ajfand SCHOLARLY, PEER REVIEWED AFRICAN JOURNAL OF FOOD, AGRICULTURE, NUTRITION AND DEVELOPMENT Volume 17 No. 4 November 2017 PUBLISHED BY AFRICAN SCHOLARLY SCIENCE COMMUNICATIONS TRUST ISSN 1684 5374

Afr. J. Food Agric. Nutr. Dev. 2017; **17(4):** 12547-12556 **DOI: 10.18697/ajfand.80.16550**

EFFECT OF DIFFERENT STRESS TREATMENTS ON MATURE GREEN TOMATOES (*Solanum lycopersicum*) TO ENHANCE FRUIT QUALITY

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AFRICAN JOURNAL OF FOOD, AGRICULTURE, NUTRITION AND DEVELOPMENT
Volume 17 No. 4
November 2017
PUBLISHED BY AFRICAN SCHOLARLY SCIENCE COMMUNICATIONS TRUST
ISSN 1684 5374

ABSTRACT

Tomato (*Solanum lycopersicum*) is a crop of immense economic importance and is grown worldwide. Its fruit is of great nutritional importance as it forms a major part of human diet. Global production of tomato has been estimated at over 153 million metric tonnes in 2009. Consumption of tomato is believed to benefit the heart among other things, as it contains lycopene, one of the most powerful natural antioxidants. The present study was conducted to find the effect of multiple stresses; salt, mannitol, drought and methyl jasmonate on fruit quality of tomato as determined by the evaluation of the content of lycopene, beta-carotene, sucrose and total phenolics. Levels of antioxidants in the tomato after exposure to different stresses during the mature green stage of fruit development were assessed at the Rice Genomics laboratory in the Department of Crop, Soil and Environmental Sciences at the University of Arkansas, USA. Seeds of the tomato cultivar M82 were germinated in the dark for 48 hours. The seedlings were then transplanted into commercial potting soil before being transferred to the greenhouse. The plants were watered every other day until the mature green stage of fruit development (85-90 days post germination). The plants were then divided into five groups and treated with 200mM NaCl, 200 mM mannitol, drought, 100 µM methyl jasmonate and tap water for a period of 72 hours. Afterwards, the stress treatments were removed and fruits allowed to ripen. After ripening the fruits were assayed for lycopene, beta-carotene, phenolics and sucrose content. Tomato plants (*S. lycopersicum*) subjected to salt stress showed the highest increase in lycopene (2.8x) while for other stresses the increase was by 1.1-1.2x. Beta-carotene content was increased by 2.5-2.7x after salt and drought stress were applied. The highest level of phenolic compounds (2.3x) was observed after treatment with methyl jasmonate. Salt stress increased the sucrose content by 3.2x. Thus the application of stress at the mature green stage of fruit development leads to an increase in bioactive compounds in tomato. This condition has the potential for production of enhanced fruit quality in tomato.

Key words: Tomato (*Solanum lycopersicum*), stress, antioxidant compounds, lycopene, beta-carotene, sucrose, phenolics

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INTRODUCTION

Biotic and abiotic stress factors are a huge concern for agricultural production worldwide. It is estimated that by the year 2050, salt stress alone will cause 50% loss in arable land [1]. The negative effects of stress on agriculture are as a result of retarded growth of plants due to decline in photosynthesis [2]. Biomass accumulation is in turn impaired leading to huge losses in yield [3]. For some fruit crops, however, the quality of fruits has been documented to increase as a result of exposure to moderate stress [4, 5]. This is because during stress, plants produce antioxidants as a means of combating damage from free radicals. Antioxidants include compounds such as beta carotene and lycopene which have health benefits for humans [6].

Tomato (*Solanum lycopersicum*) is a crop of immense economic importance worldwide [7]. Tomato is grown worldwide because of its fruit that is of great nutritional importance and forms a major part of human diet. Global production of tomato was estimated at over 153 million metric tonnes in 2009 [8]. Tomato consumption has been linked to reduced risks of cancer especially prostate cancer and reduced occurrence of cardiovascular diseases [9]. This is because tomato contains high amounts of antioxidants such as vitamins A, C and E, carotenoids, flavonoids and phenolic acids [10]. The nutritional importance of tomato has seen it being named the second most important vegetable crop after potato [11].

