Global Journal of Science Frontier Research: D
Agriculture and Veterinary
Volume 18 Issue 7 Version 1.0 Year 2018
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals
Online ISSN: 2249-4626 & Print ISSN: 0975-5896

A Study on Proline and Glycine Betaine Contents as Salinity and Drought Tolerant Indicators in Solanum Lycopersicum L. (Cultivar: Roma)

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GJSFR-D Classification: *FOR Code: 070199*

Strictly as per the compliance and regulations of:

A Study on Proline and Glycine Betaine Contents as Salinity and Drought Tolerant Indicators in Solanum Lycopersicum L. (Cultivar: Roma)

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I. Introduction

Osmolyte accumulation (OA) is frequently cited as a key putative mechanism for increasing yields of crops subjected to drought and salinity conditions (Levitt 1980). The hypothesis is that OA results in a number of benefits that sustain cell and tissue activity under water-deficit conditions. It has been proposed as an effective tolerance mechanism for water deficits and salinity, which could be enhanced in crops by traditional plant breeding, marker-assisted selection or genetic engineering, to generate drought-tolerant crops (Shibli *et al*., 2007). Under conditions where water deficits and salinity threaten crop survival, yields are so low that even large fractional yield gains offer little practical benefit to growers. Indeed, the often-cited benefit of turgor maintenance in cells is likely to result in crop behaviour that is exactly opposite to what is beneficial to crops (Shibli *et al*., 2007). The one clear mechanism identified in this review for beneficial yield responses to OA is in the maintenance of root development in order to reach water that may be available deeper in the soil profile (Zhu 2001).

Glycine betaine (GB) and proline are two major organic osmolytes that accumulate in a variety of plant species in response to environmental stresses such as drought, salinity, extreme temperatures, UV radiation and heavy metals. Although their actual roles in plant osmo tolerance remain controversial, both compounds are thought to have positive effects on enzyme and membrane integrity along with adaptive roles in mediating osmotic adjustment in plants grown under stress conditions (Ashraf and Foolad 2005).

Pepper (*Capsicum spp.*) are tropical woody vines and herbaceous plant having aromatic herbage minute flower spikellets Members of genus Capsicum are shrubs, perennials or annual herbs. The roots are often rhizomatous and leaves can be either simple with entire margin and are positioned at the base of or along the stem and alternate, opposite or whorled in arrangement (Singh *et al.,* 2012). Vegetable crops are mainly produced from irrigated agriculture and the process of soil salinization is dramatically exacerbated and accelerated by crop irrigation which imports large quantity of salts that were not there before. The majority of crop plant are relatively salt sensitive and unable to tolerate high level of salinity (Zhang *et al.,* 2014).

II. Materials And Methods

The field experiment was conducted in botanical garden Department of Biological Sciences Usmanu Danfodiyo University, Sokoto Nigeria and the laboratory experiment was carried out in the Biology laboratory Department of Biology Federal University Birnin Kebbi, Nigeria.

a) Plant Growth Condition

The seeds of *Capsicum annuum* were collected and surface sterilized in 5% sodium hypochlorite and washed with distilled water. The seeds were sown in the nursery bed in green house, and then uniformly germinated seedlings were 14 days old were selected and transferred to polygene bags containing the mixture of sand and organic manure (3:1) adopting the method of Gumi *et al*. (2013)

b) Solution Formation and Salt stress Inducement

Sodium chloride (NaCl) was weighed and dissolves in water to make 30, 60 and 90 mM concentrations of salt which were used to water the plants. The seedlings were divided in to 8 groups, first represent control, second, third and fourth received 30, 60 and 90 mM of NaCl treatments. The fifth represent control watered twice daily (morning and evening), sixth

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watered once in a day, seventh watered once in two days and eighth watered once in three days (Gumi *et al*., 2013). Each treatment was replicated three times and each replicate consist of three plant and the plant were subjected to the treatment for 27 days.

c) Determination of Free Proline Content

Aliquots of ground plants material is heated for 20 min in pure ethanol as well as in water. The resulting mixture was left overnight at 4°C, and centrifuged at 14000 rpm (5 min). The cold extraction procedure was repeated on the pellet and supernatants pooled and used for the analysis (Carillo *et al*., 2008). In 1.5 ml screw-cap tubes, 1000 μl of reaction mix was pipette with 500 μl ethanolic extract. Proline standard completed with up to 400μl of ethanol: water (40:60 v/v). The sealed tubes, were mixed and heated at 95°C in water bath for 20 min and centrifuge (1 min, 10000 rpm), contents were transferred to a 1.5 ml cuvette tubes and read at 520 nm using spectrophotometer. Free proline content was calculated using the following equation.

