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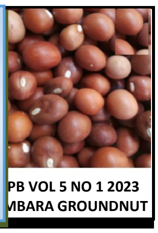

Effect of Silicon Application Methods on Drought Tolerance, Growth and Yield of Some Varieties of *Vigna unguiculata* (L.) Walp in Gombe State

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ABSTRACT

One of the things that limits crop yield is drought. It has been demonstrated that silicon (Si) plays a significant part in reducing environmental stress in cowpea. To investigate the impact of silicon (Si) on plant development, drought tolerance, physiology, and anatomy, the cowpea white variety and cowpea brown variety were chosen. Two cowpea types were sown in each pot during the trial, which was carried out in August 2021 at Gombe State university. Four treatments—a zero treatment, silicon (Na_2SiO_3) in soil, silicon (Na_2SiO_3) in seed, and silicon (Na_2SiO_3) in water—were used in the experiment, which was set up in a completely randomized block design. Vegetative, physiological, and reproductive factors are among those measured. The cowpea white treatment's findings for the vegetative parameters include the greatest averages for plant height (17.2), leaf count (14), stem diameter (1.9), and trifolium count. The greatest chlorophyll concentration of any treated silicon variety found in seed is 0.68, while the highest chlorophyll stability index of any variety found in soil is 50% for the cowpea white variation. Cowpea white of the treatment silicon in seed has the lowest transpiration rate of 0.1 mL, whereas cowpea white and brown of the treatment silicon in soil have the highest relative water contents (91.4% and 89.7%, respectively). The cowpea brown treatment silicon seed's reproductive properties had the highest mean flower count (3.7), compared to the white type, there were 1.7 pods, 17.1 pods, 5.7 pods, 12.0 seeds per pod, and 49g of total biomass. The cowpea white variety benefits from the Na_2SiO_3's effects on physiology and vegetative parameters, whereas the brown variations benefit from it in terms of reproduction.

INTRODUCTION

Despite being a common element that makes up about 28% of the earth's crust and is the second most abundant element in soil after oxygen, most sources of silicon in soil exist as crystalline amino silicate, which is no longer available to plants because it is insoluble in water [1]. Mono silicic acid, which is commonly found in soil solutions bathing roots at concentrations ranging from 0.1 to 0.6 mm, is the form of silicon that is available to plants [2]. However, all terrestrial plants contain silicon in varying amounts, ranging from 0.1 to 10 percent of the dry weight of the shoot. Despite this, silicon has recently been recognized as a significant element due to its abundance and ubiquity in all soils and plants. With the exception of the equisetaceae, no terrestrial higher taxon has a mineral element, and no plant physiologists have paid it any consideration [3].

According to [4] and [5], one of the most abiotic stresses in agriculture is drought stress, which negatively affects plant growth and metabolic functions such as water retention, photosynthetic assimilation, and nutrient uptake. By reducing leaf transpiration, which mostly happens through the stomata and partially through the cuticle, silicon can reduce water stress. The silicon is deposited behind the cuticle of the stressed plant, generating a cuticle bilayer, which reduces transpiration. Silicon can decrease the rate of transpiration in rice with a thin cuticle by 30% [6].

Cowpea, also known as black-eyed pea or southern pea, is a crop with many uses because it feeds people, cattle, soil, and other plants [7], in the United States, a sizable amount of premium cowpea is farmed for the dry bean, canning, and fresh markets. According to [8], the seeds and pods of cowpea plants

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provide about 25% of high-quality protein content in the form of amino acids, carbs, folic acid, chlorophyll, carotenoids, phenolics, and necessary minerals. It is widely adapted and grown in about 65 nations spread throughout the six continents, especially in the dry region of countries with tropical and subtropical climates [9]. However, the average worldwide output in a farmer's field is less than the anticipated maximum yield of cowpea [10] 6000 kg hectare 1. Furthermore, despite cowpeas' innate ability to adapt to water shortages, the FAO recorded a 19% reduction in productivity between 2012 and 2017 [10]. Cowpea output deficiencies and drought stress have previously been connected in research [11,12]. The main aim of this study is to evaluate the effect of silicon on plant growth and drought tolerance on selected cowpea (*Vigna unguiculata* (l.) Walp) in Gombe State Nigeria.

