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ORIGINAL ARTICLE

Glycine Betaine Enhances Growth Markers, Nutritional Quality and Antioxidant Activities in Garden Egg (*Solanum aethiopicum* L.) Under regulated Water Conditions

[1]Wale A. Ojewumi✉, [2]Olamide O. Fawibe, [2]Ayodotun S. Ayoola, [3]Nneka S. Egonu, [2] Faith O. Akinyemi [4] Kehinde T. Omolokun, [5]Adeyemi A. Adegoke, [4]Racheal Tope-Akinyetun, and [4]Oluwaseyi D. Adelugba
[1]Department of Plant and Environmental Biology, Federal University of Agriculture and Technology, Okeho, Nigeria.
[2]Department of Pure and Applied Botany, Federal University of Agriculture, Abeokuta, Nigeria.
[3]Department of Plant Science and Biotechnology, University of Nigeria, Nsukka, Nigeria.
[4]Department of Botany Lagos State University, Ojo, Lagos Nigeria.
[5]Department of Botany, University of Ibadan, Nigeria.

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ABSTRACT

Mitigating the impact of water stress on crop production is a paramount concern for ensuring food security and sustaining agricultural practices. This study assessed the efficacy of four concentrations of glycine betaine (GB) (from 1 g/L to 4 g/L) on the growth, physiological responses, and nutritional quality of garden egg (*Solanum aethiopicum* L.). Morphological characters, leaf growth components, chlorophyll contents, nutritional quality, and antioxidant activities were determined in the vegetables. The garden egg treated with 200 mL 4g/L GB exhibited the tallest height (15.03 cm) and highest number of leaf (20.25) in the well-watered garden egg. However, the application of 200 mL of 3g/L GB produced significant enhancement in the leaf area (319.14 cm^2), specific leaf area (78.24 m^2/kg), leaf area index (0.92), net assimilation rate (0.007 mg·m^2/day), relative growth rate (0.12 g/m^2/day), and leaf area ratio (0.10 m^2/kg). Analysis of vitamins revealed higher Vitamin A (87.66 mg/100 g), Vitamin B_3 (1.21 mg/100 g), and Vitamin C (66.49 mg/100 g) in garden egg sprayed with 200 mL of 4 g L^{-1} GB. This treatment further induced a substantial increase in sodium, potassium, calcium, and magnesium levels as well as dry matter, fat, ash, crude protein, and carbohydrates. Activities of key antioxidant enzymes, including glutathione reductase, superoxide dismutase, catalase, glutathione transferase, and ascorbate peroxidase, exhibited a significant increase in the roots under drought conditions.

Keywords: Growth tolerance; Nutritional quality; Antioxidant activities; African eggplant; Plant physiology; Crop production.

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✉**Corresponding author**: Ojewumi Anthony Wale. Email: anthony.ojewumi@lasu.edu.ng. DOI: https://dx.doi.org/10.4314br.v24i2.26

1.0 INTRODUCTION

Drought, caused by irregular weather patterns, disrupts the water balance in natural plants ecosystems as well as agriculture. Drought manifests as a physiological condition that diminishes plant water potential, turgor pressure within cells, and disturbs their normal physiological processes (Hemati et al., 2022). The resultant impacts of these factors on cultivated plants have a negative influence on agricultural practices, food security, and the sustainable production of vegetables and other food crops (Lisa et al., 2012). In Nigeria, vegetables such as garden egg and nightshade are often grown without an irrigation system. Garden egg, commonly known as African eggplant, is a highly versatile and nutritionally rich vegetable crop. This plant species, native to various regions in Africa, plays a significant role in traditional diets and is renowned for its distinctive fruits and edible leaves, especially in Tropical Africa and Asia (Sánchez-Mata & Yokoyama, 2010; Han et al., 2021). The vegetable is predominantly cultivated by traditional farmers to increase food security and boost the economic status (Anosike & Obidoa, 2012). Despite its numerous benefits, the vegetable is neglected and underutilized due to the lack of sustainable cultivation and conservation practices (Han et al., 2021). In numerous local communities, this vegetable is often grown in open fields without adequate farming practices, thereby making it susceptible to unfavorable meteorological conditions that significantly hinder its growth, yield, and nutritional quality (Solankey et al., 2021). Many plants have evolved innate mechanisms to mitigate stress conditions using arrays of biochemical and physiological responses. High level of tolerance to desiccation during their life cycle as an ameliorative approach to water imbalance is another potent coping methods of the vegetable (Giordano et al., 2021). Also, to regulate the production of efficient and biochemical compositions of the vegetable, the usage of potent osmolytes, such as glycine betaine, that are compatible, plays an important role in counteracting the effects of osmotic stress, which is reported to characterise ameliorative potential on water stress. Findings of Aman (2022) revealed that exogenous application is a short-term approach of stimulating protective osmolytes in plants compared with conventional breeding or transgenic approaches. However, osmoprotectants such as glycine betaine, among others, are used for the amelioration of harmful effects of water stress through osmoregulation by scavenging reactive oxygen species (ROS), stabilizing sub-cellular structures, and acting as a signal molecule interacting with other metabolic pathways when plants experience drought. Also, production of beneficial substances that improved growth and metabolism of plants under stressful conditions by enhancing root development, efficient water transportation, and increasing accumulation of photosynthetic pigments has been demonstrated to alleviate water stress in plants (Nicolas-Espinosa et al., 2023).

