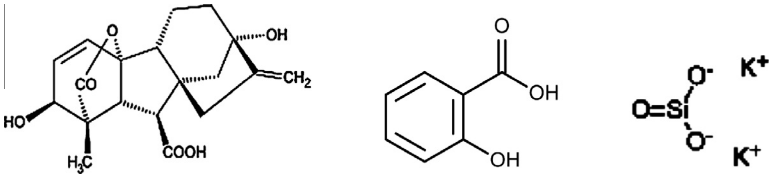

Fig. 1 Chemical structure of GA3, SA and K-silicates used in the study.

nutrient ion, particularly P, K and the micronutrients. The speciation, transformations (e.g. nitrification: ammonium to nitrate) and thus availability of certain nutrients is affected by salinity, soil moisture, texture and its nutritional status (Grattan and Grieve, 1999; Jia-minl et al., 2008). The relations between salinity and mineral nutrition of horticultural crops are extremely complex affecting the nutrient availability, competitive uptake, and transport or partitioning within the plant. The plant becomes susceptible to osmotic and specific ion injury as well as to nutritional disorders that may result in reduced yield or quality. This depends upon the salinity level, the composition of salts, the crop species, the nutrient in question and a number of environmental factors. Salt stress (S) was found to reduce the chlorophyll content and increase some enzyme activities and electrolyte leakage. It also reduced some macro and micronutrient concentrations and induces membrane permeability (Ananieva et al., 2002; Dong et al., 2006; Tuna et al., 2008a,b; Janda et al., 2012; Saidi et al., 2013). Salinity reduces N and P uptake and accumulation in crops. High levels of external Na^+ interfere with K^+ acquisition by the roots, disrupt the integrity of root membranes and alter their selectivity. Salinity may increase, decrease, or have no effect on the micronutrients (e.g. Cu, Fe, Mn, Mo and Zn) concentration in the plant shoots (Grattan and Grieve, 1999).

The effects of salinity can be minimized by improved irrigation and drainage techniques but the cost is very high which emphasizes the need for an alternative strategy. Exogenous application (Foliar application) of plant growth regulators (PGRs) such as gibberellic (GA3) and salicylic (SA) acids could overcome; to variable extents, the adverse effects of NaCl stress on the salt-affected physiological parameters. GA3 of potential economic interest could be obtained by processing of some wastes (Berry and Sachar, 1981; Slakeski and Fincher, 1992; Pastrana et al., 1995; Tuna et al., 2008a,b).

Depending on the plant species, PGRs like GA3 can improve the plant growth, ion uptake and transport, and the nutrient utilization under salt stress. They are responsible for seed germination, stem elongation, leaf expansion and flowering, and prevent chlorophyll breakdown and decreases ROS levels that lead to cell death. They stabilize microtubules in plant organs against de-polymerization (Maya-Ampudia and Bernal-Lugo, 2006; Rosenvasser et al., 2006; Tsavkelova et al., 2008; Wen et al., 2010; Janda et al., 2012; Bose et al., 2013).

Salicylic acid (SA), a naturally occurring plant phenolic is considered as a hormone like endogenous regulator. It could ameliorate the oxidative stress damaging effects of heavy metals like Cd in rice. SA strongly inhibited Na^+ and Cl^- accumulation, stimulated N, Mg, Fe, Mn and Cu concentrations of salt stressed maize plants. One of the pathways of SA biosynthesis is located in the chloroplasts in processes catalyzed by some enzymes so, it affects leaf photosynthesis (Misra and Saxena, 2009; Szepesia et al., 2009; Torre-Hernandez et al., 2010; Nazar et al., 2011; Radwan, 2012).

But the majority of results obtained with the exogenous application of PGRs cannot be generalized, since the effect may vary not only with the plant species, but also may depend on the method of administration (for example spraying, presoaking, addition to the growth medium, etc.), as well as on the time scale of the experiments. Many of the described effects in the treated plants are probably not directly due to PGRs, but may be secondary ones induced by the treatment at the site of application (Hao et al., 2012).