MATERIALS AND METHODS

Description of the study area

The experiment was conducted in botanical garden of the department of biological sciences, Gombe State University, Gombe State, Nigeria. The new site of the botanical garden was founded in 2017 and is situated adjacent to faculty of science complex, within Gombe State University, Gombe, Nigeria .it has the total size of 270 square meters or 2.7 hectares. The garden lies between latitude 10°e 18' and longitude 11° 10' 36.43"e and has an altitude/ elevation of 438-478 m above sea level (**Fig. 1**).

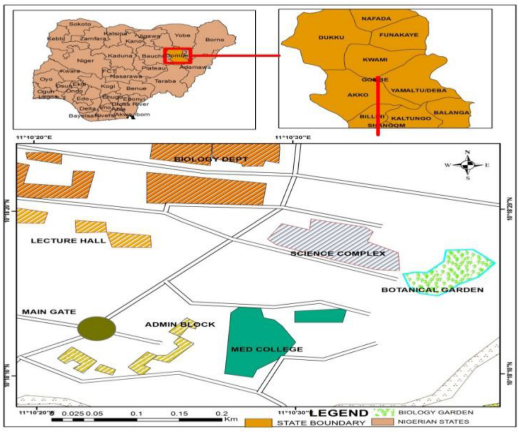

Fig. 1. Map of Gombe State University showing the location of the botanical garden.

Collection of plant material

The seed was obtained from Biu local market, Borno State. The chemical was obtained from Gombe State University.

Experimental design and treatment

The experiment was conducted under a screen house condition. The trial layout was a randomized complete block design replicated three times with an experimental plot size of 2 m x 1 m, each replicate consists of 24 pots. Cowpea seeds were sown in a pot with inter-row spacing of 0.4 m and intra-row spacing of 0.2 m. The experiment had three factors: two soil types (sandy clay loam and sandy loam soil), two cowpea lines (Yar biu; white and brown) and four treatments of silicon (Na_2SiO_3): silicon (Na_2SiO_3) in soil, silicon (Na_2SiO_3) in seed and silicon (Na_2SiO_3) in water and control for each treatment. The control was well watered while the treatments were subjected to drought that is two weeks without watering follow by one week of watering.

Procedure for application of silicon in soil

The silicon (Na_2SiO_3) was added to the mixture of sandy clay loam and sandy loam soil at dose of 800 mg per 1 kg of soil and a total of 20 kg of soil was used for this treatment. The seed was sown at a standard depth of 0.03 m from the soil surface.

Procedure for application of silicon (na_2sio_3) in seed

About a 100 g of seed were immersed in 5% hypochlorite solution for ten min to ensure surface sterility, and then washed three times with distilled water. The sodium silicate was directly applied in 100 g of seed at dose of 200 mg and stayed for up to 12 h before sowing.

Procedure for application of silicon in water

A 9 mg of Na_2SiO_3 was diluted in 1 litre of water to water the plant the treatment is repeated four times.

Data collection and analysis

The data collection is of three phases: vegetative data, physiological data and reproductive data.

Vegetative parameters

Vegetative data were taken twice a week. The number of germinated plants was recorded at 7 days after planting and germination percentage (gp) was calculated as a percentage of germinated plants per experimental unit using the formula of [13]:

$$Gp = \text{no. of } g/t \times 100,$$

where g is the number of germinated plants and t is the total number of seeds planted. The number of leaves borne on each plant at was counted and the number of branches per plant was obtained by counting the main stem of the sample plants. Plant height (cm) was measured from the main stem, from ground level to the tip of the plant using a meter ruler.

Physiological parameters

The data on chlorophyll content, chlorophyll stability index, transpiration and relative water content was recorded.

Chlorophyll content

The chlorophyll content was measured using paper chromatography where the leaves were washed with distilled water and then crushed in a mortar and pestle by adding 80% acetone, the extract were filtered using filter paper then it was dropped on 2cm line drawn on paper chromatography to create a thick concentrated pigment on the pencil line and then prepare your running solvent using petroleum ether and acetone. The paper was gently place in a beaker containing 20ml running solvent [14].

Chlorophyll stability index (CSI)

The CSI was calculated using the following formula according to [15].

$$CSI(\%) = \frac{\textbf{total chlorophyll contents (stressed)}}{\textbf{total chlorophyll contents (control)}} \times \mathbf{100}$$

Relative water content (RWC)

To determine the RWC; the youngest fully expanded leaves of plants per pot were collected, immediately weighed to record the fresh weight (FW), then rehydrated in petri dishes containing distilled water for 24 h under dim light and room temperature to get the turgid weight (TW) and subsequently the leaves were oven dried at 70 °C for 48 h to record the dry weight (DW). The RWC was calculated using the method devised by [16].

