PROMOTIONAL ACTIVITY OF INDOLE ACETIC ACID AND NAPHTHALENE ACETIC ACID ON GROWTH AND PHOTOSYNTHETIC PIGMENTS OF *Solanum lycopersicon* (TOMATO) UNDER SALINITY STRESS

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Abstract
Excessive salt in the soil leads to a series of physiological and biochemical metabolic disorders in plants. Application of bioregulators to alleviate salinity stress can be an economic and safe alternative to environment. We have previously reported that the bioregulators, indole-3-acetic acid (IAA) and naphthalene acetic acid (NAA) enhanced antioxidant enzyme activity and mineral content of salt – stressed tomato. The present study examined the effects of these bioregulators on growth characteristics and photosynthetic pigments of tomato under salinity stress. Presowing treatment of seeds of two tomato genotypes, UC 82B and Tropimech with three concentrations of IAA and NAA were done for 24 hours. Seeds from each treatment were sown in polyethylene bags containing 10kg saline soil portions (0. 100, 200 mM NaCl) in a screen house and replicated four times in a randomized block design. The 150 mg·L^{-1} IAA concentration significantly increased ($p < 0.05$) leaf area, fresh weight, and dry weight compared to the 0 mM NaCl control. The 50 mg·L^{-1} concentration of IAA and 200 mM NaCl significantly increased ($p < 0.05$) chlorophyll a content in genotype UC 82B and the chlorophyll b content was increased due to treatment with 100 mg·L^{-1} IAA at 100 mM NaCl. The 100 and 150 mg·L^{-1} concentrations of NAA at 100 and 200 mM NaCl produced the highest increase in levels of chlorophyll b and carotenoids, respectively. Treatment with these bioregulators alleviated inhibitory effects of salt stress on tomato by enhancing some growth parameters and photosynthetic pigments.

Keywords: Salt stress, growth, photosynthetic pigments, bioregulator-treated tomato, Tomato genotypes

Submitted: 18.09.2015 Reviewed: 04.11.2015 Accepted: 27.11.2015

1.INTRODUCTION

Salinity is a major abiotic stress seriously threatening to food production worldwide (Gurmani *et al*., 2011). High soil salt levels limits crop yield and restricts use of land due to presence of high amounts of Na^+, Mg^{2+}, Ca^{2+}, Cl^-, HCO_3^- and SO_4^{2-} ions (Joseph *et al*., 2010). Bioregulators act in low concentrations to inhibit, promote or modify the morphological, physiological and biochemical processes of plants. These substances can be applied directly to plant leaves, fruit and seed provoking alterations of vital and structural processes (Avila *et al*., 2008: Olaiya, 2010). Gibberellic acid (GA) for instance, is widely regarded as a growth promoting compound that positively regulates processes such as seed germination, stem elongation, leaf expansion, flower and fruit development and floral transition (Jaleel *et al*., 2008). Indole acetic acid is involved in apical dominance, and cell elongation and differentiation (Saupe, 2009). Naphthalene acetic acid inhibits ethylene biosynthesis, contributes to longevity and slows down natural shoot growth (Paul and Pieter, 1989).
Tomato (*Solanum lycopersicon*) is one of the major vegetable cash crops in the world. It is an excellent source of many nutrients and secondary metabolites that are important for human health such as folate, potassium, vitamins C and E, flavonoids, chlorophyll, β-carotene and lycopene (Adeyemi and Olorunsanya, 2012). The tomato is also one of the more intensively investigated *Solanaceous* species due to the simple diploid genome (Szczechura *et al*., 2011). However, increasing salinity levels have been reported to negatively affect germination, growth and fruit yield of the plant (Cuartero *et al*., 1999). Sodium Chloride

constitutes the most abundant salt found in environments affected by salinity (Tester and Devenport, 2003).

Arid and semi-arid lands constitute approximately one third of the world's land surface and salinity is the most important agricultural production problem (Uddin *et al*., 2011). High salt content decreases the osmotic potential of soil water reducing availability of soil water for plants (Asik *et al*., 2009), which detrimentally affects growth and yield (Ibrahim *et al*., 2007). Uptake of water by plant roots is limited by increased amounts of Na and Cl. High concentrations of soil salt reduces absorption of some micro- and macronutrients, alters hormone balance, and results in malnourished, pest and disease prone, stunted plants. Salt tolerant plant species (Ibraheem *et al*., 2011) or application of substances capable of reducing effects of salinity (Latef *et al*., 2009) need to be used in high salt areas.