Additionally, the use of PGRs must be under strict control because they can provoke several diseases. Studies indicated that they may produce organ damages, including the brain, alarming toxicity to the breast, lung, kidney, liver and neurotoxicity of experiment mice. A combination of GA3 with a high concentration of EDTA causes severe soil and ground water pollution (Young et al., 1997; Hadi et al., 2010; Troudi et al., 2012).

Silicon (Si) may be beneficial for the plant growth and photosynthetic activity. According to the literature and under the salt stress conditions, Si enhanced the K^+:Na^+ ratio against the toxic effects of Na^+. Sodium (Na^+) transportation into roots and shoots as well as shoot K^+ and Ca^{+2} concentrations was reduced by added silicate. Si application reversed the chlorosis, protected the chloroplast from disorganization, and significantly increased the pigments contents. It increases the resistance of some plant species to toxic metals such as Cd by decreasing their uptake and accumulation which damage chloroplast and root-to-shoot transport (Tuna et al., 2008a; Feng et al., 2010).

The interaction between salinity and different nutrients like nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn), zinc (Zn), copper (Cu) and silicon (Si) is complex. The interaction is highly dependent upon the plant species (or cultivar), plant developmental age, the composition and level of salinity and the concentration of nutritional element in the substrate. Therefore, depending upon plants selected and conditions of the experiment, different results can be obtained. The present study is a greenhouse pot experiment to compare the salt stress counteraction effects of gibberellic acid (GA3), salicylic acid and silicon application on some nutrients content of maize plant (*Zea mays* L. CV.).

Materials and methods

Materials

The materials used were Gibberellic acid GA3 ($C_{19}H_{22}O_6$ – Berelex, VALENT Bio-Science co.), Salicylic acid SA

($C_7H_6O_3$ – El Nasr pharmaceutical chemicals Co., Egypt.) and potassium silicates (K_2SiO_3 as liquid silicon commercial solution: K_2O – 10%; SiO_2 – 25%) Fig. 1.

The irrigation saline water used was 6000 mg L^{-1} composed of NaCl, $MgSO_4$ and $CaSO_4$ in ratios 3, 2 and 1 g L^{-1}, respectively.

Greenhouse experiment

The experiment was conducted during two summer seasons 2011 and 2012 (on the 15th and 20th May). Treatments were designed as follows (1) Control 1 (without any treatment); (2) Control 2: (irrigated by saline water without any treatment); (3) 100 mg L^{-1} GA3; (4) 100 mg L^{-1} SA, (5) 100 mg L^{-1} Si. Treatments 3, 4 and 5 were irrigated by saline water. Treatments were arranged in completely randomized block design (CRBD) with three replicates ($N = 3$).

Grains of maize (Zea mays L.) Varity of single hybrid 10 supplied from Maize Department, Field Crop Research Institute were sown. Each pot contained 5 kg soil. An agricultural soil sample (0–30 cm depth) was used for the study. It was air-dried, ground and sieved with a 2 mm sieve. Some of its properties were estimated according to Page et al. (1982) and presented in Table 1.

Calcium super phosphate (15.5% P_2O_5) was added in a rate of 0.05 g P_2O_5 kg^{-1} soil during the soil preparation. Nitrogen was applied in a rate of 0.1 g N kg^{-1} soil as urea (46% N) in three equal doses after 21; 45 and 60 days from planting.

Potassium sulphate (50% K_2O) in a rate of 0.1 g K_2O kg^{-1} soil was added in two doses after 30 and 50 days from planting. Foliar applications were applied after 30, 45 and 60 days from sowing.

All cultural practices for growing maize were done as recommended. Two leaves from the upper ground part of four randomly chosen plants per replicate after 60 days, were collected from mid-sections of the plants in order to minimize age effects to determine its chlorophyll content and chemical analysis.