Consumption of tomato is believed to benefit the heart among other things, as it contains lycopene, one of the most powerful natural antioxidants which, and especially when cooked, has been found to help prevent prostate, lung, stomach, pancreatic, colorectal, esophageal, oral, breast and cervical cancers. The tomato's medicinal properties had already been endorsed in Continental Europe as early as the 16th century [12]. Lycopenes, bioflavonoids closely related to beta carotene, are potent antioxidants present in tomatoes and seem to be responsible for the natural cancer-fighting properties of tomato. Cooking tomatoes more than doubles the effectiveness of the lycopene they contain, while a small amount of olive oil, such as what one would add in a pizza or tomato sauce, intensifies the protective effect further [12].

Owing to its immense importance, studies on improving the fruit quality of tomato have been conducted [2, 3]. The exposure of tomato to salt stress was seen to improve its lycopene content by up to 80% [1]. The effect of stress on yield and shoot height has been determined [2]. Salt stress has been found to increase sugar levels in tomato [13]. Salt stress has been the emphasis for most studies in tomato with little consideration to other forms of stress. With the exception of lycopene, there has been paucity of scientific information on the effect of stress on other antioxidants. The objective of this study was, therefore, to find out the effect of multiple stresses (salt, mannitol, drought and methyl jasmonate) on the fruit quality of tomato, looking at the content of lycopene, beta-carotene, sucrose and total phenolics.

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MATERIALS AND METHODS

The experimental part of this study was conducted from January to May 2015 at the Rice Genomics laboratory in the Department of Crop, Soil and Environmental Sciences at the University of Arkansas, USA. The plants were grown in the greenhouse in the same department.

Seed germination

Seeds of the tomato cultivar M82 were purchased from local stores and germinated in the dark for 48 hours in the laboratory. The seedlings were transplanted into 22 cm pots filled with Redi-earth potting mix (Sun Gro Horticulture Distribution Inc. Bellevue, WA). The plants were grown under greenhouse conditions with day/night temperatures of $26/22^0$C ± 1, light intensity of 600 $\mu mol^{-2}s^{-1}$ and relative humidity (RH) of 60%. The plants were watered every other day until the mature green stage of fruit development (85-90 days post germination).

Application of stress

Upon reaching the mature green stage of fruit development from seed germination, the plants were randomly divided into five treatment groups of six plants each for stress evaluation. The treatments were: i) Drought stress, that is water being withheld from the group of plants, ii) Watering treatment with 200 mM NaCl, iii) 200mM mannitol solution treatment, iv) 100 µM methyl jasmonate treatment and v) Control treatment: watered with tap water. Each stress evaluation treatment was applied for a period of 72 hours to allow for the stress response within the plant while also taking care to avoid physical injury to the plant. The stress treatments were removed after 72 hours and tap water used to water the plants until ripening of the fruits.

Collection of fruit samples

Fruit samples were collected for biochemical analyses after the fruits were fully ripened (100-120 days post-germination).

Preparation of methanolic extract

One hundred (100) mg of fruit tissue was ground in liquid nitrogen and 1ml of absolute methanol added. The mixture was centrifuged at 12000 rpm for 15 min at 4^0C. The supernatant was collected and used for subsequent biochemical analyses. This is the modified method of Alanis *et al.* [14].

Estimation of total phenolic content

To obtain an estimate of the phenolic content in the fruit tissues 0.5ml of methanolic extract was mixed with 2.5 ml of 10-fold dilution Folin- Ciocalteu reagent and 2 ml of 7.5% (w/v) sodium carbonate. The mixture was allowed to stand for thirty min at room temperature and the absorbance was measured at 760 nm [15].

Determination of carotenoid content

To determine carotenoid content, the tissue samples were thawed in the dark in a refrigerator at 4^0C to avoid carotenoid oxidation. Two milliliters of acetone: hexane (4:6) solvent were added to 0.1 g of tomato homogenate and mixed in a test tube; afterwards

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the mixture was spun at 10 000 rpm for fifteen mins at 4^0C. Automatically, two phases separated and an aliquot was taken from the upper solution for measurement of optical density at 663, 645, 505 and 453 nm in a spectrophotometer. Lycopene and beta-carotene contents were calculated according to the equations: [16]

Lycopene (mg/100 ml of extract) = -0.0458 x A_{663} + 0.204 x A_{645} + 0.372 x A_{505}- 0.0806 x A_{453}

β-carotene (mg/100 ml of extract) = 0.216 x A_{663} – 1.22 x A_{645} – 0.304 x A_{505} + 0.452 x A_{453}

Estimation of sucrose

Sucrose was estimated using anthrone reagent. A measurement of 0.1 g of tomato fruit tissue was homogenized in 5 ml of 80% (v/v) ethanol. The hexoses were destroyed by placing the reaction tubes on boiling water bath for 10 min. After cooling, 0.1 ml of 30% (w/v) KOH was added and kept at 100^0C for 10 min. When the tubes were cooled to room temperature (25^0C), 3 ml of anthrone reagent (prepared by mixing 76 ml of concentrated sulfuric acid, 36 ml of water and 0.15 g of anthrone) was added and incubated at 38^0C for 20 min. The absorbance was recorded at 620 nm, with sucrose serving as the standard. The amount of sucrose was expressed as mg g^{-1}[17].

