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RESEARCH

Physiological, biochemical and genetic studies reveal differing responses of West Africa rice genotypes under induced upland field and greenhouse drought stresses

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Abstract

Background: Drought is the single most important factor limiting rainfed upland rice production in Africa and is aggravated by climate change leading to a serious food security threat in the continent. This study aimed to assess the impact of drought stress on morpho-physiological and biochemical traits among rice genotypes and quantify the genetic variability among the genotypes under both reproductive drought stress (RS) and non-stress (NS) conditions. Further, this study aimed to select drought-tolerant genotypes and identify traits to monitor during screening for drought under field and greenhouse conditions. *Methods*: To achieve these objectives, we first screened 100 genotypes under upland reproductive stage drought field conditions as well as non-stress conditions using α-lattice design with three replications. Out of the 100, we selected 14 genotypes based on yield and yield-related traits under both conditions (RS and NS) for further screening under drought in the greenhouse condition using completely randomized block design (RCBD) with three replicates. Data on yield-related traits, relative water content (RWC), malondialdehyde (MDA), proline and genetic diversity parameters were collected. *Results*: Under the field screening, for all the investigated traits, a general reduction was observed among the genotypes under drought stress compared to non-stress conditions. Traits such as spikelet fertility (SFP), grain yield per plant (GYP), hundred grain weight (HGW), leaf rolling score, and leaf drying score recorded high broad-sense heritability (>60%) combined with high genetic advance (>20%) implying that selection could be rewarding for these traits under drought stress. Grain yield was positively associated with most yield-related traits under both water regimes. Under the greenhouse screening, RWC of all genotypes was reduced by >14%, except G11 (1.58%), G99 (8.43%), and G100 (5.43%), while increased MDA of >45% for all genotypes was observed, except G11 (25.15%), G99 (39.37%), and G100 (43.65%) under drought. *Conclusions*: Based on the correlation analysis, principal component analysis, and the multi-trait genotype-ideotype distance index (MGIDI) using relative trait values, grain yield, spikelet fertility, grain length, MDA and RWC are recommended in descending order for monitoring during screening for drought tolerance. Overall, G60, G100, G99, and G11 were selected in chronological order as genotypes with enhanced tolerance to drought. Among these genotypes, G60 (Viwornor short) is glaberrima ecotype, while G100 (UPLR-17), G99 (APO), and G11 (CRI-Enapa) are indica ecotypes.

Keywords: rice, malondialdehyde, proline content, grain yield, drought tolerance

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Introduction

Rice is one of the most important staple foods worldwide as it provides 20% of dietary energy per capita and 13% of the dietary protein for human consumption. The dietary shares of rice in developing countries are approximately 29.1% dietary protein and 29.3% calories (Sautter et al., 2006). The worldwide overall paddy rice production is 787 million tons per annum, and it is grown in over 165 million hectares of land (FAOSTAT, n.d.). In sub-Saharan Africa (SSA), the availability of improved rice varieties plays a significant role in achieving food security and reducing poverty. Between 2000 and 2014, improved varieties positively impacted poverty alleviation and food security in SSA, especially after the 2008 food crisis. The average income for the adopters of NERICA rice has increased from US$25 to US$58 per capita (Arouna et al., 2017). Apart from its importance, rice is also one of the most demanding crops for water (Bouman et al., 2007). Climate variability greatly influences water resources while droughts and floods are projected to increase in the future (Markus et al., 2019; Bhaga et al., 2020; Yang et al., 2023).

Water is intrinsically linked to agricultural and food production (Wang et al., 2012), poverty reduction, economic growth, and food security (Jägermeyr, 2020), and its deficits threaten food security by increasing losses in crop yields (Foley et al., 2011; Caparas et al., 2021). As the most demanding sector of water in the world, agriculture is subject to crop yield failures 4.5 times greater by 2030 and approximately 25 times greater by 2050 across the world's most food-producing regions (Caparas et al., 2021). Water stress, commonly called drought, impacts 80% of all farmlands in the world, causing crop failure and impairing crop growth by reducing yield productivity (Begna, 2022). Approximately 53% of rice-growing regions experience the influence of climate variability on yield, and approximately 32% of variability in rice yield is due to year-to-year climate variability (Ray et al., 2015).

Approximately 78% of the total rice production area in Africa accounting for 38% of rainfed upland rice production and 40% of rainfed lowland rice production is classified as most vulnerable to drought (Diagne et al., 2013; Dossou-Yovo et al., 2022). Drought is the most significant abiotic constraint for rice production in Africa (33%), (Van Oort, 2018), and it is one of the major abiotic stresses that constrains yield stability (Saikumar et al., 2014). The grain yield of rice severely decreases under drought stress and varied from 20 to 80% yield reduction (Pantuwan et al., 2001; Ahmadikhah and Marufinia, 2016; Hussain et al., 2022). The demand for drought-tolerant cultivars is increasing continuously making it a crucial challenge for plant breeders around the world, as climate change enhances the likelihood of increased drought risks across the crop-growing regions worldwide (Begna, 2022). To reverse the problem of yield losses due to drought stress, various tactics have been used in plant breeding, including conventional breeding using targeted secondary traits, direct selection using grain yield, quantitative trait loci (QTLs), and marker-assisted selection (MAS) approaches.