Analysis

Chlorophyll determination

Photosynthetic pigments (chlorophyll a + b) were measured in fresh leaf samples. Leaf samples (0.5 g) were homogenized with acetone (90% v/v), filtered and made up to a final volume of 50 mL. Chlorophyll concentration was calculated from the absorbance of the extract measured by JENWAY Spectrophotometer 6405 UV/Vis., using the equation proposed (Nazar et al., 2011):

- *Chl. a* (mg/g FW) = $(11.75 \times A663 - 2.35 \times A645) \times (50/500)$
- *Chl. b* (mg/g FW) = $(18.61 \times A645 - 3.96 \times A663) \times (50/500)$

(A663) and (A645) represent absorbance values read at 663 and 645 nm wavelengths, respectively.

Total NPK, Ca^{+2}, Mg^{+2}, Na^{+} and micro-nutrients

The concentrations of the different nutrients were estimated in the plant extract obtained after the wet digestion of the plant samples with conc. H_2SO_4 and $HClO_4$, acid mixture (Chapman and Pratt, 1961). The total N was determined by distillation in a Macro-Kjeldahl apparatus. Total P was estimated colorimetrically using stannous chloride mixture and measured by UV/Vis spectrophotometer, while K^{+} and Na^{+} concentrations were measured by flame photometer. The concentrations of Ca^{+2}, Mg^{+2}, Fe, Mn, Zn, Cu and Si were measured by ICP-AAS spectrophotometer (Jackson, 1973; Cottenie et al., 1982).

Statistical analysis

Statistically analyzed data are the mean values ± standard errors ($n = 3$). The one-way analysis of variance (ANOVA) was carried out to determine the statistical significance of the treatment effects with the least significant difference procedure at a significance level of 0.05 (Gomez and Gomez, 1984; Mahdy, 2011).

Results and discussion

Effect of the treatments on the chlorophyll content in the maize leaves

The total chlorophyll content of the maize leaves was strongly depressed by using the irrigation saline water as shown in Table 2. The foliar application could effectively compensate such depression in the order GA3 > Si > SA affecting the leaves content of both chlorophyll *a* and *b* (Ananieva et al., 2002).

NPK content in leaves

Table 3 presents the total NPK content of plants with different treatments. It can be said that the N content was unaffected by salinity. Additionally, it was suppressed by the application of GA3 while enhanced by SA and Si application. Phosphorous content was neither affected by salinity nor by GA3 and Si application. It was slightly enhanced by SA application. This is in agreement with previous studies (Nazar et al., 2011).

Table 1 Some properties of the studied soil sample.

Particle size distribution (g kg^{-1})					OM (%)	$CaCO_3$ (%)	pH (1:2.5)	EC_e* (dS m^{-1})
Coarse sand	Fine sand	Silt	Clay	Texture class				
747.7	190.1	48.8	10	Sand	0.25	0.89	7.99	0.45
Soluble ions* (meq L^{-1})								
Ca^{++}	Mg^{++}	Na^{+}	K^{+}	HCO_3^{-}	Cl^{-}	SO_4^{-}		
1.26	0.44	0.36	0.17	0.56	0.46	1.22		

* (1:5) soil extract.

Table 2 Effect of foliar application of GA3, SA acids and Si on the chlorophyll content of maize leaves.

	Chl *a*	Chl *b*	Total Chl	Chl *a*/*b*
Control 1	1.511^{a}	0.718^{b}	2.229	2.104
Control 2	0.576^{c}	0.298^{d}	0.874	1.933
GA3	1.604^{a}	1.017^{a}	2.621	1.577
SA	1.143^{b}	0.445^{c}	1.588	2.569
Si	1.480^{a}	0.690^{b}	2.170	2.145

The footnotes (a–d) indicate the non-significance ranges for the different treatments.

Table 3 Effect of foliar application of GA3, SA acids and Si on the macro-nutrients (NPK) content of maize leaves.

