

COMPARATIVE MORPHOLOGICAL AND PHYSIOLOGICAL RESPONSES OF THREE COWPEA (*VIGNA UNGUICULATA* L. WALP.) CULTIVARS TO INDUCED WATER, SALINITY AND COMBINED WATER AND SALINITY STRESSES

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Abstract

Cowpea (*Vigna unguiculata* L.) is a major leguminous grain mostly cultivated in semi-arid and arid regions. Thus, cowpea production is usually hampered by abiotic stresses such as drought and salinity. In this study, three local cowpea cultivars (Asontem, Kirkhouse, and Wangkae) were screened for their tolerance to water deficit stress, salt stress, and combined drought and salt stress. Plant growth, as measured by plant height and internode length, was negatively impacted by the applied stresses in all the cowpea varieties tested. The Asontem cultivar showed better growth characteristics under severe drought and salt stress. With respect to yield parameters, the Asontem cultivar recorded the highest mean seed number per pod under the various levels of drought and salt stress applied except under combined salt and drought stress treatment where Kirkhouse recorded the highest value for the same yield parameter (mean seed number per pod). Asontem and Kirkhouse accumulated more phenols than the Wangkae cultivar under severe drought stress. Kirkhouse recorded the highest phenolic content (7.677 ± 0.0150 mg GAE/L) under 50 mM NaCl whereas the highest for Asontem (7.486 ± 0.301 mg GAE/L) was detected under 150 mM NaCl concentration. It was concluded in this study that the cultivars used in this study responded differently to imposed drought and salinity stresses. It appears that Asontem and Wangkae exhibited the most and least tolerance, respectively, to drought, salinity and combined drought and salinity stresses.

Key words: Relative water content, Food security, Drought stress, Salt stress, Cowpea.

Introduction

Cowpea (*Vigna unguiculata* L.), is a major leguminous grain mostly grown in the drought-prone semi-arid or arid regions of Sub-Saharan Africa (SSA) (Hall, 2012; Kyei-Boahen *et al.*, 2017). Although cowpea originated from Africa, it is currently widely domesticated in Latin America, the Southern United States, and Asia. The crop is rich in nutrients with each grain containing about 23.4% proteins, 1.8% fats, and 60.3% carbohydrates (Haruna *et al.*, 2018). Cowpea contains other nutritional components such as iron, calcium, phosphorus, and vitamins (Mulugeta *et al.*, 2016). The crop thus, serves as a useful source of nutrition for both humans and livestock (Desoky *et al.*, 2020).

Over 8.9 million MT of cowpea is estimated to be produced annually on approximately 14.4 million hectares of land (Anon., 2020). Nigeria is the world's leading producer and consumer of cowpea, with the Niger Republic and Burkina Faso in second and third place respectively. Africa, particularly SSA, accounts for over 95% of global production. However, farmers in SSA produce less than 600 kg/ha, way below the optimum yield capacity of 1500–2500 kg/ha (Anon., 2017).

Water is vital for agricultural production, and its scarcity can have adverse effects on plant physiological and biochemical processes, resulting in yield reduction (Khan *et al.*, 2018). It has been reported that a third of the world's terrestrial areas suffer from drought due to increased global warming (Tack *et al.*, 2015). Drought stress has been identified as one of the major constraints to cowpea cultivation (Seleiman *et al.*, 2021). The stress can

affect cowpeas at both the seedling and terminal growth stages where plants may experience intermittent or protracted periods of water deficit stress leading to loss of biomass output and grain yield (Ibitoye, 2015). The incidence of drought at the flowering stage can result in a drastic reduction in cowpea yields (Boukar *et al.*, 2019). Cowpeas are also susceptible to severe water deficiency, particularly during the pod setting and grain-filling stages of reproductive growth (Hall, 2004).

Salinity stress has also emerged as a major threat to modern agriculture, adversely affecting crop productivity (Isayenkov & Maathuis, 2019). Over 20 % of agricultural lands are affected by salt stress (Saddiq *et al.*, 2021) and about one-third of irrigated lands are greatly affected by salinity stress globally (Abdel-Farid *et al.*, 2020). Cowpea production is significantly hampered by salt stress (Manaf & Zayed, 2015) because soil salinization has become a major problem in arid and semi-arid regions (Hussain *et al.*, 2019) where cowpeas are mainly cultivated.

Cowpea as a major staple crop holds great potential in meeting the food security needs of the rapidly growing population on the African continent. However, considering current climatic trends, the effects of individual as well as combined salinity and water deficit stress on cowpea production will exacerbate thereby adversely affecting global yields. To sustain and improve production to meet current and future food demands, research must focus on screening and identifying drought and salt-tolerant cowpea cultivars. The purpose of our research was to evaluate the responses of three different Ghanaian cowpea cultivars to varying degrees of drought and salt stresses as well as to combined salinity and drought stress.