Targeted secondary traits (secondary traits refer to any agro-physiological trait apart grain yield used during varietal selection) have been used for effective breeding for drought tolerance in rice, including spikelet fertility and delayed flowering (Pantuwan et al., 2002; Lafitte et al., 2003; Afiukwa et al., 2016). The required trait in rice breeding programs for drought tolerance are rice genotypes showing a delay in leaf rolling under drought with quick recovery ability from stress conditions. Leaf rolling and leaf drying have been used as criteria in the selection of drought-tolerant varieties during the vegetative growth drought stage (Thanh et al., 2006; Farooq et al., 2009; Umego et al., 2020). The number of filled grains under drought at the reproductive drought stress stage is the most important determinant of yield under drought stress condition (Yue et al., 2006), and it provides clear information on the genotypic response to stress (Lafitte et al., 2003). It can, therefore, be used for genetic studies on drought tolerance.

In the past two decades, grain yield has been used as the main criterion for the successful development and release of 17 high-yielding drought-tolerant rice varieties in Asia and Africa (Dixit et al., 2014; Kumar et al., 2014). Some of the drought-tolerant varieties released in Africa are FARO44, FARO62, NERICA1, NERICA4, and NERICA8 (Sonnino, 2009; Afiukwa et al., 2016). To date, QTLs that are linked to grain yield under drought stress have been reported in all chromosomes of rice. Robust grain yield QTLs under drought stress conditions, namely, $qDTY_{1.1}$ (Bernier et al., 2007), $qDTY_{2.1}$ (Ghimire et al., 2012), $qDTY_{3.1}$ (Venuprasad et al., 2009), $qDTY_{3.2}$ (Saikumar et al., 2014), and $qDTY_{12.1}$ (Vikram et al., 2011), have been identified using SSR markers. Four stable QTLs, namely, $qDTY_{2.4}$, $qDTY_{3.3}$, $qDTY_{6.3}$, and $qDTY_{11.2}$, have been identified for rice grain yield under reproductive stage drought stress using SNP markers through genotyping-by-sequencing (GBS)-based high-density linkage maps (Yadav et al., 2019). In the past few years, new indexes based on multi-traits have been developed to improve the selection in plant breeding including factor analysis and ideotype-design (FAI-BLUP) index (Rocha et al., 2018) and multi-trait genotype-ideotype distance index, MGIDI (Olivoto and Nardino, 2021).

Furthermore, a thorough understanding of the physiological and biochemical dissection of African indigenous landraces can help to increase genetic gain in rice productivity in West Africa. These indigenous rice genotypes embody several drought-tolerant features that are useful for rice improvement (Adjah et al., 2022). In West Africa, some physiological works have been reported on relative water content (Sangodele et al., 2014; Bimpong et al., 2018; Umego et al., 2020), chlorophyll content (Adeboye et al., 2021), and transpiration efficiency (Affortit et al., 2022) in rice under drought stress.

With the focus of remediating the yield losses to drought in mind, this study sought to contribute to selecting drought-tolerant rice cultivars with high grain yield under drought stress by assessing the effects of this stress on morphological, physiological, and biochemical traits among the rice genotypes and quantifying the genetic variability among the genotypes under both drought stress and non-stress conditions. Genotypes that are tolerant to drought stress were selected based on yield-related, physiological, and biochemical traits.

Methods

FIELD SCREENING

Field evaluation of the rice genotypes for yield and yield-related traits

This experiment was conducted at the Crops Research Institute of the Council for Scientific and Industrial Research (CSIR-CRI) in Fumesua-Kumasi, Ghana, during the long dry season (November–March) of the 2021/2022 using 100 randomly selected rice genotypes collected from the Africa Rice Center (AfricaRice) and CSIR-CRI rice genebank. Most of these genotypes were rice breeding lines or released varieties developed by AfricaRice and CSIR-CRI and were essentially grown in Africa. Few of them were breeding lines from KAFACI, South Korea, and these varieties were part of the core breeding germplasm of CSIR. Genotypes were mixed of indica, japonica, NERICA, and glaberrima ecotypes. The full name of the genotypes with their corresponding ID are presented in additional file 2. In this study, APO, a well-known drought-tolerant check, is used as a tolerant check. APO displays a reduced water use alongside with limited biomass loss under drought stress condition. Results showed that the strong antioxidant power of APO gives it the ability to maintain a stable grain yield under drought stress (Melandri et al., 2021). Reproductive stage drought stress (RS) and non-stress (NS) trials were established under upland conditions. Direct seeding was performed in upland field during 2021 wet season following an α-lattice design with three replicates in block within replicate design. The plot size was 1 x 1 m