	Content (g kg^{-1})		
	N	P	K
Control 1	14.175^{c}	1.125^{b}	13.990^{b}
Control 2	14.175^{c}	1.125^{b}	14.674^{a}
GA3	9.450^{d}	1.125^{b}	11.954^{c}
SA	20.475^{b}	1.375^{a}	9.390^{d}
Si	22.050^{a}	1.125^{b}	6.460^{e}

The footnotes (a–d) indicate the non-significance ranges for the different treatments.

Unexpectedly, potassium content was inhibited in the order Si < SA < GA3 by their foliar application. Salinity can directly affect the nutrient uptake, such as Na by reducing K uptake or by Cl^- by reducing NO_3^- uptake (Grattan and Grieve, 1999).

Ca^{+2}, Mg^{+2} and Na^{+} content in leaves

One of the most severe effects of salt stress is the absorption of Na^+ ions by plant roots. High levels of external Na interfere with K acquisition by the roots, disrupt the integrity of root membranes and alter their selectivity that must be sufficient to meet the levels of K required for metabolic processes, for the regulation of ion transport, and for osmotic adjustment. Calcium is strongly a competitive with Mg^{+2} and the binding sites on the root plasma membrane appear to have less affinity for the highly hydrated Mg^{+2} than for Ca^{+2}. Sodium chloride (NaCl) salinity had reduced leaf Mg^{+2} concentrations (Grattan and Grieve, 1999).

Table 4 Effect of foliar application of GA3, SA acids and Si on the Ca^{+2}, Mg^{+2} and Na^{+} content of maize leaves.

	Content (g kg^{-1})				
	Ca^{+2}	Mg^{+2}	Na^{+}	Na^{+}/K^{+}	Na^{+}/Ca^{+2}
Control 1	7.380^{c}	6.440^{b}	1.610^{e}	0.115	0.218
Control 2	9.400^{b}	5.750^{c}	5.050^{b}	0.344	0.538
GA3	34.950^{a}	6.560^{b}	4.790^{c}	0.401	0.137
SA	9.560^{b}	8.410^{a}	18.110^{a}	1.929	1.895
Si	7.610^{c}	5.150^{d}	3.620^{d}	0.561	0.476

The footnotes (a–d) indicate the non-significance ranges for the different treatments.

Table 5 Effect of foliar application of GA3, SA acids and Si on the micro-nutrients content of maize leaves.

	Conc. (g kg^{-1})				
	Fe	Mn	Zn	Cu	Si
Control 1	219.0^{c}	82.5^{a}	82.8^{d}	50.3^{a}	125.5^{d}
Control 2	332.5^{b}	58.0^{b}	450.0^{a}	43.0^{b}	470.0^{a}
GA3	219.3^{c}	36.8^{d}	73.8^{e}	47.3^{a}	115.0^{e}
SA	372.5^{a}	41.8^{c}	168.0^{b}	48.0^{a}	191.0^{c}
Si	149.8^{d}	35.0^{d}	105.3^{c}	32.8^{c}	196.0^{b}

The footnotes (a–d) indicate the non-significance ranges for the different treatments.

The present study (Table 4) shows that although GA3 did not reduce Na absorption, it strongly enhanced the absorption of Ca^{+2} and K^+ that could meet Na^+ higher concentration and controlled the Na^+/K^+ and Na^+/Ca^+ ratios. On the other hand, although Si could reduce Na^+ absorption due to salinity, it could not enhance the absorption of K^+ or Ca^{+2}. So, the small concentration of Na^+ absorbed has led to higher ratios of Na^+/K^+ and Na^+/Ca^+ compared to the control treatment. Plants treated by SA showed higher concentrations of most cations especially Na^+ resulting in higher ratios of Na^+/K^+ and Na^+/Ca^+ compared to the control treatment (Jia-minl et al., 2008).

Micro-nutrients content of leaves

According to Table 5, the micro-nutrients content as affected by the application of different materials is as follows:

Iron

Compared with the control, Fe the content had been increased by irrigation using saline water and by SA application, unaffected by GA3 while decreased by Si application (Feng et al., 2010; Hadi et al., 2010; Wen et al., 2010).