Material and Methods

Study location, plant materials and growing medium:

This study was conducted in the screen house of the Department of Plant and Environmental Biology, University of Ghana. Three (3) distinct local cowpea cultivars were used in this study namely: Kirkhouse and Wangkae, (developed by the Savannah Agriculture Research Institute (SARI), of the Center for Scientific and Industrial Research (CSIR)-Ghana) and Asontem, (developed by the Crop Research Institute (CRI), also of the CSIR-Ghana). Loamy soils were obtained from the Teaching Garden of the Department of Plant and Environmental Biology, University of Ghana. The soil was sterilized at 121°C and bagged at a uniform quantity into 90 polythene bags.

Experimental design: Experiments were carried out using the split-plot design. In each plot, plants were arranged in Randomized Complete Block Design (RCBD) with six (6) blocks namely normal watering (control), moderate water stress (3 days rehydration), severe water stress (5 days rehydration), 50 mM NaCl, 150 mM NaCl and severe water and salt stress (5 days rehydration and 150 mM NaCl). The variables in each block were Kirkhouse, Asontem, and Wangkae, with each having five replicates. A total of ninety (90) cowpea plants were used for the experiments.

$$\text{SMC} = \frac{\text{Initial weight (IW)} - \text{Dry weight (DW)}}{\text{Initial weight (IW)}} \times 100 \quad \text{Equation (2)}$$

Plant growth parameters: Plant height and internode length measurements were estimated using a meter rule. Plant height measurements were taken from the surface of the soil to the tip of the uppermost leaf (terminal bud) of cowpea plants for 10 consecutive weeks. Measurements for internode length were taken by recording the distance between successive plant nodes for a period of 10 weeks.

Yield parameters: Number of pods, length of pods and number of seeds per pod were determined and recorded for all three cowpea cultivars subjected to different stress treatments. Seed weight per pod was also determined using an analytical balance (AL 104; Mettler Toledo Columbus, OH, USA).

Determination of phenolic compounds: The Phenolic content of leaves was determined according to the Folin-Ciocalteu method (Blainski *et al.*, 2013). Selected leaf samples from each treatment block were ground with a mortar and pestle. Each set was then dissolved in 20 ml of ethanol for 24 hours. This was done to extract the phytochemical components. After 24 hours, the extract was filtered with a Whatman's filter paper and 2 ml of the extract was diluted with 20 ml of distilled water after which 20 µl of the diluted sample was pipetted into a test tube. Subsequently, 1.58 ml of distilled water and 100 µl of Folin-Ciocalteu reagent were added to the test tube. The mixture was steadily rotated on a shaker for 5mins and 300 µl of Na₂CO₃ was added. The mixture was re-shaken, and the solutions obtained were placed in an oven at 40 °C for 30mins. The solutions were then allowed to

Salt stress application: 58.5 g of granulated salt was weighed with a fine balance (AL 104; Mettler Toledo Columbus, OH, USA) and dissolved with 1 liter of distilled water to prepare 50 mM and 150 mM solutions.

Watering regime: Soils were well watered at a uniform rate for the first two weeks under each block, after which 100 ml of water was used to water plants under normal (rehydration every other day), moderate water stress (rehydration after every three days), and severe water stress (rehydration after every five days).

Determination of soil water content (SMC): The Gravimetric Method (Reynolds, 1970) was used to determine the soil moisture content (SMC), which is represented as a percentage of the original weight. A 1.2 cm cork-borer was used to collect soil samples along the sides of each polythene bag, swiftly dumped into Petri dishes and covered. Subsequently, the collected soil samples were weighed with a precision balance (AL 104; Mettler Toledo, Columbus, OH, USA). The soil samples were then reweighed to determine the dry weight after being dried in an oven at 60 °C for 24 hours. The SMC was then calculated as:

cool for an hour after which their absorbances were determined at 765 nm using a Spectrophotometer (SpectraMax plus 384-USA).

Data analysis

All data sets were statistically analyzed using one-way analysis of variance (ANOVA). Means were compared at a probability rate of 5% using Turkey-pairwise comparison analysis in Minitab Software (version 17).