Bioregulators can ameliorate the adverse effects of salt stress and restore normal growth and development of plants (Gurmani *et al*., 2007; Javid *et al*, 2011). There appears to be little quantitative investigation of effects of bioregulators on tomato plant tolerance and resistance to salt stress. In an earlier work (Olaiya and Anyanwu, 2013), we have demonstrated that the bioregulators, indole-3-acetic acid (IAA) and naphthalene acetic acid (NAA) enhanced antioxidant enzyme activity and mineral content of salt – stressed tomato. The present study was undertaken to examine the effects of these bioregulators on growth and photosynthetic pigments of tomato under salinity stress.

2. MATERIALS AND METHODS

2.1 Soil Material: Soil was collected from the root zone depth (0-15 cm), air-dried, ground, passed through a 5 mm mesh screen and thoroughly mixed. The soil were spread over thick plastic sheets and moistened with treatment solutions of 0 (no NaCl), 100 and 200mM concentrations of NaCl in distilled water to achieve a 13% moisture content.

2.2 Treatments: Seed of the tomato genotypes UC 82B and Tropimech, obtained from The Seed Project Company Limited, Kano, Nigeria, were surfaced sterilized with 1.0% (v/v) sodium hypochlorite (NaClO) and washed 2 – 3 times with distilled water before use. Sterilized seeds were soaked in distilled water (0, Control), IAA and NAA in concentrations of 50, 100, and 150 $mg \cdot L^{-1}$ for 24 hours at room temperature. Fifteen seeds from each treatment were sown in polyethylene bags containing about 10kg saline soil portions (0, 100, 200 mM NaCl) in a screen house and replicated four times in a randomized block design. Watering was done on alternate days with distilled water.

2.3 Determination of Growth Characteristics: After 30 days, seedling growth was assessed by harvesting five plants per treatment. Seedlings were cleaned and the height, number of leaves/plant, leaf area and fresh weight were determined. Samples were rapidly dried in a conventional oven at 80°C to constant weight, grinded to fine powder and used to determine dry weight.

2.4 Estimation of Photosynthetic Pigments: The photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) were estimated by the method of Metzzener *et al*., 1965. About 1g of the new leaves was homogenized immediately after harvesting in 10 mL cold aqueous acetone (85%), kept overnight in a refrigerator and then cold centrifuged at 15000 rpm for 20 minutes. One-half- ml of the supernatant which contained pigments, was diluted with 6ml cold aqueous acetone and the extract measured against a blank of pure 85% aqueous acetone at 452, 644 and 663nm using Lambda 25 UV/Vis spectrophotometer (Perkin-Elmer Corp. Norwalk, Connecticut). Concentrations of chlorophyll a, chlorophyll, b and carotenoids (pigment fraction) were determined using the equations: Chlorophyll a = $10.3\ E_{663} - 0.918\ E_{644}$; Chlorophyll b = $19.7\ E_{644} - 3.870\ E_{663}$ and Carotenoids = $4.2\ E_{452}$ – (0.0264 Chl. a + 0.426 Chl. b), where E is the optical density at the given wave length.

2.5 Statistical Analysis: The data were statistically analyzed using analysis of variance (ANOVA) Statistica software (Statsoft, Inc., Tulsa, OK). Duncan multiple range test

(Duncan, 1955) was used to compare the means of the treatments.

3. RESULTS AND DISCUSSION

The 50 mg·L^{-1} concentration of IAA at 0 mM of NaCl significantly increased height, number of leaves per plant, leaf area, fresh weight and dry weight compared to the control at 0 mM NaCl in the Tropimech genotype (Table 1). Concentrations of bioregulators at 100 mM and 200 mM of NaCl significantly increased ($p<0.05$) growth of the plant relative to control and the differences in the two tomato genotypes could be ascribed to genetic traits, as this is believed to govern plant growth [Scholberg *et al*., 2000]. In the UC82B tomato, the 150 mg·L^{-1} concentrations of IAA at 0 mM NaCl and the 50 mg·L^{-1} concentration of NAA at 100 mM NaCl significantly increased ($p<0.05$) the growth in comparison with the control (Table 2). This result show that the bioregulators considerably affect plant growth in salt stress conditions and is comparable to those of El-Tohamy and El-Greadly (2007) on snap bean, El-Tohamy *et al*. (2008) on eggplant, Sharaf El-Deen and Manaf (2009) on faba bean and El-Keblawy *et al*. (2010), who observed increase in the germination of Lasiurus *scindicus* seeds when treated with fusicoccin, gibberellic acid (GA_3), nitrate and thiourea. The increase in growth observed in the present work may be attributed to the growth promoting effect of the bioregulators in stimulating and accelerating cell division, increasing cell elongation and enlargement or both (Al-Khassawneh *et al*., 2006), which in turn increased the height, number of leaves per plant, leaf area, fresh weight and dry weight of the plants. The highest values of the growth parameters were obtained from the application of IAA as compared with those obtained from the other treatments.