The current study was designed to explore the osmoregulatory potential of glycine betaine (GB) on the growth, nutritional compositions, and levels of oxidative activities in garden eggs. Specifically, the study aimed to elucidate its impacts on plant growth parameters, the nutritional quality of the harvested produce, and the activity of key oxidative activities, shedding light on the mechanisms by which GB may enhance the resilience and productivity of this important vegetable crop.

2.0 MATERIALS AND METHODS

2.1 Experimental farm

A pot experiment was conducted at the screenhouse of the Department of Botany, Lagos State University, Ojo Campus (Nigeria), located between latitude 6°27'58.446" N and longitude 3°11'56" E, to explore the osmoregulatory potential of glycine betaine (GB) on growth, nutritional compositions, and levels of oxidative activities in garden eggs. Seeds of garden egg (*Solanum aethiopicum*) were purchased at Solokad, a certified agrochemical company, Agbeni, Ibadan, Oyo State, Nigeria. The soil used for this study was composed mainly of sand and silt with a moderate level of clay (Ojewumi et al., 2023). The soil was collected at a depth of 0-8 cm, 500 m apart at the botanical garden of the institution, according to the procedure of the National Soil Characterization Database of the United States Department of Agriculture cited in Ojewumi et al. (2023) with modifications. The AMS Step Soil probe (2" L x 7/8" dial) was used for soil collection. A slot was inserted vertically into the soil, and a soil core was removed. The soil collected was mixed and poured into 30 perforated planting buckets of 1.0 L, 12.5 cm external diameter, and 10.5 cm height.

2.2 Nursery and glycine betaine preparations

Seedlings of garden egg were grown for 21 days. Thereafter, the seedlings (one/bucket) were transplanted into thirty (30) planting buckets. Individual planting buckets were transferred to a greenhouse with controlled temperature and relative humidity (maximum of 28℃ and minimum of 21 ℃ and 65–75%), irrigated every two days to maintain high moisture levels in the soil. GB was imported from Doulang New Village, Zhongtang Town. Dongguan, Guangdong.

2.3 Experimental design

Thirty (30) planting buckets containing the seedlings were arranged in a completely randomized design with five replications. GB concentrations (independent variables) were manipulated by diluting 1, 2, 3, and 4 g GB in 1000 L of distilled water, respectively (Ojewumi et al., 2023). The vegetables treated with GB were not irrigated with distilled water. However, two controls outlined in this study were subjected to different watering conditions from the well-watered and the droughted vegetables as experimental conditions. Well-watered vegetables were irrigated with 200 mL of distilled water/bucket once daily using the foliar application method, allowing free drainage of excess water throughout the period of the experiment. Vegetables affected by drought conditions were irrigated with 200 of mL distilled water per bucket once a week as the "droughted." This ensured the survival of droughted vegetables. Variation in the interval of water application between the well-watered and droughted is the variation in the watering regime and soil moisture levels between the groups. Additionally, solutions containing 1, 2, 3, and 4 grams of GB per liter were diluted up to 200 mL. These solutions (200 mL/bucket) were then applied to the seedlings once daily using the foliar application method for a period of five weeks as outlined by Ojewumi et al. (2023). These treatments were named 1 g/L, 2 g/L, 3 g/L, and 4 g/L. To prevent water absorption, buckets were elevated on petri dishes above the greenhouse floor as described by Ojewumi et al. (2023). Moreover, each bucket was supplemented with a one-time application of 10g of poultry manure throughout the experiment. Weeds were removed by handpicking. These treatments were sustained for a duration of five weeks (W), during which data were collected from the vegetables in each treatment before they reached the flowering stage, usually conducted in the morning.

2.4 Analysis of morphological traits and growth components

Morphological characters, including plant height, number of leaves, and leaf area, were measured once in a week within the five weeks of the study. Plant height was measured using a ruler calibrated in cm. The biomass of the vegetables, such as the fresh and dry weight of leaves was measured using a digital mini scale (Model MT 1000, TN series) while the number of leaves was determined using the physical counting method according to Aina et al. (2018), once a week. Total leaf area was measured using the LI-3000C Portable Leaf Area Meter (Ojewumi et al., 2022). Specific leaf area was determined exclusively by the leaf's surface area relative to the dry weight of the vegetable, without considering the water content variability but in relation to the leaf's biomass and without taking into account the presence or variability of water content. Leaf area index was calculated as the projection of the canopy produced by leaves of the vegetable over the soil surface (Ismaeil et al., 2012). Relative growth rate was determined according to the method of (Houghton et al., 2013). Net assimilation rate (as dry weight of leaves of the vegetable and their corresponding leaf areas at 4 and 5 WAT) and leaf area ratio (measurement of biomass) were determined according to the methods described by Agboola et al. (2006). All traits were measured in each of the five individual plants of each treatment, as shown in the equations.

