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Antistressful Action of Two Auxin Analogues on Antioxidant Enzymes and Minerals in Tomato (Solanum Lycopersicon)

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Abstract

Salinity is a worldwide problem for agricultural soils in the production of crops. It reduces yield and limits expansion of agriculture onto previously uncultivated land. Bioregulators play important roles in the growth and development of plants. This study seeks to investigate the effect of two bioregulators, Indole-3-acetic (IAA) and Naphthaleneacetic acid (NAA) on antioxidant enzymes and minerals in the leaves of tomato (Solanum lycopersicon) plants subjected to salinity stress. Seeds of Tropimech and UC 82B tomatoes were soaked in 0, 50 and 150 mg/L of IAA and NAA for 24 hours. The seeds were planted in the screen house in three categories of polythene bags of soil to which 0, 100 and 200mM sodium chloride (NaCl) had been added respectively. After 30 days, the plants were harvested and antioxidant enzyme activity and mineral content determined. The results indicate that there was a significant increase ($p<0.05$) in the activity of catalase in the tomato genotypes at 100 mg/L concentration of IAA while the 150 mg/L concentration at 200mM NaCl gave significant increase ($p<0.05$) in peroxidase activity compared to control. Also, 50 mg/L NAA at 100mM NaCl significantly increased ($P<0.05$) catalase activity relative to control at the different salt levels investigated. The 50 mg/L and 100 mg/L concentrations of IAA gave the highest increase in Cu, Ca and K compared to the control at 0mM of NaCl. The results indicate that IAA and NAA enhanced antioxidant enzyme activity and moderately improved mineral content of tomato under salinity stress. This offers opportunities for adapting to salinity stress and represents improved development of this important vegetable food crop under such conditions.

Keywords: Bioregulator, genotypes, phytonutrients, salinity stress, Solanum lycopersicon.

Introduction

Tomato (*Solanum lycopersicon)* is one of the most popular consumed vegetables worldwide. It is low in calories, but serves as a good source of vitamins C, A, E and other antioxidants like lycopene and beta-carotene. Tomatoes contain virtually no fat and no cholesterol. They are eaten raw or cooked and are often processed to make tomato paste, sauce, ketchup or juice [1] (Chookhampaeng et al., 2008). Tomatoes are domestic and commercial crops. Their production for commercial purposes is faced with the challenge of salinization of agricultural land, due to human activities, agricultural practices and natural processes. This negatively affects soil fertility and productivity of this important cash crop in many parts of the world [2] (Cuartero and Fernandez-Munoz, 1999). Published reports indicate that over 6% of the world's land and 20% of the world's irrigated land are currently affected by salinity [3,4] (Munns, 2005; Barakat, 2011) which is a major threat to crop productivity in the arid and semi-arid regions of the world [5] (Mühling and Läuchli, 2003). Although all soils contain some amount of soluble salts of multifarious nature, when soil and environmental conditions allow the concentrations in soil profiles to a high level, soil salinity becomes severe threat to land degradation [6] (Wiebe *et al*., 2005) and crop productivity. The concentrations of sodium chloride (NaCl) in such soils typically exceed 40 mM, and much higher values are frequently found [3] (Munns, 2005), creating toxic growth conditions for most plants, including all major crop species [7] (Gurmani *et al*, 2006).

