Journal of Stress Physiology & Biochemistry, Vol. 10 No. 1 2014, pp. 203-213 ISSN 1997-0838

ORIGINAL ARTICLE

The Exogenous Amelioration Roles of Growth Regulators on Crop Plants Grow under Different Osmotic Potential

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Received October 20, 2013

The production of fresh and dry matter of maize, wheat, cotton, broad and parsley plants show a variable response to the elevation of salinity stress. The production of fresh and dry matter of shoots and roots in wheat and broad bean plants tended to decrease with increasing NaCl concentration, salt stress progressively decrease in fresh and dry matter yield of maize plants. The increase in salinization levels induced a general insignificant change in production of fresh and dry matter of both organs of parsley plants. However, salinity induced a marked increase in the values of fresh and dry matter yields of cotton plants grown at the lowest level (-0.3 MPa NaCl) and a reduction at higher salinization levels. Leaf area of unsprayed plants was excesivly decreased with the rise of osmotic stress levels especially at higher salinity levels of maize, wheat, cotton, and broad bean and parsley plants. the total pigments concentration decreased with rise of salinization levels in maize and cotton, these contents remained more or less un affected up to the level of 0.6 MPa NaCl in wheat and up to 0.9 MPa in parsley plants, there above, they were significantly reduced with increasing salinity levels. In broad bean plants the total pigments contents showed a non-significant alterations at all salinity stress. Spraying the vegetative parts of the five tested plants with 200 ppm of either GA3 or kinetin completely ameliorated the deleterious effect of salinity in fresh, dry matter, leaf area and pigment contents.

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There have been numerous studies of the effects of salinity on plants (Jamil *et al*., 2007; Duan *et al*., 2008, Hamdia and Shaddad, 2010). Recently, investigations have focused more on the mechanisms of salt tolerance in plants (Dajic, 2006; Munns and Tester, 2008). Some researchers have used PGRs for reducing or eradicating the negative effects of salinity (Kabar, 1987; Mutlu and Bozcuk, 2000 and Hamdia and Shaddad, 2010). Phytohormones suggested playing important roles in stress responses and adaptation (Sharma *et al*., 2005; Shaterian *et al*., 2005). It is thought that the repressive effect of salinity on seed germination and plant growth could be related to a decline in endogenous levels of phytohormones (Zholkevich and Pustovoytova, 1993; Jackson, 1997; Debez *et al*., 2001). Wang *et al*. (2001) clearly defined that ABA and JA will be increased in response to salinity, whereas indole-3-acetic acid (IAA) and salicylic acid (SA) are declined. For example, the exogenous application of PGRs, auxins (Khan *et al*., 2004), gibberellins (Afzal *et al*., 2005), cytokinins (Gul *et al*., 2000) produces some benefit in alleviating the adverse effects of salt stress and also improves germination, growth, development and seed yields and yield quality

Javid *et al* (2011) explain that phytohormones are chemical messengers produced in one part of plant and translocated to the other parts, where they play critical roles in regulating plant responses to stress at extremely low concentration. Phytohormones are natural products and they called plant growth regulators, when they are synthesized chemically. Plants are usually subjected to environmental factors such as drought or high soil and water salinity. The reduction in plant growth exposed to saline environments could be due to either the effects of specific ions on metabolism or adverse water relations. Different strategies are being employed to maximize plant growth under saline conditions. One of them is to produce salt tolerant genotypes of different crops. Attempts to improve tolerance to salinity through conventional plant breeding methods are time consuming, laborious and depended on existing genetic variability. In addition, many attempts have been made to overcome this disorder, including proper management and exogenous application of plant growth regulators.

Thus the present work was to throw the light on the different response of crop plants to salinity stress and the amelioration roles of growth regulators on the growth and metabolic components of some crop plants.

