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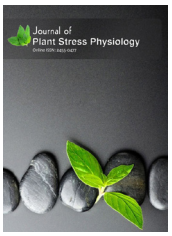
Research Article



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Oxidative defense mechanisms of proline on growth, nutritional compositions and antioxidant activities in water-stressed *Solanum aethiopicum L.*

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ABSTRACT

The growth performance of vegetables is influenced by water availability. This study explored the use of proline as an osmoregulator on growth, nutritional compositions and oxidative enzyme activities in water-stressed *Solanum aethiopicum*. Seedlings of the vegetable were subjected to 20, 40, 60, 80 and 100% proline against droughted and well-watered. Morphological and physiological characters, nutritional compositions and oxidative activities were determined in the vegetable. Plant height (20.37 cm), number of leaves (35.75 cm), Leaf area (347.55 m^2), specific leaf area (72.02 m^2 g^{-1}), leaf area index (0.71 m^2 m^{-2}) relative growth rate (0.21 mg g^{-1} day^{-1}), net assimilation rate (0.058 mg g^{-1} day^{-1}) and leaf area ratio (0.19 m^2 g^{-1}) were higher in *S. aethiopicum* seedlings sprayed with 100% proline. Crude fat (0.11%), ash (1.57%), crude fibre (1.49%), crude protein (2.44%) and carbohydrate (3.50%) were higher in the leaves of the vegetable sprayed with 100% proline. Higher vitamin A (84.21 mg/100 g), vitamin B_3 (0.56 mg/100 g) and vitamin C (10.97 mg/100 g) were observed in the leaves of the vegetable under 100% proline. Furthermore, sodium (8.93 mg/100 g), potassium (402.20 mg/100 g), calcium (121.55 mg/100 g) and magnesium (58.80 mg/100 g) were recorded in the leaves of well-watered. Higher SOD (0.88 mg g^{-1}), APX (0.95 mg g^{-1}), CAT (0.98 mg g^{-1}), GR (0.96 ug g^{-1}) and GST (14.52 mg g^{-1}) were observed in the roots of *S. aethiopicum* droughted. Although all the proline levels sustained growth components, nutritional compositions and oxidative enzymes of *S. aethiopicum* under water stress, however, 100% proline produced better ameliorative effects.

KEYWORDS: Water tolerance, water deficit, nutrient assimilation, growth rate, photosynthesis, chlorophyll

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INTRODUCTION

Solanum aethiopicum (L.) is one of the vegetables consumed in many households in Africa as an important food source for many people (Kamba *et al.*, 2013).The crop is cultivated in the humid zones of West Africa for its immature fruit, in the savanna area frequently for both its leaves and immature fruits (Tehar *et al.*, 2017). Mwinuka *et al.* (2021) described this vegetable as a neglected and underutilized horticultural species. Despite the significant contribution of the vegetable to food security in developing countries such as Nigeria, it has been regarded as an orphan due to limited knowledge and inadequate information regarding the lack of water and sustainable cultivation of the vegetable under limited water supply (Ebert, 2014). According to Emmanuel (2014), adequate water is required for sustainable cultivation of *S. aethiopicum* as it limits growth and development, production efficiency and physiological components of the vegetable. Plants react differently to water stress depending on species, duration, and degree of soil water loss (Fang *et al.*, 2011).

On the other perspective, cuticle thickness, stomatal control, the root system, hormonal balances, oxidative enzymes, protection function, osmotic modification, and tissue water content maintenance are some of the strategic coping mechanisms plants adapt to combat water stress (Fang *et al.*, 2011). Sanchez *et al.* (2012) revealed that grafting as agricultural practice against water stress increased high levels of sugars as well as essential minerals which can improve their nutritional contents under stress conditions. Although drought-tolerant plants are known to possess extensive root systems in order to absorb sufficient water necessary for growth. Seeds of some crop species have a hard seed coat which regulates the nutritional content of the seeds during drought for seed germination (Sakio, 2005).

Regulation of water vapor by varying their stomata aperture and limiting gas diffusion is another method of water stress defensive mechanism in the plant. Various environmental factors such as water supply, light intensity, and photoperiod may influence stomata opening-closing behavior. Other epidermal properties, like the number of epidermal cells, trichomes and epicuticular waxes, play important roles in gas exchange procedures in addition to stomatal characteristics to regulate water in plants (Lobato *et al*., 2021).

Despite these mechanisms, not all vegetables characterize these features as defensive mechanisms. The severe changes in climate being observed recently have triggered adverse weather conditions which have engendered several responses from vegetables against lack of water due to their succulent stems and root system which cannot withstand excess water loss. Erratic changes in climate conditions and their patterns such as unpredictable flooding and drought conditions around the globe necessitate a new approach towards sustainable cultivation of important species even during drought conditions Throughout their whole growing season, *S. aethiopicum* are extremely vulnerable to water tension as they need a large volume of water for growth, production and other cellular activities.

Despite the presence of these parameters many vegetables such as *S. aethiopicum* have gone into extinction because of their inability to cope with excessive water deficit. Therefore, water stress regulatory potentials of the vegetable need to be enhanced using osmoprotectants such as proline. Permanent or temporary water deficit limits the growth and distribution of natural vegetation and also the performance of cultivated plants (Shao *et al*., 2007) therefore this study was conducted to investigate the effects of water stress regulatory potentials of proline on growth, nutritional compositions and level of oxidative enzymes of *S. aethiopicum*.

MATERIALS AND METHODS

Study Area

The study was carried out inside the greenhouse of the Department of Botany, Lagos State University, Ojo campus, Nigeria.

Sources of Seeds

Seeds of S. aethiopicum were purchased at Gbonje market, Okeho, Oyo State, Nigeria. The seeds were identified at the Forestry Research Institute of Nigeria, Ibadan, Nigeria with voucher number FHI-5341.

