Phenotyping diverse cowpea genotypes reveals shoot traits contributions to drought recovery

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Abstract

The success of a breeding program largely depends on the presence of sufficient genetic diversity in crops to provide an avenue for selection of desirable genotypes for utilization in crop improvement. However, the primary gene pools of many crop plants are so depleted in genetic variability which is a consequence of continuous selections imposed by plant breeders. This necessitates exploring the potentials of landraces for sources of resistance to biotic and abiotic stresses. Thus, the "Wooden box techniques" was adopted to screen cowpea genotypes for their response to seedling drought stress, owing to its rapid and high throughput nature. Here, 420 cowpea genotypes were evaluated for their tolerance to seedling drought. Time course analysis of growth and agronomic traits revealed gradual cessation of growth as drought stress intensified as evidenced by reduction in trifoliate number, increase in leaf senescence and stem wilting. Multivariate analysis using principal component (PC) analysis and k-mean clustering identified 3 major clusters where PC1 and PC2 explained 46.7% of the variability in response to drought stress. The biplot analysis showed that plant height, stem greenness and trifoliate number contributed positively to PC1 while leaf senescence score was negatively related to the clustering on this axis. The comprehensive data analysis pipeline allows us to identify the relationship between the agronomic and stay-green parameters, which provides us with the understanding of traits that could be useful during the selection of lines under drought stress at the seedling stage. Our method provides an aid-to-selection for rapid screening of a large collection of cowpea lines for their response to seedling drought stress. Additionally, our results identified potential tolerant genotypes for use as parents for genetic analysis of drought tolerant traits and incorporation into breeding programs targeting the development and deployment of drought tolerant varieties.

Introduction

Cowpea (*Vigna unguiculata* (L.) Walp.) is an important part of the livelihoods of millions of small-scale farmers who rely on it for both economic and nutritional well-being (Horn et al., 2022). The dual-purpose nature of most cowpea varieties makes it an important source of green fodder or dry tillage for animals and food for humans. Like many legumes, cowpea is mostly intercropped with cereals by most smallholder farmers, thereby providing soil improvement advantages through nitrogen fixation (Horn et al., 2022; Weng et al., 2019). The crop is estimated to be grown on over 14 million hectares globally, yielding roughly 6.5 million metric tonnes each year (FAOSTAT, 2022). For 14 successive years, West Africa has been recognised as the largest cowpea producing region in Sub-Saharan Africa (SSA), culminating into 80% of total regional production (Boukar et al., 2018; Horn et al., 2022). The yield potential of cowpea is projected to increase in the next decade reaching 12.3 million tonnes by 2030 (Boukar et al., 2018; Omomowo & Babalola, 2021). However, due to many biotic and abiotic stresses affecting the crop's productivity, the maximum yield potential of cowpea has remained unattainable for decades (Omomowo & Babalola, 2021).

Drought is one of the most critical challenges posed by climate change, as well as one of the most significant threats to crop production (Carvalho, Matos, et al., 2019). Despite the fact that, cowpea is a relatively drought-tolerant legume, it is still susceptible to drought, especially during the seedling establishment, growth and development at the vegetative stage (Ravelombola et al., 2020).

Droughts are anticipated to become more frequent, intense, and last longer in many agricultural regions as a result of climate change (Olorunwa et al., 2021). The semi-arid region of SSA, which accounts for most of the cowpea production, is predicted to become drier in the future. Therefore, efforts should be made to develop more drought tolerant cowpea varieties that can cope with future climatic predictions than the existing varieties (Boukar et al., 2018). Harnessing cowpea landraces as a subject of genetic improvement would facilitate the identification of tolerant landraces with the potential for improving modern varieties. This will advance the identification of new sources of tolerant materials to be harnessed in breeding for drought-tolerant cowpea varieties (Ajayi et al., 2018).

Several methods abound for screening crop species for drought response (Carvalho, Castro, et al., 2019; Carvalho, Matos, et al., 2019; Nkomo et al., 2020). The screening methods vary depending on the crop growth stage, the number of crop lines and the objectives of the experiment. The effectiveness of drought-tolerant cowpea breeding is dependent on the screening methods deployed (Ajayi et al., 2017). Consequently, a simple and precise screening method for large cowpea lines like the "wooden box technique" has proven to be an effective drought response screening technique at the seedling stage, as demonstrated by Agbicodo et al., (2009) and Singh, (2014). Thus, we screened 420 cowpea genotypes using the wooden box technique developed by the cowpea breeding unit of the International Institute of Tropical Agriculture (IITA) (Singh, 2014). Plant height, trifoliate number, stem greenness and leaf senescence were recorded at an interval throughout the drought stress regime. Our results showed variation in the dynamic genotypic and time course response of the genotypes to drought stress.

