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Influence of Salt Stress on Proline and Glycine Betaine Accumulation in Tomato (*Solanum lycopersicum* L.)

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Abstract: Many plants accumulate high levels of free proline content (pro) and glycine betaine (GB) in response to abiotic stress, Pro and GB act as an osmoprotectant. Generally, these levels are high than those required to be used in protein synthesis. Salinity inhibition of plant growth is the result of osmotic and ionic effect and different plant species have developed different mechanisms to cope with those effects. In this study, accumulation of osmolytes of twenty tomato genotypes was evaluated in response to salinity stress. The seedlings of each genotype were divided into three groups, Sodium chloride (NaCl) dissolved in irrigation water to make variant concentration of 30 and 60 mg/L of salt concentration using electrical conductivity meter which were used to water the plants. Level of free proline and glycine betaine were measured. Data obtained were subjected to one way analysis of variance using SPSS (20) Statistical Software. Dry mass accumulation decreased with increased salt concentration in all the genotypes. However, the result differ significantly ($P < 0.05$). The highest dry mass accumulations at control were recorded on Tropimech and Giofranco F. with 6.00 and 5.97. The lowest dry mass accumulations were recorded on plant treated with 60mg/L of salt. Dangainakawa recorded the least accumulation of dry mass on plants treated with 60mg/l of salt with 0.90g followed by Dan Gombe with 1.47g respectively. The highest free proline content of 1.46 $\mu molg^{-1}$ was recorded on Dan gainakawa at plant treated with 60 mg/L of NaCl. The lowest proline content was recorded at control on Giofranco F. with 0.17 $\mu molg^{-1}$ The highest GB content in all the plants were recorded at plants treated with 60 mg/L. However, the highest GB content (1.67) among the 20 ($P<0.05$) were recorded at 60 mg/L in Rio Grande followed by Bahaushe with 1.50 $\mu molg^{-1}$. In conclusion, GB and Pro are osmoregulators produced by tomato in response to stress so as to alleviate the consequence effects of salt stress.

Introduction

Soil salinity is one of the major factors of soil degradation and is recorded 19.5% of the irrigated land and 2.1% of the dry land agriculture existing on the globe [1]. Salinity is conspicuous in arid and semi-arid areas where 25% of the irrigated land is affected by salt [2]. Salinity inhibition of plant growth is the result of osmotic and ionic effect, and different plant species have developed different mechanisms to cope with those effects [3]. Excess amount of salt in the soil adversely affects plant growth and development [4]. Several factors may contribute to reduction in growth exhibited by plant under salinity stress. Processes such as seed germination, seedling growth and vigour, vegetative growth, flowering and fruit set are adversely affected by high salt concentration ultimately causing diminishing economic yield and also quality of production [5]. Salinity also affects the diffusion both at stomata and the mesophyll [6]. Morphology, anatomy, ultra-structure and metabolism of plant species are also deeply affected by salt [7]. Salinity impairs seed germination, reduce nodules formation, retard plant development and reduce crop yield. These concentrations fluctuate because of change in water sources, drainage, evapotranspiration and solute availability [8].

All plants have evolved a cellular mechanism of salt stress survival by either avoiding or tolerating the salt stress. Plants are either dominant during salt stress or ion disequilibrium or alleviate the consequent secondary effect caused by stresses. The chemical potential of the saline solution, initially establish a water potential imbalance between the apoplast and symplast that leads to tugor decrease which if severe enough can lead to growth reduction [9]. The cellular response to turgor reduction is osmotic adjustments. The cytosolic and organellar machinery of halophytes (salt tolerant) and glycophytes (salt sensitive) is equivalently Na^+ and Cl^- sensitive; so osmotic adjustment is achieved in these compartments by accumulation of compartible osmolytes and osmoprotectants [10].