Results

Plant height: The heights of cowpea plants in all treatment blocks decreased as drought stress increased (Fig. 1A). Kirkhouse and Asontem cultivars had significantly higher plant heights ($p \leq 0.05$) under moderate drought stress compared with the Wangkae cultivar (Fig. 1A). Under severe drought stress, the Asontem cultivar recorded the highest plant height (Fig. 1A). The Cowpea cultivars did not show significant differences in plant height when treated with 50 mM NaCl (Fig. 1B). However, plant heights significantly ($p \leq 0.05$) varied between cultivars under 150 mM NaCl and the combined stress treatment (150 mM NaCl and severe drought; Fig. 1B). In the 150 mM NaCl treatment block, the Asontem cultivar recorded the highest height (Fig. 1B), whereas Kirkhouse recorded the highest height (Fig. 1B) in the combined drought and salt stress treatment block (Fig. 1B). In contrast, the Wangkae cultivar had the lowest plant height values among all treatment blocks (Fig. 1B).

Internode length: Internode lengths of cowpea cultivars were measured at 77 DAP in plants subjected to normal watering and drought stress. Internode length did not vary significantly among plants subjected to normal watering (Fig. 2A). However, Wangkae developed the longest internode under normal watering regime (Fig. 2A). In contrast, the Asontem cultivar in contrast, recorded the longest internode length under both moderate and severe watering regimes (Fig. 2A). Under salt stress, the internode lengths of Asontem, Kirkhouse, and Wangkae in the control, 50 mM, and 150 mM NaCl treatment blocks did not vary significantly (Fig. 2B). However, simultaneous application of severe drought and 150 mM NaCl induced significant ($p \leq 0.05$) variations in internode length among the three cultivars (Fig. 2B). Kirkhouse and Wangkae had the longest and shortest internode lengths, respectively, in all treatment blocks (Fig. 2B).

Soil moisture content: Results of soil moisture content analysis at 56 DAP indicated that, pots containing the Asontem cultivar had relatively higher SMC values under different watering regimes compared with those in which Kirkhouse and Wangkae were grown, although the variation in SMC values were statistically not significant (Fig. 3A). Under salt stress, Asontem and Wangkae recorded the highest SMC values for 50 mM NaCl and 150 mM NaCl treatments, respectively (Fig. 3B).

Mean pod length: The mean pod length of cowpea cultivars decreased with increasing drought stress. Asontem recorded the highest values for pod length under normal, moderate, and severe drought stresses (Fig. 4A). The pod length of Kirkhouse and Wangkae did not vary significantly from each other under normal and moderate drought stresses (Fig. 4A). Under severe drought conditions, pod development was not observed in Wangkae (Fig. 4A).

Asontem, Kirkhouse and Wangkae showed reductions in pod length when exposed to salt stress (Fig. 4B). Plants treated with 50 mM and 150 mM experienced significant ($p \leq 0.05$) variations in pod length. The Asontem cultivar recorded the highest pod length values under both

conditions (Fig. 4B). With the simultaneous application of drought and salt stress, the variations in pod length were statistically not significant (Fig. 4B).

Mean number of seeds per pod: The number of seeds per pod varied significantly ($p \leq 0.05$) between control plots and moderate watering regimes (Fig. 5A). The Asontem cultivar had the highest number of seeds under all watering regimes (Fig. 5A). Under severe drought treatment, only Asontem and Kirkhouse could produce seeds (Fig. 5A). Under salt stress treatment, the mean number of seeds per pod of Asontem, Kirkhouse, and Wangkae varied significantly ($p \leq 0.05$) across all the salt treatment levels investigated (Fig. 5B). Asontem had the highest number of seeds under all the salt concentrations tested except under combined salt and drought stress treatments, where Kirkhouse recorded the highest number of seeds (Fig. 5B).

Mean number of pods per plant: Kirkhouse and Asontem had the highest number of pods under normal watering and severe drought treatments, respectively (Fig. 6A). The Wangkae cultivar had the highest number of pods under moderate watering but failed to develop pods under severe stress (Fig. 6A). Higher number of pods were recorded for all cowpea cultivars under the control (Fig. 6B). Kirkhouse and Wangkae had the highest number of pods under 50 mM and 150 mM, respectively (Fig. 6B). There were no significant differences in the mean number of pods recorded for the three cowpeas under combined 150 mM NaCl and severe watering (Fig. 6B).

Mean seed weight per pod: Higher mean seed weights per pod were recorded for all cowpea cultivars under normal watering. (Table 1). The Wangkae cultivar recorded the highest seed weight under normal and moderate watering but failed to produce seeds under severe drought stress (Table 1). Yields for the Asontem cultivar were the highest under severe drought stress (Table 1). Under salt stress treatment, Kirkhouse recorded the highest seed weight in the 50 mM NaCl, 150 mM NaCl and combined stress treatments (Table 1).