Materials And Methods

Germplasm materials and planting

A total of 420 cowpea accessions were used in this study. These genotypes were collected from the major cowpea growing areas of Nigeria, including genotypes of Nigeria origin that were collected and stored at the Genetic Resources Centre of IITA, Ibadan, Nigeria (Fig. 1). These genotypes are widely grown across the Savanah ecologies of Nigeria including drought prone areas of the Sahel savanna. Some of these genotypes are preferred by local farmers because of their many desirable agronomic, adaptability and consumer preference traits. The genotypes were grown and evaluated for seedling drought response at the screenhouse of the Department of Botany, Ahmadu Bello University, Zaria (Lat 11°03'60.66" N & Long 7°41'59.99" E).

Wooden box experimental design

For this experiment, wooden boxes of 100 by 50 by 10 cm dimensions (length x width x height) were constructed with thick planks (4 cm thick). The boxes were tight enough to hold soil and allow excess water to drain off after irrigation. The boxes were filled with sand-loamy soil and kept raised in the screenhouse. They were then irrigated to field capacity and allowed to drain off excess water before planting. Four cowpea seeds dressed in Apron star

fungicide (Following manufacturer's recommendation) were randomly selected and planted in 4 hills at an equal distance of 5 cm in the wooden box. The experiment was laid out in a 35 x 12 alpha lattice design (lines x boxes) with two replications. The boxes were taken as Blocks. Each box was planted with the tolerant (Danila) and sensitive (TVu-7778) as checks to monitor the progress of the drought stress and determine the time of re-irrigation. After planting, the boxes were slightly irrigated until the emergence of the first trifoliate in 75% of the lines. After the emergence of the first trifoliate, the watering was stopped (27 days after last watering), and the tolerant and susceptible checks were monitored daily to ascertain the point at which the sensitive checks started wilting.

Soil samples were randomly collected at 4 cm depth from all boxes at an interval to monitor the soil drying kinetics. The samples were oven-dried at 70°C for 48 hours to determine the water content and this data was used to estimate the soil moisture stress severity for the cowpea lines.

Figure 1: Map of Nigeria showing the collection sites of the cowpea genotypes used for this study. The code for generating this map can be found here (https://rpubs.com/mjulkowska/Nigerian_cowpea_map_Ramatu_try0)

Seedling drought phenotyping and data collection

At the stop of irrigation (21 days after sowing) baseline data (plant height and the number of trifoliate) were collected on the genotypes on day 1 and 2 after water withholding water to evaluate plant conditions before the onset of drought stress. The plant height of all the genotypes was measured at 7, 12 and 15 days after the last watering, while the number of trifoliates was recorded at 6, 11, 14, 17 and 24 days after last watering. Leaf senescence and stem greenness were scored following the method described by Belko et al., (2012) and Singh, (2014).

Leaf senescence score was rated on a scale of 1 to 5 where 1= Green and fresh leaves; 2= Green and slightly wilted leaves; 3= Green-yellow and moderately wilted leaves; 4= Yellow-green and severely wilted leaves; 5= Yellow to brown leaves. Stem greenness was rated as 0 or 1, where 0= not green and 1= green. Plant recovery was scored at 16 days after resumption of watering, as the number of plants that showed green stem and new leaves after re-irrigation over the total number of plants in the plot.

Data Analysis

All data analysis and visualization were done in R 4.1.2 (R Core Team, 2013) and R studio 2021.09.0 (*http://www.rstudio.com/*). Analysis of variance (ANOVA) was first performed using general linear model function *aov()* in R on the agronomic and stay-green traits using alpha lattice design as written in this model:

$Y_{ijk} = \mu + G_j + R_i + B_i + C_i + \varepsilon_{ij}$

where y_{ijk} is the response variable from ijth wooden box experimental unit, μ is the overall mean, G_i is the effect of kth genotype, R_i is the effect of jth replication, B_i is the effect of jth box, ε_{ij} is the experimental error, and i = 1,2,3, and j = 1....420

Next, a linear mixed model using *lmer()* function of lme4 package (Bates et al., 2015) in R was deployed to estimate the genetic variance components for the traits measured. The agronomic and stay green variables were considered as the response variables by modelling the landraces, the number of boxes, number replication and box by genotypes interaction as random effect factors, as given in the best linear unbiased prediction (BLUP) equation here:

model <- lmer(y ~ (1|Rep) + (1|Box) + (1|Genotypes) + (1|Box:Genotypes), data = data1)

The broad sense heritability was calculated as a proportion of genotypic to total phenotypic variances using genetic variance components obtained from the mixed model analysis, as shown in the formula below (Falconer & Mackay, 1996).