Tomato (*Solanum lycopersicum*), Family Solanaceae, is now grown worldwide [11]. Available for its edible fruits with thousand of cultivars produce red fruits; but a number of cultivars with yellow, orange, pink, purple, green, black or white fruits are available. Tomatoes grown for canning and sauces are often elongated and have lower water content [12]. Tomato plant suffers from various physiological problems in soil (abiotic stress) around the world. Until now, the causes behind such abnormalities in tomato cultivation have not been well understood. Wada et al. [13] reported that calcium concentration of young fruit decreased quickly when fruit fresh weight reaches an average of 20g. There is paucity of information on salt responsive candidate gene in tomato which could be useful in alleviating loss and low yield in tomato production.

Theory

Selected tomato landraces genotypes were obtained from local markets around Sokoto and Zamfara metropolis and identified in the Herbarium of Ahmadu Bello University Zaria. While accessions genotypes seeds were obtained from Zamfara State Agricultural Development Project, Gusau (ZADP) and Indian Agricultural Research Institute New Delhi (IARI). The seeds of 20 genotypes of tomato grouped in to landraces and accessions. The collection locality, type and common name of the cultivar are summarized in Table 1. The experiment was laid down in a completely randomized design (CRD) consisting of three treatments (control with no concentration of NaCl, 30 and 60 mg/L of NaCl).

Table 1. Different tomato genotypes used showing type, collection locality and salt tolerant/ sensitivity

Genotypes	Type	Collection locality	Salt tolerant/Sensitive
Dangainakawa	Landrace	Market	Sensitive
Bahaushe	Landrace	Market	Sensitive
Dandino	Landrace	Market	Sensitive
Dan eka	Landrace	Market	Sensitive
Dan Gombe	Landrace	Market	Tolerant
Dan mazari	Landrace	Market	Tolerant
Dan dubu	Landrace	Market	Sensitive
Dan kwandawa	Landrace	Market	Tolerant
Ganwon Falke	Landrace	Market	Tolerant
Dan dogarawa	Landrace	Market	Tolerant
Roma	Accessions	ZADP	Tolerant
UTC	Accessions	ZADP	Tolerant
Rio grande	Accessions	ZADP	Tolerant
Giofranco F.	Accessions	ZADP	Tolerant
UC82B	Accessions	IARI	Tolerant
Indian Tomato	Accessions	IARI	Tolerant
Tomato peto	Accessions	IARI	Tolerant
Tropimech	Accessions	IARI	Tolerant
Cherry	Accessions	IARI	Tolerant
Heirloom	Accessions	IARI	Tolerant

Materials and Methods

Plant Materials

The field experiment was conducted in the Biological garden of the Usmanu Danfodiyo University Sokoto located at latitude 13^0 0'21.1428" N and longitude 5° 14' 51.1872" E, around December to first week of February 2017. The seeds of the tomato genotypes were surface sterilized by soaking in 5% sodium hypochlorite for 15 minutes and washed with sterile distilled water. The seeds were sown in nursery bed and then uniformly germinated seedlings (10 days old) were selected and transferred to the plots. Sodium Chloride (NaCl) was weight and dissolved in irrigation water to make the variant concentration of 30 and 60 mg/L using electrical conductivity meter. The seedlings of each genotype were divided into three groups: The first group represents the control where no salt was added. The second and third groups received 30 and 60 mg/L of NaCl respectively and replicated three times. After six weeks of salt treatment, the seedlings were harvested and osmolytes content were determined. Shoots and roots were left in the desiccators at 80^0C for 2 days and parameters were computed.

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 [14]. 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. The chemicals for these experiments were purchase from Inquaba Nigeria Limited.

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

Determination of Glycine Betaine

Plant material was frozen in liquid nitrogen immediately after harvesting, grinded and the pestle 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 min, 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 [14]. The chemicals for these experiments were purchase from Inquaba Nigeria Limited.

GB content was calculated as follows:

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

In plants which produce GB, it typically ranges from 0.2-1 (unstressed) to 6-13 (stressed) $\mu mol.g^{-1}$ (IPGRI 2014).

Data Analysis

Data obtained were expressed as mean of three replicates and data were subjected to one way analysis of variance (ANOVA) test. Different between means were determined by least significance difference at P< 0.05, using SPSS Statistical Package version 20.