Soil Collection and Nursery Preparation

The soil used was collected according to the National Soil Characterization Database of the United States Department of Agriculture, modified by Dhotare *et al.* (2019) and Ojewumi *et al.* (2022a, b). Topsoil was collected randomly from different fallowed lands of teaching and research botanical garden, Lagos State University, Ojo using a soil probe at a depth of 0-10 cm at 500 m apart, mixed thoroughly and poured into twenty five perforated planting buckets.

Seedlings of *S.* aethiopicum were raised in the greenhouse of the Department of Botany, Lagos State University, Lagos, Nigeria for 21 days. The seedlings (One seedling per bucket) were randomly transplanted into the planting buckets when they were 14.5 and 155 cm high and thereafter watered for 7 days to ensure acclimatization of the seedlings.

Sources of Osmolytes (Proline)

L-proline was purchased from a chemical shop at Loba Chemie Pvt. Ltd., 107 Wodehouse Road, Jehangir Villa, Mumbai-40005, India.

Preparation and Application of Proline

L-proline was prepared by modifying method of Ojewumi and Kadiri (2021). Exactly 1 g of L-proline was measured and dissolved in 1 L of water. From the 1 L of proline produced 200, 400, 600, 800, and 100 mL proline were measured using measuring cylinder and diluted in 800, 600, 400, 200 and 100 mL distilled water. These constituted 20, 40, 60, 80 and 100% proline.

Experimental Design of the Study

The planting buckets with seedlings (one seedling per bucket) were arranged in a completely randomized design with four replicates.The seedlings were applied with 100 mL of 20, 40, 60, 80, and 100% proline (Ojewumi *et al*., 2022a). Seedlings treated with 100 mL distilled water daily (Well-watered) and those treated with 100 mL distilled water once a week (droughted) served as controls. The treatments were applied using the foliar application for 5 weeks.

Data Collection

Agronomic parameters such as plant height, number of leaves, leaf area, shoot and root, fresh and dry weight of the vegetable were measured. The number of leaves was determined using physical count at two weeks interval (Kadiri, 2014). From each treatment and control, a whole stand was uprooted separately, washed and measured from the base to the root tip of the terminal bud using a thread and a meter rule calibrated in centimeters as described by Kadiri *et al.* (1999).

Determination of Total Leaf Area, Specific Leaf Area and Leaf Area Index

The total leaf area was measured using a Leaf area metre (Ojewumi *et al*., 2022b). Values of leaf area and weight of leaves were used to determine physiological parameters such as specific leaf area (SLA) and leaf area index (LAI), relative growth rate, net assimilation rate and leaf area ratio according to Alireza *et al.* (2012) as shown below

$$SLA = \frac{Leaf\ area}{\text{Corresponding weight of leaf}}$$

$$LAI = \frac{Leaf\ area}{Area\ of\ litter\ fall}$$

$$RGR = \frac{Log_e W_2 - Log_e W_1}{t_2 - t_1}$$

$$NAR = \frac{W_2 - W_1}{A_2 - A_1} - \frac{Log_e A_2 - Log_e A_1}{t_2 - t_1}$$

$$LAR = \frac{W_2 - W_1}{t_2 - t_1} - \frac{Log_e A_2 - Log_e A_1}{W_2 - W_1}$$

Where A_1 = Area of leaf at t_1, A_2 = Area of leaf at t_2, W_1 = first measured weight (g), W_2 = second measured weight (g), T_1 = initial time (weeks) and T_2 = final or second time (weeks).

Determination of Chlorophyll Contents in *S. aethiopicum* Leaves

Chlorophyll A and chlorophyll B and total chlorophyll in *S. aethiopicum* leaves were determined using the acetone extraction technique described in Gul *et al.* (2010). Two (2 g) powder of leaves of the vegetable was extracted with 10 mL of 80% acetone in a test tube wrapped with aluminum foil and then centrifuged at 5000 rpm for 10 minutes. The absorbance of the supernatant produced was measured at 663 nm and 645 nm using a spectrophotometer. The concentrations of chlorophyll a and b were calculated using the below formula:

Chlorophyll a (µg/mL) = (12.7 x A663) - (2.69 x A645) x V/W

Chlorophyll b (µg/mL) = (22.9 x A645) - (4.68 x A663) x V/W

Where:
A663 and A645 are the absorbance values obtained at their respective wavelengths.
V= the volume of the extract and w = weight of the sample used for extraction.

Proximate Contents of *S. aethiopicum*

Crude fat: Two grams of the crushed sample of *S. aethiopicum* were kept using paper thimble in a known weight fat extractor. Approximately 80 mL of C_6H_{14} was added, refluxed, allowed to cool and weighed. Crude fat was determined using the formula below (Ojewumi *et al.*, 2021).

$$\text{Crude fat (\%)} = \frac{weight\ of\ flask\ with\ fat - weight\ of\ empty\ flask}{Weight\ of\ original\ sample} \times 100$$

Crude fiber: Two grams of defatted samples of the *S. aethiopicum* were boiled in 20 mL of 1.25% H_2SO_4 for 30 min, filtered, washed in hot water and boiled again using 200 mL of 1.25% NaOH for another 30 min. The spotless beaker was dried (100+5 °C), cooled and the weights of the contents were measured. Both the spotless beakers with its content were dried using a muffle furnace (9320F-11120F) for 2-4 hours, cooled and weighed. The crude fiber was determined according to AOAC (2000) as shown below;

$$Crude\ Fiber = \frac{(weight\ \ of\ spotless\ beaker\ and\ crude\ fiber - weight\ of\ spotless\ beaker\ and\ crude\ fiber}{Weight} \times 100$$

Total carbohydrate =100-(%moisture+%effluent + % fat + %protein + %fibre) (AOAC, 1980)

Crude protein: Total nitrogen in the content was determined using Micro-Kjeldahl Method. Protein content (%) was determined using the formula below

$$\text{Protein (\%)} = \frac{v\,x\,1.4\,x\,6.25\,x\,0.1N\,HCL\,x\,vol\ \ (used)}{W \times A \times 1000} \times 100$$

where;
V = Titter value
1.4 = Weight of nitrogen expressed in gram in the formula.
6.25 = Protein factor
N = Total nitrogen content
W = Weight of sample
A = Aliquot digested sample used for distillation.