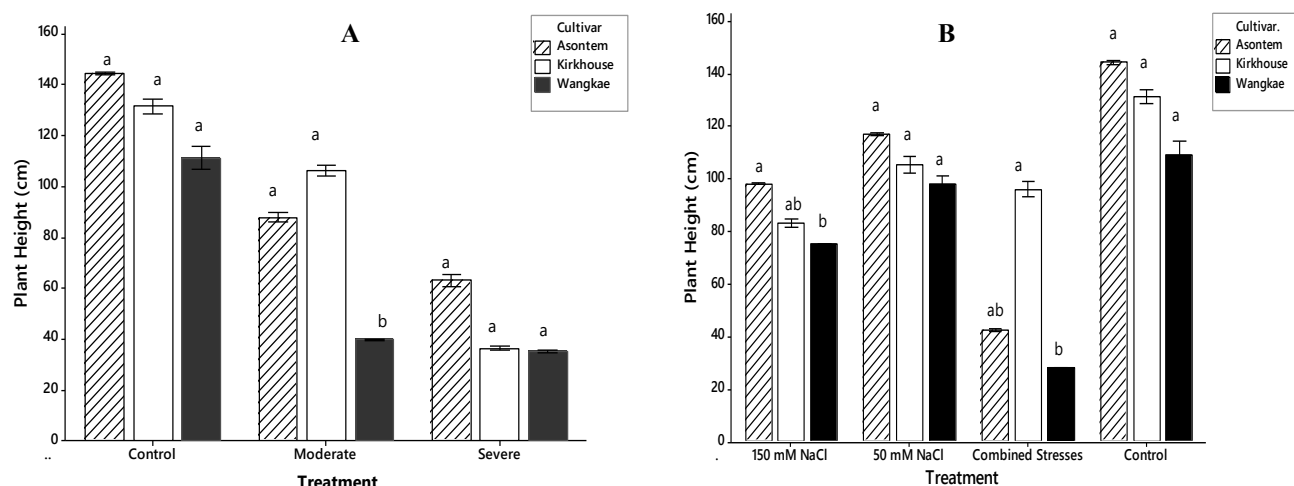


Fig. 1. Variation in mean plant height of the cowpea cultivars under normal and stress conditions at 84 DAP. (A) Cowpea cultivars under various watering regimes. (B) Cowpea cultivars under various levels of salt treatment. Means without a common letter are significantly different ($p \leq 0.05$) according to Tukey's test.

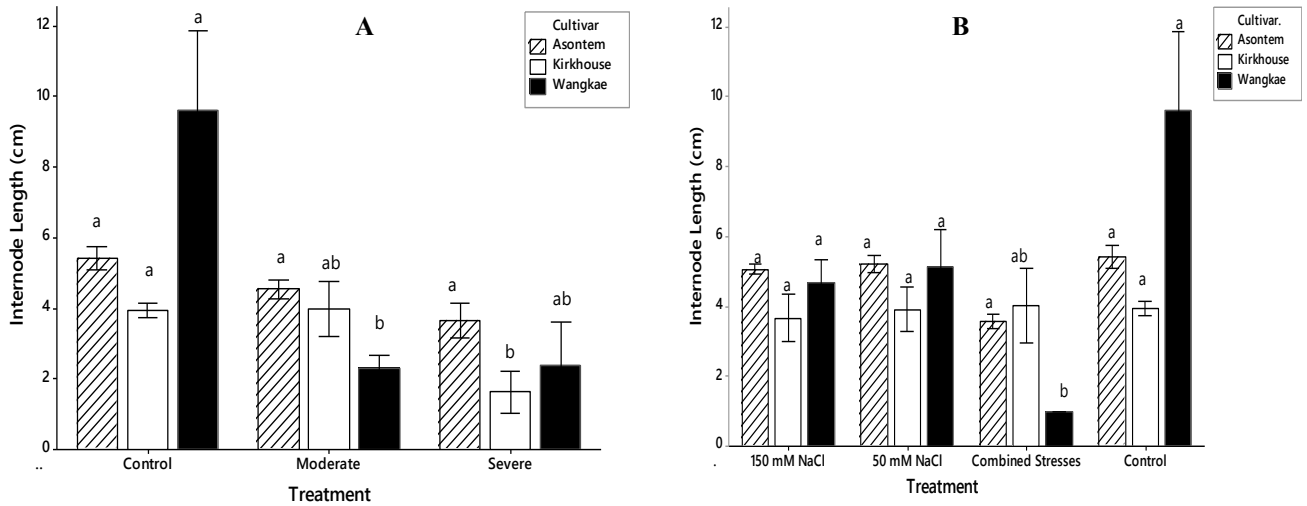


Fig. 2. Variation in mean internode length of unstressed and stressed cowpea cultivars at 77 DAP: (A) Cowpea under various watering regimes. (B) Cowpea under various levels of salt treatment. Means without a common letter are significantly different ($p \leq 0.05$) according to Tukey's test.