Result

Dry Mass Accumulation

The results on the effect of dry mass accumulations (g) of tomato genotypes were presented in Fig. 1. Dry mass accumulation decreased with increased salt concentration in all the genotypes. However, the result differ significantly (P< 0.05). The highest dry mass accumulations at control were recorded on Tropimech and Giofranco F. with 6.00 and 5.97. The lowest dry mass accumulations were recorded on plant treated with 60mg/L of salt. Dangainakawa recorded the least accumulation of dry mass on plants treated with 60mg/l of salt with 0.90g followed by Dan Gombe with 1.47g respectively.

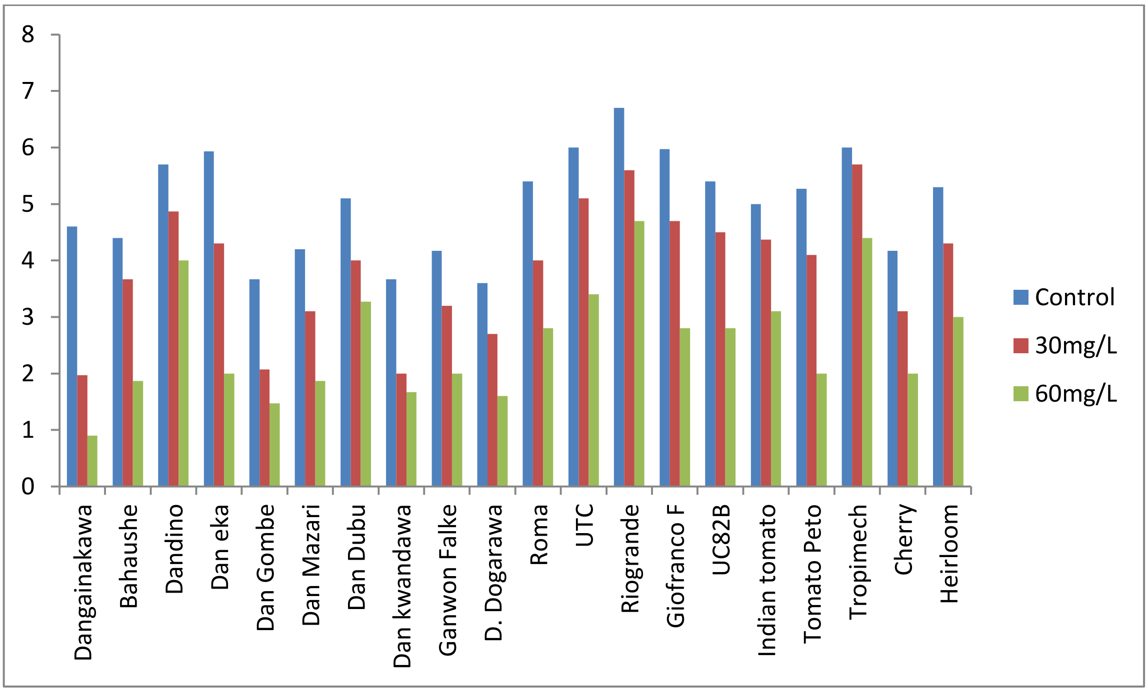

Figure 1. Effect of salt concentration dry mass accumulations in tomato

Determination of Free Proline Content

The free proline content measured as ($\mu molg^{-1}$) of tomato increased with increasing salt concentration (Fig. 2). The highest free proline content of 1.46 $\mu molg^{-1}$ was recorded on Dan gainakawa at plant treated with 60 mg/L of NaCl. The lowest proline content was recorded at control on Giofranco F. with 0.17 $\mu molg^{-1}$ (Fig. 2). The results differs significantly (P< 0.05). Out of the 20 genotype, only 2 (UTC and UC82B) are completely unstressed at both controls and treatments. The lowest proline content at 60 mg/L among the accessions was recorded on UC82B with 0.40 $\mu molg^{-1}$ (Fig. 2).