with row and hill spacing of 20 cm. Three rice seeds were directly seeded per hole with a total of 25 holes per plot per genotype. After the thinning out, each hole was made into an individual plant. All lines were tested following two water management practices in this experiment: normal irrigation (non-stress) and simulated water stress conditions by stopping irrigation (drought stress). The rain-out shelter was used to keep the rainfall off. The dimensions of the rain-out shelter were 30 × 25 m, thereby covering all the plots of the experiments together. The distance of the closest plots to the edge of the shelter is 2 m, enough to prevent these plots exposition to rain. White transparent roofing sheets with a thickness of 4 mm were used to cover the rain-out shelter in order to reduce the light shielding rate and mimic the farmers' field conditions. Drought stress was applied at early reproductive stage. The stress started 50 days after sowing and was maintained by stopping irrigation for 24 days starting from 13 November 2021 to 6 December 2021. After the drought stress period, the stressed plants were subjected to 5 days of drought stress cycle throughout the late stages of rice growth. The data on drought sensitivity traits and chlorophyll content were collected when at least 70% of the genotypes showed severe leaf rolling (SES, 2002) and the average soil moisture content was below 5%. The soil moisture TRIME-PICO TDR (IMKO, Ettlingen, Germany) was used to measure the soil volumetric water content (%, v/v) at a depth of 10 cm of the soil surface on 30 November 2021. The fields were managed following standard agronomic practices for rice growing adopted at CSIR-CRI (Asante et al., 2019).

Phenotyping

Yield-related traits

Data were collected on grain yield per plant (g) on an average of five randomly selected plants per plot per replicate. Days to 50% flowering (DTF) were recorded when 50% of the plants in each plot showed flowering. Yield-related traits such as plant height in cm, number of effective tillers, panicle length in cm, and 100-grain weight in grams were recorded for five randomly selected plants per plot per replicate at physiological maturity stage.

Drought sensitivity (DRS)

Drought sensitivity (DRS) was measured by leaf rolling at early reproductive stage, leaf drying and recovery from drought (DRR) at early reproductive stage, and spikelet fertility score at the physiological maturity stage using a 9-point scale (SES, 2002; Mukamuhirwa et al., 2019; Pandit et al., 2020).

- Leaf rolling was scored as follows: 0 (leaves healthy), 1 (leaves start to fold (shallow)), 3 (leaves folding (deep V-shape)), 5 (leaves fully cupped (U-shape)), 7 (leaves' margins touching (0-shape)), and 9 (leaves tightly rolled (V-shape)).
- Leaf drying was scored as follows: 0 (no symptoms), 1 (slight tip drying), 3 (tip drying extended up to ¼), 5 (¼ to ½ of all leaves dried), 7 (more than 2/3 of all leaves fully dried), and 9 (all plants apparently dead, length in most leaves fully dried).
- The scoring of spikelet fertility was as follows: 1 (more than 80%), 3 (61–80%), 5 (41–60%), 7 (11–40%), and 9 (less than 11%). The percentage of spikelet fertility was recorded on five randomly selected plants per plot per replicate at physiological maturity stage by counting the well-filled and unfilled grains to calculate the percentage of the well-filled grains. Based on the percentage of the well-filled grains, a score on the scale of 1 to 9 was attributed to each genotype per replicate.
- Plants recovery from drought scores were taken after 10 days following watering as 1 (90–100% of plants recovered), 3 (70–89%), 5 (40–69%), 7 (20–39%), and 9 (0–19%).

Grain dimensions

The grain length (mm) and grain width (mm) were recorded on five randomly selected grains per plot per replicate after threshing at 14% or less moisture content.

Chlorophyll content

Chlorophyll content was estimated using the chlorophyll content index (CCI) on three randomly selected plants per plot per replicate using a CCM-200plus chlorophyll content meter (Opti-Sciences, Inc.). The data were collected at the center of the second fully expanded leaves.

GREENHOUSE SCREENING

Physiological and Biochemical characterization of selected rice genotypes in response to drought stress

Plant materials

A subset of 14 genotypes was selected out of 100 based on all the traits for in-depth biochemical investigations. The genotypes consisted of seven putative drought-tolerant genotypes (G6, G62, G73, G100, G99, G65, and G11) and seven putative drought-susceptible genotypes (G5, G63, G2, G22, G53, G36, and G78). These 14 genotypes were selected based on the results obtained from the field drought screening based on relative grain yield and the multi-trait genotype-ideotype distance index (MGIDI). The seven putative drought-tolerant genotypes were among the best performing genotypes and the other seven putative drought-susceptible genotypes were among the worst performing genotypes obtained from the field screening experiment. This experiment was conducted at the Institute of Agronomy and Plant Breeding, Justus-Liebig-University Giessen, Germany.

Drought stress treatment at the vegetative stage

Twelve-day-old seedlings were transplanted into pots filled with ready topsoil for potting on 24/08/2022. The topsoil is prepared following the mixture of 30 liters of organic soil plus 10 liters of ceramic soil plus 160 grams of slow-release fertilizer (15-9-11+2MgO+TE) following (Wu et al., 2021). The moisture content of the soil in the pots was measured every 2 days during the drought stress phases and once a week during the whole experiment using a soil moisture meter (TRIME-PICO TDR, IMKO, Ettlingen, Germany). Thirty-three days after transplanting (vegetative stage before maximum tillering), drought stress was applied on 26 September 2022 by withholding the water until the plants showed clear symptoms of drought stress on 22 October 2022 (27 days after watering was withheld). Non-stressed plants were kept under well-watered conditions at 100% field capacity (FC). The 100% FC of the soil was 57.75 vol/vol, while the wilting point was 10.66 vol/vol. Pots were watered every 3 days with one liter of water per pot throughout the rice growing cycle except the drought stress pots during the vegetative stage stress period. All rice genotypes were grown in the greenhouse with an average temperature between 25 and 30°C with 70% relative humidity and light from 8 a.m. to 4 p.m. A completely randomized block design (RCRD) with three replications was used. Each replication contained both the non-stress and the drought stress trays. Each tray contained four pots of five liters each.