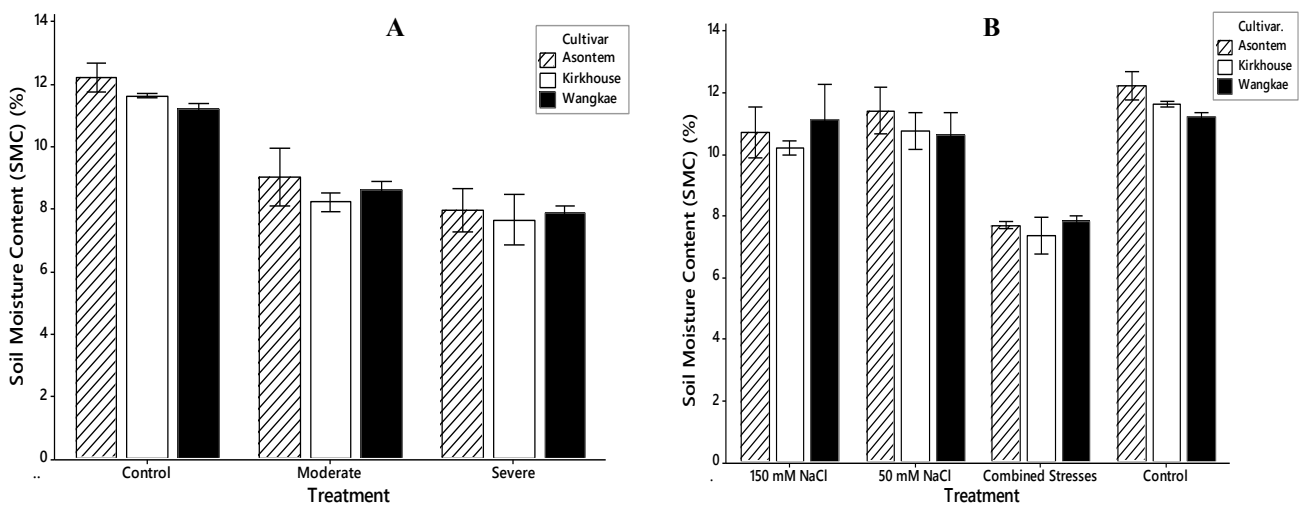


Fig. 3. Variation in mean soil moisture content (SMC) of pots in which the cowpea cultivars: were grown (A) SMC under different levels of drought at 56 DAP. (B) SMC under different levels of NaCl at 56 DAP. Means without a common letter are significantly different ($p \leq 0.05$) according to Tukey's test.

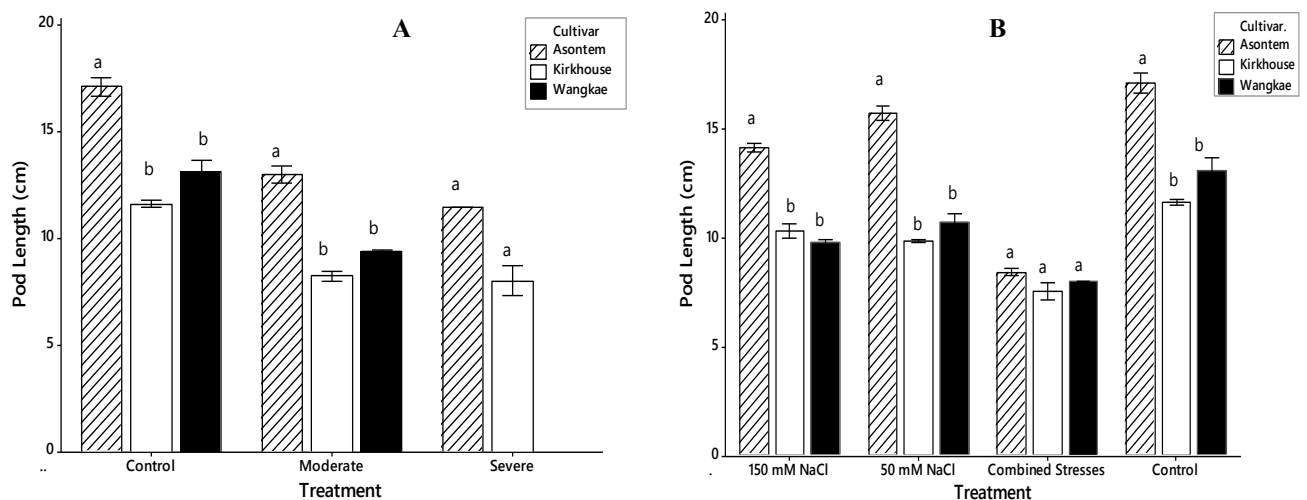


Fig. 4. Variation in mean pod length of three cowpea cultivars: (A) Cowpea cultivars under different watering regimes. (B) Cowpea cultivars under different levels of salt treatment. Means without a common letter are significantly different ($p \leq 0.05$) according to Tukey's test.

