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# Pre-sowing Seed Treatment with Proline, Glycine Betaine, and Soil Amendment with Compost as Strategies for Improving Yield and Drought Tolerance in Cowpea

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## Abstract

**Purpose** Drought is one of the major abiotic stresses limiting crop production. Compatible solutes, like proline (P) and glycine betaine (GB) enhance crop tolerance to abiotic stresses. Compost also supplies nutrients to crop and enhances crop tolerance to stress. The effects of pre-sowing seed treatments with P and GB as well as soil amendment with compost on cowpea performance under water deficit stress were investigated in this study.

**Methodology** Cowpea seeds were soaked in P and GB at 0 (distilled water: control), 2.5, 5, and 10 mM for 12 h before sowing. Compost (C) was applied at 2.5, 5, and 7.5 t ha<sup>-1</sup>. Water stress was imposed by withdrawing water for 10 (W1) or 20 (W2) days, at vegetative (VS) or reproductive (RS) stages. Data were collected on cowpea growth parameters, yield, nodulation, P, and GB accumulation.

**Results** The W2 at VS reduced cowpea growth and yield by 100% compared to the unstressed plants, while, W1 was more tolerable. Proline, GB and C enhanced tolerance, growth, and pod yield, especially with C<sub>5 t ha<sup>-1</sup></sub>, C<sub>7.5 t ha<sup>-1</sup></sub>, GB<sub>5 mM</sub>, and P<sub>2.5 mM</sub>. Water stress increased P and GB in untreated plants more than the treated plants. Compost at every rate, reduced the accumulation of proline and GB in cowpea under stress (W1), whereas proline was more in plants pre-treated with GB<sub>10 mM</sub> and P<sub>2.5 mM</sub> under W2 at VS compared to RS. At RS, compost increased P and GB accumulation and these were reduced in P treatments.

**Conclusion** Compost and seed treatment with solutes enhanced cowpea stress tolerance and improved yield.

**Keywords** Cowpea · Water deficit stress · Osmo-protectants · Yield · Nodulations

## 1 Introduction

Cowpea is an important food crop in the tropics and subtropics. It is the main source of plant protein for the growing children and adults, especially in the developing countries (Rivas et al. 2016; Ravelombola et al. 2020). Though cowpea (*Vigna unguiculata* L. Walp.) has been reported to be one of the drought tolerant crops (Bastos et al. 2011) but, its production is being threatened by extreme drought stress due to climate change (Ravelombola et al. 2020). Climate change has brought about change in rainfall pattern,

incessant drought, and abnormally high temperature, all of which pose serious threats to crop production. Drought is classified as a major limitation to food production worldwide (Shao et al. 2009; Barutcular et al. 2017). It is an important environmental stress factor that inhibits crop growth and development by disrupting crop physiological processes and causing oxidative stress through production of Reactive Oxygen Species (ROS) (Hoque et al. 2007; Verbruggen and Hermans, 2008; Rezaei et al. 2012). It leads to a decrease in plant water content and causes alteration of essential cellular processes (Lobato et al. 2008). It affects plant growth from seedling to maturity (Alidu et al. 2013). Water deficit has been reported to induce reduction in growth of different crops like pearl millet (Ashraf et al. 2001), okra (Ashraf et al. 2002), wheat (Waseem et al. 2006) maize (Ali et al. 2007; Jabeen et al. 2008), and cowpea (Ravelombola et al. 2020). Drought therefore poses a serious threat to food security.

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Plants, being sessile, has evolved different mechanisms at physiological, molecular, and cellular levels to avoid or tolerate water stress. Many plants cope with stress situation by employing enzymatic or non-enzymatic mechanisms (Hossain et al. 2012). The non-enzymatic strategy involves accumulation of different compatible solutes such as Proline (P) and Glycine Betaine (GB) (Ashraf and Foolad 2007; Ahmed et al. 2019). Proline and GB are known to function as compatible osmolytes for macromolecule protection and as scavengers of ROS under stressful conditions (Ashraf and Foolad 2007). They increase the cellular osmotic potential gradient that allows for continuous influx of water. This helps the plant to withstand water deficit while maintaining relatively high moisture content in the tissue (Sumithra et al. 2007). They improve CO<sub>2</sub> assimilation in plants, enhance photosynthetic machineries and regulate the ion homeostasis (Raza et al. 2007; Hussain et al. 2008). They play vital roles in osmotic adjustment and protection of the thylakoid membrane (Chaitanya et al. 2009). They also promote seed germination and seedling establishment under adverse environmental conditions (Hare et al. 2003; Agbicodo et al. 2009). The roles of these solutes in stress tolerance and for improving resilience to water deficit have been widely investigated and reported (Hoque et al. 2007; Verbruggen and Hermans 2008; Ahmed et al. 2019; Guo et al. 2022). Production of P and GB has been reported as osmo-protectants that confer tolerance on water stressed plants (Hanan et al. 2013; EL-Sabbagh et al. 2015a, b).

The effects of their exogenous application for pre-sowing seed priming have also been widely explored (Rezaei et al. 2012; Hanan et al. 2013; EL-Sabbagh et al. 2015a, 2015b; Ahmed et al. 2019; Farooq et al. 2019; Guo et al. 2022). Application of P and GB for pre-sowing seed priming was found to enhance tolerance in plants and ameliorate environmental stresses. Priming with P and GB increases the antioxidant enzyme activities and reduces the oxidative stress by quenching the Reactive Oxygen Species (ROS) (Cheng et al. 2018; Ghafoor et al. 2019; Ambreen et al. 2021; Guo et al. 2022). It was also reported to increase the efficiency of primary photochemistry of PSII due to better management of absorbed energy in electron transport chain of PSII (Altuntaş et al. 2020; Ambreen et al. 2021). The use of these compatible solutes for seed priming to enhance stress tolerance in crop is now a popular practice.

Meanwhile, nutrient availability is key to crop development and tolerance to adverse environmental conditions like drought. In the absence of appropriate and required nutrients for plant growth, the ability of the plant to cope with stress is usually low. Adequate nutrient supply gives the inner strength and makes available the materials needed for the synthesis of stress-inducible metabolites for tolerance. Apart from the use of chemicals to enhance stress tolerance, the use of organic amendments has been reported to enhance

tolerance by improving soil nutrient status (Adejumo et al. 2010; Abderrahim et al. 2021). Organic amendment helps to improve soil fertility and ameliorate crop stress through the modification of soil physical, chemical, and biological properties. It increases soil water retention capacity and thus, ensures continuous supply of water and nutrients to plants under stress (Tejada et al. 2009a, b). It also enhances the microbial activity and organic matter content of the soil. More importantly, it is cost effective, accessible, available, and environment-friendly. Therefore, for effective stress amelioration, maintaining soil nutrient status with the use of organic amendment is also crucial.

Crop's response to water deficit also depends on the species, plant age, water deficit level and severity (Adejumo et al. 2018). The effect of drought has been reported to be more devastating at reproductive and grain filling periods than vegetative growth stage (Ramos et al. 2003; Khalili et al. 2010). Drought during reproductive phase affects crop yield and yield attributes (Liu et al. 2018; Kuwayama et al. 2019). The severity of water deficit stress also determines crop response and the recovery rate (Adejumo et al. 2018). Effectiveness of these compatible solutes for stress tolerance is also concentration dependent and crop specific (Ambreen et al. 2021). It is therefore imperative to determine the most effective concentrations of these osmolytes (GB and P) and organic amendment as well as the most responsive growth stage(s) of the plant for their economic applications under drought stress. The optimum application rate of these osmolytes as seed primers can be combined with the appropriate soil fertility management practice for enhancing stress tolerance in crop. All these may vary from species to species and for different solutes. For instance, higher dose of proline was not effective in improving crop growth under stress (Miller et al. 2009; Ambreen et al. 2021). Seed priming with 40 mM proline or greater has been reported to cause adverse effects on plant growth and the most effective concentration of proline for growth improvement was given as 5 mM (Athar et al. 2009). The ineffectiveness of higher concentration was attributed to a feedback inhibition mechanism. Exogenous proline, if supplied in higher concentration inhibits pyrroline-5-carboxylate synthase (P5CS) enzyme which is the enzyme catalyzing the rate limiting step of proline biosynthesis (Ambreen et al. 2021). This on its own can generate ROS and cause growth inhibition of the treated crop. Conversely, in another study on wheat, seed priming with lower concentration of proline was not effective under saline soil. The effective concentration was found to be between 10 and 30 mM (Ahmed et al. 2019). It means that different crops have their tolerant or optimum levels. The optimum and effective concentration for cowpea must therefore be determined for effective and sustainable application.

Similarly, the organic amendment, despite being available, it is required in large quantity and this has a

disadvantage of bulkiness. The optimum application rate of organic amendment for drought stress amelioration must also be determined in comparison with the widely reported P and GB. Furthermore, the several studies on P, GB, or compost that have been reported for stress amelioration were studied separately in crops exposed to different stresses and at different locations and experimental conditions. There has not been comparison of these different strategies under the same location and growth conditions. This study was therefore, designed to compare the sole effects of pre-sowing seed soaking with P and GB as well as soil amendment with compost on the performance of cowpea exposed to water deficit stress. It was expected that from the results of this study, the best rate of the individual treatment that could be combined for drought stress tolerance would be selected. It is hypothesized that the combined treatment of these osmo-protectants and compost could be a sustainable method of ameliorating drought stress in crops in the face of climate change.

## 2 Materials and Methods

The pot experiment was carried out at the screen-house of the Department of Crop Protection and Environmental Biology, Faculty of Agriculture, University of Ibadan, Ibadan, Oyo State, Nigeria, located at 7°24'N 3°54'E, 234 m above sea level. The compost used for the study was prepared from *Tithonia diversifolia* (Mexican Sunflower) and poultry manure in ratio 3:1 following the procedure used by Adejumo et al. (2010). After maturity, the materials were allowed to cure, dried, and sieved using 2 mm sieve before the commencement of the experiment. Proline and GB were prepared in three concentrations 2.5 mM, 5 mM, and 10 mM, respectively. Seed soaking was carried out according to the procedure described by Hanan et al. (2013). Cowpea variety (IT07K-292-10) seeds were sourced from the International Institute of Tropical Agriculture (IITA), Ibadan, Oyo State, Nigeria. A total of 120 seeds of equal sizes were selected for uniformity. The seeds were washed with distilled water, sterilized with 1% sodium hypochlorite solution for 2 min and gently washed again with distilled water. The seeds were then divided into various groups according to the treatment applications. At 4 seeds per pot and 3 replicates, 12 seeds each were soaked in 20 ml of their respective solutions for imbibition for 12 h. The remaining 60 seeds were also divided into 5 groups and each group was soaked with 20 ml of distilled water only (these were to be planted in the compost amended soils and control soils). After 12 h, the seeds were blotted out dry on filter paper and allowed to dry at room temperature before sowing.