$h^2 = \sigma_g / [\sigma_g + (\sigma_e / n)]$

with h^2 as the estimate of heritability in a broad sense, σ_g is the total genotypic variance, σ_e is the residual variance, and n is the number of replications.

Multivariate analysis

The average value per genotype used for the multivariate analysis was calculated using the *dplyr* (https://dplyr.tidyverse.org) of *tidyverse* suite (Wickham et al., 2019). Principal component analysis (PCA) was conducted to reduce the dimension of the dataset to visualize the relationship between genotypes and identify major contributing factors to the variation observed in the response of the genotypes to seedling drought stress. The data were first scaled and PCA was performed using *prcomp()* base R function, and visualization was done using the factoextra package (Kassambara & Mundt, 2020). K-means clustering was performed on the PCA data to identify major grouping patterns of the genotypes.

Hierarchical clustering was performed using the *hclust* and a dendrogram was generated using *dendextend* packages (Galili, 2015). The visualization of the clustering and heatmap was performed using *Heatmap()* function of ComplexHeatmap package (Gu et al., 2016). The Pearson's correlation analysis was performed using *rcorr()* function of the *Hmisc()* package (Harrell & Dupont, 2021), and the correlation plot was generated with *corrplot* package (Wei & Simko, 2017). The R script for all the analyses in this study can be accessed here http://rpubs.com/Sskayode/886266.

Results

Genetic diversity among crop plants is an essential resource for enhancing the genetic base needed for developing varieties with tolerance to biotic and abiotic stress. To assess the diversity of cowpea genotypes and their tolerance to seedling drought, 420 genotypes were collected across 19 states of Nigeria, including 2 check genotypes from cowpea breeding programmes of the IITA (Figure 1, Supplemental Table 1). The evaluation protocol for seedling drought

tolerance of cowpea genotypes using the wooden box techniques is presented in Figure 2. The progress of drought stress intensity over the course of the experiment as monitored by the soil water content of each box showed a significant rapid decline from day 1 to day 7 and reached 0 gram of water per gram of soil in all the boxes on day 16 (Figure 3).

Figure 2: Image of cowpea genotypes before drought and after drought imposition in the wooden box. (A). Overview of the wooden box set up in the screenhouse (B). First trifoliate stage of the cowpea genotypes before the drought imposition. (C). Chlorosis and senescence of leaves at 15 days after the last irrigation. (D). discrimination between the tolerant and highly sensitive lines at 20 days after the last irrigation.

Figure 3: Soil drying kinetic for the seedling drought experiments across the wooden boxes. D1, D7, D13 and D16 represent days (D) 1, 7, 13 and 16 respectively, after the last watering of the boxes.

The agronomic and stay-green parameters recorded as plant height, trifoliate numbers, leaf senescence score and stem greenness showed significant variation amongst the genotypes with a sharp reduction at the latter part of the experiments when the water levels had dropped drastically (Table 1). Significant variation was observed for seedling height at 7, 12 and 15 days after drought imposition. The baseline height on day 2 after drought imposition was statistically similar among the genotypes and ranged from 1.1 to 21.1 cm, with a median of 7.0 cm and a standard deviation of 2.9 cm (Figure 4). Significant genotypic differences were found among the genotypes as the drought intensity progressed from 7 to 15 days (Table 1). Typically, the tallest genotypes were ABU_Vu150 (19.8 cm), ABU_Vu181 (18.4 cm), ABU_Vu088 (18.3 cm), ABU_Vu239 (17.7 cm), and ABU_Vu147 (17.0 cm) (Supplemental Table 2), whereas the shortest ones were ABU_Vu343 (2.9 cm), ABU_Vu369 (2.8 cm), ABU_Vu093 (2.5 cm), ABU_Vu116 (2.3 cm), and ABU_Vu179 (2.1 cm) (Supplemental Table 2). Additionally, the difference in plant height among genotypes at day 12 and day 15 was not statistically significant thus, indicating that the plant could not increase in height because of drought stress. Consequently, the drought stress has had a significant effect on the growth of the genotypes by day 12 after the last watering.

