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ARTICLE

Proline as an osmolyte modulates changes in morphological and physiological attributes of *Capsicum annuum l.* under water stress

Anthony W. Ojewumi[1*], Samuel A. Ayoola[2], Abdulqudus A. Ope[1], Toheeb M. Iyanda[1]

1Department of Botany, Lagos State University, Badagry Express Way, Ojo Lagos
2Department of Pure and Applied Botany, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria

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**Corresponding author*
Anthony W. Ojewumi (Ph.D) E-mail: anthony.ojewumi@lasu.edu.ng, ojewumianthony@yahoo.com

Tel: +2348053618237, +2348121176267

ABSTRACT
Water stress is an inevitable factor which limits growth, development, and production efficiency of vegetables such as *Capsicum annuum* (Linnaeus) (pepper). This study was carried out to investigate the use of proline as an osmolyte to ameliorate the effect of water stress on morphological and physiological attributes of *Capsicum annuum*. *C. annuum* seedlings were subjected to different levels of proline (20, 30 and 40 %) against droughted and well-watered seedlings as control in a completely randomized design. Results showed that water stress limits morphological and physiological attributes of *C. annuum*, while proline at different levels enhances it. Highest plant height (26.00±0.71 cm) was obtained in *C. annuum* sprayed with 40% proline. Well-watered seedlings of *C. annuum* produced the highest numbers of leaves (35.40±4.12 cm) and leaf length (12.60±0.68 cm). In addition, leaf area (89.40±2.04 cm2), specific leaf area (124.24±1.24 m2kg-1) and leaf area index (1.76±1.13 m2m-2) were significantly higher ($p < 0.05$) in *C. annuum* seedlings sprayed with 40 % proline compared with other treatments. Relative growth rate (0.30±0.09 mgg-1day-1), net assimilation rate (0.47±0.30 gm-2day-1) and leaf area ratio (0.58±0.11 m2kg-1) as well as, chlorophyll content (9.76±0.02 µgcm2), were higher in *C. annuum* seedlings sprayed with 40% proline. In conclusion, 20 and 30 % proline sustained morphological and physiological attributes of *C. annuum* under water stress, but 40% proline produced better ameliorative effects.

Keywords: Amelioration, Drought, Osmolyte, Pepper, Proline, Seedlings, Water Stress,

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INTRODUCTION

Capsicum annuum L. commonly known as chilli or red pepper is an economically important vegetable belonging to the *Solanaceae* family (Barceloux, 2009). It is widely cultivated around the world for its nutritional and therapeutic benefits (FA 2014). However, its cultivation is faced with several environmental factors including drought. Water stress is a severe limiting factor in crop production (Olarewaju and Showemimo, 2002) which brings about unfavorable conditions that suppresses growth, development and yield of crops like *C. annuum*. Meanwhile, inadequate, erratic and uneven rainfall distribution has been reported to majorly contribute to the low production efficiency of *C. annuum* (Olarewaju and Showemimo, 2002). In order to maintain a balanced growth and maximize yield, plants develop natural biochemical defense mechanism which enhances osmoregulation and maintains cell turgor and plant growth (Szabados and Savoure, 2010) by synthesizing osmolytes such as proline. (Ashraf and Foolad, 2007). Proline is a proteogenic amino acid and beneficial solute. When accumulated, it acts as an inorganic osmoprotectant for plants under stress and non-stress conditions (Dar *et al*., 2016). It plays a significant role in improving plant tolerance against stress, acts as signaling molecule, scavenging ionic radicals such as hydroxyl group, protein stabilization and serves as a nutrient reservoir for plants under severe environmental conditions (Verbruggen and Hermans, 2008). Several studies have highlighted the ameliorative role of proline in plants under water-deficient environments. Zouari *et al*. (2016) reported that proline enhanced growth traits and photosynthetic potentials of plants.