Relative water content and biochemical data collection

Leaf relative water content

The top-most fully expanded leaves were sampled after 27 days of drought stress on each plant per genotype per treatment under both non-stress and drought stress conditions on replicates 1, 2, and 3. The sampling proceeded quickly. The leaf samples consisting of mid-leaf sections of approximately 1×7 cm were cut with scissors from each leaf. Each sample was placed in a pre-weighed airtight 15 ml conic tubes (Falcon®). Tubes were immediately placed on ice. The tubes were weighed to obtain leaf sample weight (W). Following, the sample was immediately hydrated to full turgidity for 4 h under normal room light and temperature. Leaf samples receive water into the tube to a level of 1–2 cm. After hydration, the samples were removed from water, quickly and lightly dried with tissue paper, and immediately weighed to obtain the fully

turgid weight (TW). Samples were then dried at 80°C for 24 h and weighed (after being cooled down in a desiccator) to determine dry weight (DW). All weighing was done to the nearest mg. The leaf relative water content (RWC) was calculated for each genotype following this formula:

$$RWC(\%) = \left[\frac{(W-DW)}{(TW-DW)}\right] \times 100 \tag{1}$$

Determination of malondialdehyde (MDA)

The MDA concentration was estimated in seven out of the 14 genotypes, namely, G100, G99, G2, G11, G22, G53, and G78. These genotypes were selected based on the data obtained from the leaf relative water content. The samples (leaves + shoots) harvested were frozen immediately in liquid nitrogen and stored at -80°C. The MDA concentration was estimated using 0.1% trichloroacetic acid (TCA) and 2-thiobarbituric acid (TBA) following the protocol of Hodges et al. (1999) as modified by Höller et al. (2014). Ultrasonication was run for 5 mins to improve the extraction of MDA from the samples.

Determination of proline concentration

The proline concentration was estimated in the same seven genotypes selected previously. The proline concentration was determined according to Bates et al. (1973) as modified by Frimpong et al. (2021).

STATISTICAL ANALYSIS

Analysis of variance

Analysis of variance (ANOVA) for each trait was done by using the GLM procedure of the Statistical Analysis System (SAS) version 9.4 for Windows. To ensure normal distribution of the traits, before the analysis of variance, all the traits collected under field and greenhouse screening were transformed using z-score standardisation in Microsoft Excel. A trait with a skewness between –2 and +2 and kurtosis between –7 and +7 is normally distributed. Duncan's multiple rank test was used to separate the means among the genotypes screened in the drought stress experiments after the significance of the ANOVA.

• The z-score computation formula is below:

$$z - score = \frac{(X - \mu)}{\sigma} \tag{2}$$

Where X is a single raw data value; μ stands for the mean of the dataset; and σ stands for the standard deviation of the dataset.

• The model for measurements on a plot for alpha-lattice design (Y_{ijk}) is:

$$Y_{ijk} = \mu + R_i + B_{ij} + T_k + e_{ijk} \tag{3}$$

Where μ stands for general mean; R_i stands for fixed effect of replicate i; B_{ij} stands for random effect of block j within replicate i; T_k stands for random effect of treatment k; and e_{ijk} stands for residual effect.

• The model for measurements on a plot for randomized complete-block design (RCBD) (Y_{ij}) is:

$$Y_{ij} = \mu + R_i + T_j + e_{ij} \tag{4}$$

Where μ stands for general mean; R_i stands for fixed effect of replicate i; T_j stands for random effect of treatment k; and e_{ij} stands for residuals effect.

To test the presence of significant variability in the distribution of the traits under the field screening across both non-stress and drought stress conditions, Levene's statistic test of homogeneity of variance was carried first and it turned out that there was not homogeny in variance for all the traits under study. Since there was not homogeny in the variance, the nonparametric Mann-Whitney U test was conducted.

Genetic diversity analysis

• The coefficient of variation (C.V.%) is calculated as follows:

$$\text{Coefficient of variation} = \frac{\text{Standard deviation}}{\text{Mean}} \tag{5}$$

• Heritability in the broad sense (h_{bs}^2) was computed following Allard (1960):

$$h_{bs}^2 = \frac{\sigma_g^2}{\sigma_g^2 + \frac{\sigma_e^2}{nreps}} \tag{6}$$

Where σ_g^2 and σ_e^2 are the genotypic and error variances, respectively, and nreps is the number of replications.

Broad sense heritability (h_{bs}^2) estimates were categorized as low (0–30%), moderate (30–60%), and high (above 60%) according to Johnson et al. (1955).