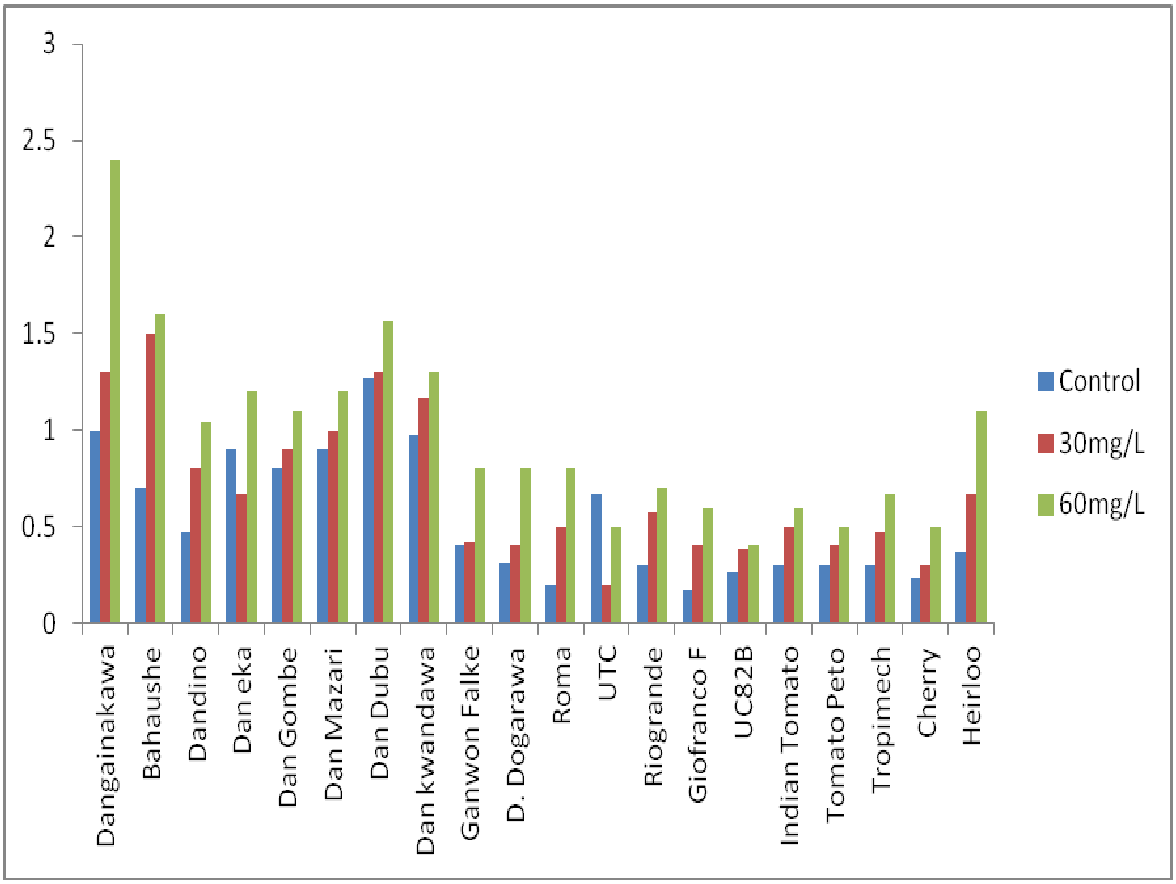

Figure 2. Effects of different concentrations of NaCl on free proline (µmolg^{-1}) content of 20 accessions of tomato

Determination of Glycine Betaine

Salt stress episode significantly differ (P<0.05) affect Glycine betaine content increase with increasing salt concentration in a concentration dependant manner. Controls had the lowest GB content followed by plants treated with 30 mg/L of NaCl. The highest GB content in all the plants were recorded plants treated with 60 mg/L (Fig. 3). However, the highest GB content (1.67) among the 20 (P<0.05) were recorded at 60 mg/L in Rio Grande followed by Bahaushe with 1.50 µmolg^{-1} (Fig. 3).