The 150 mg·L^{-1} concentrations of IAA and NAA at 0 mM NaCl significantly increased ($p<0.05$) levels of chlorophyll a, b and carotenoids compared to the control (Table 3). The 50 and 100 mg·L^{-1} concentrations of IAA at 200 mM concentration of NaCl significantly increased ($p<0.05$) levels of chlorophyll a and carotenoids respectively while the 100 mg·L^{-1} concentration of IAA at 100 mM NaCl significantly increased ($p<0.05$) the level of chlorophyll b. Results in Table 4 shows that NAA at the concentrations of 50 and 100 mg·L^{-1} under 0 mM NaCl significantly increased ($p<0.05$) the levels of chlorophyll a relative to the control in the UC82B genotype. These results resemble those of Khodary (2004) who observed that salicylic acid, alone, or in combination, with NaCl levels stimulated Rubisco and photosynthetic activities in tested plants. The reduction in photosynthetic pigments may be attributed to the toxic action of NaCl on the biosynthesis of pigments. Salinity stress has been reported to induce serious metabolic perturbations in plants, as it produces reactive oxygen species (ROS) (Arrigoni and De Tullio, 2000) thereby causing oxidative stress that can damage chloroplast thylakoid and reduce photosynthesis (Mittler, 2002). Positive correlations have been demonstrated between sensitivity to salinity and membrane damage in foxtail millet (*Setaria italica*) seedlings (Sreenivasasulu et *al*., 2000). The enhancement of photosynthetic pigments and carotenoids by the bioregulators observed in this work may therefore be linked with the possibility of exerting protective and stabilization roles on membranes, thereby improving the growth of salinity stressed tomato plants.

Table 1: Effect of bioregulators combined with NaCl on growth of tomato cv.'Tropimech*

Bioregulator (mg·L^{-1})	NaCl (mM)	Height (cm)	Number of leaves/plant	Leaf area (cm^2)	Fresh wt.(g)	Dry wt. (g)
Control 0	0	30.0±1.1b	20.0±0.6cd	13.0±0.7ef	12.3±0.5i	1.8±0.2cd
	100	0.0±0.0j	0.0±0.0g	0.0±0.0i	0.0±0.0m	0.0±0.0f
	200	0.0±0.0j	0.0±0.0g	0.0±0.0i	0.0±0.0m	0.0±0.0f
IAA 50	0	35.0±1.2a	25.0±0.4b	22.0±0.9a	21.6±0.7cd	3.0±0.1a
	100	30.0±0.8b	20.0±0.2cd	18.0±0.7bc	18.9±0.9fg	2.5±0.1ab
	200	20.0±0.5c	30.0±0.4a	16.0±0.2cd	21.0±0.7de	1.7±0.2cd
IAA 100	0	30.0±0.9b	26.0±0.6b	12.0±0.5ef	17.8±0.8gh	2.2±0.1bc
	100	22.4±0.7c	25.0±0.7b	12.0±0.6ef	17.1±0.5h	1.5±0.2cd
	200	15.3±0.6de	17.2±0.7d	12.7±0.5ef	19.8±0.3ef	1.5±0.1cd
IAA 150	0	19.0±0.5cd	24.0±0.6b	11.0±0.3ef	8.7±0.2j	1.0±0.1de
	100	10.7±0.3fg	13.0±0.6e	8.0±0.2g	7.7±0.1jk	0.6±0.1ef
	200	6.5±0.2ghi	9.0±0.2f	4.0±0.1h	3.8±0.1l	0.2±0.1f
NAA 50	0	27.0±0.7b	25.0±0.5b	19.0±0.6b	22.8±0.4bc	3.2±0.1a
	100	28.0±0.6b	25.0±0.4b	22.0±0.5a	31.6±0.6a	3.2±0.2a
	200	11.3±0.5ef	20.0±0.5cd	13.0±0.3ef	16.9±0.7h	1.5±0.1cd
NAA 100	0	21.0±0.8c	21.0±0.60c	14.0±0.5de	24.3±0.6b	3.0±0.2a
	100	9.0±0.6fgh	10.0±0.5f	10.0±0.4fg	6.3±0.3k	0.5±0.1ef
	200	3.0±0.1ij	3.0±0.3g	3.0±0.1h	1.3±0.1m	0.1±0.0f
NAA 150	0	5.0±0.2hi	3.0±0.1g	2.0±0.2hi	1.4±0.1m	0.2±0.0f
	100	4.0±0.3ij	2.0±0.1g	2.0±0.1hi	0.9±0.2m	0.1±0.0f
	200	3.0±0.1ij	2.0±0.1g	2.0±0.2hi	0.0±0.0m	0.1±0.0f

* Means with the same letters are not significantly different at P=5% according to the Duncan multiple range test (DMRT).