MATERIALS AND METHODS

Five plant species Maize (Zea mays), Wheat (*Triticum aestivum*) , Broad bean (*Vicia faba*), Cotton (*Gossypium herbaceum*) and Parsley plants (*Petroselinum crispum*) were grown in plastic pots in the soil without NaCl (control) and under salinization levels corresponding to osmotic potential of NaCl solution of, -0.3, -0.6, -0.9, and -1.2MPa. Saline solutions were added to the soil in such a way that the soil solution acquired the assigned salinization levels at field capacity. Treatments of plants with saline solutions began when seedlings were two weeks old. The salinized and non-salinized plants were irrigated every other day with 1/10 Pfeffer's nutrient solution for two weeks. Then GA_3 and kinetin (100 ppm) solutions were sprayed three times (5 intervals) by spraying the shoot system of the growing plants (each pot with 10 Cm^3 of GA_3 or kinetin solutions). The control plants were sprayed with distilled water. A week after the plants were used for analysis. Dry matter was determined after drying plants in an aerated oven at 70 C to constant mass. The leaf area was determined by using (Norman and Campbell 1994**)** and pigments contents by using the spectrophotometer method of Metzner *et al*. (1965)

RESULTS

Fresh and dry matter

The production of fresh and dry matter of maize, wheat, cotton, broad and parsley plants show a variable response to the elevation of salinity stress. In maize plants they were progressively reduced especially at higher Stalinization levels (table 1). The production of fresh and dry matter of shoots and roots in wheat plants tended to decrease with increasing NaCl concentration, however in shoots these values were prominently higher than those of control plants up to the level -0.6 MPa NaCl (table 2). However , salinity induced a marked increase in the values of fresh and dry matter yields of cotton plants grown at the lowest level (-0.3 MPa NaCl) (table 3). This stimulatory effect was much more pronounced in shoots than in roots, thereafter these values were significantly reduced with the rise of salinization levels. In shoot and root of broad bean plants the fresh and dry matter smoothly decrease with elevation of salinity levels especially at higher salinity levels (table 4). The increase in salinization levels induced a general insignificant change in production of fresh and dry matter of both organs of parsley plants, except in case of roots, where dry matter yields mostly showed a significant decrease at the different salinity levels (table 5). It is worthy to note that the percent of reduction of fresh, dry matter of shoot and root and pigments content at 1.2 MPa was 74.7, 72.7, 60, 64.7 for maize, 80.3, 84.6, 73.9, 67.1, 47.0 for wheat, 77.7, 67.0, 46.4, 71.4, 90.0 for cotton, 87.8, 80.6, 54.1, 71.4, 85.3 for cotton and 91.1, 84.6, 89.5, 71.8, 81.2 for parsley plants.

Exogenous application of GA_3 or kinetin resulted in most cases in a considerable increase in the production of fresh and dry matter yields in shoots and roots and pigments content of salt stressed maize, wheat and cotton plants as compared with control plants. This stimulatory effect was more pronounced at lower and moderate salinization levels. However, spraying with any of the two hormones (GA_3 or kinetin) resulted in a more or less comparable values with those of control in shoots especially 6Mpa and 9 MPa salinity levels. In roots these treatments considerably increased the production of fresh and dry matter up to the highest salinity levels, reach than 2-folds of control plants. In parsley plants treatment with GA_3 mostly resulted insignificant changes in fresh and dry matter at all salinity levels of shoots and roots, except of this trend, in case of roots a stimulatory effect was observed in the production of dry matter. Kinetin treatment induced a marked and regressive increase in fresh and dry matter in shoots and roots of parsley plants. This increase was higher than those of control (0.0 NaCl) especially at lower and moderate salinity levels.

Leaf area

The values of leaf area of the tested plants as by either salinization or salinization with phytohormones treatment (GA_3 or kinetin) were represented in tables 1, 2, 3, 4 and 5. The leaf area, of unsprayed plants was excessively decreased with rise of salinization levels especially at higher salinity levels. The percent of reduction in leaf area at -1.2MPa was 71.8, 64.5, 76.8, 61.7 and 68.3 for maize, wheat, cotton, and broad bean and parsley plants.

Spraying these salinized plants with any of the phytohormones (GA_3 or kinetin) mostly resulted in a marked increase in leaf area and the inhibitory effect of salinity stress was completely ameliorated especially at the relatively low and moderate salinity levels. It is worthy to mention that the values of leaf area was higher than control untreated plants in wheat plant treated with either GA_3 or kinetin at 0.9 MPa, in cotton plants GA_3 at 6 MPa and treated with kinetin at 9 &12 MPa. In broad bean plants leaf area values was higher than control plants at 1.9 with GA_3 treatments and at 0.6 MPa with kinetin treatments.