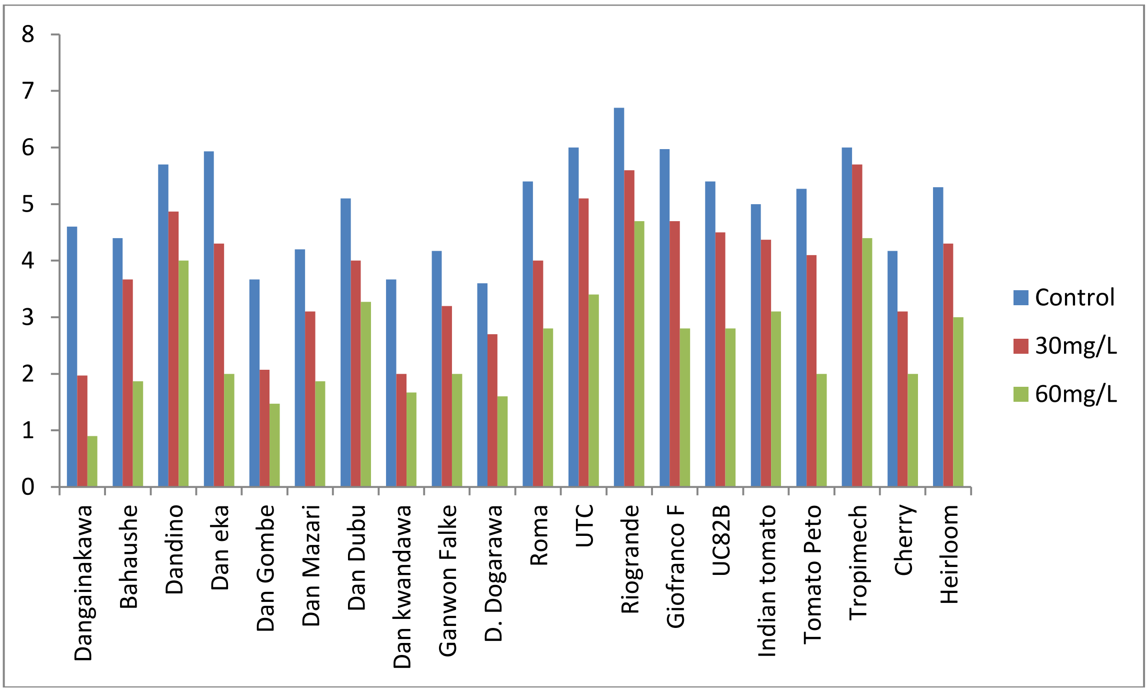

Figure 3. Effects of different concentrations of NaCl on Glycine Betaine (μmolg^{-1}) content of 20 accessions of tomato

Discussion

Growth inhibition of tomato seedlings subjected to NaCl was observed in this study. Treatments resulted to an inhibition of growth in tomato seedlings in this study. The level of free proline and glycine betaine increased in a concentration dependent manner. Pro and GB (osmoprotectant) accumulated upon exposing tomato seedlings to NaCl. Pro and GB participate in the reconstruction of chlorophyll, activate kreb cycle and constitute energy source [16]. In this study, the entire plants both control and salt treated plants showed low level of free pro and GB. Though the concentration increase with increasing salt concentration, accumulation of osmolytes compound is often a solution to overcome the negative consequences of water deficit and as adaptive mechanism for drought and salt tolerance [17]. Carillo et al. [14] reported that proline typically ranges from 0.5 is regarded as unstressed and 1 to 50 is regarded as stressed. According to IPGRI[15] 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. Salinity inhibited dry matter accumulation of tomato genotype in the current study. Similar result was reported by Amini et al. (2007) and Gumi et al. (2012) on the study of cytotoxic ions sequestration and sodium/potassium levels as salt toleranty indicators in tomato.

Conclusion

In conclusion, GB and Pro are osmoregulators produced by tomato in response to stress so as to alleviate the consequence effects of salt stress.

Conflict of Interest

The authors have no any conflict of interest.

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