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Effect of Potassium Humate on Plant Growth and Chemical Contents of Banana Plantlets Grown *in vitro* under Salinity Stress

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ABSTRACT

Salinity represents one of the most abiotic stress factors affecting plant growth and production around the world. In this experiment, *in vitro* culture of Grand Nain banana plantlets were used to study the physiological responses to salt stress and potassium humate role in reducing salt damage. Plantlets, grown in rooting media, were supplemented with different concentrations of sodium chloride (0, 1000, 3000, 5000 ppm) and potassium humate (0, 0.2, and 0.4 g/l). Salinity reduced plantlet height, fresh weight and dry weight. Leaf contents of chlorophyll a, and b were decreased while Na^+ contents increased by increasing NaCl concentrations. Also, proline contents were significantly affected by salinity. Applications of potassium humate significantly increased vegetative growth and chemical contents compared to untreated plantlets.

Key words: Musa spp., salinity, Grand Nain, *in vitro*, potassium humate

Introduction

Salinity is one of the major problems for many agricultural crops. It represents a significant factor threatening food supply due to the increased salinization of soils and ground water in recent decades (Munns and Tester, 2008). Salinity causes two different kinds of stresses a quick osmotic stress and later on an ion toxicity stress. On one hand, osmotic stress is generated when high salt concentrations disturb osmotic balance resulting in "physiological drought", and therefore, preventing plant water uptake (Yamaguchi *et al.,* 2006). Ionic stress, on the other hand, is attributed to the accumulation of NaCl ions inside plant cells and tissues resulting in ion toxicity and physiological disorders (Munns and Tester, 2008).

In vitro system offers a controlled environment which leads to perfect measurements of plant responses to salinity (Lutts *et al*., 2004).

Exogenous applications of humic substances were used to protect plants from different conditions of abiotic stress conditions such as salinity and drought (Kulikova *et al*., 2005; Hanafy *et al*., 2013).

Bananas represent a vital food crop globally, growing in more than 100 countries through the world. After rice, wheat and maize, bananas coping the fourth most important food crop in developing countries (INIBAP, 2000).

This study aimed to study the effect of potassium humate in reducing salinity stress of banana plantlets grown *in vitro*.

Materials and Methods

This experiment was carried out at Tissue Culture Laboratory, Agricultural Development System Project at Giza, Egypt. In vitro cultures of Grand Nain banana (Musa spp.) plantlets were used in this experiment. Experiments were conducted with plantlets in rooting stage. Plantlets were obtained from in vitro shoot tip cultures and grown on M&S medium (Murashige and Skoog, 1962) supplemented with 1 mg/l NAA and 1 mg/l Indole-3-butyric acid. The pH was adjusted at 5.7 perior to addition agar at 7 g/l, 30 g/l sucrose, and 1 g/l activated charcoal. Cultures were grown at 27±1 °C under photoperiod of 16/8 hrs light/ dark at 2000 Lux of light intensity conditions for 6 weeks.

NaCl and potassium humate treatments

Plantlets were grown in the rooting medium with four different concentrations of NaCl (0, 1000, 3000, and 5000 ppm). Also, potassium humate was used at concentrations (0, 0.2, and 0.4 g/l). Each treatment consisted of three replicates (jars) with 1 plantlet per jar.

Measurement of growth parameters

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After Six weeks, plantlet height and fresh weight were measured. To determine dry weight, plantlets were dried at 70°C in oven for 48 h. Chlorophyll a and chlorophyll b were determined spectrophotometrically according to Norani (1982). Sodium content was determined according to Piper (1950). Proline content (mg/g) was determined in fresh vegetative growth according to Bates *et al*. (1973).

Statistical analysis

The obtained data was statistical analyzed by using factorial experiment in randomized complete block design. Results of the measured parameters were subjected to computerized statistical analysis using MSTAT-C and the significant differences among the various treatments were compared by using LSD at 0.05 according to Snedecor and Cochran (1994).