Manganese

Salinity had reduced the Mn concentration in plant tissue. Its content had been decreased in the order: irrigation saline water > SA > GA3 > Si (Grattan and Grieve, 1999).

Zinc

Its content was increased compared with the control by the irrigation with saline water. This increase was inhibited by different treatments in the order: SA > Si > GA3. The majority of the studies in the literature have shown salinity to increase Zn concentration in the shoot tissue such as in bean (Grattan and Grieve, 1999; Guo et al., 2007; Krantev et al., 2008).

Copper

Leaves' copper content was suppressed using irrigation saline water. Both GA3 and SA could limit its decrease due to salinity but Si application has lead to further decrease in the Cu content in the maize plants. Literature indicated that the salinity influence on Cu accumulation was variable. It can cause a combination of complex interactions affecting plant metabolism or susceptibility to injury (Grattan and Grieve, 1999).

Silicon

Silicon content was highly increased due to irrigation saline water. The high content due to salinity could be suppressed by the application of Si, SA and GA3 (Guo et al., 2007; Wang et al., 2009).

In a conclusion, it can be said that the irrigation saline water caused a toxicity of the maize plants by Fe, Zn and Si due to high absorption levels of these elements compared with the control. This may be a result of different competitive and selectivity effects of different cations and anions in solution of the root distribution zone. Generally, the foliar application of GA3 was the most effective in resisting the severe increase levels of these elements due to salinity followed by Si then SA application (Tuna et al., 2008a,b; Song et al., 2009; Feng et al., 2010; Gu et al., 2011).

Silicon application decreased the heavy metal diffusive gradient in thin films (DGT) pools and the fluxes from the soil solid phase to the solution by transforming the soluble metal ions to the less soluble and slower exchanging forms such as metal silicates, phosphates and hydroxides. The Si-mediated effects on some heavy metal accumulation in rice promoted rice growth and reduced heavy metal translocation (Gu et al., 2011).

But the deficiency in Mn and Cu content due to the salinity effects could not treated effectively by the application of different materials except for Cu using GA3 and SA.

The chemical structure of applied GA3, SA and silicates may play an important role in increasing the absorption of cations (or anions) by plant tissues. Suggesting that they do not undergo complete or partial chemical degradation or dissociation in presence of the plant metabolic environment, many factors may be included. e.g. the stereochemistry of each compound, the type, nature, and site of attach of different functional groups in the compound (—OH, —COOH, —C=O) in addition to the electron cloud associated to the unsaturated cyclic moieties of the compound. They will affect its ability and affinity to chemically react with a specific ion forming a salt and/or a chelated complex in the plant cells and tissues. So, the absorption of certain cation may increase or decrease.

Conclusion

The effects of foliar application of gibberellic acid (GA3), salicylic acid (SA) and silicon (Si) on the nutritional content of maize plant leaves (*Zea mays* L. CV.) have been compared through a pot experiment using irrigation saline water. Chlorophyll, macro- and micro-nutrients contents of the plant leaves were estimated for the untreated and the treated plants by a solution of GA3 and SA or Si. Gibberellic acid was found to be the most effective for resisting the severe salinity effects on the leaves chlorophyll followed by the Si then the SA. In almost the same order, the Fe, Zn and Si toxicity due to the salinity effects on the leaves could be reduced. Copper (Cu) and manganese (Mn) deficiency may be controlled but to a limited extent by SA then by GA3. Both materials enhanced nitrogen (N) content of the leaves and potassium (K) to a lower extent. But sodium (Na^+) content as well as Na^+/K^+ ratio of the leaves was highly increased as affected by SA and GA3 and decreased by Si application. This may be due to formation of Na-salts of both acids in the plants leaves. Silicon ions may compete for Na^+ ions and hence reduce their absorption by the maize plants.

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