

Full Length Research Paper

Enzyme activity in bioregulator-treated tomato (*Solanum lycopersicon*) genotypes

Charles O. Olaiya

Department of Biochemistry, University of Ibadan, Ibadan, Nigeria. E-mail: coolaiya@yahoo.com.

Accepted 15 April, 2010

The challenges of using exogenous plant bioregulators to manipulate plant responses by researchers and producers are increasing rapidly. In previous studies, we have demonstrated that 100 mg/l concentration of the bioregulators; indole-acetic acid (IAA), indole butyric acid (IBA) and naphthalene acetic acid (NAA) is suitable for tomato growth in the field. In this work, spectrophotometric analysis was conducted to determine the level of enzymes that could affect the keeping quality of the fruits harvested at the orange-red ripe stage following pre-sowing seed treatments with 100 mg/l solutions of the bioregulators. The NHLy 11, NHLy 12, NHLy 13, NHLy 15 and NHLy 16 genotypes were used and the activities of lipoxygenase (LOX), catalase, peroxidase, alcohol dehydrogenase and acid phosphatase determined. All treated genotypes showed high LOX activity with a range of 0.06 - 1.07 $\mu\text{mol.abs.units}$ (A_{234}). The NAA-treated NHLy13 genotype had complete loss of catalase activity after 210 s. Peroxidase activity was higher in NAA-treated genotypes with values between 0.07 and 0.62 abs. units/g (A_{430}) while alcohol dehydrogenase activity suffered a decline in all IBA-treated genotypes. IAA gave consistently higher acid phosphatase activity than IBA and NAA in all the test genotypes. These findings have important implications on the biochemical changes associated with the fruits during storage and processing.

Key words: Bioregulator, seed treatment, *Solanum lycopersicon*, lipoxygenase, catalase, peroxidase, alcohol dehydrogenase, acid phosphatase.

INTRODUCTION

The tomato is a perishable food crop (Muroki et al., 1997). Its length of storage is a function of composition, resistance to attack by fungal and bacterial organisms and of external conditions of temperature and levels of gases in the environment. All these are related to enzyme activity in the fruit tissues (Hildebrand, 1989).

Lipoxygenase (Linoleate: Oxygen Oxidoreductase, E.C. 1.13.11.12) is predominant in fruits and seeds of a large number of vegetables (Pinsky et al., 1971) and is involved in a number of diverse aspects of plant physiology, including growth and development, pest resistance, senescence and wound responses (Gardner, 1991; Anthon and Barret, 2001). Catalase (CAT, E.C 1.11.1.6) is known for its ability to protect cells from the oxidising action of hydrogen peroxide (H_2O_2), a cytotoxic by-product of metabolism produced by superoxide dismutase (E.C.1.15.1.1) and flavoprotein enzymes (Thompson et al., 1987).

Peroxidase (E.C.1.11.1.7) catalyses oxyreduction between H_2O_2 and various reductants (Fernandez-Garcia et

al., 2004). The range of electron donors is wide and includes phenols, amines and alcohols. It is one of the most stable enzymes in vegetables and its thermal destruction has been used as an indicator of blanching adequacy (Schwimmer, 1981). Alcohol dehydrogenase (E.C.1.1.1.4) converts aldehydes to alcohols (Hatanaka et al., 1975). Acid phosphatases (E.C.3.1.3.2) are widely distributed in the plant kingdom. It has been suggested that they may be involved in the onset and development of senescence in fruits (Turner and Plaxton, 2001).

Bioregulators are endogenous or synthetically produced substances that can control specific biochemical and physiological functions of many plant genera and species probably by their influence on gene and enzyme interactions (Gausman, 1991). The breeding of high-yielding and nutrient rich tomato genotypes is time consuming and limited by costs (Hedin et al., 1995). Biotechnology has provided a potential for efficient development of genotypes with the desired traits. Manipulating plant responses using natural and synthetic bioregulators is an

emerging field of modern plant biotechnology with the potential of enhancing yield and phytonutrients in food crops (Cowan, 2009). In some instances, the bioregulator acts to modify plant gene expression, affecting levels of DNA, RNA, enzymes and finally their products such as proteins, carbohydrates, lipids and allelochemicals (Hedin et al., 1995). Research has shown the stimulatory effect of bioregulators on vegetative growth and yield of plants (Ibrahim et al., 2007). The 100 mg/l concentration of the bioregulators; indole-acetic acid (IAA), indole butyric acid (IBA) and naphthalene acetic acid (NAA) have been found to be suitable for tomato seedling emergence (Olaiya and Osonubi, 2009), growth and yield in the field (Olaiya, 2010). The present study examines the possible influence of this concentration of the bioregulators on enzymes in the fruit tissues that catalyse biochemical changes during storage and processing.