$$proline = (Absextract - blank) \div slope(Vol.extract \div Vol.aliquot) \times 1 \div FW$$

Where: Abs extract is the absorbance determined with the extract, Blank (expressed as absorbance) and slope (expressed as absorbance·nmol-1), Volume extract is the total volume of the extract, Vol aliquot is the volume used in the assay, FW (expressed in mg) is the amount of plant material extracted. In plant tissues, proline typically ranges from 0.5 (unstressed) 1 to 50 (stressed) μmol.g^{-1}.

d) Determination of Glycine Betaine Content

Glycine betaine content was determined by frozen the plant material in liquid nitrogen immediately after harvesting, grinded and the pestles were pre chilled in liquid nitrogen. The frozen samples were placed in the mortar and pulverized to a fine powder.

The powder was transferred/weighted to several pre cooled 1.5 mL tubes (eppis) and stored at –80 °C. The samples (40-50 mg FW) were suspended in 1 ml of MilliQ grades water, subjected to a freeze thaw cycle by freezing in liquid nitrogen and thawing at 40°C for 20 minutes, and left overnight at 4°C. Samples were then centrifuged at 14000 g, 4°C for 5 minutes. The clear supernatants were separated from the pellets. The eluted GB (retention time 4-5 min) was detected by measuring the absorbance at 200 nm using a diode-array spectrophotometer (model 7310) and quantified by a comparison of peak surface areas with those obtained with pure GB standard solutions in the range 0.05-4 mM (Carillo *et al*., 2008).

GB content was calculated as follows:

$$GB \quad content = Absorbance \quad peak \quad area \quad exact \div slope \times$$
$$Vol. \quad exact \div Vol.aliquot \times concentration \quad factor$$

Where: Absorbance Peak Area extract is the peak area (absorbance at 200 nm) determined with the extract, slope (expressed as absorbance·nmol -1) is determined by linear regression, Vol. extract is the total volume of the extract, Vol. aliquot is the volume of extract injected onto the HPLC column and FW (expressed in mg) is the amount of plant material extracted.

e) Statistical Analysis

The results were expressed as mean of three replicates and the data obtained were subjected to one way analysis of variance test. The difference between the mean were determined by least significant difference using MINITAB statistical software.

III. Results

The result on the effects of salinity and drought on Proline and Glycine Betaine Content in *Capsicum annuum* were presented in figure 1 and 2.

a) Effects of salinity on proline and Glycine betaine content of Solanum lycopersicum

The free proline and glycine betaine content significantly ($P<0.05$) affected by salt stress episodes in a concentration dependent manner. Control recorded the lowest contents of both proline and Glycine betaine content. However, the lowest contents were observed in plant treated with 90mM of salt (Figure 1). Mean comparison shows significant difference ($p<0.05$) between the control group and salt treated groups.

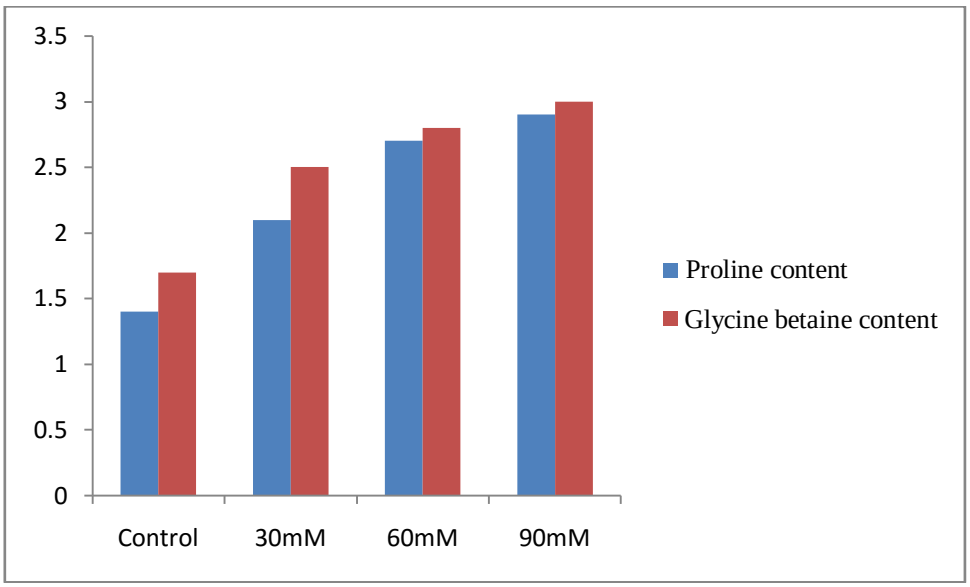

Figure 1: Effects of salinity on free proline and glycine betaine content of *Solanum lycopersicum*

b) *Effects of Drought on proline and Glycine betaine content of Solanum lycopersicum*