High salinity in soil or irrigation water is one of the major abiotic stresses globally [8] (Tanou *et al*., 2009). It decreases plant production via the osmotic and ionic balance disturbance and intensifying of the peroxidation processes [9] (Noreen and Ashraf, 2009). The production of reactive oxygen species (ROS) is thus a major biochemical change occurring in plants under environmental stress. ROS attack proteins, lipids and nucleic acids, and the degree of damage depends on the balance between formation of ROS and its removal by the antioxidative scavenging systems [10] ((Molassiotis *et al.* 2006). ROS is detoxified by both nonenzymatic and enzymatic antioxidant systems which play an important role in salt tolerance [11] (Valdeerrama *et. al*., 2007) and the detoxification may be enhanced by the application of chemicals to the plants [12] (Parida and Das, 2005). Bioregulators such as ethylene, abscisic acid, salicylic acid and steroids are involved in the regulation of plant antioxidant enzymatic system [13] (Coa *et. al*., 2005). The antioxidant enzyme system constitutes superoxide dismutase (SOD) as the primary step of cellular defense. It dismutates superoxide ions (O_2^-) to H_2O_2 and O_2. Further, the accumulation of H_2O_2 is restricted by the action of the ascorbate-glutathione cycle, where ascorbate peroxidase (APX) reduces it to H_2O. The final step is catalyzed by glutathione reductase (GR), which catalyzes the NADPH-dependent reaction of oxidized glutathione (GSSG) to reduced glutathione (GSH) [14] (Noctor et al., 2002).

Salinity stress inhibits the growth of crops [15] (Osman *et al*, 2011), activity of many enzymes [1] (Chookhampaeng et al., 2008), photosynthesis [16] (Khodary, 2004), absorption of minerals [17] (Dutt *et al*., 1991) and reduces yield [18] (Zhao et

al., 1995). It brings about an increase in the concentration of Na^+ and Cl^- , suppresses the uptake of essential minerals N, P, K, and Ca [19] (Ashraf, 2004) and facilitates the production of ROS leading to poor crop productivity. Bioregulators may act as modulator by suppressing or enhancing the stress responses of plants [20] (Popova *et al*. 1995). Thus, to reduce the effect of salinity stress on crops, bioregulators might be used as they have been suggested as possible tools for food production [21, 22] (Nickell, 1988; Olaiya, 2010).

Bioregulators are organic compounds that, in low concentrations, inhibit, promote or modify the morphological and physiological processes of plants. They are either natural or synthetic compounds that are applied directly to a target plant to alter its life processes or its structure to improve quality, increase yields, or facilitate harvesting [23] (Nickell, 1982). They influence growth and development at very low concentrations but inhibit at high concentrations [24] (Jules et al. 1981,). They include auxins, gibberellins, cytokinins, ethylene and abscisic acid. Pre–sowing seed treatment with Indole-3-acetic (IAA), Napthaleneacetic acid (NAA) and Indole butyric acid (IBA) have profound effect on improving the quality of tomato produce. Bioregulator concentrations of 100mg/L of IAA, NAA and IBA have been shown to enhance seedling emergence of some tomato genotypes to about 92.1% relative to the control [25] (Olaiya and Osonubi, 2009). Also, flavonoid and lycopene contents were significantly increased in tomato plants treated with IAA, IBA and NAA compared to control [26] (Olaiya and Adigun, 2010). In the light of these findings, we study the possible ameliorative effect of two bioregulators, IAA and NAA on antioxidative enzymes and mineral content of salt – stressed tomato.

Materials and Methods

Plant material

Seeds of Tropimech and UC 82B Tomatoes were obtained from The Seed Project Company Limited, Kano, Nigeria.

Preparation of test solutions of bioregulators

This was done by using the method of [27] Heydecker and Coolbear (1977) with slight modifications. A 37.5 mg of IAA (98% pure, Sigma) and NAA (96% pure, Sigma) were each dissolved in 10ml 60% ethanol containing 0.5% Tween 20 in different 250ml volumetric flasks. Distilled water was added to the mark in each flask to afford concentrations of 150mg/L solutions. These solutions were serially diluted with distilled water to give 100mg/L, and 50mg/L concentrations of each bioregulator.

Soil characteristics: The properties of the soil used in raising the tomato plant are shown in Table 1.

Table 1: Selected physicochemical properties of the soil used.