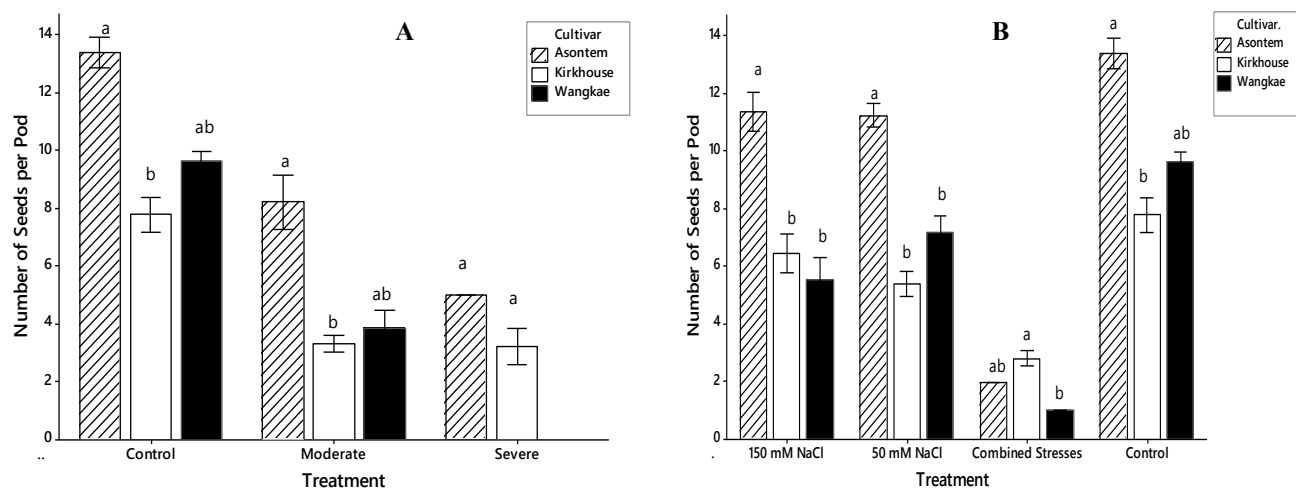


Fig. 5. Variation in mean number of seeds per pod of three cowpea cultivars:(A) Cowpea cultivars under different watering regimes. (B) Cowpea cultivars under different levels of salt treatment. Means without a common letter are significantly different ($p \leq 0.05$) according to Tukey's test.

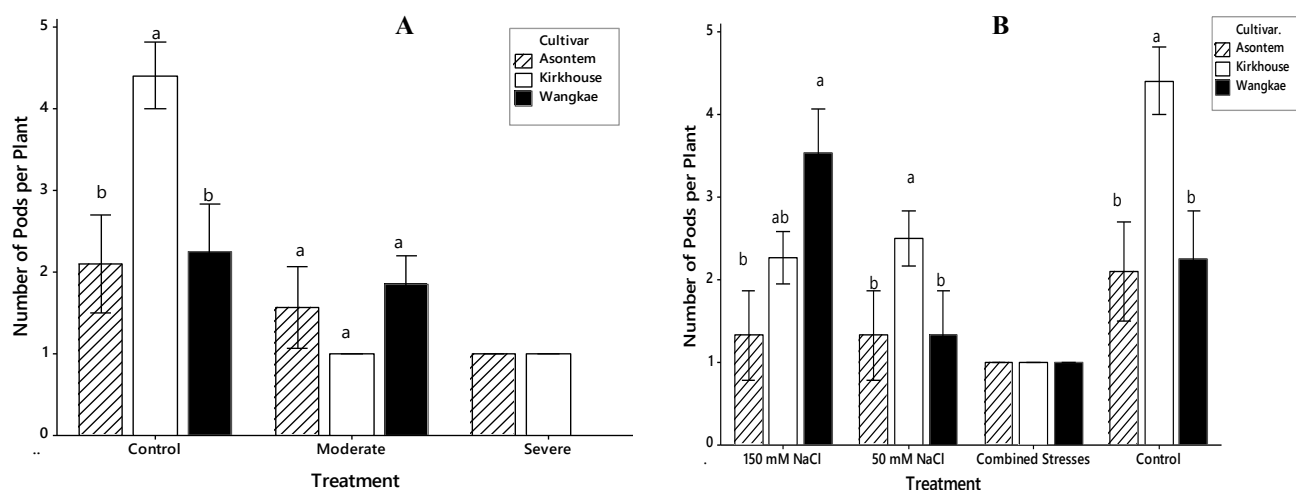


Fig. 6. Variation in mean number of pods per plant of cowpea cultivars:(A) Cowpea cultivars under different watering regimes. (B) Cowpea cultivars under different levels of salt treatment. Means without a common letter are significantly different ($p \leq 0.05$) according to Tukey's test.