## 2.1 Experimental Design and Procedure

The experimental design was a  $2 \times 2 \times 3 \times 3$  factorial fitted into completely randomized design (CRD) with three replicates. The treatments were two levels of water deficit stress imposition, which was achieved by withdrawing water for 10 days (W1) and 20 days (W2) and imposed at two growth stages, vegetative, and reproductive growth stages, three types of materials, compost (C), P (P), and GB (G) applied at three rates. Compost was applied at 2.5, 5, and 7.5 t ha<sup>-1</sup> and denoted as C1, C2, and C3. Proline and GB were applied at 2.5 mM, 5 mM, and 10 mM and labelled as G1, G2, G3, P1, P2, and P3 based on the application rates for GB and P. The seeds soaked in distilled water only without P and GB were planted in compost amended soils and were also used for the untreated stressed and unstressed control. Soil for the experiment was collected from the crop garden of the department. The soil was first air-dried, sieved using 2 mm mesh sieve and composite sample taken for physico-chemical analysis. The soil was slightly acidic with pH 6.42. The organic carbon was low (1.95%) and the cation exchange capacity was 225.94 cmol/kg. Each pot was filled with 5 kg soil and labelled according to the designated treatments. Before sowing cowpea seeds, the initial field capacity was determined following the standard procedure to know the quantity of water to be added. From the results, the 5 kg soil used for the experiment required 550 ml of water to get saturated. Each pot was then saturated to field capacity before the sowing of cowpea seeds. Thereafter, the amount of water was reduced to 300 ml and was being added at 2-day interval to every pot before the commencement of the water deficit stress. The stressed plants were deprived of water for 10 and 20 days at either vegetative or reproductive growth stage depending on the treatment, while the unstressed and those that were to be stressed at the reproductive stage were receiving continuous watering. Meanwhile, as the plants were growing, the amount of water being added also increased from 300 to 450 to meet up with the plant water requirement and evapotranspiration. Compost was also added to the soil receiving compost at different rates (The quantity applied to each soil was calculated based on the weight of the soil and each application rate, i.e., 6.25, 12.5, and 18.25 g for 2.5, 5, and 7.5 t ha<sup>-1</sup>, respectively), watered and left for 1 week before the sowing of cowpea seeds. There were two control treatments (the stressed and unstressed control plants). Thinning was done 2 weeks after planting into two plants per pot, and weeding was done when necessary. The water deficit stress at vegetative stage (V) was imposed from 2 weeks after sowing through pre-anthesis, while water deficit stress imposed at reproductive stage (R) commenced from onset of flowering through anthesis and post-anthesis stages. In all, there were 41 treatments. Instead of having control treatment for C, P, and GB, only one control was used for treated and

untreated and for stressed and unstressed to avoid repetition of results and reduce the number of experimental units.

## 2.2 Data Collection

Data were collected on percentage seeds germination or seedling emergence at 4 days after sowing. Data on morphological/growth parameters were collected on plant height (cm), total number of leaves and leaf area (cm<sup>2</sup>) starting from 2 weeks after planting and fortnightly using standard agronomic procedures and the average values determined. The leaf area was determined according to Agbogidi and Ofuoku (2005). Data on yield and yield components were collected on plant biomass (g), number of days to 50% flowering, number of pods, total number of grains per pod, and weight of pods per plant. Data on nodulation parameters were also collected on total number of root nodules, fresh and dry weight of nodules, and number of effective nodules. Effective nodules were determined per plant by counting nodules with pink color when cut open.

## 2.3 Proline and GB Determination in Fresh Leaves

Proline was estimated in the plant leaves according to the procedure of Bates et al. (1973). For this, 0.5 g of fresh leaf from each treatment was homogenized in 5 ml of 3% aqueous sulpho-salicylic acid and centrifuged at 2000 × g for 5 min. The supernatant was filtered and 2 ml of the filtrate was added to 2 ml of glacial acetic acid and 2 ml acid ninhydrin which was prepared by warming 1.25 g of ninhydrin in 30 ml glacial acetic acid and 20 ml of 6 N phosphoric acid. The mixture was heated in boiling water bath for 1 h. Thereafter, the tubes were placed in the ice bath to terminate the reaction and 4 ml of Toluene was added to the mixture and stirred for 20–30 s. The toluene layer was separated and the red color intensity was measured using UV spectrophotometer at 520 nm. The GB content in the leaf tissues was determined following the method of Grieve and Grattan (1983). Fresh leaf material (1.0 g) from each treatment was shaken in 10 ml of 0.5% toluene solution and filtered. After filtration, 1 ml of the extract was mixed with 1 ml of 2 N H<sub>2</sub>SO<sub>4</sub> and 0.5 ml of the mixture was taken in glass tube and 0.2 ml of Potassium tri-iodide (KI<sub>3</sub>) solution was added. Then, 2.8 ml ice-cooled distilled water and 6 ml of 1,2-dichloroethane (cooled at 4 °C) were added to the mixture. The upper aqueous layer was discarded and optical density of the lower layer was measured at 365 nm using UV spectrophotometer.

## 2.4 Statistical Analysis

Data collected were analyzed using DSAASTAT software. All the data presented are the means of three replicates

together with standard deviations. Analysis of variance (ANOVA) was carried out to assess the effects of drought and growth stages as well as interactions with treatments (P, GB, and compost) and means comparisons were performed using Duncan's multiple range test (DMRT) at  $P < 0.05$ . Graphs were drawn using the excel workbook. The means of the different parameters considered were used for the construction of the graphs and the standard errors were calculated for the error bars. The mean separation for the graph was carried out by using DMRT and the alphabets placed on the graph bars.

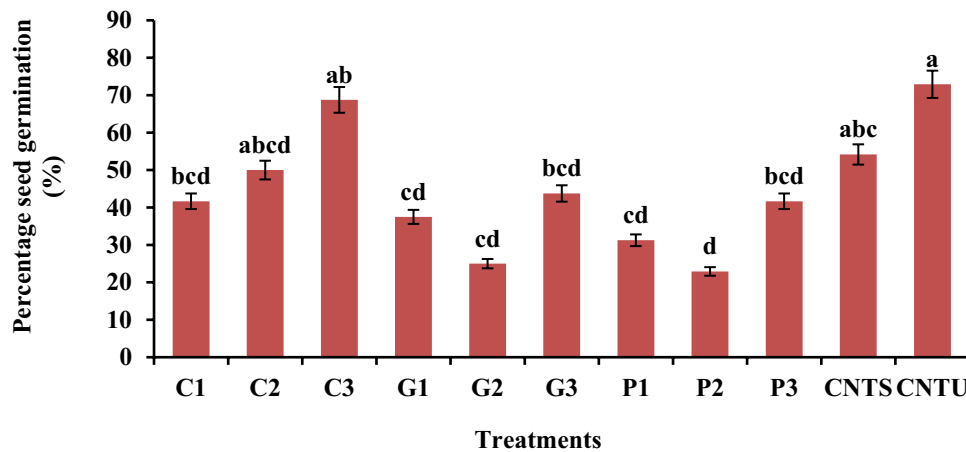
## 3 Results

### 3.1 Effect of Soil Amendment with Compost and Pre-sowing Seed Treatments with P and Gb on Percentage Seed Germination

Pre-sowing seed treatment with P or GB and soil amendment with compost had no significant effect on the seed germination at 4 days after sowing (DAS). The highest value for germination was recorded in the control seeds soaked with distilled water only having an average of 73%. This was followed by those grown on soil amended with highest rate of compost (7.5 t ha<sup>-1</sup>) with an average of 69% and the least value was observed in seeds soaked in P and GB at 5 mM. However, comparing the three treatments (C, P, and GB), compost application to the soil enhanced seed germination more than P and GB, while GB was better than P. The highest rate of 10 mM for P and GB was more effective than other rates of application and the seed germination was conversely reduced in seeds soaked in 5 mM of P and GB compared to 2.5 mM (Fig. 1).

### 3.2 Effect of Soil Amendment with Compost and Pre-sowing Seed Treatments with P and GB on Vegetative Parameters

Exposure to water deficit stress for 10 or 20 days generally reduced the vegetative growth parameters of cowpea and the reduction was more in plant exposed to 20 days water withdrawal compared to those stressed for 10 days (Fig. 2a). However, and irrespective of stress duration and growth stages, pre-sowing seed treatment with P and GB as well as soil amendment with compost increased cowpea vegetative growth parameters compared to the stressed control. Soil amendment with compost was more superior to other treatments (Fig. 3). Similarly, plants that were not stressed at vegetative stage. but exposed to stress at reproductive stage performed better than those stressed at the vegetative stage in terms of growth parameters. Cowpea plants grown on soil amended with compost at