An increase in the number of trifoliate by cowpea is an indication of growth progression, thus measuring this trait allows us to understand the growth strategies deployed by the lines under drought stress. The baseline data at day 1 after the last irrigation showed that the vast majority of the lines have one trifoliate with no statistical difference between the genotypes at the start of the drought stress. The number of trifoliate increases to a maximum value of 2.8 by days 6 and 11, after the stop of irrigation with a median value of 1.2 in both cases (Table 1), although these traits were not significantly different among the accessions. However, our data showed that by day 17, the median value of trifoliated dropped to 1.0 indicating that many lines dropped their trifoliate due to increasing water deficit. The number of trifoliate was found to be significantly different among the 420 cowpea genotypes evaluated for seedling drought tolerance at days 14 and 24 after drought imposition (Table 1). The genotypes with the highest average trifoliate number at day 24 after drought imposition were ABU_Vu170 (1.7), ABU_Vu181 (1.6), ABU_Vu119 (1.5), ABU_Vu315 (1.5), and ABU_Vu149 (1.4) (Supplemental Table 2), indicating that these lines had retained their trifoliate even under drought stress condition. The genotypes with the lowest had no trifoliate at 24 days after drought imposition including ABU_Vu356, ABU_Vu194, ABU_Vu079, ABU_Vu345, ABU_Vu266 (Supplemental Table 2), suggesting that these genotypes shed their trifoliate to reduce the impact of the stress. At 14 and 24 days after the last watering, the mean number of trifoliate for the genotypes ranged from 0 – 2.3 and 0 – 1.7, respectively (Figure 5A).

Table 1: Summary statistics of agronomic and stay green parameters measured in this study.

*Significant at 0.05

**Significant at 0.01

***Significant at 0.001

h^2: heritability in the broad sense was estimated using variance components generated from mixed model analysis using *lmer()* function of the package lme4 with the model above.

PH – Plant height, TN – Trifoliate number, LS – Leaf senescence score, SG – Stem greenness score, RR – Recovery rate and D – The days after the last watering.

Leaf senescence score is a widely used trait for assessing the effect of water deficit on plants at the seedling stage. Our data indicated significant variation in leaf senescence score among the 420-cowpea genotype at 22 days after last watering (Table 1). Leaf senescence score varied with days. At 13 days after the last watering, a median value of 3.0, and a standard deviation of 0.7 was obtained. As the drought progressed (day 25), the median of the leaf senescence score rose to 4.3. A lower leaf senescence score indicates a higher tolerance level of the cowpea lines to the drought stress. Thus, the lowest value for leaf senescence scores at day 22 were in ABU_Vu400 (2), ABU_Vu016 (2), ABU_Vu367 (2), ABU_Vu088 (2.5), and ABU_Vu359 (2.5) (Supplemental Table 2), suggesting that these genotypes might be drought tolerant as evidenced by their greenness despite the lower water availability. The genotypes with the highest leaf senescence score of 5 are presented in Supplemental Table 2, indicating that these lines were sensitive to drought stress.

Figure 4: Boxplot showing the distribution of plant height measured at 2, 7, 12 and 15 days after the last watering for all the genotypes. D – the days after the last watering

Stem greenness of cowpea seedlings is an indication of the level of photosynthetic pigments in the stem, therefore maintaining a green stem under drought stress might be a good indication of drought tolerance. At day 21 after drought imposition the stem greenness scores varied with a median of 0.7 and a standard deviation of 0.3. Significant genotypic differences for stem greenness score were identified among the 420 cowpea genotypes evaluated for drought tolerance at 21 days after drought imposition (Table 1). As the drought progresses (day 27), the stem green score shifted to a median score of 0.2. The higher

the stem greenness score of a genotype the higher the tolerance level observed. At day 27, the genotypes with the highest stem greenness scores of 1 include ABU_Vu140, ABU_Vu200, ABU_Vu102, ABU_Vu334 and ABU_Vu207 (Supplemental Table 2), indicating that these genotypes maintained green stems under drought stress condition. The genotypes with the lowest score of 0 include ABU_Vu306, ABU_Vu242, ABU_Vu191, ABU_Vu360, ABU_Vu407 (Supplemental Table 2), suggesting that these lines might have reached the permanent wilting point.

Figure 5: Density plot showing the distribution of traits measured after the later watering for all the genotypes. Distribution of (A). Average number of trifoliate (B). Leaf senescence score and (C). Stem green score for all the genotypes between 1 and 27 days after last watering. (D – the days after the last watering).