Parameter	Value
pH	7.10
Exch. Acidity	0.40
Clay (%)	12.80
Silt (%)	14.00
Sand (%)	73.20
Organic Carbon (g/kg)	48.50
Nitrogen (g/kg)	3.30
Phosphorus (mg/kg)	20.70
Potassium (cmol/kg)	1.24
Sodium (cmol/kg)	0.99
Calcium (cmol/kg)	42.61
Magnesium (cmol/kg)	12.49
Iron (mg/kg)	83.70
Manganese (mg/kg)	44.40
Zinc (mg/kg)	499.60
Copper (mg/kg)	10.70

Seedling germination

Seedling germination was carried out in a screen house behind the Biochemistry Department, University of Ibadan, Ibadan, Oyo State, Nigeria. Seeds of the two tomato genotypes were surfaced sterilized with 1.0% sodium hypochlorite (NaOCl). Before sowing, they were soaked in distilled water (Control), 50mg/L, 100mg/L, and 150mg/L concentrations of IAA and NAA respectively, for 24 hours at room temperature. Thereafter, the solutions were decanted and treated seeds were washed 2 – 3 times with distilled water. The seeds were vacuum dried for 1 hour. About 10kg soil portions of each lot were filled in polyethylene bags (20 cm diameter and 25 cm long) and 15 seeds were sown in each bag at a depth of about 10 mm. For each seed treatment (control, IAA and NAA), there were four replicates at each salinity level. Plants were watered with distilled water with alternate-day watering.

Sample preparation for antioxidant enzyme assay

1g of leaf samples was grinded in 10ml solution containing 0.1 M Potassium phosphate buffer, pH 7.5, and containing 0.5mM Ethylene diamine tetraacetic acid (EDTA). The brie was centrifuged for 20 minutes at 15000 rpm and the supernatant was collected for enzyme assays.

Determination of catalase activity: Catalase (EC 1.11.1.6) activity was determined by the method of [28] Beers and Sizer (1952) in which the disappearance of peroxide was followed spectrophotometrically at 240 nm using Lambda 25 UV/Vis spectrometer (Perkin Elmer). One unit decomposes one micromole of H_2O_2 per minute at 25°C and pH 7.0 under the specified conditions.

Determination of peroxidase activity: Peroxidase (E.C.1.11.1.7) activity was measured by using 4-aminoantipyrine as hydrogen donor [29] (Trinder, 1966). The reaction rate was determined by measuring an increase in absorbance at 510 nm using Lambda 25 UV/Vis spectrometer (Perkin Elmer) resulting from the decomposition of hydrogen peroxide. One unit results in the decomposition of one micromole of hydrogen peroxide per minute at 25°C and pH 7.0 under the specified conditions.

Determination of mineral content: The samples were oven dried at 80^0C over night and grinded to powder. Plant extract was wet digested by adding 10ml of perchloric acid mixture (HNO_3 + $HClO_4$) in the ratio (1:2). The digest is then taken and used to determine K, Na, Ca, Mg, Fe, Cu, Mn, and Zn. Perkin – Elmer Model 303 Atomic Absorption spectrometer was used to analyse all the metals [30] (Perkin-Elmer Corp., 1968) except Na. The EEL flame photometer was used to analyse Na [31] (AOAC, 1975).

Statistical Analysis

The data were statistically analyzed according to the methods described by [32] Snedecor and Cochran (1982). Duncan multiple range test [33] (Duncan, 1955) was used to compare the mean differences. $P < 0.05$ was considered statistically significant.

Results and Discussion

The effect of bioregulators on tomato antioxidant enzymes at different salinity levels