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$$RWC\ (\%) = \frac{(FW - DW)}{(TW - DW)} \times 100$$

Transpiration

The transparent polythene bags were tie on the two branches of leaves with a rubber band at 12 noon and stayed for 30 min. The water that was transpired inside the transparent polythene bag was collected with syringe and it was measured in mL.

Microscopic examination on anatomy of leaves

Procedures for sectioning of leaf sample.

The sample was held firmly, and the new razor blade was flood with water and draw across the sample to cut the sample. The pieces of the cut section were transferred to the water using brush, the thinnest section was selected and transfer to a glass slide and stained with 0.1% TBO solution for one min. The stained was removed using a filter paper and was flood with water to remove excess stained. When the stain is completely removed then add a drop of water and cover it with cover slide and examined the sample [17].

Reproductive parameters

Reproductive data were collected after harvesting from each experimental unit. Matured pods from sample plants were counted per plant weighed (g) and measured in centimeter. Hundred seed weight (g) was determined by randomly counting 100 seeds from threshed pods per treatments with a digital weighing scale.

Statistical analysis

Data on vegetative and reproductive parameters were subjected to an analysis of variance (ANOVA) using [18] computer software. Treatments were tested at least level of significance and differences between treatments mean were separated using least significant difference Tukey and Pearson correlation.

RESULTS

Table 1 shows plant height, number of leaves, flag leaf, stem diameter and number of trifoliums for both the varieties. For the white variety, treatment silicon (Na_2SiO_3) in seed (SSE) HAVE the highest mean on plant height, number of leaves, number of trifolium and stem diameter compared with the control. While, in stem diameter treatment silicon in soil has negative impact which is less than the control. For the flag leaf of white variety, treatment silicon in water (SW) has the highest mean compare with the control. Coming to the brown variety, treatment silicon (Na_2SiO_3) in water (SW) has the highest mean for all the vegetative parameters which has a positive impact over the control.

Table 1. Effect of Na_2SiO_3 on the vegetative parameters of cowpea white and brown varieties.

Varieties	Treatment	Plant height (cm)	Number of leaves	Flag leaf (cm^2)	Stem diameter (cm^2)	Number of trifolium
CW	SSO	11.0±0.29	8.0±0.3	6.2±0.3	1.4±0.04	1.9±0.09
	SSE	17.2±0.49	14.0±0.5	7.1±0.3	1.9±0.04	3.8±0.12
	SW	15.9±0.42	11.4±0.44	8.2±0.3	1.9±0.03	3.0±0.15
	Control	10.5±0.5	5.00±00	3.10±1.7	1.5±0.00	1.50±0.50
CB	SSO	10.8±0.3	8.3±0.33	5.6±0.3	1.7±0.04	2.03±0.11
	SSE	15.0±0.42	11.1±0.41	6.5±0.3	1.8±0.05	2.98±0.13
	SW	18.0±0.40	11.3±0.40	7.1±0.31	2.2±0.04	3.10±0.13
	Control	13.5±0.97	6.5±0.87	6.2±1.15	1.5±00	1.5±0.29
P<value	Variety	0.781	<.001	0.003	<0.421	<0.532
	Treatment	<0.001	<.0423	0.00	<0.001	<0.591
	Variety* treatment	<0.001	<.001	0.00	<.001	<.001

Key: CB- Cowpea Brown; CW-Cowpea White

Fig. 2 below displays the result for chlorophyll content of the treatments silicon in seed white variety, has the highest chlorophyll content than the control white variety while silicon in seed brown variety its chlorophyll content is less than the control brown which take us to conclusion. Coming to the next treatment silicon in soil white variety has high chlorophyll content compared with the control white variety meanwhile, in the same treatment but brown variety the chlorophyll content is less than the control brown variety which has the highest chlorophyll content.

For the last treatment which is silicon in soil of the white variety has high chlorophyll content than the control white meanwhile, in the same treatment but brown variety the control took over the treatment which draw us to a conclusion that the all the treatments of white varieties have the highest chlorophyll content compared with the control, control brown variety has the highest than the treatments of all the brown varieties.