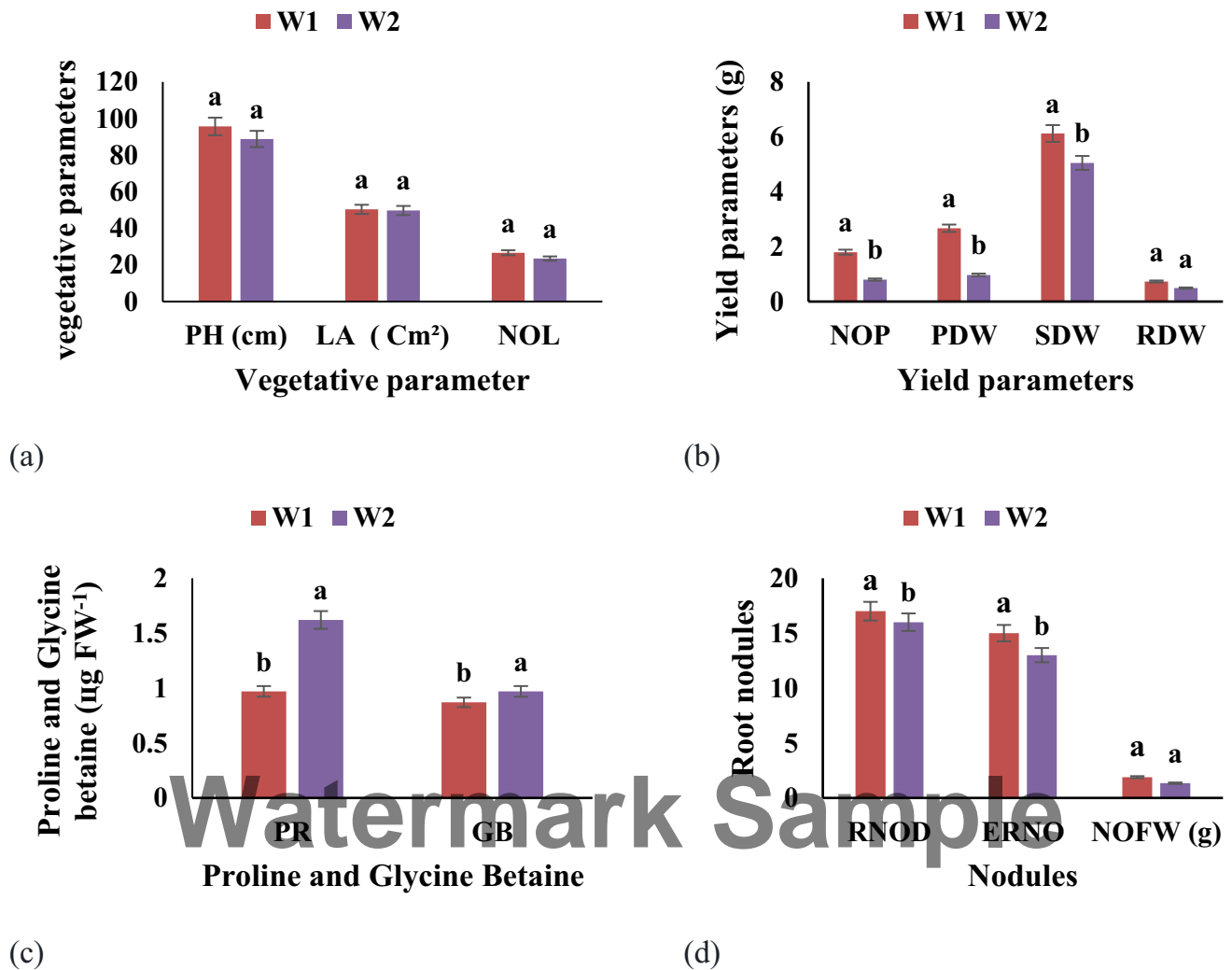
**Fig. 1** Effects of compost application and seed priming with different levels of proline and glycine betaine on percentage seed germination of cowpea 4 days after sowing. C1, C2, and C3=compost applied at 2.5, 5, and 7.5 t ha<sup>-1</sup>, respectively; G1, G2, and G3=glycine betaine applied at 2.5, 5, and 10 mM, respectively; P1, P2, and P3=proline

applied at 2.5, 5, and 10 mM, respectively; *CNTS* control stressed, *CNTU* control unstressed. Each bar represents the mean of the three replicates ( $n=3$ ) for each treatment. Bars carrying the same alphabet are not significantly different from one another at  $P<0.05$  according to Duncan multiple range test (DMRT)

7.5 t ha<sup>-1</sup> and stressed for 10 days at the reproductive stage (C3RW1) had the highest plant height. This was also followed by plants pre-treated with 2.5 mM GB and stressed for 10 days at the reproductive stage (G1RW1). The least value for plant height was recorded for untreated plant (CNTS) that was stressed at the vegetative stage for 20 days (W2) (Table 1). Total leaf area of cowpea plant was also negatively affected by water deficit stress and similar to plant height, C3RW1 also gave the highest leaf area compared to all other treatments under stressful conditions. This was followed by plants grown on soil amended with compost at 5 t ha<sup>-1</sup> and stressed at the vegetative stage for 10 days (C2VW1). The lowest value of leaf area was recorded in control plants stressed for 20 days at vegetative stage (CNTSW2) (Table 1). Similar trend was observed for the total number of leaves. Water deficit stress reduced the total number of leaves across all treatments and growth stages compared to the unstressed control. Total number of leaves was reduced by water deficit stress and the reduction was more where water deficit was imposed for 20 days duration. The treatment (C3RW1) also gave the highest number of leaves as observed for other growth parameters. The CNTS at W2 produced the lowest average number of leaves. Pre-sowing seed treatment with P and GB as well as soil amendment with compost performed better than untreated control under stress. All compost treatments were however superior to P and GB treatments except in plants pre-treated with GB at 5 mM and stressed at the reproductive stage (Table 1).

### 3.3 Effect of Pre-sowing Seed Treatments with P and GB and Soil Amendment with Compost on Biomass Accumulation in Cowpea

On biomass accumulation, irrespective of the treatments, the effect of water deficit stress was more pronounced on stressed control plants without seed pre-treatments and compost application. Water withdrawal for 20 days was also more detrimental to biomass accumulation than 10 days (Fig. 2b). Pre-sowing seed treatments and soil compost amendment at different levels enhanced shoot dry weight of plants under water deficit stress compared to untreated control. Among the treatments, plants grown on soil amended with compost at 2.5 t ha<sup>-1</sup> and stressed at the vegetative stage for 10 days (C1VW1) had the highest plant biomass and performed better than others. This was followed by plants grown on compost-treated soil at 7.5 t ha<sup>-1</sup> and stressed at the vegetative stage for 10 days (C3VW1). Control plants stressed for 20 days at the reproductive stage (CNTS RW2) had the lowest shoot dry weight. Root dry weight of plants exposed to water stress without any treatment was reduced compared to the treated and unstressed control plants. This reduction was more in plants under water deficit stress for 20 days at the reproductive stage (CNTS R W2). Though not significantly different from other treatments, root biomass was more in cowpea plants treated with highest rate of GB and exposed to water deficit stress for 10 days at the reproductive stage (G3RW1). Though, pre-sowing seed treatments and compost application had no significant effects on the plants exposed to water deficit stress for 20 days at the reproductive



**Fig. 2** Vegetative growth (a), yield parameters (b), proline and glycine betaine accumulation (c), nodule formation (d) of cowpea under different watering regimes. Each bar represents the mean of the three replicates ( $n=3$ ) for each treatment. Bars carrying the same alphabet are not significantly different from one another at  $P<0.05$  according to Duncan multiple range test (DMRT). PH=plant height; LA=leaf area; NOL=number of leaves, NOP=number of pods,

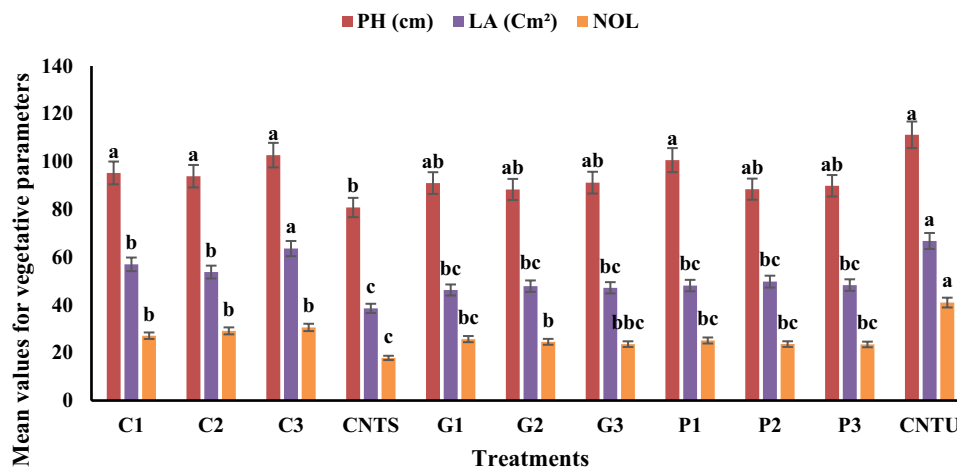
PDW=pod dry weight, SDW=shoot dry weight, RDW=root dry weight, PR=proline, GB=glycine betaine; RNOD=root nodules; ERNO=effective root nodules; NOFW=nodules fresh weight. Bars carrying the same letter for the same parameter are not significantly different from one another. W1=water regime at 10 days interval, W2=water regime at 20 days interval

stage compared with control but C2 was better than other treatments and control (Table 1). On the treatment effect, irrespective of water stress, soil amendment with compost generally enhanced biomass accumulation more than other treatments and higher rate of  $5 \text{ t ha}^{-1}$  performed better than lower rate.

### 3.4 Effect of Pre-sowing Seed Treatments with P and GB and Compost on Yield and Yield Components of Cowpea Under Water Deficit Stress

The number of days to 50% flowering of cowpea plants was affected by water deficit as compared to plants under

well-watered conditions. However, pre-sowing seed treatment with lower rate of GB hastened flowering in cowpea by reducing the number of days to flowering. Among all the treatments, cowpea pre-treated with GB (5 mM) and exposed to water deficit at reproductive stage for 10 days (G2RW1) had the average value of 38.00 days to 50% flowering. This was followed by cowpea pre-treated with higher concentration of GB (10 mM) and exposed to water deficit at vegetative stage for 20 days (G2VW2). Water deficit stress delayed flowering and untreated stressed plants at vegetative stage under 20 days water withdrawal (CNTS V W2) used 57.67 days to achieve 50% flowering (Table 2). As observed for other parameters, longer stress duration of



**Fig. 3** Sole effects of treatments on vegetative growth parameters of cowpea exposed to water deficit stress at vegetative and reproductive growth stages. Each bar represents the mean of the three replicates ( $n=3$ ) for each treatment. Bars carrying the same alphabet with the same legend and for the different treatments are not significantly different from one another at  $P<0.05$  according to Duncan mul-

tipule range test (DMRT). C1, C2, and C3=compost applied at 2.5, 5, and 7.5 t ha<sup>-1</sup>, respectively; G1, G2, and G3=glycine betaine applied at 2.5, 5, and 10 mM, respectively; P1, P2, and P3=proline applied at 2.5, 5, and 10 mM, respectively; CNTS=control stressed, CNTU=unstressed control