Selote and Khanna-Chopra. (2004) and Hassan *et al.* (2018) opined that proline contents increased in drought-tolerant species in response to drought treatment which in turn provided ameliorative measures against oxidative damage induced by reactive oxygen species in the plants.

Vegetables such as *C. annuum* require high amounts of water for proper growth and development, and production efficiency. Moreover, this crop has evolved a range of mechanisms to resist drought stress in its natural environment. Ascertaining the required amount of osmolyte such as proline needed to mitigate the effects of drought on the vegetable needs clarification. This will serve as developmental expedite in the cultivation of the spices under water deficit. Hence, the need to explore the application of proline as osmolytes against drought in order to ensure all-year-round cultivation and effective performance of *C. annuum*.

MATERIALS AND METHODS

Experimental Site: The experiment was carried out at greenhouse of teaching and research Botanical Garden, Lagos State University, Ojo Campus, Lagos, Nigeria. The greenhouse is located on latitude 6.4668. N 6° 28'0.760' and longitude 3.19917 E 3° 11'57.006'. The daily temperature of the greenhouse was 28 °C during the day and 21°C during the night with a relative humidity of about 65-75%.

***Plant Materials*:** *C. annuum* fruits (Cayenne cultivar) used for this study were obtained from local farmers at Badagry, Lagos State, Nigeria. The seeds were identified at Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria with voucher number FHI-5238. Seeds were extracted from the fruits manually and air-dried at room temperature.

***Soil Collection and Sterilization*:** Soil used was collected based on the procedures described by the National Soil Characterization Database of the United States Department of Agriculture, modified by Vijay *et al.* (2019). Top soil was collected from the botanical garden of Lagos State University, Ojo using a soil probe at a depth of 0-10 cm at 500 m apart. The collected soil was sterilized by autoclaving at 132 °C for 30 minutes.

***Nursery Preparation and Raising of C. annuum Seedlings*:** Twenty-five planting buckets (each of 5.75 cm^3 in volume) perforated at the sides and bottom to allow proper drainage of water and aeration without depleting the soil quantity were obtained and filled with about 10 kg of the sterilized soil. Air-dried seeds of *C. annuum* were first sown in a nursery for a period of 21 days. The seedlings after reaching 13.5-14.0 cm were randomly selected and transplanted (one per bucket) into the planting buckets. The seedlings were thereafter watered every day for one week to ensure acclimatization and establishment of the seedlings.

***Source of Osmoprotectants (Proline)*:** L-Proline was purchased from a chemical shop at Loba Chemie Pvt. Limited, 107, Wodehouse Road, Jehangir Villa, Mumbai-400005, India.

***Preparation and Application of Osmoprotectants*:** L-Proline was prepared by dissolving 0.5 g of L-proline in 1 liter of water (Ojewumi and Kadiri, 2021). The different levels of L-Proline (20, 30 and 40 %) used in this study were determined by diluting 200, 300 and 400 mL of proline in 1000 mL of distilled water.

Experimental layout/ design: The experiment was a factorial design arranged in completely random design (CRD) with five replicates. The *C. annuum* seedlings were treated with 300 mL of 20, 30, and 40 % proline at three days intervals. 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 to the seedlings using a foliar application for 3 months according to method of Ojewumi *et al.* (2022)

***Determination of Morphological*:** Morphological parameters such as plant height, leaf length, leaf breadth, stem girth, and leaf area were measured by standard procedures. The number of leaf was determined by physical counting at two weeks intervals (Kadiri, 2014). Plant heights were determined by measuring the shoot heights from the ground level to the terminal bud of the plant using a thread

and a meter rule calibrated in centimeters (Kadiri, 1999). The stem girth was determined using a Vernier caliper calibrated in millimeters (mm) while leaf length and breadth were determined using a ruler in centimeters according to Kadiri (1999).