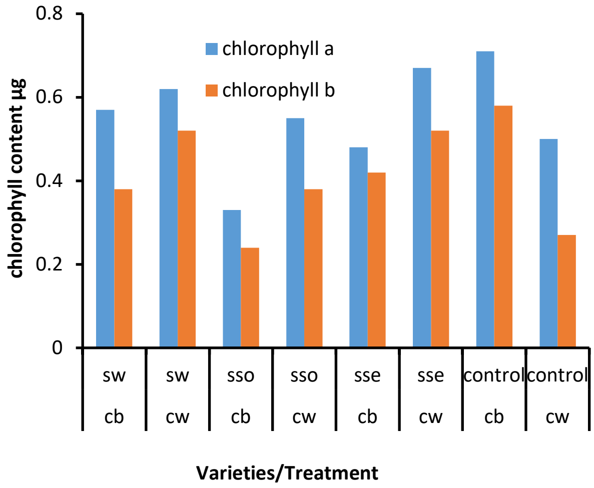

Fig. 2. Effects of Na_2SiO_3 on chlorophyll content of the cowpea varieties.

Chlorophyll stability index

Fig. 3 below shows the chlorophyll stability index for the treatments. The highest in silicon chlorophyll stability index was recorded in silicon in soil (SSO) white variety (50%) and the lowest was recorded in silicon in soil brown (11%).

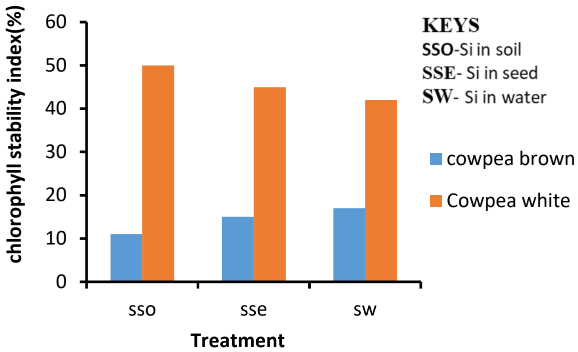

Fig. 3. Chlorophyll Stability Index.

It can be noted in the **Fig. 4** below that the relative water content of the treatment silicon in soil of both the white and the brown variety has the highest relative water content of (89.7% and 91.4%) compared to the control of both varieties. In the

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treatment silicon in seed both the two varieties also have the highest relative water content (83.1% and 62.1%) compared to both control which has a relative water content of (52.5% and 61%). Coming to the last treatment which is silicon in water for both the varieties it also, has the highest relative water content of (79.7% and 81.4%) compared to the control of both the varieties which has a relative water content of (52.5% and 61%). Among the treatments silicon in soil has the highest and silicon in seed has the lowest relative water content.

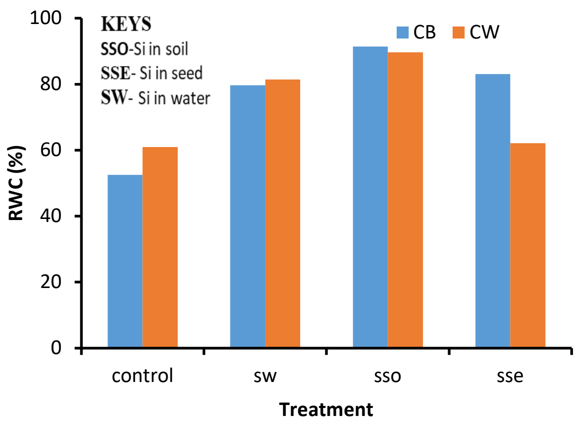

Fig. 4. Effect of Na_2SiO_3 on relative water index.

Fig. 5 shows the rate of transpiration, the control of the cowpea white variety has the highest rate of transpiration of (2 mL). Among the treatments silicon in water of cowpea brown variety has the highest transpiration rate of (0.9 mL) and the lowest rate of transpiration was recorded in treatment silicon in seed white variety of (0.1 mL). This comes to the conclusion that control has the highest rate of transpiration than the treatments.

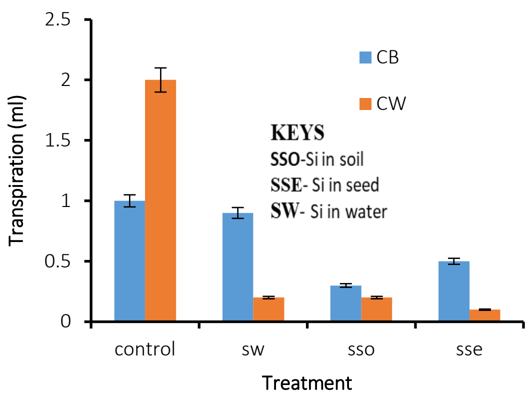

Fig. 5. Effects of Na_2SiO_3 on transpiration rate.