Determination of Mineral Elements in *S. aethiopicum*

One gram of the sample was assayed for calcium, potassium, magnesium, zinc, iron, phosphorus and sodium using Atomic Absorption Spectrophotometer (Perkin-Elmer Model 2280). Total phosphorus and iron were determined calorimetrically.

Determination of Carotene in *S. aethiopicum*

Carotene was determined according to the method of AOAC (2000) and Ojewumi and Oyebanji (2020). Two (2 g) of powder sample of the vegetable was weighed into a flat bottoms reflux flask, 10 mL of distilled water was added and shaken after which 25 mL of alcoholic KOH solution was added and a reflux condenser attached. The mixture was heated using a boiling water bath for one hour, shaken, cooled and about 30 mL of water was added followed by hydrolysate, transferred into a separator funnel and the solution was extracted thrice with 250 mL quantities of chloroform. Also, 2 g of anhydrous sodium sulphate was added to the extract to remove traces of water. The mixture was then filtered into 100 mL volumetric flasks and made up to mark with chloroform.

The standard solution of B-carotene ranged from 0- 50 µg/mL was prepared with chloroform by dissolving 0.003 g of standard L-carotene in 100mL of chloroform. The above gradients of different standard solutions prepared were determined with reference to their absorbance from which the average gradient was taken to calculate Vitamin A (B carotene in µg/100 g) using Spectrophotometer (Metrohm Spectronic 21D Model) at 328 nm.

Niacin: Five 5 g of the sample was treated with 50 mL of 1 N H_2SO, and shaken for 30 minutes. Thereafter, 3 drops of the ammonia solution were added to the sample and filtered. Afterwards, 10 mL of the filtrate was added into a 50 mL volumetric flask and 5 mL of 0.02 N H_2SO_4, 470 nm (AOAC, 2000; Hussian *et al*., 2006, 2011).

Ascorbic acid: One gram of the sample was weighed in a 25 mL conical flask. Then 10 mL of oxalic acid (0.05 M)-EDTA (0.02 M) solution was added and placed in the sample for 24 hours to provide the required reaction time. After 24 hours, the samples were filtered through using 0.45pm filter paper. Then 2.5 mL of each sample was transferred to a separate 25 mL volumetric brown flask, after which 2.5 mL of the oxalic acid (0.05 m)-EDTA (0.02 m) solution was added. Subsequently, metal phosphoric acid was added separately with acetic acid (0.5 mL), 1, 50, (5% v/v) solution (1 mL) and ammonium molybdate solution (2 mL) each volumetric brown flask and the volume made up to 25 mL with distilled water. The absorbance was measured at 760 nm in a UV/visible spectrophotometer.

Tocopherol: One (1 g) of powder sample was weighed into a conical flask of 250 mL and filtered after which 10 mL of absolute alcohol and 20 mL of IM alcoholic H_2SO_4 was added. The condenser and flask were wrapped in Aluminum foil and refluxed for 45 minutes and cooled for another 15 minutes. Fifty (50 mL) of distilled water was added to the mixture and transferred to a 250 mL separating funnel covered with Aluminum foil. The unsaponifiable matters in the mixture were extracted with 5 x 30 mL dimethyl ether. The combined extracts were washed free of acid and dry evaporated at a low temperature and the residues obtained were dissolved in 10 mL absolute alcohol. Aliquots of the sample's solutions and standards (0.3-3.0 mg vitamin E) were transferred to a 20 mL volumetric flask and 5 absolute alcohol was added, followed by 1 mL concentrated Nitric acid. The flasks were placed on a water bath at 90 °C for exactly 3 minutes from the time the alcohol began to boil, volume with absolute alcohol and absorbance was taken at 470 nm against a blank containing absolute alcohol and 1 mL concentrated Nitric acid (HNO_3) was treated in a similar manner (AOAC, 2000).

$$\text{Tocopherol (µg/100 g)} = \frac{\text{Absorbance of sample} \times \text{Gradient factor} \times \text{Dil. Factor Wt of sample}}{\textit{weight of sample}}$$

Determination of Pantothenic Acid in *S. aethiopicum*

Three (3 g) samples of the vegetable were weighed and shaken with 200 mL deionized water for 5 min, diluted using distilled water and the mixture was filtered into a 100mL volumetric flask. Thereafter, 5 mL of a liquor filtrate produced was pipetted into a beaker. Five (5 mL) of 12% potassium bromide and 10 mL of $KMNO_4$ were added, mixed in glass and transferred to a stopper flask put in a boiling water bath for 10 mins. The hot solution was cooled after which 20% freshly prepared H_2SO_4 was added, heated again for another 10 min and allowed to cool.

The yellow precipitate was obtained. The precipitate was dried in an oven at 100 °C for 30 minutes, dissolved in a hot pyridine solution and mixed to form a homogenous suspension. Also, the suspension formed was filtered into a 50 mL volumetric flask and made up to mark with pyridine solution. The Aliquot of the solution above was pipetted into 150 mL of flask 40 mL of deionised water and 5 mL of 5 M NaOH solution were added separately to develop the blue colour. The absorbance of sample and standard pantothenic and solution of range 10 ug/mL to 50 pg/mL prepared from µg/mL stock pantothenic acid were read at 570 nm.

$$\text{Pantothenic acid (µg)} = \frac{\textit{Absorbance of sample} \times \textit{average gradient factor} \times \textit{gradient factor}}{\textit{weight of samples}}$$