20 days was also more deleterious on pod formation than 10 days water withdrawal (Fig. 2b). Drought stress at both growth stages and for different durations affected the pod formation as reflected in the untreated but stressed control plant. The number of pods produced in this treatment was significantly low compared to those grown under well-watered conditions which produced the highest number of pods. This effect was more devastating on plants exposed to water deficit stress for 20 days duration at vegetative stage. These plants had no pod, while those stressed for 10 days had an average of one pod/plant. The unstressed control plants had average of 6 pods/plant. Meanwhile, compared to the control plants under stress, seed pretreatments with P and GB as well as soil amendment with compost-enhanced pod formation. Regardless of the stress duration and among all the treatments, compost was also more effective than P and GB except for pod dry weight that was enhanced by G2. On the interactive effects of stress duration, treatments, and crop growth stage, plants grown on soil amended with compost at 5 t ha<sup>-1</sup> and stressed for 10 days duration at the reproductive stage (C2RW1) had the highest pod number. Ironically, seeds pre-treated with GB at 5 mM and exposed to water deficit for 20 days at the vegetative stage (G2VW2), produced the highest number of pods in this group. This was followed by plants grown on compost amended soil at 5 t ha<sup>-1</sup> and exposed to stress at the vegetative stage for 10 days (C2VW1) (Table 3). Pod weights were also reduced significantly in untreated but, stressed plants as compared to unstressed plants. The highest value of pod weight was also recorded in plants grown under well-watered conditions. Soil amendment with compost and exogenous application

of P and GB to cowpea exposed to water deficit enhanced pod weight. Pod weight was more in cowpea grown on soil treated with C2 and exposed to water deficit for 10 days at the vegetative growth stage (C2VW2). This was followed by those pre-treated with P1. GB at 5 mM and exposed to water deficit for 20 days at the vegetative stage, but G2VW2 was better than P2VW2. Number of grains per pod under well-watered conditions was higher than those of untreated control plants exposed to water-stress conditions. However, different levels of pre-sowing seed treatments and compost application enhanced grain yield compared to stressed control. For grain yield, pre-sowing seed treatment with 2.5 and 5 mM of P increased the number of seeds per pod in plants exposed to water deficit for 10 days duration at vegetative growth stage while, compost application at rates 1 and 2 increased the number of grains per pod of cowpea exposed to W1 at reproductive growth stage (Table 3).

### 3.5 Effect of Pre-sowing Seed Treatments with P and GB and Compost on Nodulation

Generally, the total number of root nodules, effective nodules and weight were affected by water deficit stress especially in plant stressed for 20 days (Fig. 2d). Root nodule formation was affected by water deficit stress especially in plant stressed for 20 days at the reproductive stage (CNTSRW2). On the interactive effects of various treatments, stress duration and growth stage, plants treated with GB at 2.5 mM and exposed to water deficit for 10 days at the vegetative stage (G1VW1) had the highest value (Table 4). This was followed by plants treated with GB at 5 mM and exposed

**Table 1** Interactive effects of soil amendment with compost and pre-sowing seed soaking with proline and glycine betaine on vegetative parameters and dry matter accumulation of cowpea exposed to water deficit stress at different growth stages

Water regimes	Growth stages	Treatments	PH (cm)	LA (cm <sup>2</sup> )	NOL	SDW(g)	RDW (g)	
W1	Veg	C1	87.44 ± 2.0b	62.50 ± 0.8a	26.59 ± 0.5bc	11.66 ± 4.6a	0.66 ± 0.3abc	
		C2	84.29 ± 5.0b	52.29 ± 2.7b	28.24 ± 1.2b	6.47 ± 1.1bcd	0.64 ± 0.3abc	
		C3	99.68 ± 1.7a	65.46 ± 1.1a	31.87 ± 1.7ab	9.84 ± 3.2ab	0.77 ± 0.4abc	
		P1	95.28 ± 1.7a	46.25 ± 7.1 cd	26.18 ± 1.6bcd	5.30 ± 0.4 cd	0.53 ± 0.2bc	
		P2	83.44 ± 1.8b	49.98 ± 1.0bc	22.48 ± 0.7df	5.23 ± 5.7 cd	0.41 ± 0.2bc	
		P3	85.99 ± 2.1b	44.45 ± 0.9d	22.83 ± 1.2def	6.44 ± 0.7bcd	0.93 ± 0.5ab	
		G1	80.35 ± 3.3bc	40.46 ± 0.1e	28.57 ± 3.6ab	8.55 ± 4.6abc	1.18 ± 0.9a	
		G2	84.89 ± 2.3b	47.30 ± 0.6 cd	26.16 ± 1.2bcd	7.25 ± 1.1bcd	0.73 ± 0.4	
		G3	84.59 ± 1.5b	45.69 ± 2.5d	24.08 ± 1.0cdef	7.25 ± 2.1bcd	0.72 ± 0.1abc	
		CNTS	74.83 ± 1.8c	35.82 ± 0.3f	17.83 ± 0.7 g	3.18 ± 0.8d	0.30 ± 0.1c	
		CNTU	111.22 ± 2.0a	66.79 ± 1.5a	41.00 ± 1.5a	14.65 ± 2.3a	1.08 ± 0.2a	
		Rep	C1	109.40 ± 8.0ab	55.44 ± 0.5b	33.06 ± 2.5b	6.43 ± 1.9ab	1.22 ± 0.5ab
	C2		106.72 ± 9.4abc	53.93 ± 2.2bc	31.50 ± 4.5bc	8.36 ± 1.3a	0.80 ± 0.4bcd	
	C3		114.07 ± 4.6a	65.63 ± 1.3a	39.44 ± 1.2a	6.17 ± 1.3ab	0.75 ± 0.2bcd	
	P1		111.38 ± 4.3ab	48.39 ± 0.6de	29.17 ± 1.2 cd	4.82 ± 0.9ab	0.65 ± 0.1 cd	
	P2		104.59 ± 3.1bc	49.41 ± 0.6 cd	27.06 ± 2.0de	4.51 ± 0.0ab	0.48 ± 0.1d	
	P3		100.74 ± 1.9 cd	51.02 ± 3.1 cd	25.94 ± 1.4de	4.36 ± 0.4ab	1.13 ± 0.3abc	
	G1		111.70 ± 4.2ab	47.75 ± 2.4de	25.17 ± 1.4e	4.57 ± 1.2ab	0.44 ± 0.1d	
	G2		95.84 ± 2.6d	49.99 ± 1.2 cd	23.94 ± 1.7e	4.07 ± 0.2ab	0.35 ± 0.0d	
	G3		104.73 ± 6.2bc	51.27 ± 1.2 cd	23.67 ± 2.9ef	5.28 ± 1.3ab	1.55 ± 0.3a	
	CNTS		92.36 ± 3.7d	43.85 ± 0.5e	19.94 ± 0.5f	2.79 ± 0.5b	0.32 ± 0.1d	
	CNTU		111.22 ± 2.0a	66.79 ± 1.5a	41.00 ± 1.5a	14.65 ± 2.3a	1.08 ± 0.2a	
	W2		Veg	C1	79.03 ± 1.1bcd	54.73 ± 1.2ab	24.90 ± 1.2abc	4.58 ± 1.8b
		C2		81.30 ± 5.2abc	49.43 ± 1.5 cd	27.04 ± 3.4a	8.88 ± 3.0a	0.97 ± 0.3a
C3		87.23 ± 0.8a		58.00 ± 1.0a	25.22 ± 2.1ab	6.81 ± 2.5ab	0.48 ± 0.1ab	
P1		86.70 ± 3.4ab		40.94 ± 1.6f	23.26 ± 1.7bc	3.95 ± 1.5b	0.83 ± 0.9ab	
P2		75.14 ± 1.8 cd		53.71 ± 2.7ab	21.18 ± 1.0 cd	5.86 ± 1.1ab	0.73 ± 0.1ab	
P3		75.73 ± 0.5 cd		47.46 ± 1.2de	19.50 ± 1.0de	6.35 ± 0.7ab	0.61 ± 0.0ab	
G1		73.94 ± 0.7 cd		44.77 ± 4.5ef	23.97 ± 1.2bc	6.03 ± 2.3ab	0.44 ± 0.3ab	
G2		79.89 ± 1.7abcd		46.40 ± 1.5de	22.42 ± 0.4bcd	5.05 ± 2.3ab	0.52 ± 0.2ab	
G3		75.88 ± 0.9 cd		41.37 ± 3.0f	22.25 ± 1.2bcd	7.01 ± 2.7ab	0.46 ± 0.1ab	
CNTS		71.86 ± 1.4d		31.97 ± 1.8 g	15.10 ± 1.2e	3.89 ± 1.3b	0.29 ± 0.3b	
CNTU		111.22 ± 2.0a		66.79 ± 1.5a	41.00 ± 1.5a	14.65 ± 2.3a	1.08 ± 0.2a	
Rep		C1		105.17 ± 5.2ab	55.34 ± 2.5 cd	24.00 ± 2.4bc	4.81 ± 1.5a	0.62 ± 0.2a
		C2	103.28 ± 3.4abc	59.45 ± 1.6b	29.89 ± 2.2a	6.02 ± 1.7a	0.59 ± 0.0a	
		C3	109.87 ± 8.0a	65.32 ± 2.2a	25.89 ± 2.8b	3.66 ± 0.3a	0.33 ± 0.0a	
		P1	109.11 ± 5.1a	56.84 ± 3.4bc	21.94 ± 0.7c	4.64 ± 0.3a	0.35 ± 0.2a	
		P2	90.67 ± 0.8ef	48.52 ± 0.4ef	23.83 ± 2.7bc	4.79 ± 0.2a	0.48 ± 0.1a	
		P3	97.07 ± 2.8cde	50.37 ± 0.8ef	25.61 ± 0.6bc	3.65 ± 1.7ab	0.35 ± 0.1a	
		G1	98.09 ± 8.0bcde	52.12 ± 0.9de	25.17 ± 1.6bc	3.80 ± 1.0ab	0.26 ± 0.1a	
		G2	92.73 ± 1.5de	47.79 ± 2.9f	25.78 ± 1.8bc	4.27 ± 0.8a	0.36 ± 0.1a	
		G3	99.57 ± 4.9ef	50.42 ± 0.2ef	24.44 ± 1.2bc	4.31 ± 1.4a	0.32 ± 0.1a	
		CNTS	84.13 ± 2.7f	42.66 ± 0.4 g	17.06 ± 0.9d	2.63 ± 0.4b	0.23 ± 0.3a	
		CNTU	111.22 ± 2.0a	66.79 ± 1.5a	41.00 ± 1.5a	14.65 ± 2.3a	1.08 ± 0.2a	