MATERIALS AND METHODS

Sample collection

The five tomato genotypes NHLy 11, NHLy 12, NHLy 13, NHLy 15 and NHLy 16 were subjected to pre-sowing seed treatments in 100 mg/l of IAA, IBA, NAA and distilled water (control). They were grown in three replications under prevailing field conditions on prepared plots at the Vegetable Experimental Site of National Horticultural Research Institute (NIHORT), Ibadan (long 3°50' - 52E and lat. 7°23' - 25N of the equator). The fruits were harvested at the orange-red ripe stage, sampled on the basis of similar shape and size, stored at 4°C and analysed not later than 48 h after harvest.

Preparation of enzyme extracts and the assay methods

Crude enzyme extracts for the assays were prepared according to the method of Aluko and Ogbadu (1986). Ten grammes (10 g) of the outer locule tissue of each genotype was homogenised with 30 ml of the appropriate ice-cold buffer, filtered and centrifuged at 48000 x g for 15 min (MSE high speed centrifuge). The supernatant was stored at 2 - 5°C and aliquots of these were assayed for enzyme activity in a Spectronic 20 (Bausch and Lomb, Rochester, NY, USA) spectrophotometer.

Lipoxygenase activity assay

Lipoxygenase was assayed according to the method of Grossman and Zakut (1979). Pure linoleic acid (10 µl) was suspended in 25 ml of 0.1 M sodium tetraborate containing 0.1% Tween 20 by sonication (Sekhar and Reddy, 1982). The substrate (0.1 ml) was shaken vigorously with 2.9 ml of 0.1 M phosphate buffer of pH 4 - 5, in a spectrophotometer cuvette. The reaction was started by adding 0.1ml of enzyme extract and the increase in absorbance at 234 nm was measured every 30 s.

Catalase activity assay

The method of Bergmeyer (1974) was used for the determination of Catalase activity. A 1.74 g anhydrous potassium phosphate was dissolved in about 60 ml of distilled water. The pH was then adjusted to 6.8 with HCl and the final volume was brought to 100 ml. Immediately before use, 1.13 ml of 30% H₂O₂ was added to

the buffer and the pH was readjusted to 6.8. 1 ml of the enzyme extract was added and the absorbance was read at 240 nm in a Bausch and Lomb 'Spectronic 20' spectrophotometer.

Peroxidase activity assay

Peroxidase activity was determined using the method described in the Worthington enzyme manual (Worthington, 1971). A 0.5 ml of enzyme extract and 1.5 ml of phosphate buffered pyrogallol, pH 6.0 were incubated at 25°C and 1.10 ml of 30% hydrogen peroxide added. The increase in absorbance was determined spectrophotometrically at 430 nm. One arbitrary unit of peroxidase activity is defined as an increase in optical density equal to the value given in one minute.

Alcohol dehydrogenase activity assay

Alcohol dehydrogenase was assayed by the method of Amador and Wacker (1965). To 1ml of enzyme extract was added 1 ml of 0.3 µM NADH and 1.3 ml of Tris-HCl buffer (30 mM, pH 7.4). The mixture was then subjected to incubation for 10 min at 25°C and 20µmol acetaldehyde was added. The decrease in absorbance was then monitored at 2 min intervals.

Acid phosphatase activity assay

The acid phosphatase assay was conducted by the method of Amador and Wacker (1965). A 0.5 ml of enzyme extract and 4.5 ml buffered substrate were incubated at 37°C for 1 h. The tubes were then placed in ice, and 1 ml of 30% trichloroacetic acid (TCA) solution added. The mixture was then filtered using a Whatman No.1 filter paper. A control for each sample was prepared by addition of 4.5 ml buffered substrate and 1ml of TCA at zero time. Molybdate reagent (0.5 ml), aminonaphthosulphonic acid (0.2 ml) and 0.3 ml distilled water were added to 4 ml of each trichloroacetic acid filtrate. After 1 h, the absorbance was read at 660 nm against the water - reagent solution. The absorbance of each reaction mixture minus that of the corresponding extract control was converted to units of activity with a phosphate calibration curve. One unit of acid phosphatase activity is defined as the release of 1 mg of Pi/100 ml of extract/h.

Statistical analysis

Analysis of variance (ANOVA) Statistica Software (Statistica, 1997) was used to analyse the data obtained. A significant level of 0.05 was used for statistical tests

RESULTS AND DISCUSSION

The determination of enzyme activities is important in that it gives an indication of the metabolic and functional state of living tissues in samples under investigation (Schormuller, 1974). In this work, significant variation in the activities of the enzymes, following bioregulator application was noticed and this could affect the keeping quality of the fruits.