Proline and glycine betaine content significantly ($P<0.05$) affected by drought in *Capsicum annuum.* Control recorded the lowest contents of 1.4 and 1.7μMol^{-1} respectively followed by Plants watered once in a day, the lowest contents of 2.9 and 3 μMol^{-1} were recorded in plants watered once in three days. Means comparison did not shows significant difference ($P>0.05$) between the drought induced plants. However, the result shows a significant difference between the control and stress induced plants.

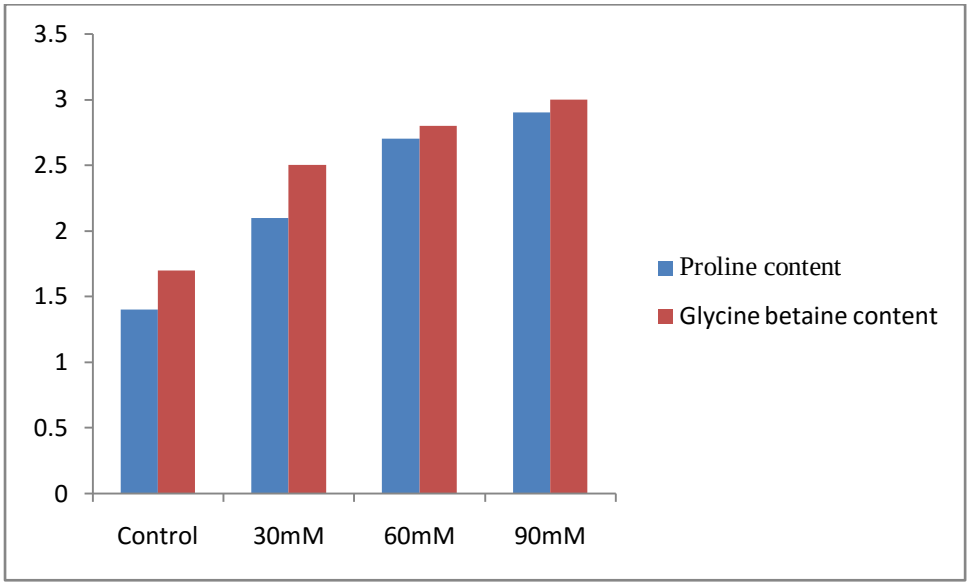

Figure 2: Effects of drought on free proline and glycine betaine content of *Solanum lycopersicum*

IV. Discussion

In this research, the contents of free proline and glycine betaine increase with increasing salt concentration. Similarly, highest proline and glycine betaine contents were recorded in plants watered once in three days, the contents decrease in plant watered once in two days. The accumulation of proline and glycine betaine is often proposed as a solution to overcoming negative effects of water deficit and salinity in crop production. This has been proposed as an adaptive mechanism of salt and drought tolerance (Gumi *et al*., 2013). In this study, the content of proline and glycine betaine did not differ significantly among the drought induced plants, but differ from the control. This explain that higher proline and glycine betaine content

accumulated in stressed plants. This findings agreed with the finding of Gumi *et al*. 2013, Ja'afar *et al*. 2018 and Asraf and Haris, 2004. Carillo *et al*. reported that proline typically ranges from 0.5 is regarded as unstressed and 1 to 50 is regarded as stressed. According to IPGRI descriptors of tomato, Plants which produce GB typically ranges from 0.2-1 is regarded as unstressed and GB content between 6-13 is considered as stressed. This infers that *Solanum lycopersicum* (cultivar: Roma) is a salt and drought tolerant cultivar to some extent.

V. Conclusion

From these findings, Solanum lycopersicum produced low proline and Glycine betaine content under salinity and drought conditions. This could be possible indicator of salinity and drought tolerance in the cultivar studied.

Acknowledgment

Our appreciation and gratitude to Dr. A.M. Gumi of the Department of Biological Sciences Usmanu Danfodiyo University Sokoto for his support in analysis and discussing the finding of this research.

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