Statistical analysis

Multiple pairwise t-test at P≤0.05 in Microsoft Excel 2010 was utilized for comparing the control with each of the treatments.

RESULTS

Estimation of total phenolic compounds, beta-carotene and Lycopene content

Tomato plants subjected to salt stress showed the highest level of lycopene (2.8x) in contrast to the others that showed an increase by 1.1-1.2x (Figure 1A). The data in the figure represent the mean of three observations (n=3). The vertical bar at the top represents the standard error in each case. Different letters indicate statistical difference at P≤0.05. Salt stress and drought stress increased the β-carotene content by 2.5-2.7x, while for methyl jasmonate and mannitol the increase was by 1.1-1.2x (Figure 1B). The plants treated with 100 µM methyl jasmonate showed the highest level of phenolic compounds in comparison to the control (2.3 x) followed by NaCl and mannitol (1.1-1.2x). Drought stress did not affect the phenolic content (Figure 1C).

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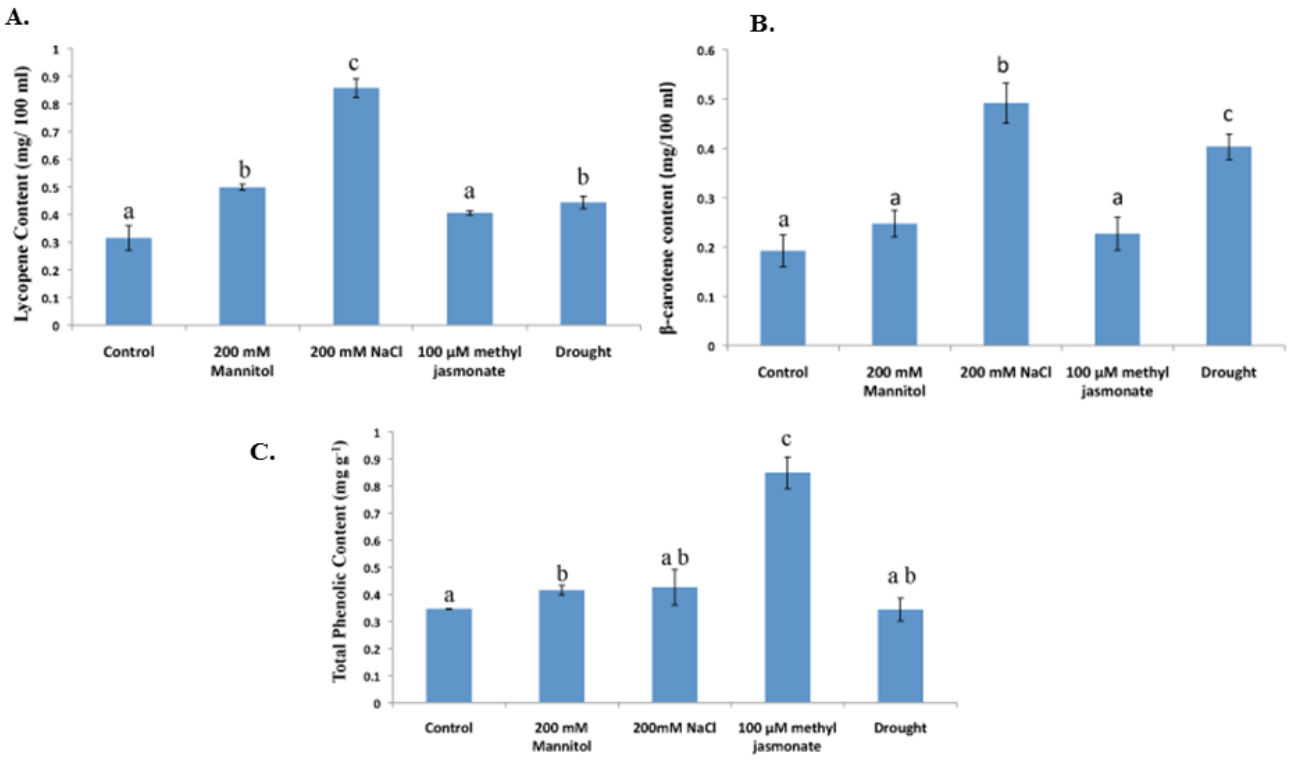