Lipoxygenase (LOX) activity

Lipoxygenase causes off - flavour effects in many foods

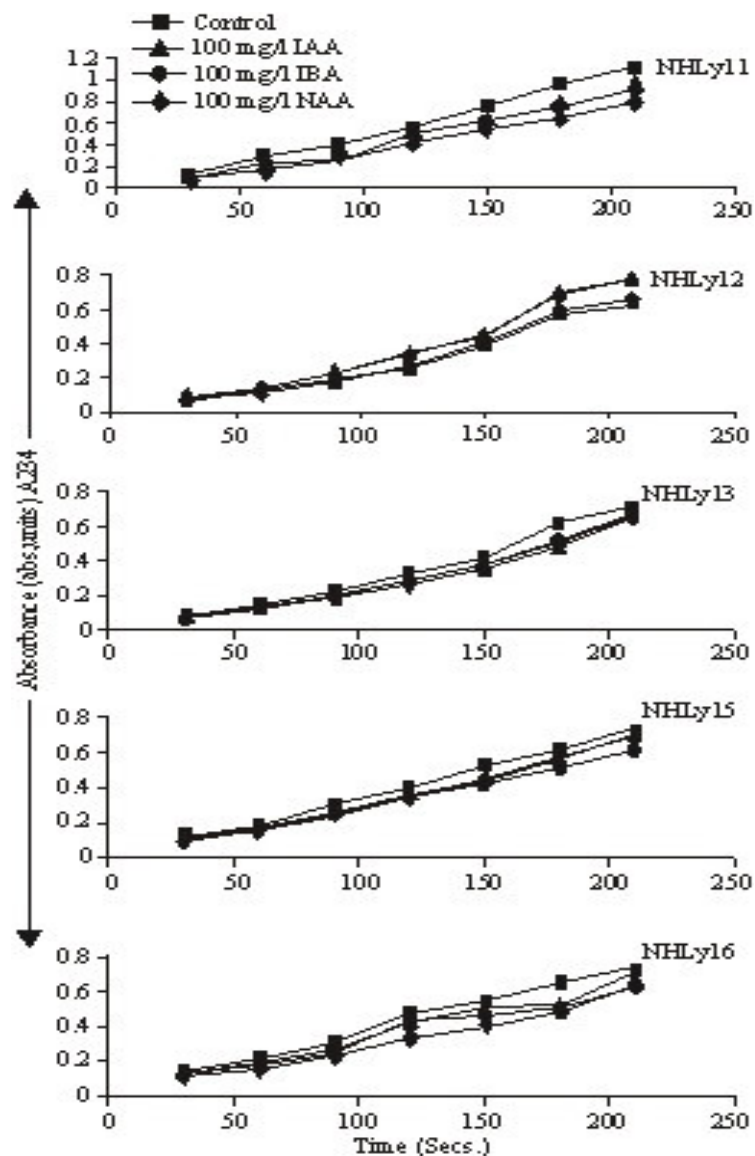


Figure 1. Bioregulator effect on lipoxxygenase activity in tomato fruits.

including fruits and vegetables (Halpin and Lee, 1987). It plays an important role in the genesis of volatile flavour aroma compounds in many plant foods, including tomato, cucumber, and banana (Eskin et al., 1977). The enzyme degrades linoleic and linolenic acids into volatiles such as hexanal and cis-3-hexanal (Kazeniak and Hall, 1970). In this study, the treated genotypes showed relatively high lipoxxygenase activity in comparison with control, ranging from 0.06 - 1.07 $\mu\text{mol.abs.units}$ (A_{234}). Although all the genotypes showed high lipoxxygenase activity, the IAA-treated NHLy11 genotype gave the highest activity (Figure 1). This suggests that endogenous damage and off-flavour developments are likely to be more profound in this genotype since these phenomena have been associated with high lipoxxygenase activity (Shibata and Axelrod, 1995; Brash, 1999).

Catalase activity

In contrast to the LOX activity, catalase activity in the NHLy11, NHLy15 and NHLy16 genotypes in IAA and IBA treatments showed lower values than in the NHLy12 genotype (Figure 2). The NAA-treated NHLy13 genotype had complete loss of catalase activity after 210 s. The inactivation of catalase activity and the loss of activity obtained in the NAA-treated NHLy13 genotype suggest that this genotype would be more vulnerable to tissue disruption and damage during handling, transportation and storage (Figure 1).