The data in Tables 2 and 3 show the effect of IAA and NAA on antioxidant enzymes in the tomato genotypes at different salt levels. The 50 mg/L concentrations of IAA at 0 mM of NaCl gave a significant increase ($P<0.05$) in catalase activity of the Tropimech tomato compared to the control. The 50 mg/L and 150 mg/L concentrations of IAA and the three concentrations of NAA gave significant increase in peroxidase activity of the tomato compared to the control at 0mM NaCl. Also, the 50 mg/L concentration of NAA at 100mM NaCl moderately stimulated catalase activity compared to the control while the 150mg/L concentration of IAA at 200mM NaCl significantly increased ($P<0.05$) peroxidase activity compared to control at the different salt levels studied. In the UC 82B tomato, 100mg/L IAA at 100mM NaCl moderately enhanced catalase activity while the 50mg/L concentration at 200mM NaCl significantly increased ($P<0.05$) peroxidase activity compared to the controls. Similarly, the 50mg/L concentration of NAA at 100mM NaCl significantly increased ($P<0.05$) catalase activity relative to control at the different salt levels investigated. Though there were variations in the stimulatory effect of different concentrations of the bioregulators on the antioxidant enzymes, a significant increase ($P<0.05$) in peroxidase activity was obtained, in comparison with control, even at the high concentration of 200mM NaCl in the tomato genotypes studied. These results show semblance to the findings of [34] Khosravinejad et al. (2008) on the response of Afzal plants to increased salt concentrations. Salinity stress results in the generation of excessive reactive oxygen species (ROS) which leads to cell toxicity, membrane dysfunction and cell death. Tolerance to salinity in higher plants correlates to the level of antioxidant systems and substrates [35, 36] (Jahnke and White, 2003; Jebara et al., 2005). Such plants have been reported to defend against the ROS by enhancement of antioxidative

enzymes [37] (RodiguezRosales et al., 1999). Therefore, the observed increase in the activities of catalase and peroxidase in the present work indicates induction of antioxidant enzymes in the tomato leaves. This protects the plant cells from injury by scavenging reactive oxygen species [38] (Asada and Kiso, 1973). These enzymes have a possible synergy to commonly resist oxidative damage caused by salt stress [39] (Dai et. al, 2009). Enhanced activity of antioxidative enzymes was also reported by [40] Harinasut et al (2003) in the leaves of mulberry grown under salt stress (150 mM NaCl) conditions. The changes in the activity of these enzymes are correlated with oxidative stress tolerance of plants [41] (Lee et al. 2001). Consequently, variations in their levels can serve as signals for the modulation of ROS scavenging mechanisms and ROS signal transduction [42] (Mittler 2002).

Table 2: Effect of bioregulators on antioxidant enzymes of Tropimech tomato depending on salinity*.

Bioregulator (mg/L)	**NaCl (mM)**	**Catalase (Units/mg protein/min)**	**Peroxidase (Units/mg protein/min)**
Control	0	0.340i	0.026k
	100	0.000r	0.000q
	200	0.000r	0.000q
IAA 50	0	1.961b	0.072c
	100	1.208d	0.050f
	200	0.393g	0.052e
IAA 100	0	0.022q	0.019o
	100	0.290j	0.018p
	200	0.313i	0.022l
IAA 150	0	0.267k	0.0442j
	100	0.625f	0.019n
	200	1.667c	0.144a
NAA 50	0	0.136m	0.057d
	100	4.103a	0.022m
	200	1.076e	0.048g
NAA 100	0	0.213l	0.133b
	100	0.000r	0.000q
	200	0.107o	0.041j
NAA 150	0	0.125n	0.045i
	100	0.000r	0.000q
	200	0.077p	0.000q

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

Table 3: Effect of bioregulators on antioxidant enzymes of UC82B tomato depending on salinity*.

Bioregulator (mg/L)	**NaCl (mM)**	**Catalase (Units/mg protein/min)**	**Peroxidase (Units/mg protein/min)**
Control 0	0	2.587c	0.046de
	100	1.393f	0.021de
	200	0.727k	0.106bcd
IAA 50	0	0.307n	0.031de
	100	0.192p	0.169ab
	200	1.011h	0.213a
IAA 100	0	1.216g	0.032de
	100	5.726a	0.029de
	200	2.721b	0.020de
IAA 150	0	1.740e	0.090bcde
	100	0.403m	0.038de
	200	0.301o	0.160abc
NAA 50	0	0.728k	0.033de
	100	0.849i	0.023de
	200	1.951d	0.085bcde
NAA 100	0	0.811j	0.0757cde
	100	0.000q	0.000e
	200	0.681l	0.019de
NAA 150	0	0.000q	0.000e
	100	0.000q	0.000e
	200	0.000q	0.000e