Table 2: Effect of bioregulators combined with NaCl on growth of tomato cv. 'UC82B'*

Bioregulator (mg/L)	NaCl (mM)	Height (cm)	Number of leaves/plant	Leaf area (cm^2)	Fresh wt.(g)	Dry wt. (g)
Control 0	0	24.9±1.2ab	12.4±0.8fg	8.0±0.7efg	15.9±0.6e	1.6±0.2cde
	100	23.2±1.1bc	19.6±0.9abc	8.7±0.6ef	18.0±0.5d	1.4±0.3def
	200	14.0±0.7f	16.0±0.5cdef	6.0±0.2fg	19.4±0.9d	1.0±0.1gh
IAA 50	0	25.5±1.4ab	22.0±1.1a	14.0±0.7bcd	19.4±0.9d	1.8±0.2bc
	100	20.0±1.1d	20.1±1.0abc	12.0±0.5cd	21.1±0.8c	1.6±0.3cd
	200	9.0±0.7ih	9.0±0.3gh	8.0±0.3efg	4.4±0.3ij	0.2±0.1lm
IAA 100	0	25.0±1.1ab	19.0±0.7abc	16.0±0.3b	13.2±0.4f	1.3±0.1ef
	100	21.3±1.0cd	17.0±0.3bcde	14.0±0.4bcd	14.3±0.5f	1.2±0.1fg
	200	7.2±0.3i	8.0±0.1h	8.0±0.2efg	5.0±0.2i	0.3±0.1kl
IAA 150	0	26.9±1.1a	21.8±1.1a	21.0±1.2a	26.9±1.4a	2.9±0.2a
	100	17.0±0.7e	17.0±0.8bcde	11.0±0.6de	10.4±0.7g	0.7±0.1ij
	200	8.0±0.1i	7.3±0.4h	6.0±0.3fg	3.4±0.3j	0.1±0.0m
NAA 50	0	20.0±1.0d	14.3±0.7def	12.0±0.5cd	10.1±0.6g	0.9±0.2hi
	100	20.00.9d	21.00.9ab	15.0±0.4bc	22.6±0.8b	1.9±0.3b
	200	11.0±0.5gh	13.0±0.7efg	7.0±0.3fg	6.8±0.3h	0.5±0.1jk
NAA 100	0	20.0±0.8d	18.0±0.8abcd	14.0±0.7bcd	14.3±0.7f	1.4±0.3def
	100	12.0±0.7fg	13.3±0.5ef	8.0±0.5efg	7.2±0.2h	0.5±0.2jk
	200	8.0±0.3i	9.0±0.3gh	7.0±0.2fg	3.9±0.1ij	0.1±0.0m
NAA 150	0	8.0±0.3i	6.00hi	5.0±0.3gh	3.6±0.2ij	0.1±0.0m
	100	4.0±0.1ij	3.3±0.2ij	2.0±0.1hi	1.6±0.3k	0.1±0.0m
	200	2.0±0.1j	2.0±0.1j	1.5±0.1i	1.3±0.1k	0.1±0.0m

*Means with the same letters are not significantly different at P=5% according to the DMRT.

Table 3: The effect of bioregulators combined with NaCl on photosynthetic pigments of tomato cv. 'Tropimech'*