The fact that there is a difference between the genotypes in the level of enzyme activity is of concern in comparative studies. Currently, there is great interest in understanding post-harvest disorders in relation to

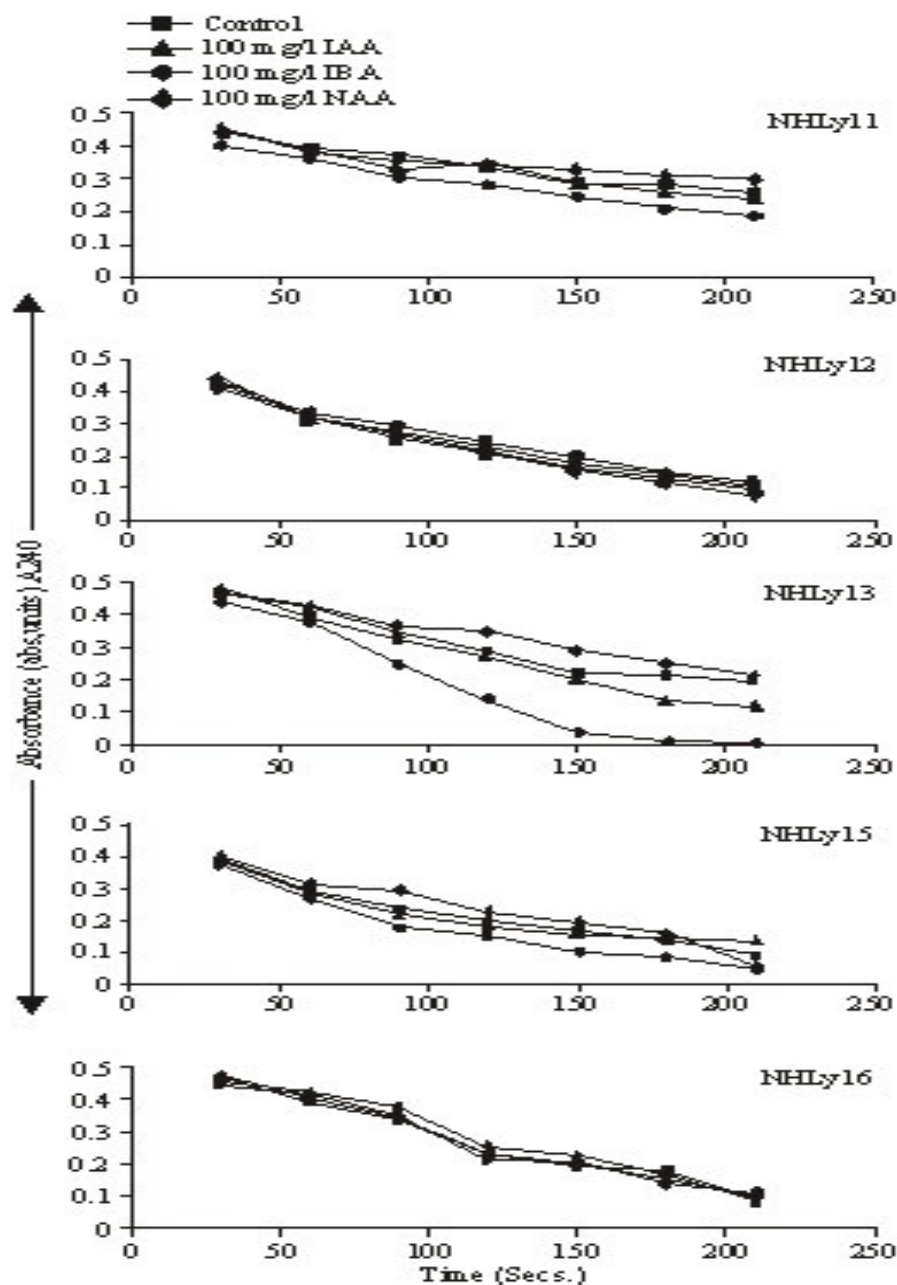


Figure 2. Bioregulator effect on catalase activity in tomato fruits.

oxidative injury (Gong et al., 2000) and the results reported here have several implications on the interpretation of catalase activity in plant tissues, specifically, in tomatoes.