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

The effect of bioregulators on tomato minerals at different salinity levels

The effects of the bioregulators on tomato minerals at different salt concentrations are shown in Tables 4 and 5. There was significant decrease in Ca, Mg, Na, Mn, Cu and Zn contents at the different concentrations of bioregulators relative to control at 0mM NaCl, especially in the UC 82B tomato genotype but the 100mg/L concentrations of

IAA and NAA significantly increased (P<0.05) the K and Fe contents compared to the control at the same salinity level. The treatments of 100mg/L IAA (at 100 and 200mM NaCl) and 50mg/L NAA (at 200mM NaCl) gave the highest increase in Mn, Fe and Cu levels in comparison with control at these salt levels. The 100mg/L concentration of NAA significantly increased (P<0.05) the Zn content compared to the control at 0mM NaCl (Table 5). The Fe and Mn levels tend to increase within the group with increase in bioregulator and salt concentrations. This result is consistent with the work of [43] Akman (2009) who observed increasing Fe and Mn contents with increasing salinity in wheat. Also, [44] Khan et al. (2007) reported that the content of Na^+ and Cl^- in plants treated with 0.1mM salicylic acid (SA) and grown at 50mM NaCl was less compared to the control. In the present work, 100mg/L IAA at different salt concentrations increased the level of Na (Table 4). These results suggest that auxins like IAA and NAA might play a role in increasing nutrient level in plants under salt stress.

Table 4: Effect of bioregulators on minerals of Tropimech tomato depending on salinity*.

Bioregulator (mg/L)	NaCl (mM)	Ca (%)	Mg (%)	K (%)	Na (%)	Mn (%)	Fe (%)	Cu (%)	Zn (%)
Control	0	0.99000d	0.33850c	1.31027c	0.03375l	0.00748e	0.11725i	0.00106d	0.01150h
	100	0.00000j	0.00000n	0.00000o	0.00000n	0.00000h	0.00000p	0.00000j	0.00000l
	200	0.00000j	0.00000n	0.00000o	0.00000n	0.00000h	0.00000p	0.00000j	0.00000l
IAA									
50	0	0.84533e	0.26505g	1.03070h	0.01295m	0.00525f	0.11200k	0.00940a	0.01120j
	100	0.99500cd	0.25350h	1.17800f	1.44250a	0.00523f	0.04800n	0.00038h	0.00908k
	200	1.00750cd	0.39425b	1.24673e	0.52500c	0.03680g	0.03925o	0.00053g	0.01128ij
100	0	1.27500b	0.11498m	2.13175a	0.05500j	0.00868d	0.07375m	0.00130c	0.01453e
	100	0.98000d	0.39300b	1.25575d	0.25775h	0.00553f	0.08425l	0.00071f	0.01188g
	200	1.45000a	0.41150a	1.30950c	0.45050d	0.01005c	0.17950e	0.00069f	0.02195a
150	0	0.76750f	0.27450f	1.33000b	0.05200j	0.01745a	0.23550b	0.00059g	0.01303f
	100	0.86500e	0.28150e	0.87800k	0.43950e	0.00883d	0.11475j	0.00028i	0.01148hi
	200	1.02000c	0.30000d	0.97000i	0.62000b	0.01017c	0.15000g	0.00180b	0.01600d
NAA									
50	0	0.69700g	0.21975j	1.15375g	0.03650l	0.00983c	0.16550f	0.00078f	0.01440e
	100	0.71300g	0.22175j	0.09047n	0.18000i	0.00865d	0.21200c	0.00103de	0.01440e
	200	0.74600f	0.24400i	0.72350l	0.35250f	0.01248b	0.29950a	0.00095e	0.01805c
100	0	0.48400i	0.19650f	0.94350j	0.04525k	0.00978c	0.18300d	0.00051g	0.01865b
	100	0.61700h	0.18450l	0.25850m	0.34300g	0.00853d	0.13775h	0.00030hi	0.01820c
	200	0.00000j	0.00000n	0.00000o	0.00000n	0.00000h	0.00000p	0.00000j	0.00000l
150	0	0.00000j	0.00000n	0.00000o	0.00000n	0.00000h	0.00000p	0.00000j	0.00000l
	100	0.00000j	0.00000n	0.00000o	0.00000n	0.00000h	0.00000p	0.00000j	0.00000l
	200	0.00000j	0.00000n	0.00000o	0.00000n	0.00000h	0.00000p	0.00000j	0.00000l