Results and Discussion

Growth characteristics:

Plantlet height

Data in table (1) showed a significant difference between the four NaCl concentrations on banana plantlet height. By increasing NaCl concentrations, plantlet height decreased from 16.89 to 11.78 cm. On the other hand, potassium humate applications increased plantlet height compared with untreated ones. The highest plantlet height was obtained with 0.2 g/l potassium humate. For the interaction between salt stress and potassium humate, the best result in plantlet height was noticed from 0.2 g/l potassium humate under 1000 and 3000 ppm NaCl. This reduction in plantlet height may be due to the adverse effect of salinity on cell growth. Salinity affects the rate of cell division by slowing the expansion rate or decreasing its duration (Volkmar *et al*., 1998). While humic substances promote plant growth through improving water and nutrient uptake (Zandonadi *et al.,* 2013).

Table 1: Effect of Sodium Chloride (NaCl) and Potassium humate treatments on plantlet height (cm), plantlet fresh weight (g) and plantlet dry weight (g) of banana during rooting stage

NaCl (ppm) (A)	Potassium humate (g/l) (B)			Mean
	0	(0.2)	(0.4)	
Plantlet height (cm)				
0	16.00	17.67	17.00	16.89 a
1000	13.67	16.00	15.67	15.11 b
3000	13.00	16.00	14.00	14.33 b
5000	11.33	12.33	11.67	11.78 c
Mean	13.50 b	15.50 a	14.58 ab	
L.S.D. 0.05 for A = 1.33 B= 1.15 A×B= 2.30				
Fresh weight/plantlet (g)				
0	5.63	7.10	6.20	6.31 a
1000	4.20	5.80	5.80	5.27 b
3000	4.03	4.97	4.43	4.48 c
5000	2.67	3.33	3.20	3.07 d
Mean	4.13 b	5.30 a	4.91 a	
L.S.D. 0.05 for A = 0.77 B= 0.67 A×B= 1.34				
Dry weight/plantlet (g)				
0	0.260	0.340	0.360	0.320 a
1000	0.180	0.313	0.290	0.261 b
3000	0.173	0.240	0.217	0.210 c
5000	0.120	0.150	0.130	0.133 d
Mean	0.183 b	0.261 a	0.249 a	
L.S.D. 0.05 for A = 0.031 B= 0.027 A×B= 0.054				

Fresh and Dry weight

Salinity led to a significant reduction in fresh and dry weight of banana plantlets (Table 1). Using 5000 ppm NaCl recorded the biggest reduction in fresh weight (3.07 g) and dry weight (0.13 g) of banana plantlets. Using 0.2 g/l potassium humate recorded the highest value of fresh weight (5.30 g) and dry weight (0.26) compared with untreated plantlets. For the interaction between salinity and potassium humate, the best results in fresh weigh (5.80 g) were noticed with 0.2 and 0.4 g/l potassium humate under 1000 ppm NaCl. Salt stress reduces the water uptake capacity of the plant, and this causes a reduction in the biomass production and

decrease plant fresh and dry weight (Munns 2002). On the other hand, humic substances improve water absorption and stimulate biomass accumulation (Zandonadi *et al*., 2007).

Chemical Contents:

Leaf Na^+ content

Na^+ concentrations in banana plantlets were significantly increased with increasing salinity levels (Table 2). The highest Na^+ content (1.063 %) was obtained from 5000 ppm. Applications of potassium humate were significantly decreased Na^+ content in leaves. Adding 0.4 g/l potassium humate decreased Na^+ to the lowest value (0.590 %). Potassium humate decreased Na^+ uptake under different levels of salinity. By using 0.4 g/l potassium humate, Na^+ content was decreased from 0.510 to 0.429 % under 1000 ppm NaCl and from 0.971 to 0.829 % under 3000 ppm NaCl.

These results are in agreement with other in vitro studies of banana grown under salt stress conditions (Haq *et al*., 2011). Also, Hanafy *et al.* (2013) found that using 1 and 2% of humic acid reduced Na^+ uptake of cotton Plants grown under saline soil conditions.