Determination of Total Leaf area, Specific Leaf Area and Leaf area Index of C. Annum: The total leaf area was determined using LI-3000 C Portable Leaf Area Meter by DMP Ltd. Specific leaf area (SLA) and leaf area index (LAI) were determined using the below formula

$$SLA = \frac{\text{Leaf area(LA)}}{\text{Corresponding weight of leaf(WL)}}$$ Daffonchio, *et al.*, 2015).

$$LAI = \frac{\text{Leaf area(LA)}}{\text{Area of litter fall}}$$ (Jasper *et al.*, 1989)

Determination of Physiological Parameters of C. Annum:

Determination of Growth Indices: The values obtained for dry weights, leaf areas and time of harvest of the leaves were used to determine net assimilation rates (NAR), relative growth rates (RGR) and leaf area ratio (LAR) of *C. annum* leaves using the below formula: (Alireza *et al.,* 2012, Kadiri, 1999).

$$RGR = \frac{\text{LogeW2} - \text{LogeW1}}{\text{t2}-\text{t1}}$$

$$NAR = \frac{W2 - W1}{A2 - A1} \cdot \frac{\text{LogeA2} - \text{LogeA1}}{t2 - t1}$$

$$\backslash LAR = \frac{W2-W1}{t2-t1} \cdot \frac{\text{LogeA2}-\text{LogeA1}}{W2-W1}$$

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 Leaves of C. annum

One gram of fresh leaves from each treatment was obtained and crushed separately with 40 mL of 80 % (vlv) acetone using a clean mortar and pestle. The resulting crushed material was filtered through Whatman No. 1 filter paper in Buchner Funnel. The residual pulp was re-extracted with 20 mL aliquot solution of 80 % acetone and filtered into the previous filtrate. The optical density of the chlorophyll extract in a 10 mm cell was determined using a spectrophotometer at 652 nm. Chlorophyll content in the leaves was determined using the below formula (Kadiri, 2014):

$$\text{Mg total chlorophyll/g tissue} = \frac{O.D.652X1000XV}{34.5\times1000\times W}$$

Where: O.D = Optical density reading of chlorophyll extract at 652 nm, V = the final volume of the chlorophyll extract and W = the fresh weight (g) of the leaf tissue extracted.

Statistical analysis

Data obtained were analysed using statistical analysis system version 9.4 ((SAS, 2013)). One-way analysis of variance (ANOVA) was conducted to determine significant difference between parameters. Means were separated using Duncan's Multiple Range Test at $p < 0.05$.

RESULTS

***Effects of different Levels of Proline on Morphological Parameters of C. annum under Water Stress*.** The effects of different levels of proline on the morphological parameters of *C. annum* is shown in Figures 1-6. From the results obtained, it was observed that proline at different levels had no significant ($P>0.05$) effects on plant height, number of leaves, leaf length, and breadth of leaves, of *C. annum* at 2 and 4 weeks after treatment (WAT). However, there was a significant increase in the plant height, number of leaves, leaf length, and breadth of leaves of the pepper sprayed with 40% proline and well-watered at 8 WAT. Meanwhile, at the 8 WAT, it was observed that leaf breadth was highest in *C. annum* (3.50±0.01cm) made to receive water every day (well-watered) (Fig 1-4). The results also revealed that petiole length (4.6 cm) and stem girth (0.85 ± 0.3 cm) of *C. annum* well-watered and the ones made to receive 40% proline every day were significantly higher compared with other treatments while the lowest values of the parameters were observed in droughted seedlings of *C. annum* (Fig 5 and 6).