The recovery rate which is the number of plants that showed growth, and development of new leaves after rewatering varies from 0.0 to 50% with a mean of 1.3% (Supplemental Figure 1, Table 1), the genotypes with the highest recovery rate were ABU_Vu005 (37.5%), ABU_Vu298 (41.7%), ABU_Vu221 (50.0%), ABU_Vu201 (50.0%), ABU_Vu213 (50.0%) (Supplemental Table 2), indicating that these genotypes maintained growth and regeneration after drought stress condition. Out of 420 genotypes evaluated only 26 showed recovery rates ranging from 12.5% to 50%.

We next estimate the broad sense heritability to ascertain the proportion of genetic factors and the effect of the environment on the observed phenotypic responses to seedling drought stress in the cowpea lines. The data revealed that the highest broad sense heritability was found in plant height measured on day 7 (42.1%), day 15 (38.0%) and day 12 (36.5%), and after the last watering while, the least broad sense heritability was recorded in leaf senescence score at day 16 and 22 (0%) (Table 1).

To identify the major source of variation of cowpea genotypes to seedling drought stress, we conducted a principal component analysis (PCA), which identified 19 components that contribute to the variation observed in the genotypes' response to seedling drought stress (Figure 6A). The first and second components account for 46.7% of the variation observed and these components divided the genotypes into three major clusters using K-mean clustering (Figure 6B). The first cluster is made up of 68 accessions of which the members showed an average recovery rate of 1.5% (Supplemental Figure 3). The second and third clusters had 183 and 150 accessions, respectively. On average, the accessions in cluster 2 had a recovery rate of 0.8% (Supplemental Figure 3), which shows that they are sensitive to seedling drought. Next, further examination of the principal components showed that PC1 was positively associated with plant height, trifoliate numbers, stem greenness and negatively related to leaf senescence score, while PC2 was positively associated with stem greenness and negatively associated with plant height and leaf senescence scores (Figure 6C and Supplemental Figure 2).

Figure 6: Principal component analysis of the traits measured for all the cowpea genotypes. (A). Scree plot showing all the principal component and the percentage variance explained by each. (B). K-means clustering analysis for all the genotypes based on the agronomic and stay green scores. (C). The contributions of the agronomic and stay green score to the PC1 and PC2 principal axis of the dimensionality reduction plot. The length of the arrows indicates level of the contribution to the principal component axes, the orange-red colour means a high contribution of the variable to the dimension, while blue colour indicates the low contribution of the variable to the Dimension. PH – Plant height, TN – Trifoliate number, LS – Leaf senescence score, SG – Stem greenness score, RR – Recovery rate, and D – The days after the last watering.

To identify the relationship between all the genotypes based on all the traits measured in addition to the dimension reduction PCA and k-mean clustering previously mentioned in Figure 6. Hierarchical clustering analysis was performed using Ward's method which minimizes the Euclidean distance between the points in the matrix. The hierarchical clustering identified 6 major clusters (Figure 7). Clusters 1 was made up of the highest number of genotypes (235) with moderate plant height, and no recovery rate. Cluster 6 consisted of 3 genotypes with moderate plant height and higher recovery rate (Supplemental Figure 4), indicating that the cowpea genotypes deployed have tolerance mechanisms to maintain growth activities under stress.

Our experiment collects many agronomic and stay-green traits which have the potential to help our understanding of the plants coping strategies under seedling drought stress. To provide insight to the interdependent association between these agronomic parameters as well as the time course measurement of these traits, we conducted Pearson's correlation analysis. Our data showed that there was a significant positive correlation between plant height, stem greenness, number of trifoliate and the recovery rate of the cowpea genotypes (Figure 8). However, leaf senescence scores were negatively correlated with plant height, stem green and trifoliate numbers. Interestingly, the recovery rate was only significantly positively correlated with the plant height at day 15, stem greenness at day 27, and trifoliate number at day 24. This indicates that recovery is only a function of the plant to maintain growth at the peak of drought than the earlier points after the stop of irrigation.

Figure 7: Hierarchical clustering analysis and heat map for the cowpea genotypes based on the on the agronomic and stay green scores. PH – Plant height, TN – Trifoliate number, LS – Leaf senescence score, SG – Stem greenness score, RR – Recovery rate, and D – The days after the last watering.

Figure 8: Pearson's correlation analysis between the traits measured and recovery rate of the genotypes in this study. (PH – Plant height, TN – Trifoliate number, LS – Leaf senescence score, SG – Stem greenness score, RR – Recovery rate, and D – The days after the last watering).