Each value represents the mean of the three replicates ( $n=3$ ) for each treatment. Means followed by the same letter(s) in the same column under vegetative or reproductive stage for each watering regime are not significantly different from each other according to Duncan multiple range test ( $P<0.05$ ). W1 10 days of water withdrawal, W2: 20 days of water withdrawal. C1, C2, C3=compost at 2.5, 5, and 7.5 t ha<sup>-1</sup>; P1, P2, P3=Proline at 2.5, 5, and 10 mM; G1, G2, G3=glycine betaine at 2.5, 5, and 10 mM. CNTS=stressed control, PH=plant height, LA=leaf area, NOL=number of leaves, SDW=shoot dry weight, RDW=root dry weight, CNTU=unstressed control. Veg=vegetative stage, Rep=reproductive stage

**Table 2** Interactive effects of soil amendment with compost and pre-sowing seed soaking with proline and glycine betaine on number of days to 50% flowering of cowpea exposed to water deficit stress at different growth stages

Treatments	Vegetative stage		Reproductive stage	
	W1	W2	W1	W2
C1	51.67 ± 2.3 bc	55.33 ± 3.4 ab	46.33 ± 1.4 ab	46.00 ± 2.4 abc
C2	56.67 ± 2.3 a	51.33 ± 5.8 bcde	41.33 ± 2.1 cde	46.33 ± 1.4ab
C3	48.00 ± 0.9 cd	51.33 ± 3.4 bcde	40.33 ± 1.4 cde	44.00 ± 5.9 bcd
G1	50.33 ± 1.0 bc	54.67 ± 5.2 abc	43.00 ± 1.5 bc	45.00 ± 1.8bcd
G2	44.67 ± 2.7d	39.67 ± 2.3 f	38.00 ± 1.5 de	41.33 ± 1.4 cde
G3	47.33 ± 0.5 cd	50.00 ± 0.9 cde	42.33 ± 1.9 bcd	43.67 ± 1.9 bcd
P1	52.67 ± 1.4ab	54.67 ± 1.4 abcd	39.67 ± 2.1cde	41.00 ± 3.2 de
P2	43.33 ± 3.1 d	48.67 ± 1.4 e	49.67 ± 1.0 a	46.33 ± 3.1 ab
P3	45.67 ± 2.3 d	49.33 ± 2.3 e	40.33 ± 1.4cde	43.00 ± 0.9 bcd
CNTU	36.67 ± 2.1 e	39.67 ± 2.3 f	36.67 ± 2.1 e	36.67 ± 2.1e
CNTS	57.00 ± 1.8a	57.67 ± 1.0 a	50.00 ± 2.4 a	50.33 ± 2.1 a

Each value represents the mean of the three replicates ( $n=3$ ) for each treatment. Means followed by the same letter in the same column are not significantly different according to Duncan multiple range test ( $P<0.05$ ). W1 10 days of water withdrawal, W2 20 days of water withdrawal. C1, C2, C3=compost at 2.5, 5, and 7.5 t ha<sup>-1</sup>; P1, P2, P3=proline at 2.5, 5, and 10 mM; G1, G2, G3=glycine betaine at 2.5, 5, and 10 mM. CNTS stressed control, CNTU unstressed control

to water deficit for 10 days at the vegetative stage (G1V10). Similarly, among those exposed to water deficit stress for 20 days at vegetative stage, plants treated with GB at 5 mM (G2VW2) recorded the highest value. For those stressed at the reproductive stage, the root nodules of plants grown on soil amended with compost at 7.5 t ha<sup>-1</sup> and exposed to water deficit for 10 days (C3RW1) was the highest. Similar trend was observed on the number of effective root nodules (Table 4). Nodule fresh and dry weights were also reduced under water deficit stress compared to plants grown under well-watered conditions (data not shown). Seed treatments and compost application to soil increased nodule fresh and dry weights under stressful conditions.

### 3.6 Effect of Pre-sowing Seed Treatments with P and GB and Soil Amendment with Compost on Leaf P and GB Contents of Cowpea Leaf

Generally, water deficit stress for 20 days caused an increase in P and GB contents of cowpea leaves compared to unstressed control plants (Fig. 2c). Proline accumulation was affected by the duration of water deficit at both growth stages. The P and GB contents in plants exposed to water deficit for 20 days were higher than those exposed to water deficit for 10 days duration. On the overall effects of treatments on proline content of the cowpea leaf exposed to water deficit, plants treated with the highest level of GB gave the highest P content in cowpea leaf (Fig. 4). This was followed by that of G1 and CNTS, while compost application reduced P accumulation. Similarly on the interaction of treatments, stress duration, and crop growth stages, G3-treated plants that were exposed to water deficit for 20 days at the vegetative stage had the highest P content (Fig. 5a). Among the

plants exposed to water stress at reproductive stage, plants treated with lower level of GB and exposed to water deficit for 20 days had the highest P content in the leaf (Fig. 5b). The least value of P was observed in unstressed control plants at both growth stages. For the GB concentration in the cowpea leaf exposed to stress, results also showed that moderate or severe water deficit stress at both growth stages, increased GB concentration in cowpea leaves compared to the unstressed control plants (Fig. 2c). On the treatment effects, the accumulation of GB was also more in the CNTS but was however enhanced by P1 and P2 treatments and its accumulation was reducing as GB concentration increased. As observed for P, compost application also reduced the GB content in plant compared with control (Fig. 4). The interaction between the stress duration and crop growth stages showed that GB accumulation was also duration dependent and was more in plants stressed at vegetative stage for 10 days (Fig. 6a, b).

## 4 Discussion

Water stress is capable of causing 100% reduction in crop production, thereby posing serious threat to food security and human existence. In this study, water stress imposed at different growth stages reduced cowpea growth and yield. The exogenous application of osmo-protectants like P and GB has, however, been widely reported to be effective in crop drought stress tolerance (Hoque et al. 2007; Verbruggen and Hermans 2008; Rezaei et al. 2012). They are known for ameliorating stresses in crops and for modulating plant water relations under drought stress. The results of this study also confirmed that pre-sowing seed treatment

**Table 3** Interactive effects of soil amendment with compost and pre-sowing seed soaking with proline and glycine betaine on the yield of cowpea exposed to water deficit stress at different growth stages