Pigment contents

The results presented in tables 1,2,3,4,5 demonstrated that the total pigments concentration decreased with rise of salinization levels in maize and cotton, these contents remained more or less un affected up to the level of 0.6 MPa

NaCl in wheat and up to 0.9 MPa in parsley plants, there above, they were significantly reduced with increasing salinity levels. In broad bean plants the total pigments contents showed a non-significant alterations at all salinity stress. The percent of reduction at 1.2 MPa was 64.7, 47.0, 90.0, 85.3 and 81.2.

Hormonal treatment mostly increases the concentration of total pigment contents in maize especially in kinetin treatment. In wheat GA_3 treatments induced non-significant changes while a marked and progressive increase in case of kinetin treatments, cotton, broad bean and parsley plants. Salinity with hormonal treatments resulted a stimulatory effect in the biosynthetic pigments over those of control plants (0.0) of both broad bean and parsley plants, and in GA_3 especially in broad bean plants.

Table 1 : Effect of salinization levels and treatment with GA_3 or Kinetin (200 mg kg^{-1}) on fresh, dry matter [g $plant^{-1}$] of shoot and root , leaf area and total pigments maize plants.

Treatment	NaCl mM	Shoot		Root		Leaf area
		f.m.	d.m.	f.m.	d.m.	
0	0.0	29.3	4.4	4.5	1.2	223.8
	0.3	28.2	3.9	3.7	0.92	221.1
	0.6	23.9	3.7	4.0	0.93	188
	0.9	21.4	3.9	3.9	0.94	166.9
	1.2	21.9	3.2	2.4	0.72	160.7
GA_3	0.0	35.6	5.2	4.9	1.3	230.8
	0.3	31.6	8.5	6.2	1.5	230.9
	0.6	29.0	6.1	7.9	1.7	210.0
	0.9	29.6	4.5	5.9	1.6	170.9
	1.2	25.5	4.4	4.2	1.2	170.5
Kinetin	0.0	39.6	8.4	5.2	1.7	248.1
	0.3	36.3	7.5	5.6	1.4	230.6
	0.6	36.2	7.3	5.2	0.97	200.5
	0.9	36.1	7.1	4.8	0.94	184.1
	1.2	29.2	5.9	3.7	0.92	180.2
L.S.D. 5%		2.3	0.84	0.88	0.13	4.9

Table 2 : Effect of salinization levels and treatment with GA_3 or Kinetin (200 mg kg^{-1}) on fresh, dry matter [g $plant^{-1}$] of shoot and root, leaf area and total pigments wheat plants.

Treatment	NaCl mM	Shoot		Root		Leaf area
		f.m.	d.m.	f.m.	d.m.	
0	0.0	7.6	1.3	2.3	0.450	99.3
	0.3	9.6	1.8	1.8	0.436	93.9
	0.6	8.8	1.5	1.8	0.401	88.5
	0.9	5.5	1.1	1.6	0.371	67.2
	1.2	6.1	1.1	1.6	0.302	63.9
GA_3	0.0	15.3	3.2	2.8	0.558	150.1
	0.3	13.2	2.5	2.3	0.555	130.1
	0.6	10.5	2.0	2.1	0.590	114.2
	0.9	11.3	2.8	3.2	0.618	115. 0
	1.2	11.7	2.3	2.9	0.668	116.9
Kinetin	0.0	12.1	2.9	3.3	0.811	110.6
	0.3	12.4	2.6	3.2	0.776	112.0
	0.6	11.4	2.0	2.3	0.523	131.7
	0.9	8.5	1.6	2.3	0.523	115.7
	1.2	8.3	1.2	2.3	0.470	73.5
L.S.D. 5%		1.55	0.115	0.793	0.014	2.3

Table 3 : Effect of salinization levels and treatment with GA_3 or Kinetin (200 mg kg^{-1}) on fresh, dry matter [g $plant^{-1}$] of shoot and root , leaf area and total pigments cotton plants.