Determination of Pyridoxine in *S. aethiopicum*

One gram (1 g) of the sample was extracted using 0.5 g ammonium chloride, 45 mL of chloroform and 5 mL of absolute ethanol. The mixture was mixed in a separating funnel for 30 minutes, after which 5 mL of deionized water was added. The chloroform layer containing the pyridoxine was filtered into a 100 mL volumetric flask and made up to mark with chloroform. Also, 0-10 ppm vitamin B6 standard solutions were prepared and treated as described above, and their absorbance was measured using a spectrophotometer at 415 nm. Vitamin B6 in the sample was determined using the formula below.

$$\text{Pyridoxine (µg)} = \frac{\text{Absorbance of sample X gradient factor X gradient factor}}{\text{weight of sample}}$$

Determination of Phylloquinone in *S. aethiopicum*

Five (5 g) of the sample was weighed and 30 mL of Butyl alcohol was added and shaken to obtain a homogeneous solution and the resulting mixture was filtered into a 100 mL volumetric flask and made up to mark with butyl alcohol. 10 mL aliquot of the filtrate was pipetted into a 30 mL centrifuge tube and 4 drops of 2, 4- dinitrophenyl hydrazine was added to develop the blue colour later changed to bluish green upon addition of 3mL of alcoholic ammonia. Standard solutions of vitamin K from 0-20 µg/mL were prepared and treated to obtain a gradient factor. The Absorbances of standards and samples were read at 480 mm.

$$\text{Phylloquinone (µg)} = \frac{\text{Absorbance of sample X average gradient factor X gradient factor}}{\text{weight of samples}}$$

Determination of antioxidants activity in root and leaves of *Solanum aethiopicum*

Catalase activity (CAT)

The catalase activity was assayed by measuring the initial rate of disappearance of H_2O_2 (Shishira *et al.*, 2016). Catalase reaction solution contained 50 mmol L^{-1} sodium-potassium phosphate buffer (pH 7.0), 10 mmol L^{-1} H_2O_2 and 20 µL enzyme extract in a final assay volume of 1 mL. The decrease in H_2O_2 was measured following the changes in the absorbance of the reaction solution at 240 nm. The concentration of CAT was calculated using an extinction coefficient $\varepsilon = 0.036$ L $mmol^{-1}$ cm^{-1} One unit of CAT is defined as the enzymatic activity that catalyses the degradation of 1 µmol H_2O_2 per minute.

Glutathione reductase (GR)

Glutathione reductase activity was determined using a reaction solution containing 50 mmol L^{-1}sodium-phosphate-buffer (pH 7.5), 5 mmol L^{-1} EDTA, 1 mmol L^{-1} NADPH, 1 mmol L^{-1} oxidized glutathione (GSSG) and 300 µL enzyme extract in a final assay volume of 1 mL. NADPH oxidation was determined at 340 nm (Foyer *et al.*, 1991). The activity was calculated using an extinction coefficient $\varepsilon = 6.22$ L $mmol^{-1}$ cm^{-1} for NADPH. One unit of GR is defined as the enzyme activity that oxidizes 1 µmol NADPH per min.

Superoxide dismutase (SOD)

Superoxide dismutase activity in the root and leaves of the vegetable was evaluated by measuring its ability to inhibit the photochemical reduction of nitro-blue tetrazolium (NBT) to blue formazan by flavins under illumination Masia (1998). Superoxide dismutase reaction solution contained 50 mmol L^{-1} sodium-potassium phosphate buffer (pH 8.0), 300 µmol L^{-1} methionine, 1.5 mmol L^{-1} NBT, 120 µmol L^{-1} M1 riboflavin, 100 mmol L^{-1} Na^2 EDTA, 300 µmol L^{-1} potassium cyanide and 100 µL enzyme extract in a final assay volume of 1 mL. The riboflavin was added last. The reaction was started by illuminating the test tubes under 4 fluorescent lamps for 10 min. The absorbance was measured by spectrophotometry at 560 nm. One unit of SOD activity is defined as the amount of enzyme that inhibits 50% of NBT photo-reduction versus a blank cell containing no enzymatic extract.

Superoxide Peroxidase (SP)

The peroxidase activity was determined using 4-methylcatechol as substrate. The increase in the absorption caused by the oxidation of 4-methylcatechol by H_2O_2 was measured at 420 nm spectrophotometrically. The reaction mixture contained 100 mM sodium phosphate buffer (pH 7.0), 5 mM 4-methylcatechol, 5 mM H_2O_2 and 500 µL of crude extract in a total volume of 3.0 mL at room temperature. One unit of enzyme activity was defined as 0.001 change in absorbance per min, under assay conditions.

Glutathione S-transferase

Glutathione S-transferase (GST) activity was assayed with 1 mM 1-chloro-2,4-dinitrobenzene and 1 mM reduced GSH in 1 mL phosphate buffer (pH 6.5). The reaction was monitored and an increase in OD_{340} was measured. Also, activities on other substrates were assayed according to Shishira *et al.* (2016).

Statistical Analysis

A one-way analysis of variance (ANOVA) was performed using a statistical analysis system software program. The means and standard errors were calculated. The significant differences were determined using Duncan's Multiple Range Test (DMRT) at $p < 0.05$.

RESULTS

Effects of Varying Levels of Proline on Morphological Characters of *Solanum aethiopicum*

Effects of varying levels of proline on *S. aethiopicum* height are presented in Figure 1. Results showed that proline levels had non-significant effects on *S. aethiopicum* height at 1 and 2 weeks after treatment whereas the treatment produced a significant increase in the parameter with the inclusion of various percentages of the treatments between 3-5 WAT.

The highest height (20.37 cm) was observed in the vegetable sprayed with 100% proline. A similar significant increase ($p<0.05$) was noticed in the effect of the treatments in number of leaves. The highest number of leaves (35.75) was observed in the vegetable sprayed the treatments (Figure 2) and least of the parameters stated above were recorded in *S. aethiopicum* droughted.