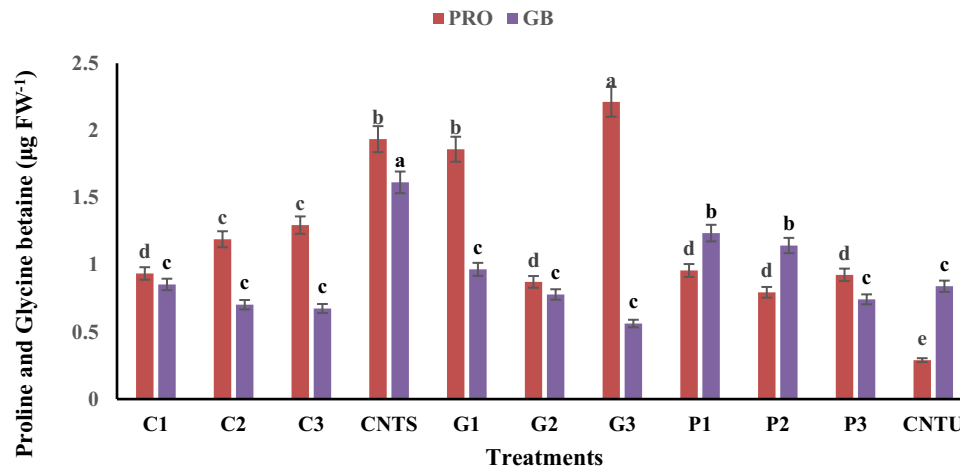
	Number of pods						Pod weight (g)						Grains per pod									
	VS		RS		VS		RS		VS		RS		VS		RS		VS		RS			
	W1	W2	W1	W2	W1	W2	W1	W2	W1	W2	W1	W2	W1	W2	W1	W2	W1	W2				
C1	1.33 ± 1.0c	2.00 ± 1.5ab	0.00 ± 0.0c	2.00 ± 3.1a	1.27 ± 1.0b	3.08 ± 2.4ab	0.0 ± 0.0c	1.90 ± 2.9a	3.50 ± 2.7ab	5.89 ± 4.6a	0.00 ± 0.0a	1.56 ± 2.4a	3.33 ± 3.1b	1.33 ± 1.0ab	5.67 ± 4.4a	1.00 ± 0.0c	0.71 ± 1.1a	3.31 ± 2.6ab	6.00 ± 5.0a	3.65 ± 3.0a	1.67 ± 2.6a	
C2	0.00 ± 0.0d	1.33 ± 2.1ab	0.00 ± 0.0c	0.00 ± 0.0b	0.00 ± 0.0b	0.89 ± 1.4b	0.00 ± 0.0c	0.00 ± 0.0c	0.00 ± 0.0b	0.00 ± 0.0b	0.00 ± 0.0b	0.00 ± 0.0a	0.00 ± 0.0a	0.00 ± 0.0b	1.50 ± 2.3a	0.00 ± 0.0a	0.00 ± 0.0a	0.00 ± 0.0a	0.00 ± 0.0a	0.00 ± 0.0a	0.00 ± 0.0a	
C3	0.00 ± 0.0d	0.33 ± 0.5b	2.67 ± 4.1ab	0.33 ± 0.5b	0.00 ± 0.0b	0.34 ± 0.8b	3.22a ± 5.0b	0.56 ± 0.9a	1.00 ± 1.7b	4.33 ± 6.7a	1.92 ± 3.0a	4.00 ± 6.2a	1.67 ± 1.4c	4.00 ± 3.6a	3.67 ± 2.9ab	0.67 ± 1.0ab	0.81 ± 1.2a	3.27 ± 2.5ab	4.64 ± 3.6a	5.62 ± 4.4a	1.33 ± 2.1a	
G1	1.67 ± 1.4c	4.00 ± 3.6a	3.67 ± 2.9ab	0.67 ± 1.0ab	3.91 ± 3.1a	5.53 ± 4.8a	5.94 ± 4.8a	0.00 ± 0.0a	4.50 ± 3.6ab	2.00 ± 3.1a	5.73 ± 4.9a	0.00 ± 0.0a	3.00 ± 2.7b	1.00 ± 1.5ab	4.33 ± 3.1ab	0.00 ± 0.0b	0.00 ± 0.0b	8.17 ± 2.8a	2.67 ± 4.1a	6.62 ± 5.4a	0.00 ± 0.0a	
G2	3.00 ± 2.7b	1.00 ± 1.5ab	3.67 ± 3.1ab	0.00 ± 0.0b	2.71 ± 2.7a	1.53 ± 2.4ab	3.16 ± 3.0ab	0.00 ± 0.0a	8.17 ± 2.8a	2.67 ± 4.1a	6.62 ± 5.4a	0.00 ± 0.0a	2.00 ± 2.4b	0.33 ± 0.5ab	4.33 ± 3.6a	0.00 ± 0.0b	0.00 ± 0.0b	7.53 ± 6.7a	0.00 ± 0.0a	0.00 ± 0.0a	0.00 ± 0.0a	
G3	2.67 ± 1.0b	0.33 ± 0.5ab	4.33 ± 3.6a	0.00 ± 0.0b	4.23 ± 0.3a	0.54 ± 0.8b	3.78 ± 2.9ab	0.00 ± 0.0a	0.00 ± 0.0a	0.00 ± 0.0a	0.00 ± 0.0a	0.00 ± 0.0a	0.00 ± 0.0a	0.00 ± 0.0b	0.00 ± 0.0c	0.00 ± 0.0c	0.00 ± 0.0a	7.53 ± 6.7a	0.00 ± 0.0a	0.00 ± 0.0a	0.00 ± 0.0a	
P1	2.00 ± 2.4b	0.00 ± 0.0b	0.00 ± 0.0c	0.00 ± 0.0b	3.44 ± 3.1a	0.00 ± 0.0b	0.0 ± 0.0b	0.00 ± 0.0a	0.00 ± 0.0a	0.00 ± 0.0a	0.00 ± 0.0a	0.00 ± 0.0a	0.67 ± 1.0c	0.33 ± 0.5b	1.67 ± 1.4bc	0.00 ± 0.0b	0.81 ± 1.3b	1.67 ± 2.6b	4.00 ± 6.2a	4.00 ± 3.1a	0.00 ± 0.0a	
P2	0.67 ± 1.0c	0.33 ± 0.5b	1.67 ± 1.4bc	0.00 ± 0.0b	0.81 ± 1.3b	0.61 ± 0.9b	2.16 ± 1.7ab	0.00 ± 0.0a	0.00 ± 0.0a	0.00 ± 0.0a	0.00 ± 0.0a	0.00 ± 0.0a	1.00 ± 1.5c	0.00 ± 0.0b	0.33 ± 0.5c	1.33 ± 1.4a	0.80 ± 1.2b	1.10 ± 1.7b	0.00 ± 0.0a	2.33 ± 3.6a	5.07 ± 5.0a	
P3	1.00 ± 1.5c	0.00 ± 0.0b	0.33 ± 0.5c	1.33 ± 1.4a	0.80 ± 1.2b	0.00 ± 0.0b	1.61 ± 2.5b	0.79 ± 0.6a	1.10 ± 1.7b	0.00 ± 0.0a	2.33 ± 3.6a	5.07 ± 5.0a	6.66 ± 3.5a	6.66 ± 3.5a	6.66 ± 3.5a	6.66 ± 3.5a	7.30 ± 5.1a	9.5 ± 2.5a	9.5 ± 2.5a	9.5 ± 2.5a	9.5 ± 2.5a	
CNTS	6.66 ± 3.5a	6.66 ± 3.5a	6.66 ± 3.5a	6.66 ± 3.5a	7.30 ± 5.1a	7.30 ± 5.1a	7.30 ± 5.1a	7.30 ± 5.1a	7.30 ± 5.1a	7.30 ± 5.1a	7.30 ± 5.1a	7.30 ± 5.1a	7.30 ± 5.1a	7.30 ± 5.1a	7.30 ± 5.1a	7.30 ± 5.1a	7.30 ± 5.1a	7.30 ± 5.1a	7.30 ± 5.1a	7.30 ± 5.1a	7.30 ± 5.1a	7.30 ± 5.1a
CNTU	6.66 ± 3.5a	6.66 ± 3.5a	6.66 ± 3.5a	6.66 ± 3.5a	7.30 ± 5.1a	7.30 ± 5.1a	7.30 ± 5.1a	7.30 ± 5.1a	7.30 ± 5.1a	7.30 ± 5.1a	7.30 ± 5.1a	7.30 ± 5.1a	7.30 ± 5.1a	7.30 ± 5.1a	7.30 ± 5.1a	7.30 ± 5.1a	7.30 ± 5.1a	7.30 ± 5.1a	7.30 ± 5.1a	7.30 ± 5.1a	7.30 ± 5.1a	7.30 ± 5.1a

Each value represents the mean of the three replicates ( $n = 3$ ) for each treatment. Means followed by the same letter(s) in the same column under vegetative or reproductive stage for each watering regime are not significantly different from each other according to Duncan multiple range test ( $P < 0.05$ ). W1 10 days of water withdrawal, W2 20 days of water withdrawal. C1 compost (2.5 t ha<sup>-1</sup>), C2 compost (5 t ha<sup>-1</sup>), C3 compost (7.5 t ha<sup>-1</sup>), G1 glycine betaine (2.5 mM), G2 glycine betaine (5 mM), G3 glycine betaine (10 mM), P1 proline (2.5 mM), P2 proline (5 mM), P3 proline (10 mM), CNTS stressed control, CNTU unstressed control, VS = vegetative stage, RS = reproductive stage

**Table 4** Interactive effects of soil amendment with compost and pre-sowing seed soaking with proline and glycine betaine on total number of root nodules and effective root nodules of cowpea exposed to water deficit stress at vegetative and reproductive stages

Treatments	Vegetative stage						Reproductive stage					
	W1			W2			W1			W2		
	TNRN	NOERN	TNRN	NOERN	TNRN	NOERN	TNRN	NOERN	TNRN	NOERN	TNRN	NOERN
C1	10.67 ± 1.9d	8.67 ± 4.6c	11.33 ± 1.0 d	11.33 ± 1.0c	18.67 ± 9.6b	15.67 ± 8.0b	12.00 ± 2.4bcd	9.33 ± 8.1bc				
C2	19.33 ± 4.6 cd	9.00 ± 3.4c	12.67 ± 1.4d	12.33 ± 1.9c	14.33 ± 4.6b	9.67 ± 9.0b	38.67 ± 6.6b	23.00 ± 17.9b				
C3	18.50 ± 8.2 cd	21.83 ± 6.5c	18.50 ± 5.9 cd	18.17 ± 5.8c	24.33 ± 8.1 b	24.67 ± 4.5b	6.17 ± 1.6 cd	6.33 ± 1.4bc				
G1	44.17 ± 7.9b	39.83 ± 12.3b	13.67 ± 1.0 cd	13.00 ± 4.4c	13.67 ± 1.0b	12.67 ± 2.3b	15.00 ± 1.8bcd	12.67 ± 2.3bc				
G2	28.00 ± 8.8c	23.33 ± 6.7c	50.50 ± 24.4 b	46.17 ± 21.4b	15.67 ± 1.4b	14.67 ± 1.0b	6.67 ± 1.9 cd	4.67 ± 1.4bc				
G3	22.67 ± 4.4 cd	16.33 ± 7.2c	18.50 ± 5.9 cd	17.83 ± 2.5c	14.83 ± 3.9 b	13.33 ± 4.4b	10.00 ± 5.6bcd	8.33 ± 3.1bc				
P1	19.33 ± 3.1 cd	18.00 ± 3.2c	17.50 ± 5.1 cd	12.33 ± 2.3c	11.67 ± 1.4b	11.67 ± 1.4b	22.67 ± 5.8bc	20.33 ± 2.3bc				
P2	14.33 ± 4.6 cd	12.67 ± 5.8 c	11.67 ± 1.4d	8.67 ± 1.5c	8.17 ± 6.8 b	6.67 ± 5.5b	16.33 ± 3.6 bcd	14.67 ± 4.2bc				
P3	17.67 ± 7.3 cd	16.50 ± 5.9c	29.33 ± 4.9 c	17.50 ± 8.4c	16.83 ± 4.3b	16.33 ± 3.7b	11.33 ± 9.2 bcd	9.33 ± 8.1bc				
CNTU	83.00 ± 5.5a	75.67 ± 10.1a	83.00 ± 6.5a	75.67 ± 10.1a	83.00 ± 6.5a	75.67 ± 10.1a	83.00 ± 6.5a	75.67 ± 10.1a				
CNTS	9.83 ± 2.7d	4.83 ± 3.8c	9.33 ± 2.7 d	8.67 ± 2.1c	7.33 ± 3.2b	6.33 ± 1.4b	3.67 ± 2.9 d	3.00 ± 2.4c				

Each value represents the mean of the three replicates ( $n = 3$ ) for each treatment. Means followed by the same letter(s) in the same column under vegetative or reproductive stage for each watering regime are not significantly different from each other according to Duncan multiple range test ( $P < 0.05$ ). W1 10 days of water withdrawal, W2 20 days of water withdrawal, C1 compost (2.5 t ha<sup>-1</sup>), C2 compost (5 t ha<sup>-1</sup>), C3 compost (7.5 t ha<sup>-1</sup>), G1 glycine betaine (2.5 mM), G2 glycine betaine (5 mM), G3 glycine betaine (10 mM), P1 proline (2.5 mM), P2 proline (5 mM), P3 proline (10 mM), CNTS stressed control, CNTU unstressed control, TNRN = total number of root nodules, NOERN = number of effective root nodules



**Fig. 4** Sole effects of treatments on proline and glycine betaine accumulation in cowpea leaf exposed to water deficit stress at vegetative and reproductive growth stages. Each bar represents the mean of the three replicates ( $n=3$ ) for each treatment. Bars carrying the same letter with the same legend are not significantly different from one another at  $P<0.05$  according to Duncan multiple range test