$$Specific\ leaf = \frac{leaf\ area\ (cm2)}{Correspomding\ dry\ weight\ of\ leaf\ (g)} \quad (1)$$

$$\text{LAI}\ (m2m-2) = \frac{\text{Leaf area per plant}}{\text{Plant ground area}} \quad (2)$$

$$\text{RGR}\ (g\,g-1\ day) = \frac{Log_eW_2 - Log_eW_1}{t_2 - t_1} \quad (3)$$

$$\text{NAR}(g\,m-2\ day-1) = \frac{W_2 - W_1}{A_2 - A_1} \times \frac{Log_eA_2 - Log_eA_1}{t_2 - t_1} \quad (4)$$

$$\text{LAR}\ (m2kg-1) = \frac{W_2 - W_1}{t_2 - t_1} \times \frac{Log_eA_2 - Log_eA_1}{W_2 - W_1} \quad (5)$$

Where LAI = leaf area index, RGR = relative growth rate, NAR= net assimilation ratio, LAR= leaf area ratio, 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)

2.5 Analysis of proximate contents in garden egg leaves

At the flowering stage (five weeks after treatments), leaves of garden egg were collected and assayed for proximate contents such as moisture, dry mater, crude fibre, total carbohydrate crude protein, crude fat, and ash according method AOAC (2000).

2.5.1 Moisture

Moisture in garden egg leaves was evaluated using hot air oven technique for 1h at 130 °C

$$\text{Moisture}\ (\%) = \frac{W1(g) - W2(g)}{W1\ (g)} x100 \quad (6)$$

Where; W_1 - weight of sample before drying, W_2 - weight of sample after drying.

Dry mater determination was obtained as shown below;

$$\text{Dry mater}\ (\%) = 100 - \text{moisture} \quad (7)$$

2.5.2 Crude fibre

Crude fibre was assayed by boiling 2 g of defatted sample of garden egg leaves in 20 mL of 1.25% H_2SO_4 for 20 min. Filtered and washed contents were boiled using 200 mL of 1.25% NaOH. Spotless beaker was dried (100 ± 5°C), cooled and the weights of the contents were determined. Spotless beakers and its contents were dried (100 ± 5°C) for 3 h, cooled and weighed. Crude fiber was determined according to AOAC (2000). as shown below;

$$\text{Crude Fiber}\ (\%) = \frac{(W3 - W2)(g)}{W1\ (g)} \times 100 \quad (8)$$

Where; W1: Weight of the sample, W_2: weight of spotless beaker, W_3: weight of spotless beaker and crude fiber

2.5.3 Total carbohydrate

The total carbohydrate in the sample was determined as shown below.

Total carbohydrate (%) = 100 – moisture (%) + fat (%) + protein (%) +fibre (%) (9)

2.5.4 Total nitrogen
The total nitrogen was determined using Micro-Kjeldahl (Ojewumi & Oyebanji, 2022).

2.5.5 Protein content
Protein content (%) was determined as shown below

$$\text{Protein (\%)} = \frac{\text{T X 1.4 X 6.25 X 01NHCL X volused}}{\text{W(g) x A}} \text{X100} \quad (10)$$

Where; T: titter value, W: weight of sample, A: aliquot digested sample used for distillation

2.5.6 Crude fat
Crude fat was determined by preparing two grams of the plant and kept using paper thimble in a known weight fat extractor. Exactly 70 mL of C_6H_{14} was added, refluxed and weighed. Crude fat was determined using the mathematical relationship as shown below:

$$\text{Crude fat (\%)} = \frac{W1(\text{g}) - W2(\text{g})}{W3(\text{g})} X100 \quad (11)$$

Where: W_1: weight of flask with fat, W_2: weight of empty flask, W_3: Weight of original sample

2.5.7 Ash content
Ash was determined by adding ten grams of the sample to a known crucible, weighed and dried at 550 °C for 4 h. The sample was reweighed and ash was determined as shown below;

$$\text{Ash (\%)} = \frac{\text{Weight of Ash(g)}}{weightofsamples\text{ (g)}} \quad (12)$$

2.6 Analysis of mineral and vitamin content
2.6.1 Minerals
Leaf samples were assayed for calcium, potassium, magnesium, zinc, phosphorus, and sodium using an Atomic Absorption Spectrophotometer (Perkin-Elmer Model 2280). Phosphorus content of the digest was determined calorimetrically according to the method of Sahaptin & Bassiri (1975).

2.6.2 Vitamins
Vitamin A, Pantothenic acid (vitamin B5), vitamin B6, vitamin C (ascorbic acid), Vitamin E (tocopherol) and vitamin K were determined according to the method of AOAC (2000).

2.6.3 Chlorophyll a and b
Chlorophyll a, chlorophyll b, and total chlorophyll were determined using a spectrophotometry method as described by Dawood et al. (2014).

2.7. Enzymatic activity assay
Glutathione reductase (GR) 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). Activity was calculated using an extinction coefficient ε = 6.22 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) activity 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 (pH8.0), 300 µmol L^{-1} methionine,1.5 mmol L^{-1} NBT, 120 µ mol L^{-1} mL 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^{-1}. The riboflavin was added last. The reaction was started by illuminating the test tubes under 4 fluorescent lamps for 10 min. The absorbance of illuminated solution was measured using spectrophotometry at 560 nm. One unit of SOD activity is defined as the amount of enzyme that inhibited 50 % of NBT photo-reduction versus a blank cell containing no enzymatic extract. *Catalase* (CAT) activity was assayed by measuring the initial rate of disappearance of H_2O_2 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 ε = 0.036 ·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 min. The *peroxidase activity* was determined using 4-methylcatechol as substrate. The increase in the absorption caused by 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 oxidative activity was defined as 0.001 change in absorbance per min, under assay conditions.