Discussion

Drought stress negatively affects cowpea growth and development at the establishment and vegetative stage (Muchero et al., 2008; Olorunwa et al., 2021). Increasing drought intensity caused reduced turgor pressure within the cells leading to wilting and senescence (Shao et al., 2008). For this reason, drought is highly devastating to crop production activities. Reductions in crop yield have been attributed to the damaging impact of drought stress world-wide (Fahad et al., 2017). Drought is expected to increase in many agricultural areas of the world which will lead to a significant reduction in the yield of major crops if adequate intervention is not provided. Sub-Saharan Africa is projected to be one of the regions that will be greatly affected by drought (Ofori et al., 2021; Thompson et al., 2010), and this is where the largest production of cowpea happened. This anticipated drought intensity in agricultural land is compounded by climate change events (Ofori et al., 2021). Many drought-tolerant varieties of cowpea have been identified and incorporated into breeding programs

(Ogbuinya, 1997). However, the increasing intensity of drought has boosted the drive to identify and develop even more drought tolerant varieties due to the prevailing harsh conditions. Thus, large scale screening and phenotyping efforts can provide an opportunity to identify potential germplasm for breeding cowpea resilient to drought stress. Many rapid phenotyping methods have been developed to allow for the screening of large number of accessions to understand their level of tolerance to drought at seedling stage (Agbicodo et al., 2009; Nkomo et al., 2020; Ravelombola et al., 2018, 2020). In this study, we explored the diverse collection of cowpea genotypes for their agro-morphological responses to seedling drought stress using wooden box phenotyping protocols. Our main aim was to identify seedling drought tolerant lines and genotypic components of the seedling responses to drought stress. The wooden box technique has been shown in previous studies to be useful for evaluating a large number of genotypes such as Recombinant inbred lines (RILs) and landraces (Agbicodo et al., 2009; Alidu et al., 2019; Cui et al., 2020; Ravelombola et al., 2018, 2020; Verbree et al., 2015).

In this study, we observed that drought stress caused progressive declines in the growth rate of cowpea genotypes to slow down as the stress becomes intense with significant variation among the genotypes, as evident in the reduction in plant height and number of trifoliates. A similar decline in the growth rate of cowpea under drought stress at seedling was also observed in 36 cowpea breeding lines evaluated at the breeding program at the University of Arkansas (Cui et al., 2020), suggesting that growth performance could be reliably used to discriminate the seedling drought stress responses in cowpea. Reduction in trifoliate under stress have been proposed to be a stress coping strategy by cowpea (Cui et al., 2020; Ravelombola et al., 2020). As exemplified in this research, few drought-tolerant plants stopped growing, and shed their leaves to conserved moisture by reducing surface area for transpiration to survive the period of drought. Although, the extent to which drought stress affects transpiration rate is beyond the scope of this study. Thus, the reduction in trifoliate numbers and the cease in growth rate under drought stress might be due to the inability of many genotypes to coordinate growth at cellular levels due to a reduction in available water level in the soil.

Our data showed significant variation in leaf wilting scores, where genotypes with high senescence scores were lost and failed to recover after rewatering. Wilting increased with an increase in the intensity of drought while the variation among the genotypes for the wilting score reduced with an increase in drought intensity. This suggests that genotypic sensitivity to drought could be at an early or later growth stage. Stem greenness was a good indicator of drought tolerance in cowpea. The stem greenness varied considerably between genotypes at day 21, however by day 27, the vast majority of genotypes had lower stem greenness scores, suggesting that most genotypes have reached the permanent wilting point. The cowpea plant's ability to maintain green stem has been proposed as one of the tolerance mechanisms to seedling drought stress (Ravelombola et al., 2020). Tolerant lines can the ability to mobilize moisture from the stem and carried out photosynthesis to remain alive for a longer time under drought stress (Ogbuinya, 1997). The low variability in the response to drought stress by day 27 in this study might be attributed to the severity of the drought stress applied.

Our seedling drought phenotyping protocol provides an opportunity to measure some agronomic and stays green traits over time. This data richness coupled with time scale measurement provided us with the opportunity to improve our understanding of the relationships between different traits and the early response orchestrating the drought tolerance in cowpea. For this purpose, we deployed correlation analysis to identify trait(s) with the highest contribution to recovery from drought stress after rewatering. Statistical correlation has been widely employed to understand the relationship between traits and plant performance under stress, and to reduce the complexity of multiple traits from phenotyping experiments (Abiola T Ajayi et al., 2018; Abiola Toyin Ajayi et al., 2017; Mohammed et al., 2022). Our correlation analysis highlighted plant height, trifoliate number, and stem greenness as important positive contributors to plant survival under drought stress conditions. The higher rate of leaf wilting negatively affected the recovery rate in cowpea genotypes screened in this study. Leaf senescence is a robust parameter that measured how negatively photosynthesis is impacted. With prolonged exposure to drought stress, the senescence increases, which reflects a reduction in the photosynthetic capacity of the plant, therefore, leading to a decline in growth and reduced ability to recover from drought stress.