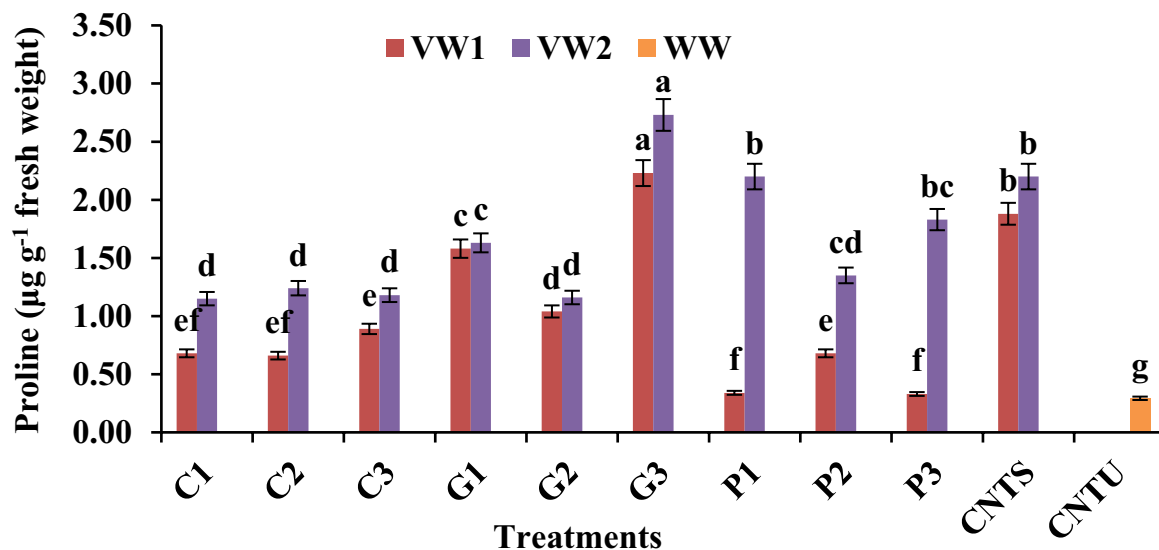
(DMRT). C1, C2, and C3=compost applied at 2.5, 5, and 7.5 t ha<sup>-1</sup>, respectively; G1, G2, and G3=glycine betaine applied at 2.5, 5, and 10 mM, respectively; P1, P2, and P3=proline applied at 2.5, 5, and 10 mM, respectively; CNTS=control stressed, CNTU=unstressed control

with P and GB was effective in enhancing cowpea growth and development under water deficit stress. Effectiveness of seed priming with these osmolytes on stress amelioration in crop has been linked to different mechanisms. These include increase in endogenous accumulation of osmolytes, improved seed germination and seedling establishment, enhanced antioxidant machinery, improved photosynthetic performance, upregulation of stress responsive genes, and faster recovery from stress (Zhang et al. 2014; Farooq et al. 2019; Aswathi et al. 2021). In this study, however, the percentage germination of the primed seeds was lower than those of unprimed seeds. This was contrary to the previous report that seed priming expedites the pre-occurrence of metabolic events necessary for seed germination and hence, reduces the time-gap between seed sowing and seedling emergence (Athar et al. 2009; Ambreen et al. 2021; Johnson and Puthur 2021). The variation observed was also confirmed by the report of Zhang et al. (2014) and the effect of compatible solutes on seed germination was found to be crop specific and concentration dependent (Zhang et al. 2014). The delay in seedling emergence according to Hare et al. (2003) and Miller et al. (2009) was attributed to the negative feedback inhibition of exogenous osmo-protectants on the rate limiting enzyme of proline biosynthetic pathway. Excess priming with these compatible solutes has also been reported to interrupt the amylase enzyme activity which consequently affects seed germination (Farooq et al. 2006). In another report, priming has been described as a mild stress to the seeds which in turn triggers stress responsive genes and proteins for stress tolerance (Chen and Arora 2013). It is

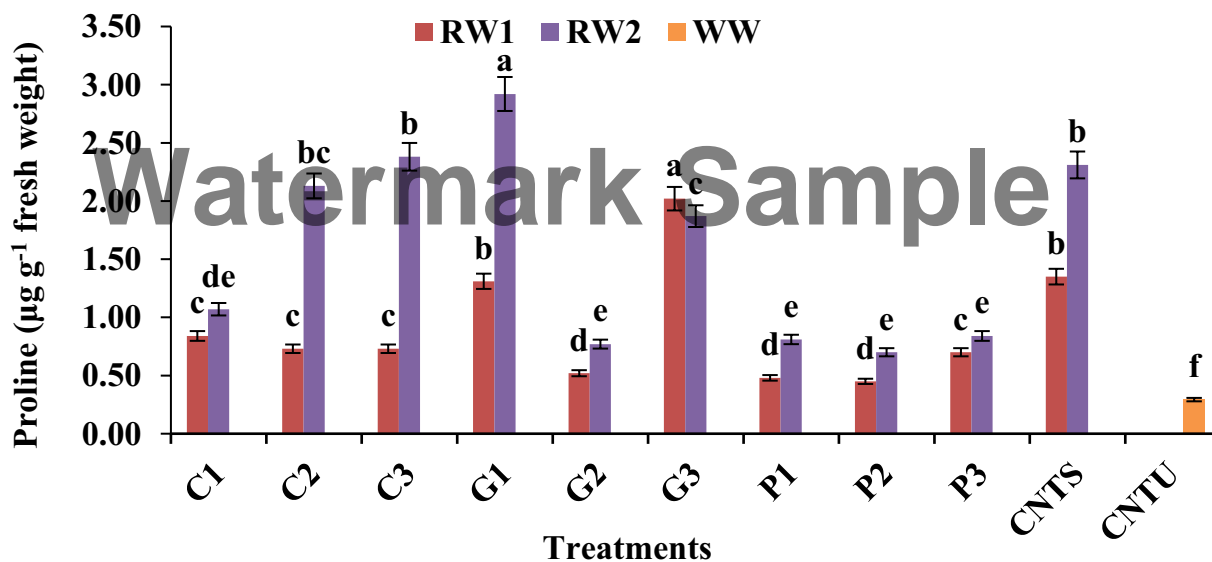
not impossible that the mild stress might in turn be responsible for the delay in seed germination of the primed seeds.

In this study, water deficit conditions reduced all the growth parameters considered, especially the leaf area of cowpea. Many studies have also indicated that leaf area growth decreased in response to water stress in many species of soybean (Zhang et al. 2004; Abdalla and El-Khoshiban 2007). Inadequate water supply to plant at vegetative stage has been reported to limit the productive potential of plant due to a decrease in plant vegetative growth which in turn affects reproductive development (Sibel and Birol, 2007; Liu et al. 2018). The decline in leaf area under water stress conditions is mainly attributed to a decline in turgor pressure (Prasad et al. 2008). The decrease according to Banon et al. (2006) could, therefore, be due to reduction in cell elongation, volume, and growth. Comparative studies on the effect of different plant parts have also shown that water stress reduced the linear growth of the shoots of the stressed plants relative to that of unstressed plants (Kumar et al. 2004). This was confirmed in this study and growth parameters of the cowpea plants that were stressed at the reproductive stage were better than those of the plants exposed to water stress at vegetative stage. Anjum et al. (2003) reported that water deficit stress is a very important limiting factor at the initial phase of plant growth and establishment. It was also found that plant height and leaf number were reduced following imposition of water stress at vegetative, flowering and pod filling stages, in Bambara groundnut (Vurayai et al. 2011). The reason could be attributed to the fact that those stressed only at reproductive stage had enough water at their vegetative stage to carry out more physiological processes and

(a)



(b)



**Fig. 5** Interactive effects of treatments on proline accumulation in cowpea exposed to water deficit stress at vegetative (a) and reproductive (b) growth stages. Each bar represents the mean of the three replicates ( $n=3$ ) for each treatment. Bars carrying the same letter with the same legend are not significantly different from one another at  $P<0.05$  according to Duncan multiple range test (DMRT). VW1:

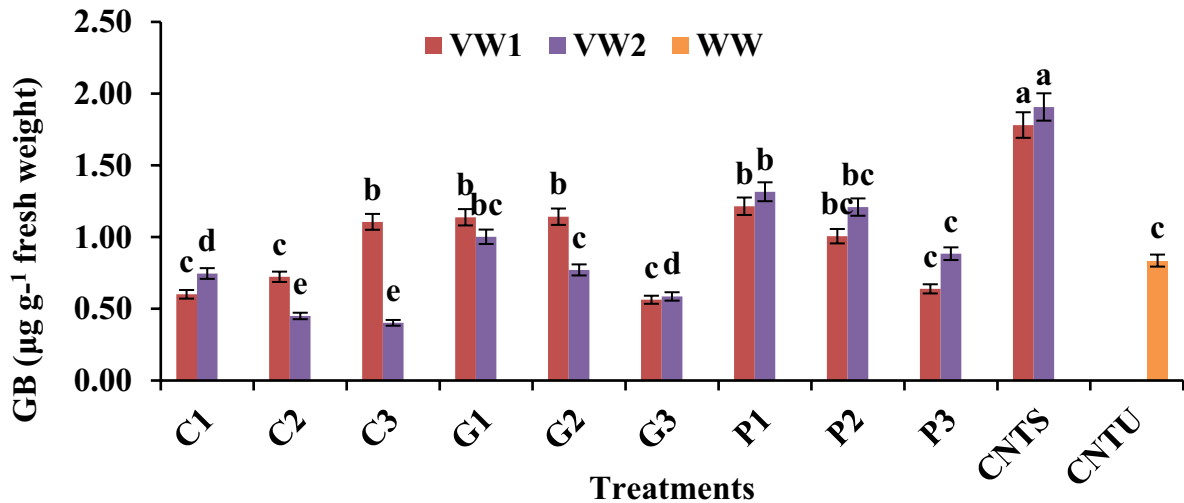
10 days water withdrawal, VW2: 20 days water withdrawal, WW: well-watered (no deficit); C1, C2, and C3=compost applied at 2.5, 5, and 7.5 t ha<sup>-1</sup>, respectively; G1, G2, and G3=glycine betaine applied at 2.5, 5, and 10 mM, respectively; P1, P2, and P3=proline applied at 2.5, 5, and 10 mM, respectively; CNTS=control stressed, CNTU=unstressed control

produce many metabolites. Whereas, when water stress was imposed at reproductive stage the plant had already finished its phenological developments and reserved more nutrients and metabolites to tolerate stress than those stressed at the vegetative stage.