Peroxidase activity

Figure 3 shows the effects of bioregulator treatments on peroxidase activity in tomato genotypes under study. All the treatments enhanced peroxidase activity relative to control, except in the IBA-treated NHLY15 genotype. In comparison with IAA and IBA, peroxidase activity was

relatively higher in the NAA-treated genotypes with values between 0.07 and 0.62 abs.units/g (A_{430}). Increased peroxidase activity has been suggested to be directly responsible for disease resistance in plant tissues (Polyak-Feher et al., 1992; Mika and Luthje, 2003). The high peroxidase activity recorded in the NAA treatments especially in the NHLY12 genotype is of interest, as their fruits are expected to be more resistant to infections and diseases during handling and storage. The decline in peroxidase activity observed in some of the tomato genotypes is consistent with findings by previous workers (Baardseth, 1978; Barrett and Therakulkait, 1995) who

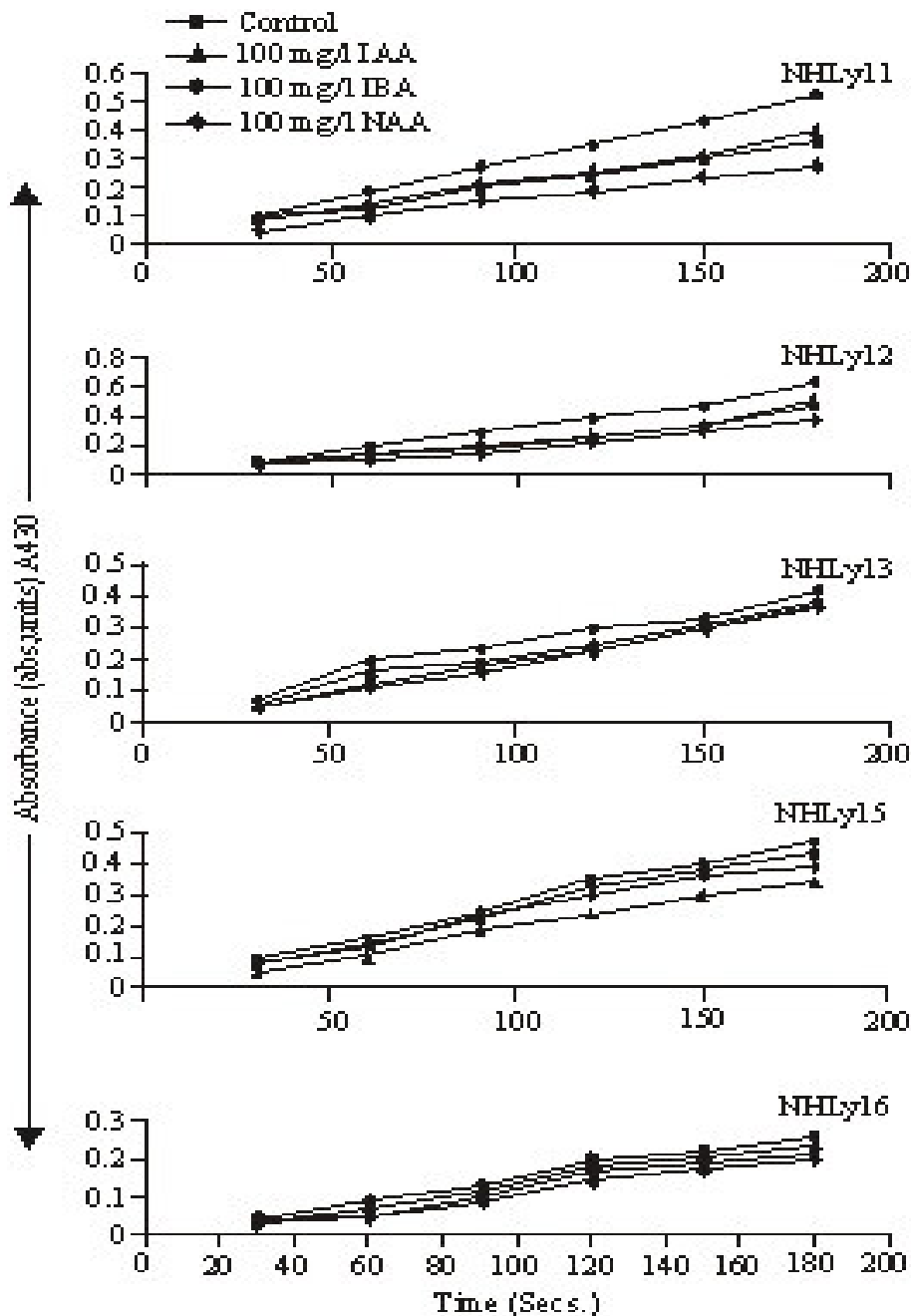


Figure 3. Bioregulator effect on peroxidase activity in fruits of tomato genotypes.

reported decline in peroxidase activity in vegetables during frozen storage. Peroxidase action, as observed in this work, suggests a conferment of protective effects on membrane integrity through the inhibition of lipid breakdown and attenuation of ethylene action.