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

Table 5: Effect of bioregulators on minerals of UC82B tomato depending on salinity*.

Bioregulator (mg/L)	NaCl (mM)	Ca (%)	Mg (%)	K (%)	Na (%)	Mn (%)	Fe (%)	Cu (%)	Zn (%)
Control	0	1.05900b	0.32300c	1.46750d	0.55500b	0.01018bc	0.15440c	0.00052bc	0.01033ab
	100	1.51000a	0.43567a	2.24675a	0.41700d	0.00785def	0.07700f	0.00050c	0.01220a
	200	0.44000k	0.12000n	0.52000k	0.62100a	0.00800def	0.02000h	0.00020g	0.01000bc
IAA									
50	0	0.81750e	0.17375k	0.00012n	0.04050m	0.00750def	0.02263h	0.00031f	0.00431g
	100	0.68800g	0.23132g	1.57350b	0.23375j	0.00706ef	0.09625e	0.00029f	0.00670ef
	200	0.54000i	0.14675m	0.41000l	0.31400f	0.00928cd	0.15125c	0.00020g	0.01058ab
100	0	0.86900d	0.26650d	1.51775c	0.04125m	0.00653f	0.06925f	0.00049c	0.00828cde
	100	1.04250b	0.36275b	1.43450e	0.28400h	0.01000bc	0.12075d	0.00065a	0.01003bc
	200	0.61000h	0.25000e	0.54000j	0.40000e	0.01205a	0.15050c	0.00020g	0.00800de
150	0	0.87800d	0.24650f	0.99175i	0.03825m	0.00878cde	0.07700f	0.00047cd	0.00698ef
	100	0.87700d	0.25175e	1.01700h	0.28750g	0.01185ab	0.22525a	0.00041e	0.01072ab
	200	0.279001	0.08050o	0.16327m	0.29000g	0.00404g	0.04600g	0.00018g	0.00515fg
NAA									
50	0	0.94300c	0.22000h	1.27450f	0.048501	0.00938cd	0.08000f	0.00056b	0.00788de
	100	0.75100f	0.21575i	1.11475g	0.26850i	0.00781def	0.14325c	0.00043de	0.00725e
	200	0.47000j	0.160331	0.51000k	0.45000c	0.01292a	0.17000b	0.00030f	0.00507fg
100	0	0.62900h	0.20325j	0.98575i	0.11750k	0.00945cd	0.17820b	0.00050c	0.00943bcd
	100	0.00000m	0.00000p	0.00000n	0.00000n	0.00000h	0.00000i	0.00000h	0.00000h
	200	0.00000m	0.00000p	0.00000n	0.00000n	0.00000h	0.00000i	0.00000h	0.00000h
150	0	0.00000m	0.00000p	0.00000n	0.00000n	0.00000h	0.00000i	0.00000h	0.00000h
	100	0.00000m	0.00000p	0.00000n	0.00000n	0.00000h	0.00000i	0.00000h	0.00000h
	200	0.00000m	0.00000p	0.00000n	0.00000n	0.00000h	0.00000i	0.00000h	0.00000h

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

Conclusion

The results obtained clearly shows that IAA and NAA has a stimulating effect on the salinity stress tolerance of tomato through enhanced antioxidant enzyme activities and increased level of some mineral nutrients. These bioregulators prevent the unsavoury effects of salinity stressed tomato and could therefore be potential tools to improve the development and quality of this vegetable food crop, particularly under moderate sodium chloride salinity levels.

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