• The genetic variability parameters such as estimate of the phenotypic (σ_p^2), genotypic (σ_g^2), and environmental (σ_e^2) variances, the genotypic coefficient of variation (GCV), error coefficient of variation (ECV), and expected genetic advance (GA) were calculated following the exact method of Asante et al. (2019) and classified as low (< 10%), moderate (10–20%), and high (> 20%).

Association analysis

Graphics were made using R and Microsoft Excel. Principal component analysis (PCA), and Pearson correlation (SAS software) were done to understand variations and relationships among various traits and treatments. The visualization of the Pearson correlation using graphics was generated using Visplore data analysis tools (Available at: https://visplore.com/, accessed 22 June 2024), while the visualization of the PCA biplot was generated with R software.

• Pearson's correlation coefficient was calculated following this formula:

$$r_{xy} = \frac{n\sum xy - (\sum x)(\sum y)}{\sqrt{\left[n\sum x^2 - (\sum x)^2\right]\left[n\sum y^2 - (\sum y)^2\right]}} \tag{7}$$

Where r_{xy} stands for Pearson's correlation coefficient; x is the variable 1; y is the variable 2; n is the sample size; and Σ represents a summation of all values.

Classification of the genotypes into their tolerance classes

The classification of the genotypes into their tolerance classes was done based on the relative value for each trait per genotype between non-stress and drought stress management following this formula:

$$\begin{aligned} Relative\ trait\ value\ (drought\ risk\ index) = \\ \frac{trait\ value\ under\ drought - stress}{trait\ value\ under\ non - stress} \end{aligned} \tag{8}$$

If the trait relative value is equal to 1, there is no drought stress, and if it is equal to 0, complete crop failure due to drought stress. The relative grain yield was considered first followed by other yield-related and physiological traits. Correlation analysis and PCA were conducted among the different traits using trait relative values to determine the relationship and select priority traits to involve in selecting the drought-tolerant genotypes.

The multi-trait genotype-ideotype distance index (MGIDI) implemented in R, was also used to select the genotypes showing tolerance to drought (Olivoto and Nardino, 2021). To compute the

MGIDI, the trait relative value (drought risk index) was used as input data following the formula:

$$MGIDI_i = \sqrt{\sum_{j=1}^{f} \left(y_{ij} - y_j \right)^2} \qquad (9)$$

Where $MGIDI_i$ is the multi-trait genotype-ideotype distance index for the ith row/genotype/treatment; y_{ij} is the score of the ith row/genotype/treatment in the jth factor $(i = 1, 2, …, g; j = 1, 2, …, f)$, being g and f the number of rows/genotypes/treatments and factors, respectively; and y_j is the jth score of the ideotype. The row/genotype/treatment with the lowest MGIDI is then closer to the ideotype and therefore presents desired values for all the p traits; g, f, and p represent the number of rows/genotypes/treatments, respectively.

Results

FIELD SCREENING

During this study, clear symptoms of drought stress were seen in the field at 18 days after the stress, with an average volumetric soil moisture content less than 5% vol (2.05% vol) at 10 cm of soil depth. The phenotypic performance of some of the genotypes showing drought sensitivity (DRS) like leaf rolling is presented in Fig. 1.

Agronomic and physiological performance of the rice genotypes under both water regimes

Analysis of variance and mean performance

The analysis of variance (ANOVA) revealed the presence of significant differences among the rice genotypes for spikelet fertility percentage, days to flowering, and plant height under non-stress condition (Additional file 1-S1), while under drought stress, significant differences were observed in grain yield per plant, plant height, hundred grain weight, leaf rolling score, and leaf drying score (Additional file 1-S2). To test the presence of significant variability in the distribution of the traits across both non-stress and drought stress conditions, a Mann-Whitney U test across both water regimes was conducted. The Mann-Whitney U test revealed the presence of significant differences in the distribution of all the traits across both non-stress and drought stress conditions except for grain length and grain width indicating a major role that the environment will be playing in the expression of these traits in this germplasm (Table 1). Under drought stress, a wide range of variation among the 100 rice genotypes was noted for all the traits except days to flowering, which had a CV of 7.38%. The largest CV was observed for recovery from drought stress (74.68%), followed by grain yield per plant with a CV value of 71.72% (Additional file 1-S2).