The table below shows the relationship between the physiological parameter, within the table it shows that there is a high correlation between chlorophyll a and chlorophyll b whereas in the other parameters it shows no correlation or not significant. **Table 3** below shows the result for the statistical analysis of the reproductive parameter. The reproductive parameters are number of flowers, number of pods, pod length, pod weight and number of seed per pod for both the variety and the treatments. For the white variety, in treatment SSE (silicon in seed) has the highest mean in terms of number of flowers, pod length and pod weight compared with the control, and it has negative impact on number of pods for both the treatment and the control. While, in terms of number of seeds per pod treatment SSO (silicon in soil) has the highest mean than the control. For the cowpea brown variety, in treatments, silicon in seed (SSE) has the highest mean in terms of number of flowers, number of pod and pod weight which is more than the control. While, in treatment SSO (silicon in soil) has high impact on pod length. The treatment has a negative impact on the number of seed per pod because the control has the highest mean.

Table 3. Effect of Na_2SiO_3 on the reproductive parameters.

Variety	Treatment	No of Flower	No of Pods	Pod Length	Pod Weight	No Seed Per Pod
CB	SSE	3.7±0.9	1.7±0.7	17.1±1.7	5.7±0.33	12.0±1.15
	SW	1.00±0.77	1.00±0.33	16.3±6.6	5.00±1.2	10. 7±3.8
	SSO	1.33±0.00	1.00±0.00	12.7±0.00	4.7±60.50	10.0±0.00
	Control	3.00±0.00	1.00±0.00	17.0±00	2.08±0.00	15.00±0.00
CW	SSE	0.50±0.00	0.00±0.00	1.50±0.00	2.50±0.00	0.5±0.00
	SSO	0.33±0.00	0.00±0.00	1.5±0.00	0.33±0.00	0.67±0.00
	SW	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
P<Value	Treatment	0.66	0.01	0.577	0.058	0.436

Key: CB- Cowpea Brown, CW-Cowpea White

The comparison between the cowpea varieties in term of reproductive parameters that was analyzed shows that silicon applied in form of sodium silicate (na_2sio_3) has positive impact on the cowpea brown more than the cowpea white (**Table 4**).

Table 4. Effect of Na_2SiO_3 on reproductive parameter of the two cowpea varieties.

Variety	No.Of Flower	No. Of Pods	Pod Length	Pod Weight	No. Of Seed/Pod
CB	2.2±0.33***	1.5±0.2***	18.0±1.7**	4.3±0.6n.s	13.7±1.2*
CW	2.0±0.31*	1.0±00**	14.2±0.93*	4.1±0.4n.s	9.9±0.7n.s
Pvalue	0.001***	.001***	0.003**	0.040*	0.05*

Key: CB- Cowpea Brown; CW-Cowpea White

Total biomass (g)

The highest total biomass mean (49 g) was recorded in the cowpea brown variety (**Fig. 6**) of the treatment silicon in seed and the lowest biomass was recorded in the control of cowpea brown variety (29 g).

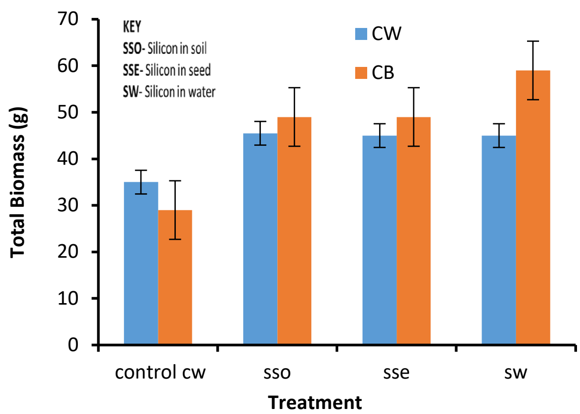

Fig. 6. Effect of Na_2SiO_3 on total biomass (g)

DISCUSSION

The germination characteristics are particularly important since they are linked to the plant's capacity for growth and resistance to adverse circumstances after planting. As a consequence, the cowpea brown variant had the greatest percentage of germination rate of all the cowpea kinds. The outcomes were consistent with those of [19], who conducted a germination test on several cowpea lines and came to the conclusion that seed size and viability are the variables that determine germination on various cowpea lines. Numerous studies demonstrate that silicon is essential for plant growth [20,21]. Additionally, [22] discovered that diverse sources of Si fertilizers had a substantial impact on the majority of growth metrics. Even while Si can encourage plant development and more photosynthesis by encouraging an upright posture, this is widely known from a scientific perspective [23].