Effects of varying Level of Proline on Physiological Attributes of *S. aethiopicum*

Table 1 revealed that the varying levels of osmolyte produced significant variations in the physiological attributes of the

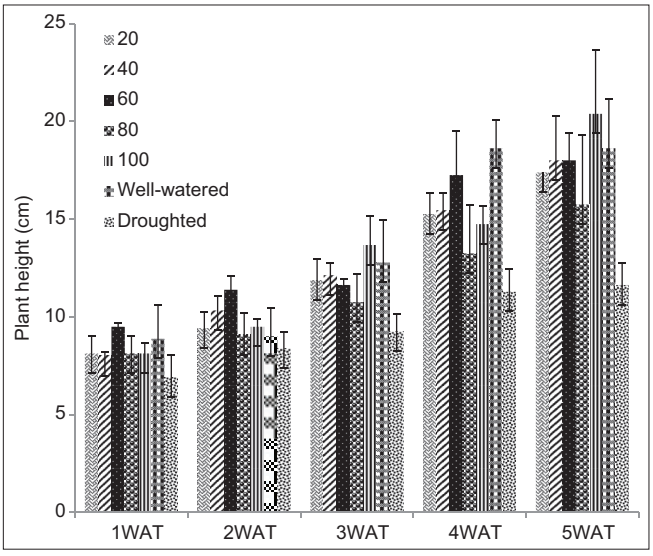

Figure 1: Effects of varying percentage of proline on height of *S. aethiopicum*

vegetable. Higher leaf area (347.55 cm^2), specific leaf area (72.02 $m^2 kg^{-1}$) and leaf area index (0.71 $m^2 m^{-2}$) were recorded in the leaves of the vegetable spayed with 100% proline, followed by values of the parameter in the well-watered vegetable. RGR (0.21 mg g^{-1} day^{-1}), NAR (0.058 mg g^{-1} day^{-1}) and LAR (0.19 $m^2 g^{-1}$) were higher in the well-watered vegetable followed by the vegetables treated with 100% proline.

Effects of varying Levels of Proline on Chlorophyll Contents of *S. aethiopicum* Leaves

Investigation on the effects of the osmolyte on chlorophyll contents and its types on the leaves of the vegetable showed that the treatments produced significant variations in the pigment. Chlorophyll a (1.55 mg/g), Chlorophyll b (2.12 mg/g) and total Chlorophyll (3.74) were significantly higher in the leaves of *S. aethiopicum* sprayed with 100% proline followed by 80% proline while the least of them were observed in droughted (Table 2).

Influence of Varying Levels Proline on Nutritional Compositions of *S. aethiopicum* Leaves

Table 3 revealed that the proximate contents of the leaves of *S. aethiopicum* are affected by the level of inclusion of proline. Hundred (100%) proline produced a significant increase in dry matter (9.87%), fat (0.7 %), ash (1.57%), crude fibre (1.49%), crude protein (2.44%) and carbohydrate (3.20%) of leaves of the vegetable. This was preceded by the values of the parameters in the leaves of the vegetable well-watered while the least of them were noticed in the vegetables droughted.

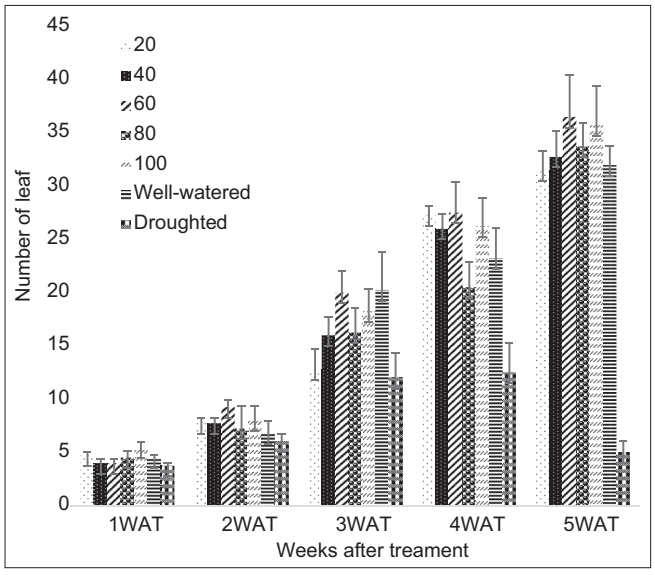

Figure 2: Effects of varying percentage of proline on number of leaf of *S. aethiopicum*

In addition, the highest carotene (84.21 mg/100 g), vitamin B_3 (0.56 mg/100 g), vitamin C (10.97 mg/100 g) and vitamin K (64.15 mg/100 g) were observed in the leaves of *S. aethiopicum* sprayed with 100 % proline while vitamin B_5 (0.06 mg/100 g) and vitamin E (0.79 mg/100 g) were recorded in the leaves of the vegetables sprayed with 80% proline (Table 4).

Furthermore, sodium(8.93 mg/100 g), Potassium (402.20 mg/100 g), calcium (121.55 mg/100 g), magnesium (58.80 mg/100 g), phosphorus (90.87 mg/100 g) and zinc (0.97 mg/100 g) were higher in the leaves of the vegetables well-watered followed by 100% proline while the least of them were observed in the vegetables droughted (Table 5).

Effects of Varying Levels of Proline on Oxidative in *S. aethiopicum* Leaves and Roots

Table 6 showed the effects of proline levels of concentrations of oxidative enzymes in the roots of *S. aethiopicum*. Results had it that higher SP (20.10 u/mL) was recorded in plants sprayed with 100% proline while SOD (0.88 mg g^{-1}), APX (0.95 mg g^{-1}), CAT (0.98 mg g^{-1}), GR (0.96 ug g^{-1}), GST (14.52 mg g^{-1}) and were observed in the roots of the vegetables droughted.