Conclusion

In summary, our data provide a broad overview of the seedling drought stress responses of 420 cowpea genotypes. In this study, we classified the different traits as a negative or positive contributor to recovery and drought stress response in cowpea. Hence, further investigations into the genetic basis and the trait contributing to the recovery rate that was observed in this study can provide an avenue for identifying molecular markers for improving seedling tolerance cowpea to support the food security of sub-Saharan Africa and the world at large in the face of climate uncertainty.

Declarations

Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

Data Availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Table 1

Table 1: Summary statistics of agronomic and stay green parameters measured in this study.

Trait	Min	Max	Mean	Median	SD	F value (*pvalue)	h^2
PH_D2	1.1	21.1	7.3	7.0	2.9	0.999^{ns}	22.6
PH_D7	2.0	21.9	8.4	8.0	3.1	1.485^{*}	42.1
PH_D12	1.3	24.0	9.2	9.2	3.2	1.574^{*}	36.5
PH_D15	2.1	19.8	9.2	9.0	3.1	1.503^{*}	38.0
TN_D1	0.0	2.3	0.7	0.6	0.5	1.065^{ns}	14.8
TN_D6	0.0	2.8	1.2	1.2	0.5	1.132^{ns}	8.5
TN_D11	0.0	2.8	1.2	1.2	0.5	1.027^{ns}	2.8
TN_D14	0.0	2.3	0.9	1.0	0.4	1.532^{*}	27.0
TN_D17	0.0	3.0	0.9	1.0	0.4	1.257^{ns}	9.2
TN_D24	0.0	1.7	0.5	0.5	0.4	1.620^{*}	24.5
LS_D13	1.0	5.0	3.1	3.0	0.7	1.019^{ns}	14.8
LS_D16	1.0	5.0	3.6	3.7	0.8	1.218^{ns}	0.0
LS_D19	2.0	5.0	3.9	4.0	0.7	1.098^{ns}	0.0
LS_D22	2.0	5.0	4.2	4.3	0.7	2.120^{***}	19.6
LS_D25	2.5	5.0	4.3	4.3	0.5	1.334^{ns}	16.6
SG_D21	0.0	1.0	0.6	0.7	0.3	1.685^{**}	35.7
SG_D24	0.0	1.0	0.5	0.5	0.3	1.309^{ns}	26.9
SG_D27	0.0	1.0	0.2	0.2	0.3	1.195^{ns}	10.6
RR	0.0	50.0	1.3	0.0	6.1	1.387^{*}	5.8

*Significant at 0.05

**Significant at 0.01

***Significant at 0.001

h^2: heritability in the broad sense was estimated using variance components generated from mixed model analysis using *lmer()* function of the package lme4 with the model above.