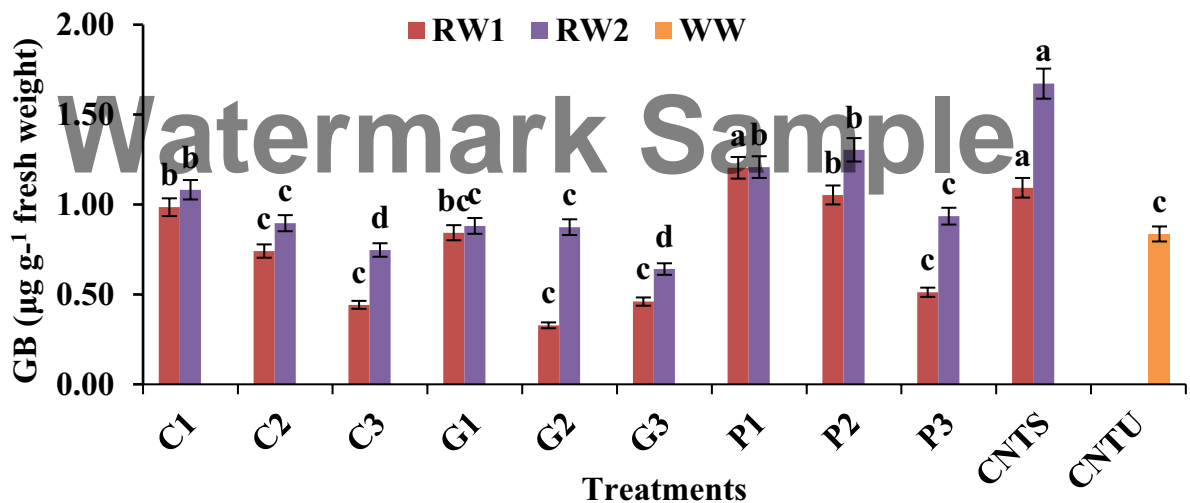
Water deficit stress significantly decreased biomass accumulation in cowpea as was observed in vegetative parameters. The reduction in biomass of cowpea under water deficit

stress conforms with the findings of other researchers (Anyia and Herzog 2004; Haji et al. 2009; Saruhan et al. 2011). The effect of water stress on biomass was more evident on plants exposed to water deficit for 20 days at vegetative stage. Reduction in leaf area by water stress could be responsible for the reduced crop yield as a result of reduction in photosynthesis (Rucker et al. 1995). In the present study, pre-sowing seed treatment with GB and compost application were,

(a)



(b)



**Fig. 6** Interactive effects of treatments on glycine betaine accumulation in cowpea exposed to water deficit stress at vegetative (a) and reproductive (b) growth stages. Each bar represents the mean of the three replicates ( $n=3$ ) for each treatment. Bars carrying the same letter with the same legend are not significantly different from one another at  $P < 0.05$  according to Duncan multiple range test (DMRT).

VW1: 10 days water withdrawal, VW2: 20 days water withdrawal, WW: well-watered (no deficit); C1, C2, and C3 = compost applied at 2.5, 5, and 7.5 t ha<sup>-1</sup>, respectively; G1, G2, and G3 = glycine betaine applied at 2.5, 5, and 10 mM, respectively; P1, P2, and P3 = proline applied at 2.5, 5, and 10 mM, respectively; CNTS = control stressed, CNTU = unstressed control

however, found to improve the plant biomass compared to other treatments. The high yield recorded from the use of compost under stressful conditions as compared to control in this study confirms the reports of previous researchers (Ganuga et al. 1998; Adejumo et al. 2011; Nguyen et al. 2012). Water also plays an essential role in root nodulation in plants, and shortage of it results in reduced number of nodules in the cowpea plant

roots was negatively affected by water stress and this correlated with the duration of water deficit. Untreated but stressed plants exposed to water deficit for 20 days duration at the reproductive stage recorded the least number of root nodules. Serraj (2003), reported that root nodulation in legumes synchronizes with the availability of necessary soil components, including soil moisture and nutrients. However, seed pre-treatment and soil amendment with compost

increased the number of root nodules under stressful conditions. Alyemini et al. (2016) also reported that the exogenous application of P and GB enhanced nodule formation. The efficiency of seed pretreatment with osmolytes could be due to their ability to induce tolerance in stressed crop. Hayat et al. (2012), reported that proline, which is a multifunctional amino acid, apart from acting as an excellent osmolyte was also known for stabilizing subcellular structures such as proteins and cell membranes, scavenging free radicals, balancing cellular homeostasis, and signaling events and buffering redox potential under stress conditions.

Compost application, however, outperformed the seed priming treatments with P and GB. The positive effects of compost in improving soil fertility, soil structure and in enhancing crop tolerance to drought stress have been previously reported (Adejumo et al. 2011; Duo et al. 2018). Farrell and Jones (2009) also reported that compost increased the water holding capacity of the soil and cushion the effect of drought stress on crop by increasing the soil water holding capacity and soil nutrients (Adebayo et al. 2011). The potential of compost in increasing soil organic matter and water holding capacity of the soil might have brought about increase in plant growth parameters under water stress, which in turn enhanced cowpea yield (Sun et al. 2003; Chaturvedi et al. 2010; EL-Sabagh et al. 2015b, c). The effectiveness was found to also depend on the quantity of compost applied to the soil as previously reported (Adejumo et al. 2011) and this in-turn varied based on the different parameters. It was observed that highest application rate of  $7.5 \text{ t ha}^{-1}$  improved vegetative growth and biomass accumulation in the shoot while, the lower rate of  $5 \text{ t ha}^{-1}$  was more effective for cowpea's pod and grain production. The performance of compost could be linked to higher nutrient availability in the soil containing highest compost rate which in-turn probably made enough nutrients available for vegetative development at the expense of reproductive development.

It is worthy of note that, contrary to the common report that the reproductive stage is the most sensitive stage to water stress in cowpea (Ahmed and Suliman, 2010), variation was observed in this study. Though the reduction was more in the untreated plants in response to water stress but, the yield of the plants stressed at vegetative stage was more affected in this study. As observed for vegetative growth, imposition of water stress at the reproductive stage was found to produce higher number of pod than those under water deficit at the vegetative stage. This agrees with Vurayai et al. (2011), who reported that water stress during vegetative growth reduced yield components in Bambara. The enhanced yield of the cowpea plants stressed at reproductive stage could be attributed to the increase in plant growth at vegetative stage before stress imposition (Vurayai et al. 2011). However, soaking cowpea seeds in different concentrations of P, GB, and compost application

contributed to increase in yield and yield components of cowpea under stressed conditions at both growth stages as compared to stressed control. Compost application has been reported as one of the important measures to enhance seed yield under water stress condition (EL-Sabagh et al. 2015b). Surprisingly, higher rate of compost did not contribute to yield in terms of pod formation in the present study. As explained earlier, higher rate of compost might be favoring vegetative growth more than pod formation. Application of compost or seed pre-treatment seemed to be insufficient under prolonged water deficit especially at the reproductive stage. According to Ahmadian et al. (2011), under water shortage conditions, nutrient absorption and water uptake are often limited, and this could have led to reduction in growth, decreased leaf expansion, reduction in light absorption, and photosynthetic potential of plant under severe water stress.

It is well established that exogenous application of GB might have some advantages as it improves water deficit tolerance in plants (Zhang et al. 2014; Ahmed et al. 2019, 2021). As was also observed, it has also been reported earlier that the rate and timing of GB application significantly affect drought tolerance ability of sunflower (Iqbal et al. 2008). This could be responsible for the early recovery observed in plants treated with GB at 5 mM and exposed to water deficit at the vegetative stage. According to the report of Rezaei et al. (2012), GB applied at the vegetative growth stage was effective in ameliorating the adverse effects of water deficit stress on tomato due to GB induced improvement in plant water status. Water deficit stress increased P and GB contents in the leaves of cowpea. Their accumulation correlates closely with the intensity and duration of water stress (Reddy et al. 2004). It means that cowpea, like other reported crops also uses high P accumulation as a mechanism for drought tolerance. High P accumulation has been reported to play an adaptive role in the plant for survival under stress (Ashraf and Foolad 2007; Hoque et al. 2007; Verbruggen and Hermans 2008). High level of P in the cell enables the plant to maintain a low water but high osmotic potential that allows plants to take up water from the environment (Kumar et al. 2003). In this study, due to the lowering of oxidative stress, plants grown on compost amended soil and those pre-treated with exogenous P had reduced amount of P in the leaf. Seed priming with GB and P, however, enhanced endogenous P and GB accumulation, respectively in the leaf of cowpea under water stress conditions. It therefore means that external P and GB application induced endogenous synthesis of these osmolytes.

## 5 Conclusion

It could be concluded from this study that, the effects of water stress on cowpea growth and yield were duration and growth stage dependent. Cowpea growth and yield were

affected by longer duration of water stress for 20 days, especially at the vegetative stage. However, crop adaptation to drought under climate change could be enhanced by seed pretreatment with proline and glycine betaine as well as soil amendment with compost. In the present study pre-sowing seed treatment with proline and glycine betaine was able to mitigate the drought stress in cowpea at any growth stage. The detrimental effects of stress like oxidative stress must have been reduced with these treatments, hence, the increase in the growth and yield of treated cowpea plants more than the untreated control. By increasing the soil physico-chemical properties as well as tolerance, soil amendment with compost also ameliorated the drought stress in cowpea by improving the growth and yield of cowpea under drought stress. Their ameliorative roles were also confirmed with the reduction in the endogenous proline and glycine betaine contents of the stressed but pre-treated cowpea plants. The effectiveness of seed treatment with proline and glycine betaine as well as soil amendment was found to be concentration dependent, with the application of 2.5- and 5-mM rates of these osmolytes being more effective than the highest rate of 10 mM. Similarly, from the study, the optimum application rates of compost were found to be 5 and 7.5 t ha<sup>-1</sup>. As a way of improving the stress tolerance, higher rate of glycine betaine also enhanced cowpea tolerance by increasing the production of endogenous proline and glycine betaine in the treated plants more than the untreated plants. The higher proline and glycine betaine contents produced in the leaves of untreated, but stressed cowpea plants could, however, mean that cowpea is also accumulating these osmolytes to adapt and overcome stressful conditions. Therefore, the adverse effects of water deficit stress on cowpea can be alleviated by the exogenous application of these osmolytes and soil amendment with compost. The mechanisms being employed by these chemicals and organic amendments, however, need to be further understood at the molecular level for their effective utilization in crop production under stressful environments.

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## Declarations

**Competing Interests** The authors declare that they have no competing interests.

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