Alcohol dehydrogenase activity

In all the treatments, alcohol dehydrogenase suffered a decline in comparison with control in most tomato geno-

types. Slight enhancement of the enzyme activity was only noticed in the NAA-treated NHLy12 and NHLy15 genotypes (Figure 4). Aldehydes produced from lipoxygenase-mediated reactions can destroy proteins through the formation of Schiff bases with their amino groups (Gardner, 1979). The reduction of these aldehydes to alcohols by alcohol dehydrogenase helps in preventing this type of activity. Increased alcohol dehydrogenase activity is associated with ethanol production, a condition which favours anaerobic metabolism (Toyosaki, 1992). Increased conversion of aldehydes from lipoxygenase-

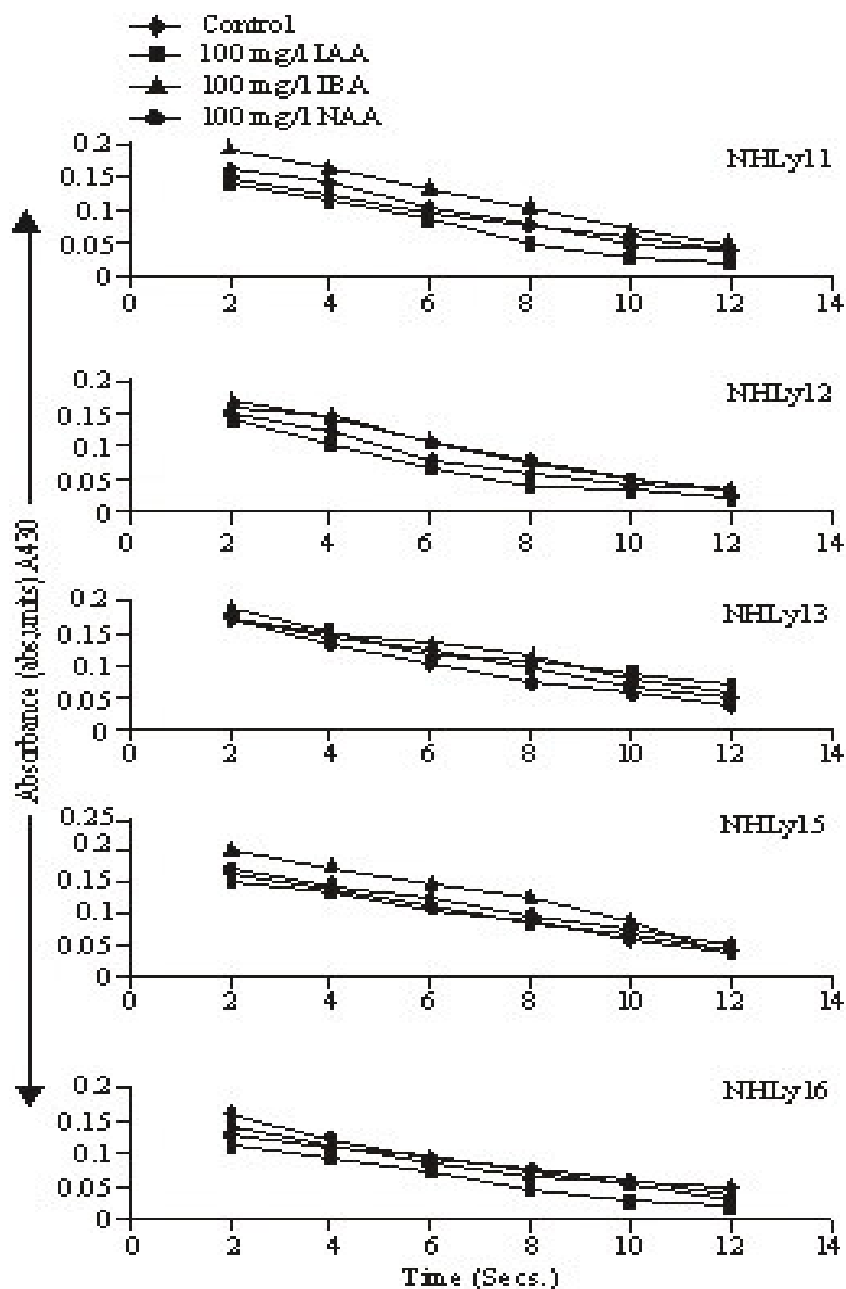


Figure 4. Bioregulator effect on alcohol dehydrogenase activity in fruits of tomato genotypes.

mediated reactions to alcohols by alcohol dehydrogenase is therefore expected to be more profound in the NAA - treated NHLY12 and NHLY 15 genotypes.