The mean performance under both non-stress and drought stress is presented in Additional file 2. For all the investigated traits in this study, a general reduction was observed among the genotypes under drought stress compared to non-stress. The G6 performed best for the grain yield per plant (15 g) and hundred grain weight (4.02 g) under drought stress, with the best relative grain yield, relative spikelet fertility score, and 12-day delays to reach 50% of all plants flowering on the plots. The G6 was followed by G16, G59, G62, and G79, which recorded a grain yield of 10 g per plant each. The G6 also recorded the highest relative hundred grain weight, highest leaf rolling score with the lowest leaf drying score,
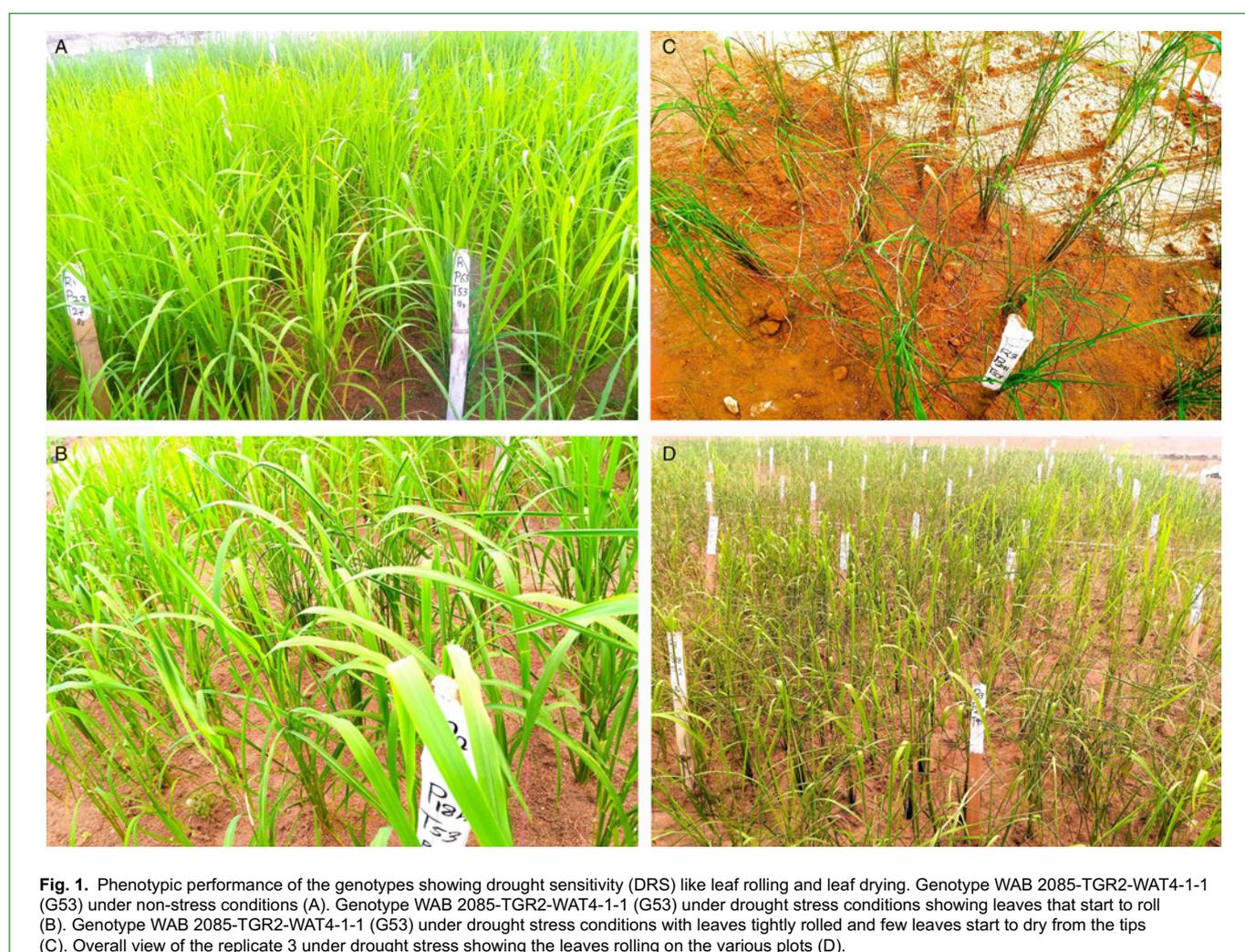
Fig. 1. Phenotypic performance of the genotypes showing drought sensitivity (DRS) like leaf rolling and leaf drying. Genotype WAB 2085-TGR2-WAT4-1-1 (G53) under non-stress conditions (A). Genotype WAB 2085-TGR2-WAT4-1-1 (G53) under drought stress conditions showing leaves that start to roll (B). Genotype WAB 2085-TGR2-WAT4-1-1 (G53) under drought stress conditions with leaves tightly rolled and few leaves start to dry from the tips (C). Overall view of the replicate 3 under drought stress showing the leaves rolling on the various plots (D).

Table 1. Distribution of 11 morpho-physiological traits across non-stress and drought stress conditions among 100 rice genotypes evaluated at Fumesua-Kumasi, Ghana, in 2021–2022.

Null hypothesis	*p-value* (Mann-Whitney U test)	Decision
The distribution of chlorophyll content index is the same across non-stress and drought stress conditions	<0.0001	Reject the null hypothesis: there were significance differences in the distribution of these traits across non-stress and drought stress conditions
The distribution of spikelet fertility score is the same across non-stress and drought stress conditions		
The distribution of grain yield per plant (g) is the same across non-stress and drought stress conditions		
The distribution of days to flowering is the same across non-stress and drought stress conditions		
The distribution of tiller number is the same across non-stress and drought stress conditions		
The distribution of panicle number is the same across non-stress and drought stress conditions		
The distribution of plant height (cm) is the same across non-stress and drought stress conditions		
The distribution of panicle length (cm) is the same across non-stress and drought stress conditions		
The distribution of hundred grains weight (g) is the same across non-stress and drought stress conditions		
The distribution of grain length (mm) is the same across non-stress and drought stress conditions	0.092	Retain the null hypothesis: there were no significance differences in the distribution of these traits across non-stress and drought stress conditions
The distribution of grain width (mm) is the same across non-stress and drought stress conditions	0.115	