Effects of different Levels of Proline on Physiological Parameters of C. annum under Water Stress

AT 8 and 10 WAT, growth indices such as leaf area, specific leaf area, and leaf area index of leaves of *C. annum* were significantly influenced by different levels of proline. Leaf area (89.40±2.04 cm), specific leaf area (148.16±2.38 m^2kg^{-1}) and leaf area index (1.76±1.13 m^2m^{-2}) were had highest values in *C. annum* sprayed with 40%. Also, the relative growth rate (0.03±0.09$mgg^{-1}day^{-1}$), net assimilation rate (0.47±0.03$gm^{-2}day^{-1}$) and leaf area ratio (0.58±0.11 m^2kg^{-1}) were highest in *C. annum* sprayed with 40% proline followed by those observed in well-watered and lowest in droughted seedlings (Table 5). Chlorophyll contents (9.76±0.02μgcm^2) was significantly higher ($p < 0.05$) in *C. annum* leaf sprayed with 40% proline while least chlorophyll content (7.52±0.02μgcm^2) was obtained in droughted *C. annum* (Figure 7).

DISCUSSION

Water deficit is one of the major abiotic factors threatening the sustainable cultivation and productivity of many plants (Akula *et al.*, 2011, Liliane *et al.,* 2020). Plants under this unfavorable condition exhibit symptoms of injury due to oxidative damage caused by reactive oxygen species (ROS) on the tissue of plants (Couée *et al.,* 2006). The phenomenon has also been reported to have the ability to reduce several plant metabolites and photosynthetates production. Adaptations of plants to tolerate stress or their natural coping mechanisms against the condition' depend on genetically modified traits (Ashapkin *et al.,* 2020). The mechanisms may fluctuate with changes in climatic conditions, soil conditions and species of plants involved (Dos Santos *et al.,* 2022). This condition therefore, results in the inhibition of the growth or synthesis of some non-toxic compounds which are used to increase osmotic potential, improve metabolic processes, and ultimately increase activity of some oxidative enzymes and production of some osmolytes (Turkan *et al.,* 2005).

In the present findings, an increase in the morphological parameters of *C. annuum* such as plant height, number of leaves, leaf length, and breadth of leaves under 40 % proline compared to other treatments

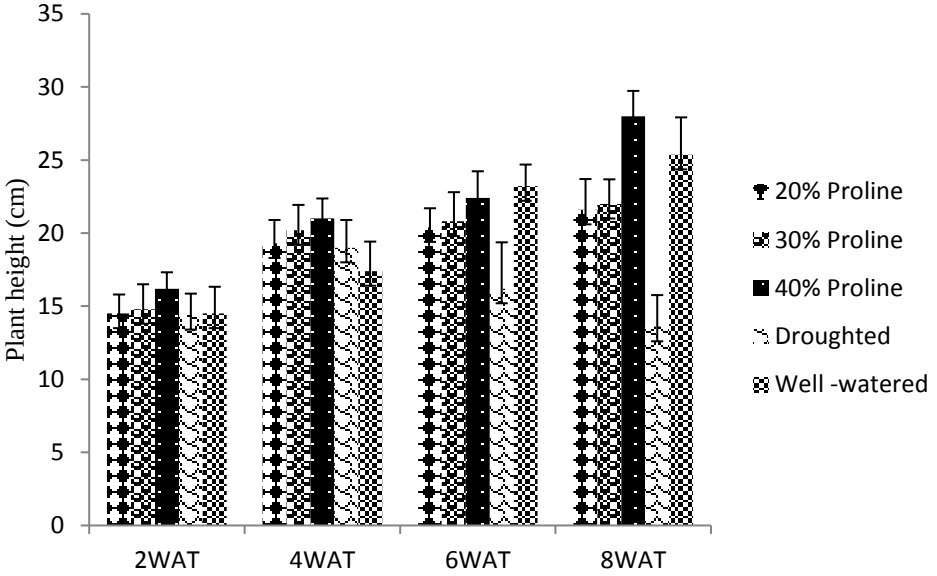


Fig. 1: Effect of different levels of proline on plant height of *C. annuum* under water stress at various weeks