Acid phosphatase activity

The effect of bioregulator treatments on acid phosphatase activity was generally not significant ($p > 0.05$) in the five genotypes studied. Relative to control, the enzyme activity was enhanced in all treatments except in the

NAA-treated NHLY12 genotype (Table 1). Of note is the fact that IAA gave consistently higher effects on the acid phosphatase activity than IBA and NAA in all the test genotypes. High acid phosphatase activity in plant tissues has been suggested to cause increased rate of hydrolysis of ribose nucleotides which can impair the synthesis of ribonucleic acids (Bozzo et al., 2004). A high level of acid phosphatase can also lead to increased rate of hydrolysis of membrane ATP, phosphatidyl serine and phosphatidyl ethanolamine, thereby destroying membrane integrity, which could cause accelerated senescence

Table 1. Bioregulator Effects on the Acid Phosphatase Activity in Tomato Genotypes

Treatment	NHLY11				NHLY12				NHLY13				NHLY15				NHLY16			
	T ₀	T ₁	T ₂	T ₃	T ₀	T ₁	T ₂	T ₃	T ₀	T ₁	T ₂	T ₃	T ₀	T ₁	T ₂	T ₃	T ₀	T ₁	T ₂	T ₃
A ₆₆₀ *	0.26	0.35	0.29	0.31	0.19	0.24	0.02	0.18	0.24	0.28	0.26	0.25	0.29	0.36	0.3	0.03	0.27	0.83	0.32	0.29
Activity (mg/100ml Pi)h ⁻¹ x 10 ³	50.0	68.0	56.0	60.0	37.0	46.0	39.0	35.0	46.0	5.0	50.0	49.0	56.0	70.0	58.0	58.0	52.0	74.0	62.0	56.0

*Absorbance at 660 nm.

T₀ = Control; T₁ = 100 mg/l IAA; T₂ = 100mg/l IBA; T₃ = 100 mg/l NAA.

(Deleo and Sacher, 1970). The results obtained in this study have indicated that there was no significant difference ($p > 0.05$) between the levels of acid phosphatase found in the test genotypes relative to control. The highest activity obtained in the IAA-treated NHLY16 genotype suggests that this genotype will exhibit a higher degree of senescence than others will (Rhodes, 1980), during damage to fruit tissue or during storage.

This study provides information on the usefulness of these bioregulators in improving the post-harvest quality of the tomato fruit. However, the biochemical and molecular mechanisms underlying bioregulator-induced responses in plants and harvested plant parts remain unclear and is still a subject of investigation (Madhavan and Benedict, 1991, Cowan, 2009).