DISCUSSION

Erratic climate changes in abiotic stress are some of the major constraints affecting the production and losses of vegetables. A typical example of such factor is extreme temperatures which often results in water deficiency. It disturbs plant growth, reduces productivity and in severe cases, leads to the total death of plants (Raza *et al.*, 2023). To these effects, plants have developed numerous natural strategies to mitigate the detrimental effects of the phenomenon both on fauna and flora. Results of the present findings connote that morphological parameters (plant height and number) of *S. aethiopicum* leaves subjected to water stress were influenced by various levels of the osmolyte most especially 100% proline. This observation may suggest that 100% proline provided the plants with better water stress coping mechanism as scavenger and consequently

Table 1: Effects of varying levels of proline physiological attributes of *Solanum aethiopicum*

Proline levels (%)	LA (cm^2)	SLA (m^2kg^{-1})	LAI (m^2m^{-2})	RGR (mgg^{-1} day^{-1})	NAR (gm^{-2} day^{-1})	LAR (m^2kg^{-1})
20	260.01 ± 48.83^{ab}	62.02 ± 6.71^{ab}	0.53 ± 0.10^{ab}	0.09 ± 0.03^{a}	0.01 ± 0.00^{b}	0.08 ± 0.00^{a}
40′	241.71 ± 0.36^{ab}	59.22 ± 6.82^{b}	0.49 ± 0.00^{ab}	0.06 ± 0.01^{a}	0.01 ± 0.00^{b}	0.09 ± 0.00^{a}
60	289.92 ± 68.94^{ab}	57.18 ± 3.13^{b}	0.59 ± 0.14^{ab}	0.09 ± 0.06^{a}	0.01 ± 0.00^{ab}	0.14 ± 0.00^{a}
80	218.67 ± 36.78^{ab}	56.93 ± 4.48^{b}	0.45 ± 0.08^{ab}	0.21 ± 0.01^{a}	0.01 ± 0.00^{b}	0.06 ± 0.00^{a}
100	347.55 ± 27.39^{a}	72.02 ± 0.33^{a}	0.71 ± 0.06^{a}	0.17 ± 0.01^{a}	0.01 ± 0.00^{ab}	0.18 ± 0.00^{a}
Well- watered	188.76 ± 62.20^{b}	64.69 ± 4.77^{ab}	0.43 ± 0.11^{a}	0.09 ± 0.01^{ab}	0.01 ± 0.00^{a}	0.190 ± 0.00^{a}
Droughted	198.12 ± 34.05^{ab}	54.35 ± 13.42^{b}	0.40 ± 0.07^{b}	0.05 ± 0.02^{a}	0.01 ± 0.00^{ab}	0.04 ± 0.00^{a}

Mean values (± Standard Error) with different superscripts in columns are significantly different ($p < 0.05$) LA=Leaf area, SLA=Specific leaf area, RGR=Relative growth rate, LAR=Leaf area ratio, NAR=Net assimilation rate

improved the growth characteristic of the vegetable. Exogenous application of proline at 100% to stressed vegetable might have improved the water stress capacity of the vegetable through osmo-adjustment (Deivanai *et al*., 2011; Dawood & Sadak, 2014).

The significant increase observed in the number of leaves of the vegetable sprayed with 100 % proline might be due to the ability of proline to promote axillary bud formation (Liu *et al*., 2020) or enhance the formation of meristematic cells which might have resulted in cell differentiation and multiplication of its parts such as leaves and stems (Anjum *et al*., 2011; Khanna-Chopra *et al*., 2019). In addition, the results of this study indicate an increase in the physiological attributes of *S. aethiopicum* plants sprayed with 100% proline. The increase observed in leaf area and specific leaf area and leaf area index could indicate that the osmolyte characterize ability not only to act as osmoprotectants or source nutrients for plants for the vegetables under water stress but it could also increase appreciably unit of biomass production (Semida *et al*., 2020), light interception and photosynthetic rates (Semida *et al*., 2020), and leaf longevity, water use efficiency and precipitation, energy conversion and water balance of the vegetable (Bahadur *et al*., 2011). This submission is in agreement with the findings of Ali *et al*. (2007) who opined that the application of proline enhanced growth components and maintained the nutrient status of plants by promoting the uptake of K^+, Ca^+, P and N in Zea mays plants exposed to drought stress.

Furthermore, the increase noted in relative growth rate, net assimilation and leaf area ratio could imply that proline could ameliorate the adverse effect of low water availability and

Table 2: Effects of varying levels of proline on chlorophyll contents of *Solanum aethiopicum*

Proline levels (%)	Chlorophyll types (mg/g)		
	Chlorophyll A(μg cm^{-2})	Chlorophyll B (mg m^{-2})	Total Chlorophyll
20	1.12±0.00^{c}	0.93±0.03^{cd}	2.01±0.00^{d}
40	1.21±0.00^{b}	0.94±0.00^{cd}	2.02±0.00^{d}
60	1.25±0.03^{b}	1.00±0.01^{c}	2.26±0.03^{c}
80	1.52±0.03^{a}	1.17±0.02^{b}	2.69±0.03^{b}
100	1.55±0.03^{a}	2.12±0.03^{a}	3.67±0.03^{a}
Well-watered	1.14±0.00^{c}	0.91±0.01^{d}	2.05±0.01^{d}
Droughted	1.09±0.00^{c}	0.83±0.02^{e}	1.91±0.00^{e}

Mean values (± Standard Error) with different superscripts in columns are significantly different ($p < 0.05$)

Table 3: Effects of varying levels of proline on proximate of *Solanum aethiopicum*

