African Journal of Plant Science Vol. 4(6), pp. 167-171, June 2010
Available online at http://www.academicjournals.org/ajps
ISSN 1996-0824 ©2010 Academic Journals

Full Length Research Paper

Chemical manipulation of tomato growth and associated biochemical implications on flavonoid, lycopene and mineral contents

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Approved 18 December, 2009

The consumption of tomato (*Solanum lycopersicon*) has been linked with reduced risk of chronic degenerative diseases. Therefore, this study was carried out to investigate the biochemical implications of bioregulator application on flavonoids, lycopene, calcium, magnesium, potassium, sodium, and phosphorus and iron contents of tomato. Seeds of the tomato, genotype JM 94/47 were subjected to pre –germination treatment by soaking in 60, 100 and 140mg/L of indole-acetic acid (IAA), indole butyric acid (IBA) and naphthalene acetic acid (NAA) and planted. The ripe tomato fruits were harvested at the orange red ripe stage and some biochemical parameters were investigated. The analysis results showed that flavonoid and lycopene content were significantly ($p < 0.05$) increased in tomato plants treated with IAA, IBA and NAA compared to control. The flavonoid content ranged from 0.081 to 0.169 mg/100 g while the 100 mg/L NAA resulted in the highest lycopene concentration of 1.16 µg/100 gfwb and assayed minerals were also increased in tomatoes treated with all bioregulators. These results indicate that pre – sowing seed treatment with 1AA, IBA and NAA have profound effect on improving the quality of tomato, especially the phytonutrients examined.

Key words: Bioregulators, flavonoids, lycopene, minerals, *Solanum lycopersicon.*

INTRODUCTION

Bioregulators are chemicals that affect the expression of biological responses in plant tissues. They are endogenous and exogenous substances that influence the growth, development and composition of plants. They act in low concentrations and are without any biocidal or nutritive action (Rademacher, 2000). They are readily absorbed by plants, penetrating the living surface cells of most plant parts. Bioregulators have been instrumental in understanding morphological and growth phenomena *in vitro*. They are potential tools for elucidating biochemical pathways in plants (Ribnicky et al., 1996) and may be classified into one of two groups namely, phytohormones or synthetic bioregulators (Schott and Walter, 1991). The phytohormones include abscisins (such as abscisic acid), ethylene (such as ethephon), auxins (such as 2,4-dichlorophenoxy acetic acid), cytokinins (such as kinetin and benzylaminopurine) and gibberellins (such as gibberellic acid). Indole acetic acid, indole butyric acid, naphthalene acetic acid and 2,4,6–tichlorobenzoic acid are examples of synthetic bioregulators. Some physiological and biochemical roles of bioregulators in plants include: plant development, embryogenesis, growth, flowering, fruiting and senescence, plant defence mechanisms, seed germination and dormancy, abiotic stress caused by heat or cold, salinity, drought or water logging, nutrient deficiency or excessive nutrients, pollutants; tropic and nastic movements, growth regulator biosynthesis, transport and metabolism and molecular mechanism of signal transduction (Trolinder, 1991; Ibrahim et al., 2007).

Millions of people throughout the developing countries of the world have inadequate food supply or have nutrient deficiencies in their diets and as such suffer from starvation and malnutrition of various types (NAS, 2004). The tomato is the most widely cultivated vegetable crop in Nigeria (FOS, 1995). It is economically and commercially

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Table 1. Effects of bioregulator application on total flavonoids content of tomato*

Conc.(mg/L)	Total flavonoids (mg/100g)**		
	IAA	IBA	NAA
60	0.081±0.012a	0.089±0.015a	0.112±0.013b
100	0.150±0.050c	0.131±0.016b	0.169±0.011c
140	0.127±0.042b	0.104±0.095b	0.139±0.017c

* Means of three determinations ± S.E.
**Values within same columns followed by same letters are not significantly different (p>0.05).

important throughout the world. The tomato is rich in health - promoting phytochemicals (carotenoids, anti-oxidant vitamins, flavonoids, polyphenols and minerals). Mounting evidence suggests that the consumption of fresh and processed tomato products is associated with reduced risk of cancer (Ames et al., 1993; Baysal et al., 2000) and heart diseases (Pandey et al., 1995). Specifically, the carotenoids are known to act as antioxidants, exhibiting anti-ulcer effects and activating the immunological systems (Scalfi et al., 2000) while the flavonoids have been linked to the lowering of the risk of heart disease (Hertog et al., 1997). The different minerals are also essential as cofactors for enzymes, as well as for maintaining body homeostasis (Gibson and Hotz, 2001).