REFERENCES

- Aluko R, Ogbadu GH (1986). Analysis of eggplant varieties for enzymes related to their organoleptic properties. *Trop. Sci.* 26: 163-171.
- Amador E, Wacker WEC (1965). Enzymatic methods used for diagnosis. In: *Methods of Biochemical Analysis* Vol. XIII. Ed. David Glick, Interscience Pubs, USA, pp. 265-356.
- Anthon GE, Barret DM (2001). Colorimetric Method for the determination of Lipoygenase Activity. *J. Agric. Food Chem.* 49: 32-37.
- Baardseth P (1978). Quality changes of frozen vegetables. *Food Chem.* 3: 271-282.
- Barrett DM, Therakulkait C (1995). Quality indicators in blanched frozen, stored vegetables. *Food Technol.* 49: 63-65.
- Bergmeyer HU (1974). Principles of enzymatic analysis. In *Methods of enzymatic analysis*, ed. Bergmeyer HU. Academic Press, New York, p. 94.
- Bozzo G, Raghothama K, Plaxton WC (2004). Structural and kinetic properties of a novel purple acid phosphatase from phosphate starved tomato (*Lycopersicon esculentum*) cell cultures. *Biochem. J.* 377: 419-428.
- Brash AR (1999). Lipoygenases: occurrence, functions, catalysis. *J. Biol. Chem.* 274(34): 23679-23682.
- Cowan AK (2009). Plant growth promotion by 18:0-*lyso*-phosphatidylethanolamine involves senescence delay. *Plant Signal Behav.* 4(4): 324-327.
- Deleo P, Sacher AJ (1970). Senescence: Association of synthesis of acid phosphatase with banana ripening. *Plant Physiol.* 46: 208-211.
- Eskin NAM, Grossman S, Pinsky A (1977). Biochemistry of lipoygenase in relation to food quality. *Crit. Rev. Food Sci. Nutr.* 9(11): 1-40.
- Fernandez-Garcia N, Carvajal M, Olmos E (2004). Graft union formation in tomato plants: peroxidase and catalase involvement. *Ann. Bot.* 93: 53-60.
- Gardner HW (1979). Lipid hydroperoxide reactivity with proteins and amino acids: A review. *J. Agric. Food Chem.* 27: 220-229.
- Gardner HW (1991). Recent investigations into the lipoygenase pathway of plants. *Biochim. Biophys. Acta.* 1084: 221-239.
- Gausman HW (1991). *Plant Biochemical Regulators*, Marcel Dekker Inc. Pubs., New York, p. 323.
- Gong Y, Toivonen PMA, Wiersma PA, Lu C, Lazz OL (2000). Effect of freezing on the activity of catalase in apple flesh tissue. *J. Agric. Food Chem.* 48: 5537-5542.
- Grossman K, Zakut R (1979). Determination of the activity of lipoygenase. *Methods Biochem. Anal.* 25: 303-329.
- Halpin BE, Lee CY (1987). Effect of blanching on enzyme activity and quality changes in green peas. *J. Food Sci.* 52: 1002-1005.
- Hatanaka A, Kajiwara R, Harada T (1975). Biosynthetic pathway of cucumber alcohol. *Phytochemistry*, 14: 2589-2592.
- Hedin PA, Tang B, Creech PG (1995). Effects of bioregulators on development and reproduction of root-knot nematodes in cotton plant roots. *Mississippi Agric. For. Exp. Station Bull.* p. 1028.
- Hildebrand DF (1989). Lipoygenases. *Physiol. Plant*, 76: 249-253.
- Ibrahim ME, Bekheta MA, El-Moursi A, Gaafar NA (2007). Improvement of growth and seed yield quality of *Vicia faba* L. plants as affected by application of some bioregulators. *Aust. J. Basic Appl. Sci.* 1(4): 657-666.
- Kazeniak SJ, Hall RM (1970). Flavour chemistry of tomato volatiles. *J. Food Sci.* 35: 519-530.
- Madhavan S, Benedict CR (1991). Regulation of rubber transferase activity by 2-(3,4-Dichlorophenoxy)-triethylamine in plants of *Parthenium argentatum*. In *Plant Biochemical Regulator*, Ed. Gausman HW, Marcel Dekker Inc. Pubs., New York. pp. 27-43.
- Mika AO, Luthje S (2003). Properties of Guaiacol peroxidase activities isolated from corn root plasma membrane. *Plant Physiol.* 132(3): 1489-1498.
- Muroki NM, Manitim GK, Karuri EG, Tolong HK, Carter E (1997). The development of nutritionally improved foods: Nutribusiness strategy. *J. Nutr. Educ.* 29: 335-342.
- Olaiya CO (2010). Pre-sowing Bioregulator Seed Treatments increase the Seedling Growth and Yield of Tomato (*Solanum lycopersicon*). *Journal of Plant Growth Regulation*. DOI: 10.1007/s00344-010-9142-3. In Press.
- Olaiya CO, Osonubi O (2009). Effects of Pre-sowing Seed Treatments on Tomato *Lycopersicon esculentum* (L.) Mill) Seedling Emergence. *Int. J. Eng. Technol.* 1(4): 321-323.
- Pinsky A, Grossman S, Trop M (1971). Lipoygenase content and antioxidant activity of some fruits and vegetables. *J. Food Sci.* 36: 571-572.
- Polyak-Feher K, Racz-Horvath B, Keeskes T (1992). Effects of blanching and ripeness on green pea peroxidase activity. *Acta Alimentaria*, 21: 261-274.

- Rhodes MJC (1980). The maturation and ripening of fruits. In: Senescence in plants (ed Thimann KV), C.R.C Press, Boto Raton, Flo. pp. 157-205.
- Schormuller J (1974). The importance of enzymatic analysis in food stuff chemistry. In: Methods of enzymatic analysis, ed. Bermeyer HU. Academic Press, London. p. 97.
- Schwimmer S (1981). Source book of food enzymology. AVI Publishing Co. Inc., Westport, CT. p. 110.
- Sekha BPS, Reddy GM (1982). Studies on Lipoxygenase from rice (*Oryza sativa* L.). J. Sci. Food Agric. 33: 1160-1163.
- Shibata D, Axelrod B (1995). Plant lipoxygenases. J. Lipid Mediat. Cell Signal, 12: 213-228.
- Statistica (1997). Statistical Manual, Statsoft, USA.
- Thompson JE, Lagge RL and Barber RF (1987). The role of free radicals in senescence and wounding. New Phytol. 105: 317-344.
- Toyosaki T (1992). Antioxidant effect of riboflavin in enzymic lipid peroxidation. J. Agric Food Chem. 40: 1727-1730.
- Turner WL, Plaxton WC (2001). Purification and characterisation of banana fruit acid phosphatase. Planta, 214: 243-249.
- Worthington XX (1971). Enzyme Manual, Worthington Biochemical Corp., Freehold, New Jersey.