Treatment	NaCl mM	Shoot		Root		Leaf area
		f.m.	d.m.	f.m.	d.m.	
0	0.0	31.9	8.8	6.7	2.1	130.4
	0.3	37.7	13.4	6. 9	2.8	124.6
	0.6	28.4	6.7	5.9	2.0	118.4
	0.9	26.6	6.4	3.6	1.6	100.2
	1.2	24.8	5.9	3.2	1.5	100.1
GA_3	0.0	35.9	10.7	7.9	2.6	114.7
	0.3	39.7	12.7	7.8	2.4	140.7
	0.6	42.9	13.5	7.7	2.8	136.4
	0.9	30.6	11.1	7.8	2.8	120.2
	1.2	28.9	11.5	7.7	2.7	118.9
Kinetin	0.0	37.3	12.6	7.1	3.3	136.3
	0.3	35.5	10.4	7.8	3.1	131.4
	0.6	34.9	10.4	7.8	3.1	127.9
	0.9	34.7	11.3	7.1	2.5	135.4
	1.2	30.9	10.2	6.1	2.4	138.9
L.S.D. 5%		2.5	1.5	0.68	0.369	5.3

Table 4 : Effect of salinization levels and treatment with GA_3 or Kinetin (200 mg kg^{-1}) on fresh, dry matter [g $plant^{-1}$] of shoot and root , leaf area and total pigments broad bean plants.

Treatment	NaCl mM	Shoot		Root		Leaf area
		f.m.	d.m.	f.m.	d.m.	
0	0.0	29.2	3.6	3.7	0.429	140.4
	0.3	28.4	3.5	3.8	0.433	116.5
	0.6	27.6	3.1	3.2	0.393	103.9
	0.9	24.7	3.0	3.0	0.369	96.6
	1.2	25.6	2.9	2.0	0.369	86.6
GA_3	0.0	29.7	3.6	6.6	0.533	149.6
	0.3	29.2	3.5	6.7	0.628	149.8
	0.6	32.8	4.9	8.3	0.746	157.8
	0.9	28.9	3.9	8.8	0.794	165.7
	1.2	25.7	3.2	6.4	0.654	130.2
Kinetin	0.0	30.0	3.2	6.8	0.710	147.3
	0.3	30.0	3.5	6.7	0.719	147.2
	0.6	29.3	3.9	6.7	0.617	150.1
	0.9	25.4	3.2	5.6	0.632	132.4
	1.2	25.3	3.1	5.9	0.579	130.2
L.S.D. 5%		1.6	0.254	1.4	0.017	6.9

Table 5 : Effect of salinization levels and treatment with GA_3 or Kinetin (200 mg kg^{-1}) on fresh, dry matter [g $plant^{-1}$] of shoot and root, leaf area and total pigments of parsely plants.

Treatment	NaCl mM	Shoot		Root		Leaf area
		f.m.	d.m.	f.m.	d.m.	
0	0.0	6.8	1.3	1.9	0.685	61.9
	0.3	6.6	1.3	1.8	0.519	57.1
	0.6	6.5	1.3	1.8	0.544	36.2
	0.9	7.3	1.3	1.8	0.518	45.2
	1.2	6.2	1.1	1.7	0.492	42.3
GA_3	0.0	6.8	2.6	2.2	0.841	65.0
	0.3	6.9	1.6	2.0	0.696	60.3
	0.6	6.6	1.3	1.9	0.719	67.7
	0.9	6.5	1.4	1.8	0.459	57.5
	1.2	5.2	1.3	1.7	0.529	54.0
Kinetin	0.0	7.6	1.8	3.6	1.3	65.8
	0.3	7.4	1.6	3.1	1.0	52.6
	0.6	8.2	1.8	2.6	0.804	50.2
	0.9	7.9	1.3	2.2	0.800	50.4
	1.2	7.2	1.3	2.4	0.732	38.1
L.S.D. 5%		1.3	0.1	0.280	0.011	5.1