Table 1. Variation in mean seed weight of cowpea cultivars under drought, salt and combined drought and salt treatments.

Treatment blocks	Cultivar		
	Kirkhouse ± se	Asontem ± se	Wangkæ ± se
Normal watering (Control)	1.22 ± 0.0952a	0.93 ± 0.1525b	1.305 ± 0.1775a
Moderate water stress	0.4675 ± 0.0457b	0.642 ± 0.126b	1.143 ± 0.207a
Severe water stress	0.367 ± 0.257a	0.410 ± 0.00a	--
50 mM NaCl	1.09 ± 0.0682a	0.734 ± 0.0559b	1.002 ± 0.1997a
150 mM NaCl	0.982 ± 0.244a	0.604 ± 0.0611b	0.942 ± 0.252ab
Severe water stress and 150 mM NaCl	0.3225 ± 0.1343a	0.2525 ± 0.015a	0.270 ± 0.00a

Means without a common letter are significantly different ($p \leq 0.05$) according to Tukey's test

Table 2. Variation in mean phenolic content of cowpea cultivars under various stress treatments.

Treatment blocks	Total phenol (mg GAE/ L)		
	Kirkhouse ± se	Asontem ± se	Wangkæ ± se
Normal watering (Control)	2.405 ± 0.369b	3.695 ± 0.149a	4.00 ± 0.546a
Moderate water stress	6.178 ± 0.439b	6.475 ± 0.234ab	7.1287 ± 0.129a
Severe water stress	7.407 ± 0.247a	7.573 ± 0.355a	7.285 ± 0.109a
50 mM NaCl	7.677 ± 0.0150a	7.399 ± 0.302a	7.616 ± 0.302a
150 mM NaCl	7.102 ± 0.921a	7.486 ± 0.301a	5.743 ± 0.303a
Severe water stress and 150 mM NaCl	4.688 ± 0.341b	5.281 ± 0.209ab	5.743 ± 0.303a

Means without a common letter are significantly different ($p \leq 0.05$) according to Tukey's test

Phenolic content of leaves: Phenol accumulation increased with increasing drought levels (Table 2). Under normal and moderate watering regimes, Wangkæ recorded the highest amount of phenol with Kirkhouse showing the lowest amount (Table 2). However, under severe drought conditions, Asontem and Kirkhouse accumulated more phenols than the Wangkæ cultivar (Table 2). Plants subjected to salt stress treatment recorded higher amounts of phenols compared to unstressed plants. The variation observed in phenolic contents of cowpea cultivars exposed to 50 mM NaCl and 150 mM was not statistically significant (Table 2). Kirkhouse recorded the highest phenolic content under 50 mM NaCl with Asontem recording the highest phenolic levels under 150 mM NaCl treatment. Interestingly, phenolic levels in the Asontem and Kirkhouse cultivars subjected to the combined salt-drought stress were lower than those in the 150 mM NaCl treatment alone.

Discussion

In this study, a decrease in internode length with a corresponding reduction in plant height was observed in all the three cowpea cultivars after drought stress induction (Fig. 1A, 2A). The observed decrease in height could be due to the oxidative damage caused by the stress to plants (Yadav *et al.*, 2021). Notably, the Asontem cultivar maintained higher plant height values under water stress compared to Wangkæ and Kirkhouse (Fig. 1A).

Plant growth is considered an important marker of salinity stress tolerance (Nxele *et al.*, 2017). Salt stressed cowpea cultivars showed a decrease in internode length and plant height (Fig. 2A, 1A) in this work possibly due to the effect of osmotic stress caused by NaCl (Isayenkov & Maathuis, 2019). The observations made were consistent with the report of Nkrumah *et al.*, (2021), who observed a reduction in the plant height of groundnuts under NaCl stress.

The simultaneous occurrence of drought and salinity stress has been shown to have negative impacts on plant growth (Abrar *et al.*, 2020). In the current study, a decrease in height was observed in all the three cowpea cultivars subjected to combined salt and drought stress (Fig. 1B). However, the reduction in height observed in the combined stress blocks was more severe than that observed in the individual stress treatments (Fig. 1B).