Bioregulators have been suggested as possible tools for food production (Belakbir et al., 1998; Olaiya, 2006). Therefore, the purpose of this study was to determine the biochemical implications of bioregulator application on flavonoids, lycopene, Calcium (Ca), Magnesium (Mg), Potassium (K), Sodium (Na), Phosphorous (P) and Iron (Fe) contents of tomato.

MATERIALS AND METHODS

Seeds of the tomato, genotype JM 94/47 obtained from the National Horticultural Research Institute (NIHORT), Idi-Ishin, Ibadan, Nigeria (long $3°50^1 - 52^1$E and lat. $7°23^1 - 25^1$N of the equator) were subjected to pre-germination treatment by soaking in 60, 100 and 140 mg/L of Indole – acetic acid (IAA: 98% pure, Sigma), Indole butyric acid (IBA: 98% pure, Sigma) and Naphthalene acetic acid (NAA: 96% pure, Sigma). Seedlings were raised in the nursery in seedling trays (300 x 200 x 60 mm) and then transplanted into well – aerated polythene bags containing loamy, well drained, fertile and good moisture retaining capacity soil having 1.0 – 1.5% organic matter. A control was set up by using distilled water in place of the bioregulators. A randomized block design with three replications for each treatment was used. The plants were grown according to normal cultural practices without application of insecticide or fertilizer in a screen house. Ripe tomato fruits were harvested at the orange – red ripe stage and stored in reasealable plastic bags at 20°C until use.

Total flavonoids: Total flavonoids content was determined using the method of Mackay et al., 1979. About 0.5 g of finely ground fruit sample was weighed into 100 ml beaker and 80 ml of 95% ethanol added and stirred with a glass rod to prevent lumping. The mixture was filtered through a Whatman No.1 filter paper into a 100 ml volumetric flask and made up to mark with ethanol. 1 ml of the extract was pipetted into a 50 ml volumetric flask; four drops of conc. HCl and 0.5 g of magnesium turnings were added to develop a magenta red colouration. Standard flavonoid solutions of range 0 – 5 ppm were prepared and similarly treated with conc. HCl and magnesium turnings to develop a magenta red colouration. The absorbance of magenta colouration of sample and standard solutions were read at 520 nm in a digital spectronic 21D spectrophotometer.

Lycopene content: Lycopene content was determined by the method of Sims and Gamon (2002) with slight modifications. About 1 g of the tomato fruit sample was weighed into a 250 ml beaker and crushed with a glass rod. 25 ml of HPLC acetone was added and shaken for 10 min. 25 ml of methanolic NaOH solution was added and a reflux condenser attached. The mixture was heated in a boiling water bath for 1 h with frequent shaking. The mixture was rapidly cooled and 50 ml of distilled water added. The solution was extracted thrice with acetone and 1 g K_2SO_4 added to remove traces of water.

The organic layer was carefully removed and filtered into a 100 ml volumetric flask and made up to mark with HPLC acetone. Standard solutions of lycopene of range 0 – 50 µg/ml were prepared and treated similarly. The absorbance of extracted samples and standard solutions of lycopene were read on a spectronic 21D spectrophotometer at 340 nm.

Mineral contents: The ash of the tomato fruit sample was digested using 2 M HCl and the amounts of Calcium, Sodium and Potassium in the digests were determined using a flame photometer (PFP7; Jenway), while phosphorous, Magnesium and Iron were measured using spectrophotometer (spectronic 21D).

Statistical analysis

All data obtained were analysed using analysis of variance (ANOVA) Statistica Software (Statistica, 1997). A significant level of 0.05 was used for statistical tests.