Bioregulator (mg/L)		NaCl (mM)	Chlorophyll a (mg/g fw)	Chlorophyll b (mg/g fw)	Carotenoids (mg/g fw)
Control	0	0	4.54±0.12ef	3.07±0.23fg	1.43±0.13i
		100	2.10±0.00k	2.60±0.00k	2.00±0.00m
		200	1.91±0.11hi	2.48±0.32h	1.91±0.12f
IAA	50	0	7.99±0.51cd	4.84±0.12b	2.48±0.12b
		100	5.19±0.23e	3.51±0.13ef	1.56±0.11h
		200	9.73±0.21a	4.75±0.13b	2.38±0.15c
IAA	100	0	4.49±0.17f	2.55±0.12gh	1.61±0.11h
		100	1.79±0.13hi	5.89±0.23a	1.75±0.13g
		200	4.95±0.32ef	4.39±0.14bc	3.19±0.12a
IAA	150	0	7.70±0.34cd	3.73±0.21de	1.95±0.11f
		100	7.36±0.23d	4.15±0.31cd	2.02±0.13e
		200	8.21±0.34bc	3.94±0.23cde	1.96±0.12ef
NAA	50	0	3.35±0.21g	3.78±0.24de	1.15±0.24j
		100	7.67±0.31cd	4.90±0.33b	1.47±0.12i
		200	2.45±0.23h	1.59±0.15i	0.78±0.10k
NAA	100	0	8.84±0.32b	4.40±0.21bc	2.22±0.13d
		100	2.00±0.00k	2.00±0.00k	1.00±0.00m
		200	1.09±0.11j	0.95±0.10j	0.43±0.14l
NAA	150	0	8.84±0.31b	3.85±0.21cde	1.91±0.13f
		100	2.00±0.00k	2.10±0.00k	1.00±0.00m
		200	1.30±0.21ij	1.00±0.11j	0.45±0.10l

*Means with the same letters are not significantly different at P=5% according to the DMRT.

Table 4: Effect of bioregulators combined with NaCl on photosynthetic pigments of tomato cv. 'UC82B'*

Bioregulator (mg/L)		NaCl (mM)	Chlorophyll a (mg/g fw)	Chlorophyll b (mg/g fw)	Carotenoids (mg/g fw)
Control	0	0	6.40±0.33j	3.44±0.12h	1.63±0.15k
		100	6.77±0.24h	3.52±0.21h	2.00±0.23d
		200	6.77±0.35h	4.81±0.26c	1.23±0.12n
IAA	50	0	7.28±0.62s	3.46±0.12h	2.21±0.16b
		100	2.23±0.34p	2.14±0.24m	1.76±0.11gh
		200	5.84±0.31m	3.15±0.15j	1.68±0.23j
IAA	100	0	5.89±0.23l	2.65±0.23k	1.72±0.24i
		100	8.24±0.33a	4.74±0.36c	1.77±0.32fg
		200	6.15±0.45k	4.70±0.22c	0.84±0.26o
IAA	150	0	4.30±0.23n	5.39±0.34b	1.61±0.22kl
		100	7.65±0.42e	4.02±0.23f	2.06±0.34c
		200	6.36±0.35j	3.32±0.33i	1.93±0.23e
NAA	50	0	6.51±0.32i	3.53±0.33h	1.58±0.34l
		100	7.06±0.31f	4.16±0.36e	1.81±0.42f
		200	7.77±0.33c	4.39±0.33d	1.76±0.35ghi
NAA	100	0	6.95±0.20g	3.97±0.41f	2.00±0.24d
		100	6.11±0.32k	6.16±0.33a	0.46±0.12q
		200	7.93±0.42b	3.78±0.24g	1.72±0.21hi
NAA	150	0	2.15±0.30q	1.32±0.11n	0.64±0.12p
		100	1.76±0.11r	6.19±0.32a	1.28±0.13m
		200	2.89±0.21o	2.32±0.22l	2.89±0.24a

*Means with the same letters are not significantly different at P=5% according to the DMRT.

To survive stress, plants employ intricate mechanisms to perceive external signals and activate optimal responses to environmental conditions (Olaiya *et al*., 2013). However, the mechanisms underlying plant responses to salinity are still not fully understood. Salt-induced cellular accumulation of damaging reactive oxygen species (ROS) has been shown to be involved (Jiang and Zhang, 2001; Ashraf and Ali, 2008) and coupled with this, is the possibility of osmotic adjustment by the plants (Farkhondeh *et al*., 2012). A full understanding of the mechanisms involved in salt-tolerance of tomato will help in production of this antioxidant – rich vegetable food crop for the health benefit of the populace, especially in high saline areas.

4. CONCLUSIONS

Pre-sowing seed treatment of tomato with low concentrations of IAA and NAA can increase the capacity of tomato to survive the lethal effects of salt. This is particularly relevant to food crop growers and producers in high saline environments where productivity is low. The bioregulators have good effect when applied to plants at low to moderate salt soil concentration. Possible negative impacts of bioregulators on human health can be avoided when used for seed treatment and at low concentrations.

ACKNOWLEDGEMENTS

The Authors wish to thank The Seed Project Company Limited, Kano, Nigeria for the supply of the tomato genotypes used in this work.

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