2.8 Statistical analysis
A one-way analysis of variance (ANOVA) was performed using the software SAS 9.3 (SAS Institute Inc., Cary, NC, USA). Significant differences were determined using Tukey's HSD test at $p < 0.05$ for each week after treatment (WAT) individually.

3.0 RESULTS

3.1 Effect of glycine betaine on morphological characters
Effects of varying concentrations of glycine betaine on the morphological characters of garden eggs under water-regulated conditions. The impact of different levels of glycine betaine (GB) concentrations on the height of garden egg plants is illustrated in Figure 1. The findings indicate that the height of the garden egg plants was notably influenced by the varying concentrations of GB from 2 to 5 weeks after treatment (WAT). The tallest plants, reaching 15.03 cm, were observed in the group treated with 200 mL of 4 g/L GB (Figure 1). On the other hand, the highest number of leaves (20.25) (Table 1) and stem girth (12.95 cm) (as shown in Table 2) were found in the well-watered group, closely followed by those treated with 200 mL of 4 g/L GB. Conversely, the plants subjected to drought conditions exhibited the lowest values for these parameters.

Table **1**. Effects of varying concentrations of glycine betaine on number of leaves of garden egg under water-regulated conditions

GB (g/l)	1WAT	2WAT	3WAT	4WAT	5WAT
1	3.75±0.02^{a}	5.25±0.05^{b}	6.75±0.06^{b}	10.0 ±0.14^{bc}	12.00±0.11^{c}
2	2.75±0.02^{a}	5.75±0.07^{b}	6.00±0.06^{b}	10.7±0.15^{bc}	13.50±0.13^{c}
3	3.25±0.07^{a}	5.2±0.06^{b}	6.50±0.07^{b}	10.00±0.11^{bc}	13.75±0.14^{c}
4	3.5±0.06^{a}	5.75±0.05^{b}	8.75±0.09^{ab}	12.50±0.13^{b}	17.7±0.20^{b}
Droughted	3.75±0.05^{a}	5.00±0.07^{b}	6.50±0.08^{b}	6.25±0.08^{b}	6.12±0.08^{d}
Well-watered	3.5±0.05^{a}	8.25±0.09^{a}	10.75±0.10^{a}	16.75±0.12^{a}	22.25±0.16^{a}

Means ± standard errors in columns with different superscripts are significantly different at p < 0.05 using Tukey's HSD Test. GB = Glycine betaine, WAT= Weeks after treatment

Table **2**. Effects of varying concentrations of glycine betaine on stem girth of garden egg under water-regulated conditions

GB (g/l)	1WAT	2WAT	3WAT	4WAT	5WAT
1	0.15±2.28^{a}	1.00±0.09^{a}	1.20±0.18^{a}	1.45±0.26^{ab}	1.53±0.26^{ab}
2	0.58±0.13^{a}	1.18±0.16^{a}	1.35±0.16^{a}	1.55±0.13^{ab}	1.48±0.15^{ab}
3	0.80±0.04^{a}	1.03±0.05^{a}	1.33±0.08^{a}	1.45±0.15^{ab}	1.58±0.14^{ab}
4	0.90±0.08^{a}	1.08±0.17^{a}	1.15±0.19^{a}	1.08±0.18^{b}	1.70±0.14^{a}
Droughted	0.80±0.00^{a}	1.03±0.05^{a}	1.03±0.16^{a}	1.25±0.17^{ab}	1.13±0.21^{b}
Well-watered	0.85±0.12^{a}	1.30±0.17^{a}	1.48±0.19^{a}	1.83±0.21^{a}	1.95±0.20^{a}

Means ± standard errors in columns with different superscripts are significantly different at p < 0.05 using Tukey's HSD Test. GB = Glycine betaine, WAT= Weeks after treatment

3.2 Effects of varying concentrations of glycine betaine on the physiological parameters of garden egg under water-regulated conditions

Table 3 revealed that 200 mL of 3 g/L produced highest leaf area (264.12 cm), specific leaf area (78.24 cm^2) and leaf area index (0.92 $m^2.kg^{-1}$) in the vegetable treated with 200 mL of 3 g/L GB. This trend was consistent in net assimilation rate (0.007 $mg^{-2}day^{-1}$), relative growth rate (0.12 gm^2day^{-1}) and leaf area ratio (0.10 m^2kg^{-1}) of garden egg treated with 200 mL of 3g/L GB. Chlorophyll a (1.72 mg/gm) and Chlorophyll b (2.17 mg/gm) and total Chlorophyll (3.89) showed a significant (*p<0.05*) enhancement in vegetable treated with 200 mL 3g/L GB compared with other concentrations (Figure 2).