Table 2: Effect of Sodium Chloride (NaCl) and potassium humate treatments on Na^+ contents (%) of banana through rooting stage

NaCl (ppm) (A)	Potassium humate (g/l) (B)			Mean
	0	(0.2)	(0.4)	
0	0.131	0.118	0.104	0.118 d
1000	0.510	0.449	0.429	0.463 c
3000	0.971	0.842	0.829	0.881 b
5000	1.161	1.029	0.999	1.063 a
Mean	0.693 a	0.609 b	0.590 b	
L.S.D. 0.05 for A = 0.031 B= 0.027 A×B= 0.054				

Leaf chlorophyll contents

Data in Table (3) showed significant differences between different salinity levels on leaf chlorophyll contents. By increasing Nacl concentrations to 5000 ppm, chlorophyll A was decreased 3.57 mg/g, while chlorophyll B decreased to 1.27 mg/g. On the other hand, a remarkable promotion was detected on leaf Chlorophyll contents of plantlets that received potassium humate at 0.2 g/l and 0.4 g/l rather than those untreated. The highest Chlorophyll A and Chlorophyll B values were obtained from 0.2 g/l potassium humate (6.40 and 2.60 mg/g, respectively).

There are reductions in chlorophyll A and B concentrations due to NaCl treatments. This reduction may be related to the adverse effect of salinity on chlorophyll synthesis or due to the degradation of chlorophyll under salinity conditions (Santos, 2004). However, humic substances increase chlorophyll contents by stimulation the enzymes related to photosynthetic process (Nardi *et al.,* 2002).

Table 3: Effect of Sodium Chloride (NaCl) and potassium humate treatments on Chlorophyll A and B (mg/g) contents of banana through rooting stage

NaCl (ppm) (A)	Potassium humate (g/l) (B)			Mean
	0	(0.2)	(0.4)	
Chlorophyll A (mg/g)				
0	7.33	8.70	8.90	8.31 a
1000	6.30	7.10	7.20	6.87 b
3000	5.40	5.80	5.60	5.60 c
5000	3.20	4.00	3.50	3.57 d
Mean	5.56 b	6.40 a	6.30 a	
L.S.D. 0.05 for A = 0.37 B= 0.32 A×B= N.S				
Chlorophyll B (mg/g)				
0	3.20	3.70	3.90	3.60 a
1000	2.70	3.10	3.30	3.03 b
3000	1.60	2.10	1.70	1.80 c
5000	1.10	1.50	1.200	1.27 d
Mean	2.15 b	2.60 a	2.53 a	
L.S.D. 0.05 for A = 0.24 B= 0.21 A×B= N.S				

Proline content

Results presented in Table (4) reveal significant differences on proline content in response to salinity levels. The highest value in proline content (5.86 mg/g) was observed under 1000 ppm NaCl, meanwhile the lowest one (2.66 mg/g) was recorded under 5000 ppm NaCl. In plantlets submitted to different levels of potassium humate, proline content was significantly increased with respect to control plantlets. The effect of potassium humate on proline content was significantly greater under 0.2 g/l (4.63 mg/g) followed by 0.4 g/l (4.40 mg/g) compared to untreated plantlets (3.98 mg/g).

Proline plays an important role in defense mechanism against abiotic stress. Some plants synthesized proline in order to cope with salt stress. Proline accumulation is vital in maintaining the osmotic adjustment of plant cell under osmotic stress, and thereby enhance plant salinity tolerance (Hong *et al.*, 2000, and Chinnusamy *et al*., 2005). High concentrations of NaCl decreased proline content in leaves (Table 4). This decline may be due to proline degradation caused by high salinity in sensitive plants such as banana. Similar results were recorded by Lutts *et al*. (1999), who found that, proline contents were increased under 50 mM NaCl and decreased by increasing NaCl concentrations to 100 mM in some rice cultivars.

Table 4: Effect of Sodium Chloride (NaCl) and potassium humate treatments on Proline content (mg/g) of banana through rooting stage

NaCl (ppm) (A)	Potassium humate (g/l) (B)			Mean
	0	(0.2)	(0.4)	
0	4.60	4.80	4.93	4.78 b
1000	5.40	5.83	6.33	5.86 a
3000	3.53	5.00	3.67	4.07 c
5000	2.40	2.90	2.67	2.66 d
Mean	3.98 c	4.63 a	4.40 b	
L.S.D. 0.05 for A = 0.087 B= 0.076 A×B= 0.151				

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