DISCUSSION

The fresh and dry matter yields, leaf area of maize, wheat, cotton, broad bean and parsley plants were generally lowered by increasing osmotic stress. This inhibitory effect may be attributed to the effects of salinity on several facets of plant activities such as enzyme activity (Seckin *et al*., 2009), DNA, RNA, protein synthesis (Anuradha and Rao, 2001) and mitosis (Tabur and Demir, 2010). However, plant species differ in their sensitivity or tolerance to salt stress (Ashraf and Harris, 2004), osmotic adjustment Hamdia and El-Komy (1998), hormonal balance (Jackson, 1997; Debez *et al*., 2001; Iqbal and Ashraf (2013) and photosynthesis (Amuthavalli and Sivasankaramoorthy 2012). It is worthy to note that lower concentrations of NaCl stimulated the growth of wheat shoot and broad bean root and cotton shoot and root plants. The productions of pigments were generally reduced with rise of osmotic stress in maize, wheat, cotton, broad bean and parsley plants. This reduction may be due to the lower enzyme activity of pigment biosynthesis or increase the enzyme of pigment degradation. There is strong evidence that salt affects photosynthetic enzymes, chlorophylls and carotenoids (Stepien and Klobus, 2006). The decrease in chlorophyll 'a' and 'b' in the *Panicum accessions* might have been due to salt-induced acceleration of chlorophyll enzymes degradation (Hernandez *et al*., 1993; 1995; Hernandez & Almansa, 2002), and/or disorder of chloroplast structure and related proteins (Singh & Dubey, 1995 and Sabir *et al*., 2009). The reduction in leaf area of maize, wheat, cotton, broad bean and parsley tested plants, under saline conditions were of also due to reduced growth as a result of decreased water uptake, toxicity of sodium and chloride in the shoot cell as well as reduced photosynthetic pigments similar to work obtained by Ali *et al.* (2004).

Exogenous application of GA_3 or kinetin (200 ppm) were promoted, generally the growth criteria (fresh and dry matter yield) of the five tests plants and thus alleviated to some extent the suppressive effect of salinity. This observed increase in fresh matter and dry matter of salt stressed plants after hormonal treatments may indicate that the phytohormonal applications increased the plant efficiency of water uptake, conservation and utilization (Javid *et al*., 2011). The promotion in dry matter production could be attributed to a rapid increase in cell division, cell division, cell enlargement and accumulation of building units. Gibberellic acid (GA_3) has been reported to be helpful in enhancing wheat and rice growth under saline conditions (Parasher and Varma, 1988; Prakash and Prathapasenan, 1990; Tuna *et al*., 2008). Maggio *et al*. (2010) reported that GA_3 treatment in tomato reduced stomatal resistance and enhanced plant water use at low salinity. GA_3-priming induced increase in wheat grain yield was attributed to the GA_3-priming-induced modulation of ions uptake and partitioning (within shoots and roots) and hormones homeostasis under saline conditions (Iqbal and Ashraf, 2010). Cytokinins can also enhance resistance to salinity and high temperature in plants (Barciszewski *et al*., 2000). Seed enhancement (seed priming) with cytokinins is reported to increase plant salt tolerance (Iqbal *et al*., 2006a). CKs are often considered as ABA antagonists and auxins antagonists/synergists in various processes in plants (Pospisilova, 2003). It was hypothesized that cytokinins could increase salt tolerance in wheat plants by interacting with other plant hormones, especially auxins and ABA (Iqbal *et al*., 2006b, Chakrabarti & Mukherji 2003). CKs retard senescence having effect on membrane permeability to mono and divalent ions, and localized induction of metabolic sinks (Letham, 1978). Salt stress suppressed the level of endogenous phytohormones in plants (Nagvi, 1999; Shaddad, 1990). Further evidence for the role played by salt stress in modifying plant metabolism can be obtained from the data of pigment content, these results clearly demonstrate that, the biosynthesis of pigments in salt stressed plants differ according to the species used. In broad bean and parsley plants the biosynthesis of pigments was generally unaffected by salinity stress especially at lower salinity levels (0.3 KPa & 0.6KPa). However in maize, wheat and cotton plants there was a significant decrease in pigment contents at all salinity levels. It is adopted the view that osmotically increased water stress enhances the decay of chlorophyll (Iqbal *et al*., 2006). There was a general increase in pigments contents with phytohormones treatments at all 5 tested plants, especially in cotton and parsley plants. This may be due to the inhibition of pigment degradation or stimulation of protochlorophyll (ide) synthesis by phytohormones (Iqbal *et al*., 2006, Pazuki *et al*., 2013)

Therefore an alternative strategy of ameliorate salt stress could be by exogenous application of plant growth regulators. So, focusing on using of phytohormones such as GA_3 or kinetin, which has important effects on regulation of plant reaction to environment and control of some metabolic changes and more or less similar between the five tested plants. It has been reported that GA_3 or kinetin *treatment* reduces the adverse effects of salt stress of the maize, wheat, cotton, broad bean and parsley plants

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