RESULTS AND DISCUSSION

The total flavonoids content ranged from 0.081 to 0.169 mg/100 g in treated plants and was found to be signify-cantly higher ($p < 0.05$) than that of the control, especially at the 100 and 140 mg/L treatment with the bioregulators (Table 1). Similar findings was reported by Mitchell et al. (2007) who observed an increase in total flavonoid content over time in archived tomato samples in both organic and conventional systems. Bongue-Bertles-man and Philips (1995) also found that nitrogen – deficient tomato plants had significantly greater ($p < 0.05$) total flavonoids content in their leaves. There was however a

Table 2. Effects of bioregulator application on lycopene content of tomato*.

Conc.(mg/L)	Lycopene (µg/100 gfwb)**		
	IAA	IBA	NAA
60	0.52±0.10	0.59±0.03	0.67±0.06
100	0.82±0.05	0.91±0.08**	1.16±0.08**
140	0.61±0.07	0.68±0.01	0.89±0.10
Control	0.31±0.07	0.31±0.078	0.31±0.07

* Means of three determinations ± S.E.
**Significantly different from other values in the column.

decrease in total flavonoids content at the higher concentration of 140 mg/L of the bioregulators. This observation suggests an interference with one or more enzymatic processes in the flavonoid biosynthetic pathway. Epidemiological studies revealed that flavonoids protect against cardiovascular diseases (Hertog and Hollman, 1996) and to a lesser extent against cancer (Knekt et al., 2002) and other age – related diseases such as dementia (Commenges et al., 2000).

Flavonoids demonstrate potent in vitro antioxidant activity (Van Acker et al., 1995). The huge increase in antioxidant activity of blood seen after the consumption of flavonoid – rich foods is not caused directly by the flavonoids themselves but most likely is due to increased uric acid levels that result from expelling flavonoids from the body (Linus Pauling Institute, 2007). It is noteworthy that most attempts to assign health – promoting activity mechanistically to the antioxidant action of individual flavonoids in foods have been unsuccessful (Williamson and Manach, 2005).

Lycopene contents of fruits of treated tomato plants varied from 0.52 ± 0.10 to 1.16 ± 0.08 µg/100 g fresh weight and are generally higher in comparison with control (Table 2) but the increase seems not to be concentration dependent. The appearance of the tomatoes revealed that the 100 mg/L NAA- treated samples were redder than the others as they tend to contain more lycopene, the red carotenoid pigment in tomatoes. Reports indicate that, although flavonoids are abundant in tomato fruit, lycopene is one of the major compounds affecting the antioxidant activity of tomato (Chang and Liu, 2008).

Lycopene has been reported to quench singlet oxygen (Di Mascio et al., 1991). The singlet oxygen – quenching properties of lycopene and its ability to trap peroxyl radicals results in the reduction of the risk of developing atherosclerosis and coronary heart disease as it prevents oxidation of low – density lipoprotein (LDL), cholesterol and thus contributing protection against carcinogenic substances (Levy et al., 1995).

Important minerals highly implicated in human nutrition, namely calcium (Ca), phosphorous (P), potassium (K), sodium (Na), Magnesium (Mg) and iron (Fe) were analysed in samples of the tomato fruits. The diets of impoverished people especially in the developing world are often monotonous and of poor quality (Gibson and Hotz, 2001). Mineral elements are very essential for various metabolic and immune processes in the body. Magnesium is an enzyme cofactor; sodium is required for nerve and muscle function while iron is a constituent of haemoglobin and cytochromes. It has long been known that inadequate provision of the bone-forming minerals Ca and P, due to low dietary intakes or to reduced bioavailability, could be a contributing factor in the poor growth performance of children in the developing world (Keller, 1988; Prentice and Bates, 1993). The effects of chemical treatments on the mineral content in fruits reported in literature have been contradictory (Bar-Yosef and Sagiv, 1982) giving both positive (Carswell et al., 1997) and negative (Olienyk et al; 1997) reports. In the present study, bioregulator application produced no significantly different effects ($p > 0.05$) relative to control, on all the mineral contents in test tomato samples. However, elevated levels of the minerals were noticed especially at the 100 mg/L concentration of the bioregulators (Table 3). Belakbir et al., 1996 also reported no significant differences within treatments for Fe level of leaf or fruit in pepper plants treated with eight bioregulators in southeast of Spain.