PH – Plant height, TN – Trifoliate number, LS – Leaf senescence score, SG – Stem greenness score, RR – Recovery rate and D – The days after the last watering.
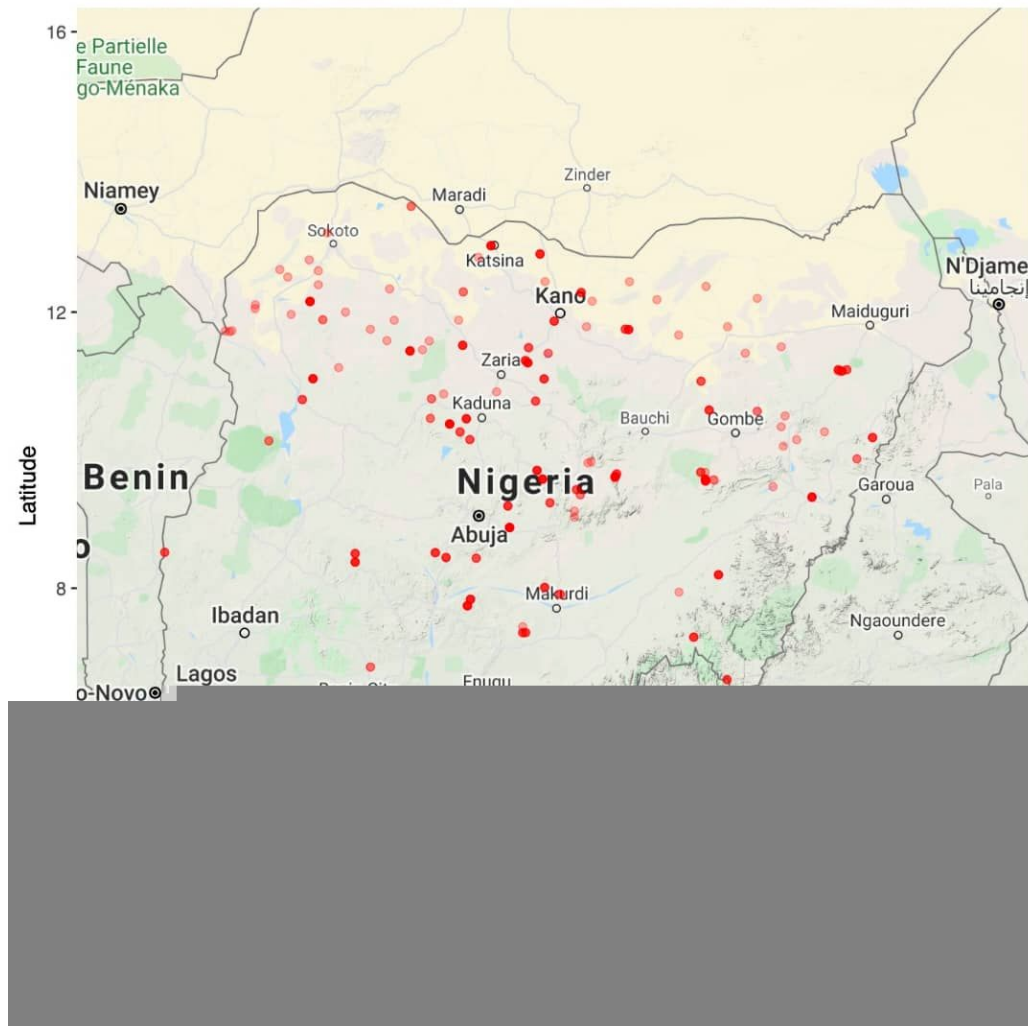
Figures

Figure 1

Map of Nigeria showing the collection sites of the cowpea genotypes used for this study. The code for generating this map can be found here (https://rpubs.com/mjulkowska/Nigerian_cowpea_map_Ramatu_try0)

Figure 2

Image of cowpea genotypes before drought and after drought imposition in the wooden box. (A). Overview of the wooden box set up in the screenhouse (B). First trifoliate stage of the cowpea genotypes before the drought imposition. (C). Chlorosis and senescence of leaves at 15 days after the last irrigation. (D). discrimination between the tolerant and highly sensitive lines at 20 days after the last irrigation.

Figure 3

Soil drying kinetic for the seedling drought experiments across the wooden boxes. D1, D7, D13 and D16 represent days (D) 1, 7, 13 and 16 respectively, after the last watering of the boxes.

Figure 4

Boxplot showing the distribution of plant height measured at 2, 7, 12 and 15 days after the last watering for all the genotypes. D – the days after the last watering

Figure 5

Density plot showing the distribution of traits measured after the later watering for all the genotypes. Distribution of (A). Average number of trifoliate (B). Leaf senescence score and (C). Stem green score for all the genotypes between 1 and 27 days after last watering. (D – the days after the last watering).

Figure 6

Principal component analysis of the traits measured for all the cowpea genotypes. (A). Scree plot showing all the principal component and the percentage variance explained by each. (B). K-means clustering analysis for all the genotypes based on the agronomic and stay green scores. (C). The contributions of the agronomic and stay green score to the PC1 and PC2 principal axis of the dimensionality reduction plot. The length of the arrows indicates level of the contribution to the principal component axes, the orange-red colour means a high contribution of the variable to the dimension, while blue colour indicates the low contribution of the variable to the Dimension. PH – Plant height, TN – Trifoliate number, LS – Leaf senescence score, SG – Stem greenness score, RR – Recovery rate, and D – The days after the last watering.

Figure 7

Hierarchical clustering analysis and heat map for the cowpea genotypes based on the on the agronomic and stay green scores. PH – Plant height, TN – Trifoliate number, LS – Leaf senescence score, SG – Stem greenness score, RR – Recovery rate, and D – The days after the last watering.

Figure 8

Pearson's correlation analysis between the traits measured and recovery rate of the genotypes in this study. (PH – Plant height, TN – Trifoliate number, LS – Leaf senescence score, SG – Stem greenness score, RR – Recovery rate, and D – The days after the last watering).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- 2022.06.21.supplementarymaterialscowpeaseedlingdrought.docx