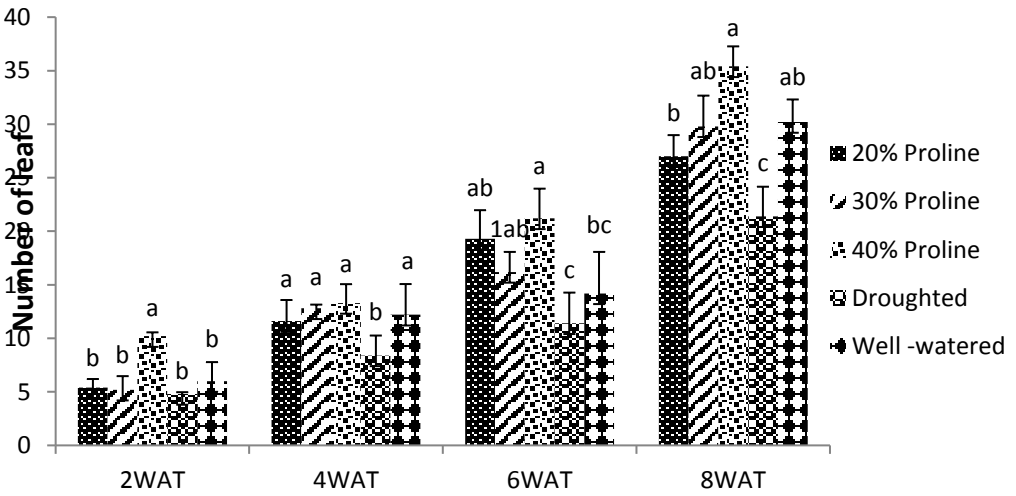


Fig. 2: Effect of different levels of proline on number of leaf of *C. annuum* under water stress at various weeks

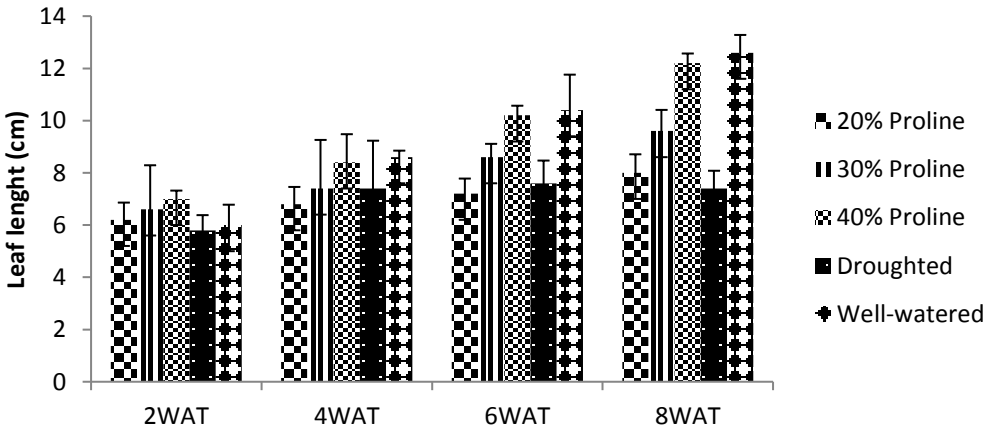


Fig. 3: Effect of varying levels of Proline on Leaf Length of *C. annuum* under water stress at various weeks