The results also show that the calcium concentration in the fruits ranged from 0.030 to 0.060%. The role of calcium in the maintenance of firmness of fruits has been established. Its requirements in fruits are related to cell wall stability and membrane integrity (Belakbir et al., 1998). The present results suggests that the 100 mg/L IAA -treated plants with calcium level of 0.060% will likely exhibit the highest degree of firmness since calcium concentration and fruit firmness are positively correlated (Song and Fujiyama, 1996).

The results of the determination of the K/Na ratio revealed values between 1.46 in the 60 mg/L NAA treatment to 2.14 in the 100 mg/L IAA treatment (Table 4). In comparison with control, the IAA treatment gave an increase in this ratio at the 100 and 140 mg/L concentrations; IBA in the 60 and 100 mg/L concentrations while NAA gave an increase only at the 140 mg/L concentration. Since the K/Na ratio in fruits and vegetables is closely linked with the treatment of hypertension (Golden and James, 1994), these findings would be helpful in alleviating the menace of hypertension in the consumers

Table 3. Effects of bioregulator treatments on mineral content of tomato*.

Bioregulator conc. mg/L)		Potassium (%)	Calcium (%)	Magnesium (%)	Sodium (%)	Phosphorus (%)	Iron (%)
IAA	60	0.0094±0.06	0.035±0.06	0.062±0.07	0.00605± 0.02	0.128±0.03	0.058±0.02
	100	0.0188±0.03	0.060±0.03	0.069±0.06	0.00880±0.07	0.175±0.04	0.063±0.01
	140	0.0116±0.01	0.045±0.06	0.067±0.03	0.00715±0.05	0.156±0.03	0.060±0.05
IBA	60	0.0092±0.08	0.030±0.03	0.058±0.06	0.00441± 0.16	0.099±0.10	0.054±0.02
	100	0.0127±0.01	0.045±0.02	0.066±0.05	0.00715±0.03	0.157±0.03	0.059±0.02
	140	0.0099±0.03	0.035± 0.08	0.062±0.07	0.00654±0.08	0.123±0.07	0.057±0.04
NAA	60	0.0088±0.03	0.037±0.07	0.063±0.08	0.00605± 0.07	0.167±0.03	0.097±0.07
	100	0.0154±0.07	0.055±0.03	0.072±0.10	0.00998±0.05	0.204±0.04	0.065±0.05
	140	0.0115±0.02	0.040±0.06	0.068±0.02	0.00715±0.03	0.181±0.09	0.062±0.02
Control		0.00771±0.05	0.030±0.05	0.059±0.07	0.00495± 0.07	0.066±0.05	0.056±0.03

*Means of three determinations ± S.E.

Table 4. Effects of bioregulator treatments on K/Na ratio of tomato.

Conc.(mg/L)	K/Na ratio*		
	IAA	IBA	NAA
60	1.55 b	2.09 a	1.46 b
100	2.14 a	1.78 ab	1.54 b
140	1.62 ab	1.51 b	1.61 a
Control	1.56 b	1.56 b	1.56 b

*Means in the same column followed by the same letter are not significantly different ($p > 0.05$).

and the 100 mg/L IAA - treated plants with a ratio of 2.14 would be most appropriate in this regard.

Conclusion

The observed differences can be correlated with differences in the bioregulators. These comparisons are free of compounding factors such as soil type differences and variable crop management histories, harvesting and sample handling.

The effects of exogenous application of bioregulators on the nutrient value of the tomato fruit, especially the ones examined in this study, and by implication on human health can easily be imagined and further investigations into the mechanism of action of the bioregulators appear to be worthwhile.

ACKNOWLEDGEMENT

The authors appreciate Mr. O. O. Afolabi of the Institute of Agricultural Research and Training (I.A.R&T), Moor Plantation, Ibadan for his technical assistance.

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