Soil moisture is vital for plant growth and serves as an indicator of drought stress (Ajaz *et al.*, 2018). Soil moisture can negatively affect seed yield, seed number, and total dry weight in plants (Wijewardana *et al.*, 2019). In the present study, all cowpea cultivars had lower soil moisture content under salt and drought stresses (Fig. 3A, B). However, Asontem under both stress conditions, maintained soil moisture better than Wangkæ and Kirkhouse (Fig. 3A, B). The coupling of drought and salinity stress resulted in the reduction of soil water content (Fig. 3B), which further caused a reduction in the osmotic and soil water potential as suggested by Abrar *et al.*, (2020) thereby negatively affecting the water absorption capacity of all the cowpea cultivars. For this reason, a decrease in SMC was observed for all the cowpea cultivars under combined stress. Nonetheless, Asontem under combined drought and salt

stress maintained relatively higher SMC values as compared to Wangkæ and Kirkhouse (Fig. 3B).

Yield component traits are good indicators of drought stress tolerance in cowpea (Iwuagwu *et al.*, 2017). In this study, cowpea cultivars under drought stress showed decreased pod length, number of pods per plant, and number of seeds per pod (Fig. 4A, 5A, 6A). Dadson *et al.*, (2005) reported a significant reduction of the seed yield of cowpeas under water deficit stress conditions similar to those used in this experiment. The observed reduction in pod and seed numbers under the stress elements could be due to flower abortions caused by drought stress (Boukar *et al.*, 2019). The study further revealed a decrease in the seed weight of drought stressed cowpea cultivars (Table 1). The Asontem cultivar recorded the highest seed weight under severe drought stress possibly as a result of the cultivar's ability to maintain photosynthate assimilation and carbohydrate accumulation under severe water deficiency (Yahaya, 2019).

The ability of a plant to maintain yield even under adverse environmental conditions is one of the most important determinants of salt tolerance (Negrão *et al.*, 2017). In this study, the application of salt stress adversely affected yield parameters such as pod length, the number of pods per plant, and the number of seeds per pod (Fig. 4B, 5B, 6B). These results have been confirmed by Akter *et al.*, (2022), who reported a significant reduction in the pod and grain numbers of cowpea genotypes as a result of salinity stress. Cowpea cultivars subjected to salt stress in this experiment showed differences in their responses. The Asontem cultivar recorded the highest grain yield under all the NaCl concentrations tested (Fig. 4B, 5B, 6B) indicating that, Asontem had relatively better dry matter accumulating capacity under salinity stress than Kirkhouse and Wangkæ cultivars.

The combined effects of salinity and drought stress are more detrimental to plants than their individual effects (Sahin *et al.*, 2018). In this study, we found that yield parameters such as pod number per plant, seed number per pod, and seed weight were consistently lower in the combined stress treatment block compared to the individual stress blocks (Fig. 5B, 6B, Table 1). The observed significant reduction in yield parameters could be explained by the fact that the two stresses may have had an additive effect on dry matter accumulation and hence severely impacted yields (Abdelraheem *et al.*, 2019; Ors *et al.*, 2021). Whereas Asontem demonstrated superior physiological characteristics in the individual stress blocks, Kirkhouse consistently outperformed in the combined salt and drought stress block, indicating that the cowpea cultivars did not respond in the same way to individual and combined stresses.

Phenolic compound accumulation is one of plants' adaptive mechanisms against drought-induced oxidative damage (Varela *et al.*, 2016). In this work, phenolic levels in leaves increased with increasing drought stress (Table 2). The Asontem cultivar accumulated the highest amount of phenols under drought indicating a high tolerance to drought stress.

Plants are able to tolerate osmotic stress through ion homeostasis (Meng *et al.*, 2018). Plants do this, by lowering their osmotic potentials to aid in water absorption and ionic adjustment (Dolatabadi & Toorchi, 2017). Salinity stress triggers the formation of phenolic compounds which are involved in defense against oxidative damage (Jali., 2021). Under 150 mM, the highest phenolic value was found in Asontem followed by Wangkae and Kirkhouse (Table 2), making Asontem more tolerant to salinity stress than Wangkae and Kirkhouse.

Conclusion

In this work, three cultivars (Asontem, Kirkhouse and Wangkae) were found to differ in their tolerance and susceptibility to imposed drought, salinity, and combined drought/salinity stresses. It was observed that Asontem proved to be more tolerant to all the imposed stress factors whereas Wangkae appeared the most susceptible and least tolerant to the imposed drought, salinity, and combined drought/salinity stress factors. In all the growth and developmental parameters estimated in this work, it was shown that they were affected as the intensity of drought and salinity increased. The impact of combined severe drought and high salinity stresses imposed on the three cultivars was more pronounced than the individual drought and salinity stress factors.

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