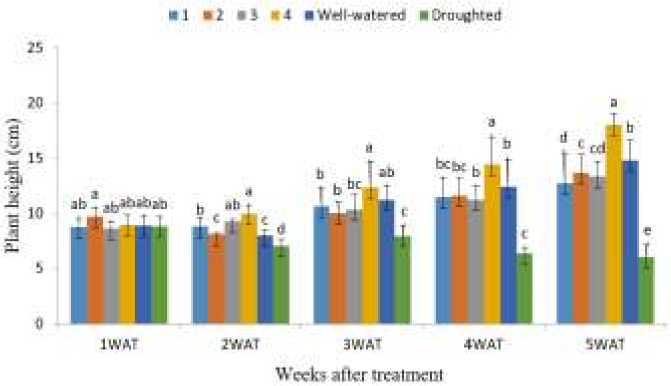

Figure 1. Effects of varying concentrations of glycine betaine on the height of garden egg under water-regulated conditions. Difference in lowercase letters on bars in each week indicate significant differences among treatments at p < 0.05.

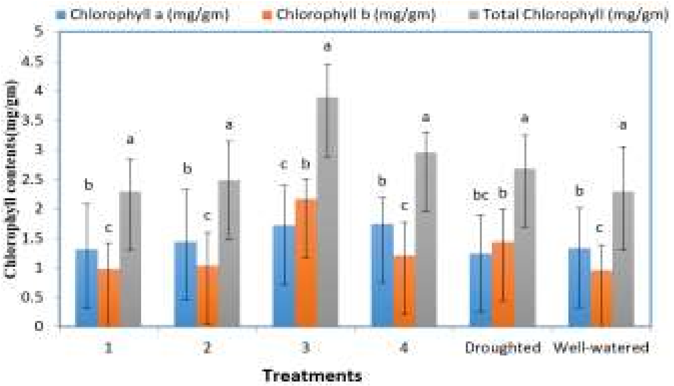

Figure 2. Effects of varying concentrations of glycine betaine on chlorophyll contents of garden egg leaves under water-regulated conditions. Deference in lowercase letters on bars in each week indicate significant differences among treatments at p < 0.05.

3.3 Effects of varying concentrations of glycine betaine on nutritional contents of garden egg under water-regulated conditions

Effects of varying concentrations of GB on vitamin contents in the leaves of garden egg represented in Table 3 revealed that 200 mL of 4 g/L GB produced highest vitamin A (87.66 mg/100 g), Vitamin B3 (1.21 mg/100 g), Vitamin B5 (0.07 mg/100 g), vitamin C (66.49 mg/100 g), vitamin K (13.53 mg/100 g) and vitamin E (1.11 mg/100 g) compared with other treatments (Table 4). The trend was consistent on influence of the treatments on mineral elements recorded in the plant. Sodium (10.95 mg/100 g), potassium (384.44 mg/100 g), calcium (113.32 mg/100g), magnesium (57.62 mg/100 g) were significantly higher in garden egg leaves treated with 200 mL of 4g/L GB (Table 5).

In addition, dry matter (11.13%), fat (1.77%), ash (2.57%) crude protein (3.47%), and carbohydrate (4.23 %). were significantly higher in the leaves of the vegetable treated with 200 mL of 4 g/L GB (Table 6). Peroxidase (21.39 $\mu molmin^{-1}mg^{-1}$) showed significant increase in the leaves of the vegetable sprayed with 4g/LGB, however, activities of glutathione reductase (1.08 $\mu mol\ min^{-1}\ mg^{-1}$), superoxide dismutase (0.98 mg^{-1}), catalase (1.08 mg^{-1}), glutathione s-tranferase (11.50 $\mu mol\ min^{-1}\ mg^{-1}$) and ascorbate peroxidise (1.28 $\mu mol\ min^{-1}\ mg^{-1}$) showed substantial increase in the leaves of the vegetable droughted compared with the plants treated with different concentrations of GB (Table 7). Similar trend was observed in activities of the enzymes in the roots of the vegetable (Table 8)

Table **3**. Effects of varying concentrations of glycine betaine on the physiological parameters of garden egg under water-regulated conditions

GB (g/l	LA (cm^2)	SLA(m2kg^{-1})	LAI (m^2m^{-2})	NAR (mg/g1day)	RGR (gm^2day-)	LAR(m^2kg^{-1})
1	264.12 ± 12.39^{ab}	64.19 ± 3.57^{ab}	0.76 ± 0.12^{ab}	0.004 ± 0.01^{b}	0.06 ± 0.01^{ab}	0.09 ± 0.01^{a}
2	246.75 ± 31.80^{ab}	51.36 ± 2.69^{b}	0.71 ± 0.10^{ab}	0.004 ± 0.01^{b}	0.07 ±0.03^{ab}	0.10 ± 0.04^{a}
3	319.14 ± 11.62^{a}	78.24 ± 9.31^{a}	0.92 ± 0.18^{a}	0.008 ± 0.01^{a}	0.12 ± 0.01^{a}	0.07 ± 0.03^{a}
4	198.84 ± 21.56^{ab}	51.98 ± 3.47^{b}	0.57 ± 0.09^{ab}	0.003 ± 0.01^{b}	0.03 ± 0.01^{b}	0.07 ± 0.03^{a}
Droughted	166.26 ± 21.21^{b}	48.261 ± 2.98^{b}	0.48 ± 0.07^{b}	0.004 ± 0.01^{b}	0.06 ± 0.02^{ab}	0.10± 0.03^{a}
Well-watered	237.71 ± 11.01^{ab}	59.88 ± 7.33^{b}	0.69 ± 0 .04^{ab}	0.003 ± 0.01^{b}	0.04 ± 0.02^{b}	0.05± 0.01^{a}