Si generally benefits plants by boosting biomass and plant growth. This may be because it helps plants absorb more nutrients, reduce their intake of harmful metals, and maintain the structure of their photosynthetic machinery [24]. According to [25], it may also be because of its duty to regulate stomatal activity, photosynthesis, and water use efficiency, all of which lead to improved vegetative growth. The most crucial pigment for photosynthesis is chlorophyll, and dry circumstances have a negative impact on it. Si treatment increased photosynthetic performance and thylakoid membrane stability in plants under drought stress, while also boosting antioxidant capacity and reducing oxidative damage [26,27].

The chlorophyll content and chlorophyll stability index of all the treated white variety plants in this study were greatly raised by the application of na_2sio_3 to plants under stress, but they were noticeably lowered in the treated brown variety, where the control took over. According to this study, the addition of 1 mM Si to rice under osmotic stress dramatically raised the concentration of chlorophyll a, [28] b, and total, while stress-induced addition of 2 mM Si markedly enhanced the chlorophylls a/b ratio. The addition of 2.5 mM K_2SiO_3 dramatically raised the concentration of chlorophylls a and b, as well as that of carotenoids, in tomato plants under osmotic stress, whereas the addition of Si to non-stressed plants had no effect on the concentration of these molecules [26] when applied to rice plants under osmotic stress, 0.5 mM Na_2SiO_3 raised chlorophyll a and b concentrations by more than 50% compared to plants not treated with Si [27] by enhancing the thylakoid membrane's integrity, stability, and functionality under drought stress circumstances and lowering the degradation of membrane protein complexes brought on by the stress, Si can sustain the chlorophyll content and photosynthetic performance [27]. According to [29], the RWC is a reliable measure of a plant's ability to withstand salt and drought conditions. The RWC has grown in this study's treatment of the white and brown variants. This outcome is consistent with earlier findings in *Glycyrrhiza uralensis* plants, which demonstrate an increase in RWC under both sd1 and sd2 following the application of Si [30]. The improvement in growth may have also been caused by the fact that our data showed si boosted RWC in cowpea plants of both kinds, which is consistent with [31] findings. According to [32], drought stress increased the buildup of intermediate molecules like reactive oxygen species, which in turn led to oxidative damage to DNA, lipids, and proteins and reduced plant growth and production. The findings for the morphological parameters (number of flowers, number of pods, pod length, weight, number of seeds per pod, and 100 seed weight) demonstrate the impact of silicon application (Na_2SiO_3) on cowpea variety, particularly the white variety, which exhibits a notable increase in yield parameter in comparison to their controls. The outcome is consistent with [33] findings that cuNP and agNPs had a significant impact on the morphological parameters of wheat plants. The outcome is consistent with that of [34]. According to [35], Si may reduce stomatal and cuticular evaporation. The formation of polar monosilicic acid and/or polymerized silicic acid in epidermal cell walls, according to [36], may create hydrogen bonds between water and hydrated silica, making it harder for water molecules to escape from leaf surfaces.

CONCLUSION

Drought stress treatments were detrimental to physiological and reproductive parameters of cowpea (*Vigna unguiculata* (l.) Walp) plant. In this study, na_2sio_3 treatment suppresses the negative effects of drought stress and this treatment enabled the plant to better maintain their chlorophyll content and leaf relative water content relative to their control. At the vegetative stage the na_2sio_3 treatments are more effective in the cowpea white variety in treatment silicon in seed while at the reproductive stage the treatment is more effective in the brown variety and in comparison, the cowpea brown variety assimilate the treatment more than the cowpea white. Therefore, it could be concluded that na_2sio_3 has a crucial role in ameliorating the adverse effects of drought stress in cowpea plants.

RECOMMENDATION

Further investigation is needed between the two varieties to know why silicon has more impact in cowpea brown than cowpea white. Application of silicon inform of na_2sio_3 prove to more effect when applied the seed before sowing, therefore, we suggested that cowpea seed should be treated with silicon before planting for productivity.

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AUTHOR CONTRIBUTIONS

HFM and GMA collected the samples from the collection sites. AAZ, and HFM developed and proofread through the manuscript. AAZ, HFM and GMA conducted the experiments in the field.

COMPETING INTEREST

Authors have declared that no competing interests exist.

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