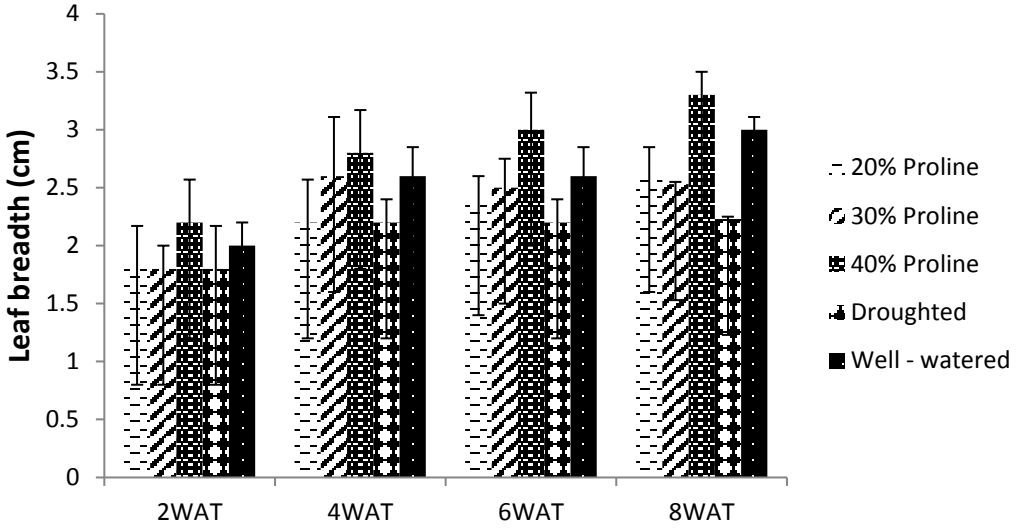


Fig. 4: Effect of varying levels of Proline on Leaf breadth of *C. annuum* under water stress at various weeks

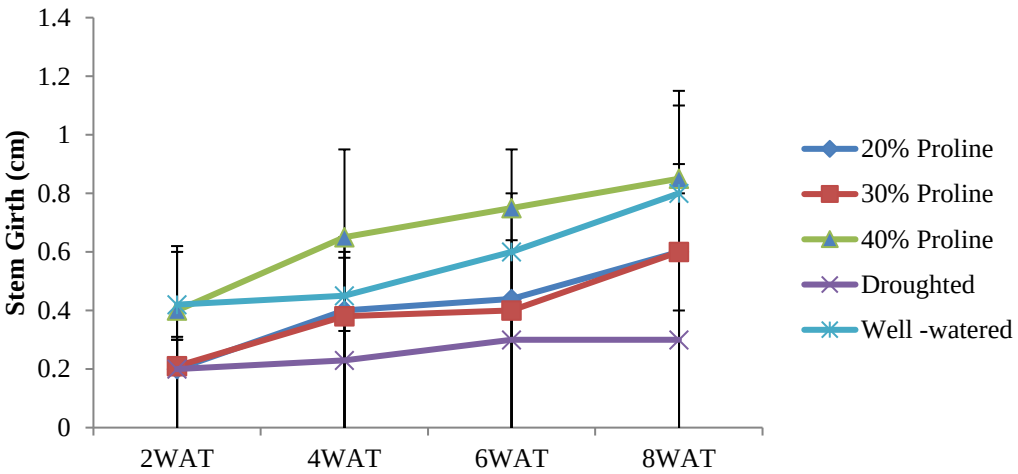


Fig.5: Effect of varying levels of Proline on Stem Girth of *C. annuum* under water stress at various weeks

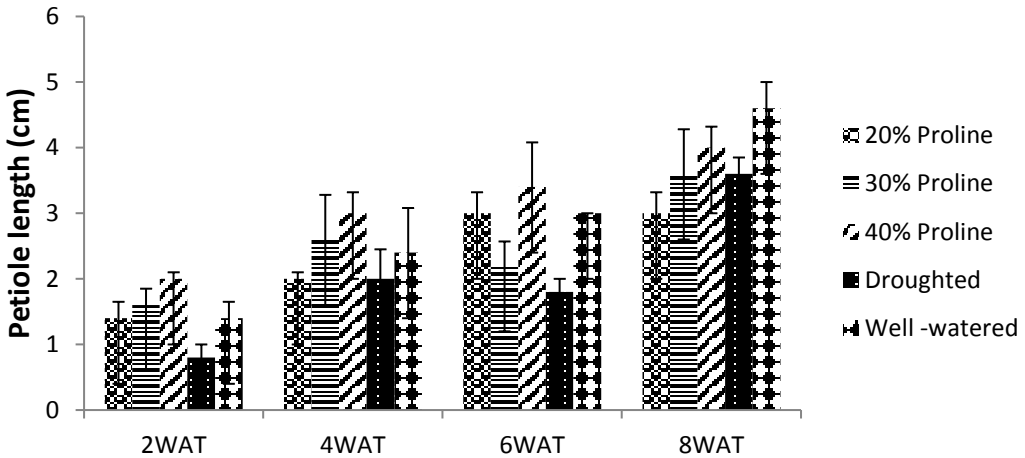


Fig. 6: Effect of varying levels of Proline on Petiole Length of *C. annuum* under water stress at various weeks