Means ± standard errors in columns with different superscripts are significantly different at p<0.05 using Tukey's HSD Test GB = Glycine betaine. LA= Leaf area, SLA= specific leaf area LAI= Leaf area Index, NAR =Net Assimilation Rate, RGR= Relative growth rate, LAR= Leaf area ratio

Table 4. Effects of varying concentrations of glycine betaine on vitamin compositions of garden egg under water-regulated conditions

GB (g/l)	Vitamin A (mg/100 g)	Vitamin B3 (mg/100 g)	Vitamin B5 (mg/100 g)	Vitamin B 6(mg/100 g)	Vitamin K (mg/100 g)	Vitamin C (mg/100 g)	Vitamin E (mg/100 g)
1	60.42±0.03^{d}	1.10±0.03^{ab}	0.05±0.003^{ab}	0.32±0.03^{a}	63.62±0.07^{c}	11.51±0.013^{d}	0.95±0.07^{ab}
2	78.28±0.04^{c}	1.21±0.03^{a}	0.07±0.01^{ab}	0.31±0.003^{a}	65.11±0.02^{b}	12.68±0.003^{c}	0.96±0.05^{ab}
3	81.86±0.03^{b}	1.18±0.03^{a}	0.07±0.002^{ab}	0.33±0.02^{a}	65.29±0.02^{b}	13.43±0.03^{b}	1.06±0.06^{a}
4	87.66±0.32^{a}	1.17±0.04^{ab}	0.08±0.015^{a}	0.32±0.004^{a}	66.49±0.03^{a}	13.53±0.005^{a}	1.11±0.03^{a}
Droughted	52.66±0.06^{f}	0.82±0.03^{c}	0.04±0.003^{b}	0.27±0.03^{a}	51.61±0.33^{e}	9.45±0.042^{f}	0.85±0.03^{b}
Well-watered	54.35±0.05^{e}	1.04±0.04^{b}	0.07±0.014^{ab}	0.32±0.03^{a}	62.05±0.04^{d}	10.75±0.03^{e}	0.92±0.04^{ab}

Means ± standard errors in columns with different superscripts are significantly different at p<0.05 using Tukey's HSD Test GB = Glycine betaine

Table 5. Effects of varying concentrations of glycine betaine on mineral content of garden egg under water-regulated conditions

GB (g/l)	Sodium (mg/100 g)	Potassium (mg/100 g)	Calcium (mg/100 g)	Magnesium (mg/100 g)	Phosphorus (mg/100 g)	Zinc (mg/100 g)
1	8.74±0.07^{bc}	315.05±0.03^{c}	100.26±0.03^{c}	47.19±0.03^{c}	75.51±0.03^{bc}	1.16±0.03^{ab}
2	9.52±0.03^{b}	348.19±0.02^{b}	108.53±0.09^{b}	49.10±0.02^{bc}	76.90±0.04^{bc}	1.15±0.04^{abc}
3	9.53±0.02^{b}	359.52±0.02^{b}	109.70±0.03^{b}	49.30±0.01^{bc}	78.39±0.07^{bc}	1.17±0.04^{a}
4	10.95±0.52^{a}	394.60±10.18^{a}	116.21±2.84^{ab}	57.62±3.59^{a}	89.52±4.99^{a}	1.17±0.01^{a}
Droughted	8.37±0.05^{c}	384.44±10.14^{a}	95.75±0.05^{c}	54.08±3.46^{ab}	84.86±4.84^{ab}	1.06±0.03^{c}
Well-watered	10.49±0.51^{a}	304.79±0.04c	113.32±2.94^{a}	46.55±0.05^{c}	75.18±0.04^{c}	1.07±0.04^{bc}

Means ± standard errors in columns with different superscripts are significantly different at p<0.05 using Tukey's HSD Test. GB = Glycine betaine

Table 6. Effects of varying concentrations of glycine betaine on the proximate contents of garden egg under water-regulated conditions

GB (g/L	Moisture (%)	Dry mater (%)	Fat (%)	Ash (%)	Crude Fibre (%)	Crude Protein (%)	Carbohydrate (%)
1	85.82±0.02^{b}	11.06±0.08^{a}	1.69±0.01^{ab}	2.53±0.02^{ab}	2.51±0.02^{a}	3.43±0.03^{ab}	4.20±0.030^{ab}
2	87.69±0.03^{a}	9.16±0.03^{c}	1.54±0.02^{bc}	2.30±0.03^{c}	2.21±0.02^{c}	2.94±0.03^{c}	3.61±0.03^{d}
3	86.61±0.03^{ab}	10.24±0.03^{b}	1.68±003^{b}	2.46±0.03^{b}	2.40±0.03^{b}	3.25±0.04^{b}	3.91±0.03^{c}
4	58.74±26.94^{c}	11.13±0.02^{a}	1.77±003^{a}	2.57±0.01^{a}	2.51±0.01^{a}	3.47±0.02^{a}	4.23±.030^{a}
Droughted	88.55±0.06^{a}	8.38±0.06^{d}	1.44±0.04^{c}	2.19±0.03^{d}	2.07±0.03^{d}	2.81±0.03^{d}	3.21±0.01^{e}
Well-watered	87.55±0.58^{a}	10.25±0.03^{b}	1.47±0.13^{c}	2.40±0.01^{b}	2.36±0.01^{b}	3.18±0.01^{b}	4.07±0.015^{b}