Figure 1: **Lycopene content (A), beta- carotene content (B) and total phenolic content (C) of the tomato plants under stress and control**

Sugars/Osmolytes

The fruits from the control plants had the lowest amount of sucrose while the highest level was in the fruits from plants that were exposed to salt stress (3.2x) followed by mannitol, methyl jasmonate and drought (1.9-2.1x) (Figure 2). Values are expressed as mean ± SE (n=3), and different letters indicate significant difference at P≤0.05.

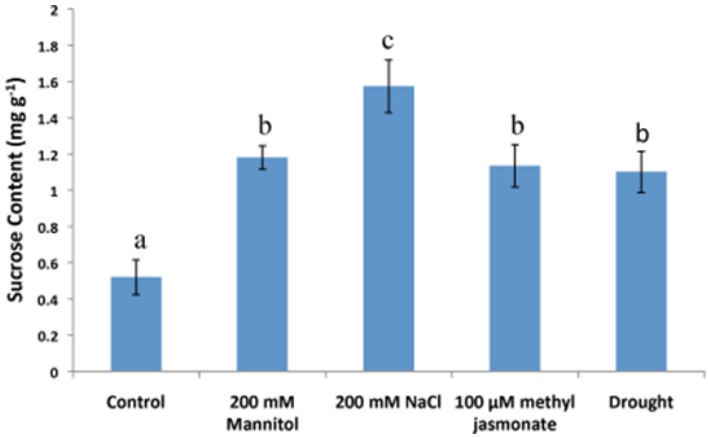

Figure 2: **Sucrose content of the fruits from plants under different stress treatments**

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DISCUSSION

In the present study, tomato plants were subjected to different stresses during the mature green stage of fruit development and the levels of antioxidants and sugar (sucrose) were evaluated. The fruits from plants that had been exposed to different stresses exhibited increase in levels of various antioxidants: lycopene, beta-carotene and phenolic compounds and in level of sucrose.

Tomato plants showed the highest level of lycopene (2.8x) under salt stress compared to mannitol, methyl jasmonate and drought which showed an increase of 1.1-1.2x. Increase in lycopene content following salt stress has been reported [1], which is in agreement with the present study. Post-harvest treatment of tomato fruits with methyl jasmonate resulted in increase in lycopene content [18]. Lycopene is a highly important bioactive compound that has the capacity to combat oxidative stress when consumed by humans [19].

Drought stress increased beta-carotene content by 2.5-2.7x, while treatment with mannitol and methyl jasmonate increased the beta-carotene content by 1.1-1.2x. Similar to lycopene, beta-carotene has an antioxidative function and as such is produced under stress conditions by plants to scavenge free radicals that damage cellular organelles [6]. Beta-carotene is known as a strong antioxidant and is the best quencher for singlet oxygen [19].

Total phenolic content was highest in fruits treated with methyl jasmonate (2.3x) followed by treatment with NaCl and mannitol (1.1-2.1x). Methyl jasmonate is a hormone produced in response to herbivory and triggers several responses in plants including production of antioxidants such as phenolic compounds [20]. The latter are associated with therapeutic tools in inflammatory diseases including cardiovascular diseases, obesity, type II diabetes, neurodegenerative diseases, cancer and aging [19].

Sucrose is one of the compatible solutes that get accumulated in plants, particularly under drought or salt stress [9]. In this study, treatment of plants with NaCl led to the highest increase in sucrose (3.2x). Drought stress, methyl jasmonate and mannitol led to increase of 1.1-1.2x in sucrose content. Salt stress led to increase in levels of fructose and glucose, while sucrose and starch were reduced in a similar study [13].

CONCLUSION

In conclusion, the present study shows that exposing tomato fruits to stress at the mature green stage of fruit development leads to increased antioxidant bioactive compounds. The study presents an alternative method of producing enhanced quality tomato fruits with potential health benefits to humans. The method is cheaper and faster than conventional breeding methods and requires minimal expertise.

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ACKNOWLEDGEMENT

This work was funded by the United States Department of Agriculture and the University of Botswana. I would like to thank Professor Joseph Allotey of the Department of Biological Sciences, University of Botswana for reading through the manuscript and making suggestions.

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