and lowest recovery from drought score. The highest relative grain yields (0.88) obtained by G6 implied that the performance under both drought stress (15 g) and non-stress conditions (17.14 g) is similar and not statistically different. The same performance applied to leaf greenness (CCI) under which G6 recorded a high relative CCI (0.99), implying that the performance under both drought stress (14.22 CCI) and non-stress conditions (14.41 CCI) is statistically similar. However, in general, CCI decreased under drought stress compared to non-stress conditions in this study. Genotype G28 recorded the highest plant height under drought stress (105.93 cm), with a relative plant height of 0.89, while G16 recorded the lowest plant height of 56 cm, with a relative value of 0.56, implying that this genotype recorded a reduction of half in height compared to non-stress conditions.

The following genotypes, G6, G10, G34, G7, G93, G3, G50, G23, G94, G88, G37, and G51 recorded hundred grain weights greater than 3.00 g under drought stress with a relative hundred grain weight greater than 1.00 for all the above-cited genotypes (Additional file 2). Relative hundred grain weight ≥1 implied that these genotypes performed better under drought stress than non-stress conditions for this trait. It is also important to note that all these genotypes recorded a leaf rolling score higher than 5.00 except G10, which recorded a leaf rolling score of 1.00. In this study, only three genotypes, namely, G10, G64, and G99, recorded the lowest leaf rolling score and recovery from drought score of 1.00 (the lower the leaf rolling and DRR, the better the performance of the genotypes under drought stress). The genotype G100 recorded a DDR=1 and leaf rolling=5, implying its ability to recover quickly from drought stress. The genotypes G44 and G74 recorded the lowest relative spikelet fertility score of 0.33, while G99, G3, G88, G90, G96, G24, G31, G70, G11, G79, G60, G71, G33, G72, G38, G28, and G6 scored 1.00 as a relative spikelet fertility score. Even though G11 recorded a low relative grain yield value of 0.07, it recorded the best relative spikelet fertility score of 1.00, fewer days of delay to flowering of 3 days, a relatively high relative CCI (0.70) and relative hundred grain weight (0.75) coupled with the lowest leaf drying and recovery from drought score of 1.00. The genotypes G77 and G41 flowered at the same time under both

non-stress and drought stress conditions at 83 and 81 days after sowing, respectively. Delays in 50% flowering dates were observed in almost all the accessions and ranged from 1 to 34 days. The following genotypes, G94, G59, G50, G68, G20, G58, G97, G79, G63, G11, G9, G62, and G57, recorded delays in days to flowering between 1 and 3 days. This made them have similar performance under both water regimes, with probable tolerance to drought stress. In contrast to the other genotypes in the present study, the genotypes G67, G5, G87, G31, and G23 flowered 17, 12, 4, 3, and 1 days, respectively, earlier under drought stress than non-stress conditions.

Overall, the results showed that grain yield and yield-related traits and physiological traits in this study recorded a significant reduction under drought stress manifested through the delay in the growth and development of the plants, especially for the drought-susceptible genotypes.

Genetic variability among the phenological, physiological and yield traits under both drought stress and non-stress conditions

For all the studied traits, the estimates of the environmental (σ_e^2) variance and error coefficient of variation (ECV) were higher than the estimated genotypic (σ_g^2) variance and the genotypic coefficient of variation (GCV) under drought stress compared to non-stress conditions, respectively. The estimates of the broad-sense heritability and Genetic advance as percentage of mean (GA%) were higher under drought stress than non-stress conditions, respectively, for the traits such as chlorophyll content index (CCI), spikelet fertility % (SPF), grain yield per plant (GYP), panicle length (PL), and hundred grain weight (HGW). Compared to the non-stress conditions, under drought stress, grain yield per plant combined high heritability (64.88%) with the highest GA% (118.52), while HGW combined highest heritability (94.69%) with high GA% (74.93). On the other hand, SPF recorded moderate heritability (50.81%) and high GA% (54.99) under drought stress. Leaf rolling score (LRS) and leaf drying score recorded both moderate heritability and high GA% under drought stress (Table 2). These results suggested that traits such as SPF, GYP, HGW, LRS,

Table 2. Estimate of genetic parameters, broad-sense heritability, and genetic advance 14 morpho-physiological traits across non-stress and drought stress conditions among 100 rice genotypes evaluated at Fumesua-Kumasi, Ghana, in 2021–2022.