Means ± standard error in columns with different superscripts are significantly different at p<0.05 using Tukey's HSD Test GB = Glycine betaine

Table 7. Effect of varying concentrations of glycine betaine on oxidative *activities* in leaves of garden egg under water-regulated conditions

GB (g/L)	Glutathione reductase(µmol min^{-1} mg^{-1})	Superoxide dismutase(µmol min^{-1} mg^{-1})	Peroxidase (µmol min^{-1} mg^{-1})	Catalase (µmol min^{-1} mg^{-1})	Glutathione S-Tranferase (µmol min^{-1} mg^{-1})	Ascorbate peroxidase (µmol min^{-1} mg^{-1})
1	0.58±0.02^{e}	0.55±0.02^{d}	15.63±0.06^{d}	0.75±0.002^{e}	8.03±0.03^{d}	0.93±0.01^{d}
2	0.81±0.002^{d}	0.74±0.02^{cd}	18.47±0.03^{c}	0.83±0.003^{d}	9.27±0.03^{c}	0.99±0.001^{cd}
3	0.88±0.02^{c}	0.79±0.005^{c}	19.60±0.03^{b}	0.85±0.003^{cd}	9.64±0.04^{bc}	1.07±0.02^{b}
4	0.92±0.00^{b}	0.85±0.03^{b}	21.39±0.05^{a}	0.91±0.02^{b}	10.08±0.02^{ab}	1.09±0.001^{b}
Droughted	1.08±0.03^{a}	0.98±0.03^{a}	19.60±0.09^{b}	1.08±0.04^{a}	11.50±0.10^{a}	1.28±0.02^{a}
Well-watered	0.55±0.03^{f}	0.39±0.003^{f}	9.43±0.01^{f}	0.75±0.03^{e}	7.57±0.02^{e}	0.62±0.003^{e}

Means ± standard errors in columns with different superscripts are significantly different at p<0.05 using Tukey's HSD Test GB = Glycine betaine

Table 8. Effect of varying concentrations of glycine betaine on oxidative *activities* in roots of garden egg under water-regulated conditions

GB (g/L)	Glutathione reductase(µmol min^{-1} mg^{-1})	Superoxide dismutase(µmol min^{-1} mg^{-1})	Peroxidase (µmol min^{-1} mg^{-1})	Catalase (µmol min^{-1} mg^{-1})	Glutathione S-Tranferase (µmol min^{-1} mg^{-1})	Ascorbate peroxidase (µmol min^{-1} mg^{-1})
1	0.70±0.02^{de}	0.68±0.009^{e}	18.27±0.02^{d}	0.93±0.01^{c}	9.90±0.01^{e}	1.01±0.03^{e}
2	0.88±0.015^{c}	0.83±0.03^{d}	19.80±0.03^{c}	1.01±0.03^{b}	10.61±0.04^{d}	1.08±0.01^{d}
3	0.93±0.002^{bc}	0.87±0.02^{c}	21.20±0.03^{b}	1.03±0.01^{b}	11.08±0.02^{c}	1.16±0.02^{c}
4	0.98±0.01^{b}	0.98±0.01^{b}	23.26±0.02^{a}	1.04±0.004^{b}	11.78±0.02^{b}	1.26±0.03^{b}
Droughted	1.21±0.02^{a}	1.18±0.006^{a}	20.42±0.01^{b}	1.23±0.004^{a}	15.64±0.03^{a}	1.45±0.02^{a}
Well-watered	0.62±0.01^{e}	0.43±0.002^{f}	11.47±0.03^{e}	0.90±0.02^{cd}	9.52±0.06^{f}	0.65±0.003^{f}

Means ± standard errors in columns with different superscripts are significantly different at p<0.05 using Tukey's HSD Test GB = Glycine betaine