Proline levels (%)	Proximate (%)						
	Moisture	Dry matter	Fat	Ash	Crude fibre	Crude protein	Carbohydrate
20	90.82±0.02^{a}	9.30±0.08^{b}	0.47±0.13^{c}	1.40±0.01^{b}	1.34±0.01^{b}	2.15±0.01^{c}	3.06±0.02^{b}
40	91.03±0.00^{a}	9.87±0.01^{a}	0.58±0.03^{bc}	1.21±0.00^{d}	1.38±0.01^{b}	2.25±0.02^{b}	3.04±0.02^{b}
60	92.69±0.03	7.40±0.03^{d}	0.54±0.02^{bc}	1.30±0.03^{c}	1.19±0.02^{c}	1.91±0.03^{d}	2.60±0.03^{d}
80	91.61±0.03^{a}	8.48±0.03^{c}	0.68±0.03^{ab}	1.46±0.03^{b}	1.38±0.03^{b}	2.22±0.04^{bc}	2.90±0.03^{c}
100	63.74±26.94^{a}	9.37±0.02^{b}	0.77±0.03^{a}	1.57±0.010^{a}	1.49±0.01^{a}	2.44±0.02^{a}	3.20±0.03^{a}
Well-watered	92.55±0.58^{a}	8.49±0.03^{c}	0.69±0.01^{ab}	1.56±0.02^{a}	1.49±0.01^{a}	2.40±0.03^{a}	3.20±0.03^{a}
Droughted	93.55±0.06^{a}	6.62±0.06^{e}	0.44±0.04^{c}	1.19±0.03^{d}	1.05±0.03^{d}	1.78±0.03^{e}	2.20±0.03^{e}

Mean values (± Standard Error) with different superscripts in columns are significantly different ($p < 0.05$)

Table 4: Effects of varying levels of proline on vitamins of *Solanum aethiopicum*

Proline levels (%)	Vitamins (mg/100 g)						
	Carotene	Niacin	Pantothenic acid	Pyridoxine	Ascorbic acid	Tocopherol	Phylloquinone
20	56.77±0.03^{e}	0.86±0.03^{abc}	0.03±0.00^{bc}	0.20±0.03^{a}	8.95±0.01^{e}	0.68±0.07^{abc}	61.28±0.07^{d}
40	63.90±0.33^{d}	0.82±0.00^{cb}	0.03±0.00^{bc}	0.14±0.00^{a}	9.21±0.01^{d}	0.64±0.00^{bc}	61.86±0.00^{c}
60	74.63±0.04^{c}	0.93±0.04^{ab}	0.05±0.01^{ab}	0.19±0.00^{a}	10.12±0.00^{c}	0.69±0.01^{abc}	62.77±0.02^{b}
80	78.21±0.03^{b}	0.94±0.03^{a}	0.06±0.02^{a}	0.21±0.02^{a}	10.87±0.03^{b}	0.79±0.06^{a}	62.95±0.02^{b}
100	84.01±0.32^{a}	0.97±0.03^{a}	0.05±0.01^{ab}	0.20±0.00^{a}	10.97±0.01^{a}	0.76±0.03^{ab}	64.15±0.03^{a}
Well-watered	50.70±0.05^{f}	0.80±0.04^{c}	0.05±0.01^{ab}	0.20±0.03^{a}	8.19±0.03^{f}	0.65±0.03^{bc}	59.71±0.05^{e}
Droughted	48.96±0.02^{g}	0.58±0.03^{d}	0.02±0.00^{c}	0.15±0.03^{a}	6.89±0.04^{g}	0.58±0.03^{c}	49.27±0.33^{f}

Mean values (± Standard Error) with different superscripts in columns are significantly different ($p < 0.05$)

Table 5: Effects of varying levels of proline on mineral of *Solanum aethiopicum*

Proline levels (%)	Mineral elements (mg/100 g)					
	Sodium	Potassium	Calcium	Magnesium	Phosphorus	Zinc
20	6.18±0.08^{e}	312.49±0.03^{f}	102.71±0.03^{f}	44.85±0.03^{e}	71.86±0.03^{ef}	0.85±0.03^{b}
40	6.46±0.00^{d}	323.57±0.00^{e}	105.57±0.00^{e}	45.87±0.00^{d}	72.05±0.01^{e}	0.47±0.01^{c}
60	6.96±0.03^{c}	345.64±0.02^{d}	110.98±0.01^{d}	46.76±0.02^{c}	73.25±0.04^{d}	0,94±0.04^{ab}
80	6.97±0.02^{c}	356.96±0.02^{c}	112.15±0.01^{c}	46.96±0.01^{c}	74.74±0.05^{c}	0.96±0.04^{a}
100	7.40±0.03^{b}	371.72±0.04^{b}	112.88±0.10^{b}	48.22±0.06^{b}	76.22±0.34^{b}	0.94±0.02^{ab}
Wall watered	8.93±0.02^{a}	402.20±0.06^{a}	121.55±0.06^{a}	58.80±0.30^{a}	90.87±0.03^{a}	0.97±0.02^{a}
Droughted	5.81±0.05^{f}	302.24±0.04^{g}	98.20±0.05^{g}	44.21±0.05^{f}	71.53±0.04^{f}	0.86±0.04^{b}

Mean values (± Standard Error) with different superscripts in columns are significantly different ($p < 0.05$)

Table 6: Effects of varying levels of proline on oxidative in leaves *Solanum aethiopicum*