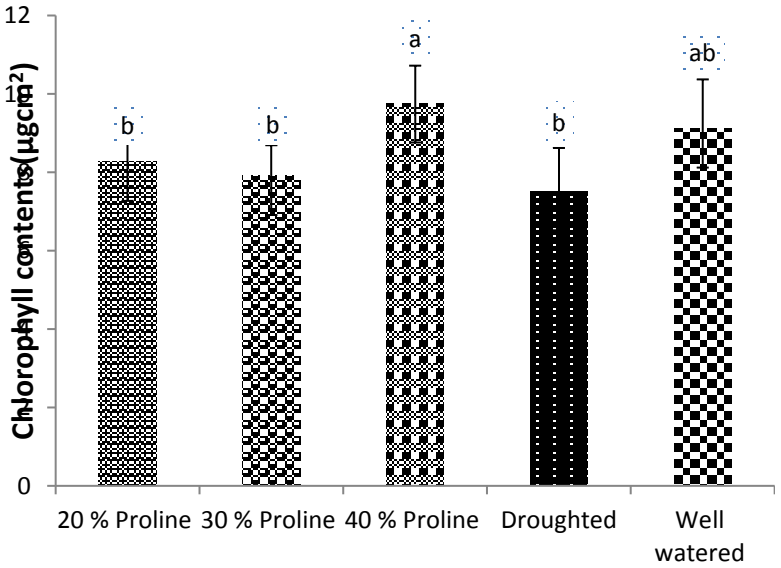


Fig. 7; Effects of different levels of proline on chlorophyll contents of *C. annuum* under water stress at various weeks

Table 1; Effect of varying levels of Proline on Growth parameters and some Physiological Parameters of C. annuum under water stress

Treatments	Physiological parameters						Growth parameters		
	8WAT LA (cm^2)	SLA(m^2kg^{-1})	LAI (m^2m^{-2})	10WAT LA (cm^2)	SLA(m^2kg^{-1})	LAI(m^2m^{-2})	RGR	NAR	LAR
20% Proline	31.00±9.41^{c}	64.12±11.78^{c}	0.37±0.05^{b}	56.48±12.12^{b}	112.67±1.23^{b}	0.62±0.24^{b}	0.12±0.02^{ab}	0.16±0.08^{c}	0.39±0.03^{c}
30% Proline	21.15±6.74^{d}	85.83±11.23^{c}	0.41±0.08^{ab}	56.86±19.72^{b}	128.98±21.26^{ab}	0.76±0.20^{c}	0.12±0.09^{ab}	0.15±0.03^{c}	0.47±0.15^{ab}
40% Proline	74.08±11.50^{a}	124.24±1.24^{a}	0.46±0.06^{a}	89.40±12.04^{a}	148.16±21.38^{a}	1.76±1.13^{a}	0.30±0.09^{a}	0.47±0.30^{a}	0.58±0.11^{a}
Droughted	30.04±4.44^{c}	44.34±11.77^{d}	0.25±0.01^{c}	48.56±9.05^{c}	65.11±11.56^{d}	0.49±0.16^{c}	0.08±0.03^{c}	0.15±0.04^{c}	0.36±0.14^{c}
Well-watered	58.35±22.73^{b}	114.45±0.24^{b}	0.44±0.49^{a}	73.32±11.63^{a}	146.67±0.18^{ab}	0.92±0.55^{ab}	0.12±0.08^{ab}	0.29±0.18^{b}	0.57±0.52^{a}