4.0 DISCUSSION

4.1 Effects of glycine betaine on morphological traits and leaf characteristics of garden egg

Glycine betain is an osmoprotectant known for its ability to mitigate abiotic stresses, particularly water deficits (Kumar et al., 2017; Zhu et al., 2022). Investigation on the effects of GB on the growth of water-stressed garden eggs indicates modulatory effects of the osmolyte on the morphological characters of the vegetable. The concentrations of the GB studied may have enhanced cellular water balance, which was reflected in the increase in morphological parameters of the vegetable. This observation is in line with contributions of Anjum et al. (2015) and Taheril et al. (2011) who speculated that GB improved the development of agronomic characters in plants even if grown under water stress. Application of 3 g/L GB influenced the leaf-related attributes such as leaf area, leaf area index, and specific leaf area. These results in relation to specific leaf area may be a reflection of the influence of 3 g/L GB on the leaves' surface area relative to its dry weight and other leaf's biomass regardless of water content variability. The observation in terms of leaf and specific leaf area also suggests that 200 mL of 3 g/L GB enhanced the formation of photosynthetic pigments and the ability of the pigments to capture light, which is often used for the improvement of photosynthetic efficiency and biomass production (Estaji et al., 2019). Lower values of the attributes observed in garden egg drought could be a clear indication of reduced nutrient availability and imbalance in water circulation and its usage in cellular and metabolic activities of the vegetable under water deficit. Effects of GB on plants cultivated under water stress conditions may be due to the ability of the osmolyte to maintain cell turgor pressure, stabilize cell membranes, prevent dehydration, and prevent cell damage (Zhu et al., 2022). A high leaf area index recorded through application of 200 mL of the GB may be a measure of leaf area per unit of ground surface, reflection of the amount of sunlight that can be captured by the community of the vegetable, or a large amount of photosynthetic surface area relative to the ground surface area. This allows the efficient use of available light for development of parts formed and general growth of plants (McKendry, 2002). Higher influence of 200 mL of the GB on relative growth rate suggests that the treatment enhanced competitive advantage and maximum productivity. In addition, higher relative growth rate, leaf area ratio, and net assimilation rate observed in the leaves of garden egg treated with 200 mL 3 g/L GB suggest that the treatment may improve the plant's ability for nutrient channelization from the source to the sink needed for growth and improvement in the overall growth rate of the vegetable (Alaei & Mahna 2023). However, lower relative growth rate and leaf area ratio recorded in droughted vegetables may denote those resources of the garden egg drought have been allocated to other physiological processes such as defense against water deficit (O'Donnell et al., 2013).

4.2 Effects of glycine betaine on the nutritional quality of garden egg

Application of GB at different concentrations had differential effects on the nutritional quality of garden eggs. Appreciable quantities of vitamins A, B3, B5, C, K, and E as well as sodium, potassium, phosphorus, calcium, and magnesium observed in the vegetable applied with 200 mL of 4 g/L GB suggests that GB has the potential to improve the nutritional attributes of garden eggs treated aside from its water stress amelioration capability (Hu et al., 2012).

4.3 Effects of glycine betaine on antioxidants and reactive oxygen species in garden egg

In addition, results of the study also demonstrated that application of different concentrations of GB modulated the level of oxidative activities in garden eggs. A higher level of glutathione reductase, superoxide dismutase, catalase, glutathione S-transferase and ascorbate peroxidase observed in the roots of the garden egg when droughted, overall suggests a significant heightened antioxidant defense mechanism. This enhanced activity is indicative of the plant's adaptive response to water stress, aimed at mitigating oxidative damage caused by reactive oxygen species (ROS) (Aminu et al., 2021; Amuji et al., 2025). In addition, significant quantities of superoxide peroxidase observed in the roots of the vegetable treated with 200 mL of 4 g/l of the GB are a clear indication that the substance can be used to regulate loss of water. It could also be inferred that 4 g/L of GB enhanced the roots and leaves of the vegetable to be involved naturally in the oxidative activities as coping strategies against water stress or defense mechanisms to alleviate oxidative stress induced by water stress such as osmotic regulation (Vendruscolo et al., 2007; Tatar & Gevrek, 2008). These findings have important implications in agriculture, particularly in arid regions where the volume of water needed by plants often exceeds water available. The ability of glycine betaine (GB) to enhance both plant growth and the plant's antioxidative defense mechanisms under water stress conditions suggests that it could serve as a valuable tool in mitigating the challenges posed by water scarcity. By promoting more efficient water use and improving crop resilience, the application of GB holds promises for sustainable agricultural practices, ultimately contributing to increased food security and the livelihoods of farmers in water-scarce regions. While this work focused on the beneficial crop-specific responses of GB, its use is not without limitations. GB production can be costly, making it less accessible for small-scale farmers, and its environmental impact depends on the source and production process. Furthermore, GB provides short-term relief from water stress but may not offer a long-term solution to water scarcity, such as in many parts of Africa. Addressing water scarcity on this continent, as well as in other regions of the world, requires a multifaceted approach, considering the erratic rainfall patterns, over-extraction of groundwater, and competition for water resources among various sectors. For this reason, in the future the use of GB should be integrated with other strategies like drought-resistant crop varieties, efficient irrigation, and sustainable soil practices to achieve the best results in garden egg production.

Conclusion

The findings of this study provide valuable insights into the response of garden egg to water stress and suggest potential strategies for enhancing the resilience of crops in the face of changing environmental conditions. The use of GB as a mitigating agent may help improve the yield and nutritional value of this important vegetable. The results underscore the potential for GB to enhance not only plant growth and nutritional quality but also the overall resilience of crops to adverse environmental conditions, paving the way for more sustainable and food-secure agriculture in the face of changing climates and water resource limitations. Hence, farmers and agricultural practitioners in water- stressed regions can considered using GB as a mitigating agent to ameliorate water deficit and improve the growth and yield of garden egg.

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Conflict of interest

The authors declare no competing interests.

Authors contribution

WAO and OOF conceived the research idea, initiated it and analysed the data generated from the study. NSE and FOA, interpreted results and wrote the draft. AAA and ODA, performed the field and collected data of the study, RTA and TKO performed laboratory experiment of the study. ASA, Read and corrected the draft.

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