Proline levels (%)	Antioxidants in the leaves					
	Superoxide dismutase	Ascorbate peroxidase	Catalase (mg g^{-1})	Glutathione reductase	GST (mg g^{-1})	SP (mg g^{-1})
20 Leaf	0.25 ± 0.02^{h}	0.43 ± 0.01^{g}	0.50 ± 0.02^{g}	0.35 ± 0.02^{ij}	$6.91\pm0.03^{l(L)}$	14.33 ± 0.06^{j}
20 Root	0.38 ± 0.01^{fg}	0.51 ± 0.03^{f}	0.68 ± 0.01^{de}	0.47 ± 0.02^{g}	8.78 ± 0.01^{g}	16.97 ± 0.02^{h}
40 Leaf	0.36 ± 0.01^{g}	0.51 ± 0.01^{f}	0.43 ± 0.01^{h}	0.41 ± 0.03^{h}	7.84 ± 0.01^{k}	15.67 ± 0.01^{i}
40 Root	0.42 ± 0.01^{ef}	0.52 ± 0.01^{ef}	0.71 ± 0.02^{d}	0.53 ± 0.03^{f}	8.97 ± 0.04^{f}	16.88 ± 0.03^{h}
60 Leaf	0.44 ± 0.02^{e}	0.50 ± 0.03^{f}	0.58 ± 0.03^{f}	0.58 ± 0.02^{e}	8.15 ± 0.03^{j}	17.17 ± 0.03^{g}
60 Root	0.53 ± 0.03^{cd}	0.58 ± 0.01^{d}	0.76 ± 0.01^{c}	0.65 ± 0.02^{d}	9.49 ± 0.04^{e}	18.50 ± 0.03^{e}
80 Leaf	0.49 ± 0.01^{d}	0.57 ± 0.02^{de}	0.60 ± 0.00^{f}	0.65 ± 0.02^{d}	8.5 ± 0.04^{h}	18.30 ± 0.03^{f}
80 Root	0.57 ± 0.02^{c}	0.66 ± 0.02^{c}	0.78 ± 0.01^{bc}	0.70 ± 0.03^{d}	9.96 ± 0.02^{d}	20.50 ± 0.03^{b}
100 Leaf	0.55 ± 0.03^{c}	0.60 ± 0.01^{d}	0.66 ± 0.02^{de}	0.69 ± 0.03^{d}	8.52 ± 0.02^{f}	20.10 ± 0.05^{c}
100 Root	0.68 ± 0.01^{b}	0.76 ± 0.03^{b}	0.79 ± 0.03^{bc}	0.75 ± 0.01^{c}	10.66 ± 0.020^{b}	21.96 ± 0.02^{a}
Well-watered Leaf	0.09 ± 0.00^{i}	0.12 ± 0.00^{h}	0.50 ± 0.03^{g}	0.32 ± 0.03^{j}	6.45 ± 0.02^{m}	$8.13\pm0.01^{k(L)}$
Well-watered Root	0.13 ± 0.00^{i}	0.15 ± 0.00^{h}	0.67 ± 0.02^{e}	0.39 ± 0.01^{hi}	8.40 ± 0.06^{j}	10.17 ± 0.03^{k}
Droughted leaf	0.68 ± 0.03^{b}	0.78 ± 0.02^{b}	0.83 ± 0.04^{b}	0.85 ± 0.03^{b}	10.38 ± 0.10^{c}	18.30 ± 0.09^{f}
Droughted root	0.88 ± 0.01^{a}	0.95 ± 0.02^{a}	0.98 ± 0.00^{a}	0.96 ± 0.02^{a}	14.52 ± 0.03^{a}	19.12 ± 0.01^{d}

Mean values (± Standard Error) with different superscripts in columns are significantly different ($p < 0.05$)

promote the growth in plants (Bahadur *et al.*, 2011). On the other perspective, the results could imply indicate roles of proline in the resources acquisition, increased growth rate, nutrient assimilation capacity (El Moukhtari *et al.*, 2020), net production efficiency of assimilatory apparatus, and as a measure of the increase in plant dry mass per unit leaf area unit time of the vegetable (El Moukhtari *et al.*, 2020).

These observations are in line with submissions of Hayssam *et al.* (2013) who opined that application of proline applied with Abscisic acid was effective in alleviating the adverse effect of water stress and enhanced growth parameters of treated plants. Chlorophyll contents in the leaves of the vegetable were also influenced by the varying levels of proline but the contribution of 100 % was more effective. The observation may predict the role of proline in the induction or formation of photosynthetic pigments such as chlorophyll a and chlorophyll b possibly as a stress coping mechanism for the vegetable despite the water deficit challenge experienced. This assertion is in line with the submission of Rasheed *et al.* (2014) who opined that exogenous application of proline increased chlorophyll contents in bread wheat seedlings and the addition of 20 mM proline under oxidative stress caused an increase in the formation of the chlorophyll and stabilized photosynthetic reactions in bread wheat (Abdelhamid *et al.*, 2013; Shahid *et al.*, 2014).

The appreciable increase observed in the nutritional attributes such as proximate, mineral and vitamins; observed in the vegetable sprayed with 100% proline may be due to the ability of the osmolyte to induce the production of chlorophyll as a precursor of glucose production through photosynthesis (Semida *et al.*, 2020). Interestingly, the results of the study clearly indicate that water stress decreased nutritional contents in *S. aethiopicum* droughted. This may have been due to a decrease in transpiration rate and stomatal conductance and low production of photosynthates (Semida *et al.*, 2020). However, the application of different concentrations of proline enhanced the accumulation of all nutritional attributes under water stress conditions with the application of proline most importantly 100% proline (Ali *et al.*, 2007).

Also, application of proline enhanced the activities of oxidative enzymes such that there was a significant increase in the proline, superoxide dismutase, ascorbate peroxidase, catalase, gluytathione, GST and SP. High observation of the enzymes in the roots may be due to their key roles in osmotic regulation Vendruscolo *et al.* (2007) and Tatar and Gevrek (2008) also reported that under drought conditions, there was an increase in proline content in different plant species. The activity of antioxidant enzymes increased in the root of stressed plants due to their ability to act as protection against oxidative stress than osmotic adjustment or as a defense system against reactive oxygen species.

CONCLUSION

The study revealed that proline at different levels ameliorated water stress in morphological and physiological attributes of *S. aethiopicum* and on level of oxidative enzymes in leaves and roots of the vegetable; however, the osmolytes at 100% are recommended for water deficit amelioration in plants.

AUTHOR'S CONTRIBUTIONS

A. W. Ojewumi conceived and designed the study. A. W. Ojewumi and L. F. Mabinuori conducted the experiment, analysed and interpreted the data. M. O. Keshinro and L. F. Mabinuori drafted the manuscript while S. C. O. Makinde revised the manuscript for intellectual contents. All the authors' proof-read the manuscript and approved it for submission.

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