Mean ± standard error followed by different superscripts in the same columns are significantly different at $p<0.05$ according to Duncan's Multiple Range Test. WAT= Weeks after treatment, LA= Leaf area, SLA=Specific leaf area, LAI=Leaf area index, RGR=Relative growth rate, NAR=Net assimilation rate, LAR=Leaf area ratio

implies that 40 % proline has better drought mitigating potential. This may be due to the roles of proline in adaptation, recovery, and signaling under stress conditions or presence of antioxidants that have the ability to scavenge the oxidative damage which might be caused by ROS.

This observation is in agreement with the findings of Ali *et al*. (2007) on maize and Hu and Hu (2012) on perennial Ryegrass. It is also consistent with the study of Kaur and Asthir (2015), who posited that proline at higher concentration increased hydraulic conductivity, enhanced water flow from roots to leaves, increased water retention and reduce transpiration rate of plants. Furthermore, higher number of leaves obtained in *C. annuum* sprayed with 40% proline indicated that a higher level of proline has a better ability to reduce excessive loss of water as one of the factors that determine leaf expansion and bud formation. This treatment might have activated actions on some growth promoters which help in the formation and development of plant organs under water deficit (Camaille et al., (2021), Vardharajula et al., 2011). This submission is in agreement with the findings of Meena et al. (2019) who posited that increase in proline level in water-stressed plants provided high energy to promote plant growth and their metabolic processes.

The increase in petiole length and stem girth observed in *C. annuum* sprayed with 40% proline might be as a result of increase osmotic potential, improvement in metabolic processes and ultimately increase in activity of some antioxidant enzymes (Hong *et al.* 2000, Turkan *et al.,* 2005; Kadri *et al.*, 2016). Variations observed in the morphological indices of *C. annuum* under water stresses compared with the ones under proline treatments may be due to differential regulation of the physiological attributes involved in growth processes of the pepper

The significant increase in the chlorophyll contents of *C. annuum* sprayed with 40% proline may infer that proline enhanced pigment formation and maintained the photosynthetic efficiency of the plants (Sivakumar *et al.,* 2000). In addition, lower quantity of chlorophyll contents evaluated in the leaves of *C. annuum* droughted may be caused by stomatal closure, non-stomatal inhibition or damage in photosynthetic machinery of the leaves (Lidia *et al.,* 2004).

Furthermore, significant increase recorded in the plant growth indices such as relative growth rate, net assimilation rate and leaf area index of *C. annuum* sprayed with 40% proline may suggest that proline improves the plant biomass and activates the production of primary metabolites in the leaf of *C. annuum*. It could also be due to the interaction of proline with some enzymes such as **proline** dehydrogenase (PDH and pyrroline-5-carboxylate synthase (P5CS) in leaves of plants which speed up energy production thereby enhancing the growth indices of *C. annuum* under water deficit (Pollard *et al*., 2000).

Conclusion

From the findings of this study, it can be concluded that proline significantly improved the morphological and physiological parameters of water-stressed *C. annuum* plants, which was associated with increased biosynthesis of chlorophyll pigments, as well as the improvement in plant biomass and leaf water relations through cellular osmotic adjustment of the accumulation of the osmolytes. As a result, better functioning of the plants occurred, which led to better growth and production efficiency of water-stressed *C. annuum* plants. Meanwhile, 20 and 30% proline sustained morphological and physiological attributes of *C. annuum* under water stress, but 40% proline produced better ameliorative effects.

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Authors contribution

AWO conceived the research idea, analyzed and interpreted data.

AAO and TMI performed the experiment, collected data and wrote the draft.

S AA read and corrected the draft.

All authors read the manuscript and approved its submission to Annals of Science and Technology

Conflict of interest

The authors declared no known conflicts of interest associated with this publication

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