Trait	Water regime	Mean	σ^2_g	σ^2_e	GCV	ECV	H^2_{bs}	GA	GA(%)
CCI	NS	20.82	8.59	731.33	14.08	129.90	3.40	1.91	9.16
	RS	14.47	2.20	29.62	10.26	37.61	18.26	2.12	14.66
SPF	NS	84.72	12.32	84.14	4.14	10.83	30.53	6.18	7.29
	RS	53.98	205.93	598.13	26.59	45.31	50.81	29.68	54.99
GYP	NS	18.77	15.33	150.32	20.86	65.31	23.43	6.21	33.09
	RS	3.28	3.23	5.24	54.74	69.75	64.88	3.89	118.52
DTF	NS	83.09	11.56	16.28	4.09	4.86	68.05	7.40	8.90
	RS	92.24	5.79	44.47	2.61	7.23	28.09	4.10	4.45
TN	NS	11.11	0.35	10.75	5.30	29.51	8.84	0.61	5.46
	RS	5.23	0.08	2.83	5.56	32.12	8.25	0.29	5.54
PN	NS	10.14	0.36	8.87	5.92	29.37	10.86	0.68	6.70
	RS	4.47	0.00	1.99	0.00	31.59	0.00	0.00	0.00
PH	NS	98.76	128.81	262.35	11.49	16.40	59.56	24.27	24.57
	RS	76.46	29.46	174.89	7.10	17.30	33.57	9.89	12.93
PL	NS	23.91	0.56	36.27	3.12	25.19	4.41	0.55	2.31
	RS	20.11	0.73	27.76	4.24	26.20	7.27	0.80	3.97
GL	NS	8.47	0.04	0.97	2.33	11.63	10.74	0.22	2.62
	RS	8.40	0.04	1.59	2.49	15.03	7.61	0.20	2.39
GW	NS	2.23	0.00	0.15	0.00	17.45	0.00	0.00	0.00
	RS	2.16	0.00	0.23	0.00	22.25	0.00	0.00	0.00
HGW	NS	2.97	0.04	0.26	6.36	17.00	29.56	0.33	11.06
	RS	2.66	0.89	0.15	35.54	14.58	94.69	1.99	74.93
LRS	RS	5.59	0.96	4.05	17.56	35.99	41.66	1.92	34.37
LDS	RS	1.29	0.12	0.75	26.43	66.77	31.97	0.61	47.30
DRR	RS	1.69	0.07	1.60	15.15	74.70	10.99	0.29	17.26

NS: non-stress condition; RS: reproductive stage drought stress; CCI: chlorophyll content index; SPF: spikelet fertility; GYP: grain yield per plant in g; DTF: days to flowering; TN: tiller number per plant; PN: panicle number per plant; PH: plant height in cm; PL: panicle length in cm; GL: grain length in mm; GW: grain width in mm; HGW: hundred grains weight in g; LRS: leaf rolling score; LDS: leaf drying score; DRR: drought recovery; σ^2_g: genotypic variance; σ^2_e: environmental/error variance; GCV: genotypic coefficient of variation; ECV: phenotypic coefficient of variation; h^2_{bs}: broad sense heritability; GA: genetic advance; GA%: genetic advance as percentage of mean.

and LDS will be rewarding when involved in the selection program among this germplasm under drought conditions.

Association analyses among the phenological, physiological and yield traits under both drought stress and non-stress conditions

The Pearson correlation based on the relative trait value or drought risk index (Bouman *et al*., 2001) is presented in Fig. 2A. Based on the drought risk index, the relative grain yield was highly positively correlated with the relative grain length, grain width, and hundred-grain weight but negatively correlated with the relative spikelet fertility score (the lower the score, the higher the spikelet fertility according to rice standard evaluation). The relative spikelet fertility score was negatively correlated with the hundred grain weight. Based on the correlation analysis using the drought risk index, spikelet fertility score, grain length, grain width, and hundred grain weight can be used to predict the grain yield under drought stress. The correlation analysis is confirmed by the principal component analysis (PCA) where the contribution of the traits was highlighted and ranked using a colour gradient with the highest contributing trait represented in red and the lowest contributing traits in blue. The PCA revealed that tiller number, panicle number, grain length, grain width, and hundred grain weight were the most contributing traits in descending order Fig. 2B. These traits were followed by spikelet fertility score and CCI.

Selection of the potential drought-tolerant genotypes based on MGIDI index under field conditions

In the current study, MGIDI index was used to select the tolerant genotypes. Out of the 14 traits inputted in the MGIDI computation model, only ten was used to compute the index. The trait used to compute the MGIDI index are relative spikelet fertility score, relative grain yield, relative plant height, relative grain length, relative leaf rolling, relative leaf drying, relative recovery from drought, relative chlorophyll content index, relative days to flowering, and relative tiller number. The following genotypes were selected in the chronological order: Viwonor short (G60), UPLR-17 (G100), SR35250-2-4-2-3 (G77), APO (G99), SA69-SARI (G79), UPL 32 (G66), Togo Marshall (G6), SR35266-2-12-4-1-SARI (G74), GR18-SARI (G65), ART75-33-1-1-B-B (G44), SR34590-HB3433-6-2-1 (G81), SR35266-2-12-4-1 (G21), SR35311-HB3497-87 (G89), AGRA-CRI-LOL-1-11 (G14), and CRI-Mpuntuo (G10). In this ranking, three genotypes G60, G100, and G77 ranked better than the tolerant-check G99. The full name of the genotypes with their corresponding ID is presented in the Additional file 2. The genotype ranking based on the MGIDI index is presented in Fig. 3. CRI-Enapa (G11) is known to be tolerant to short-term drought and was confirmed during this experiment based on the best relative spikelet fertility score, low delay in days to flowering, and efficient recovery from drought.
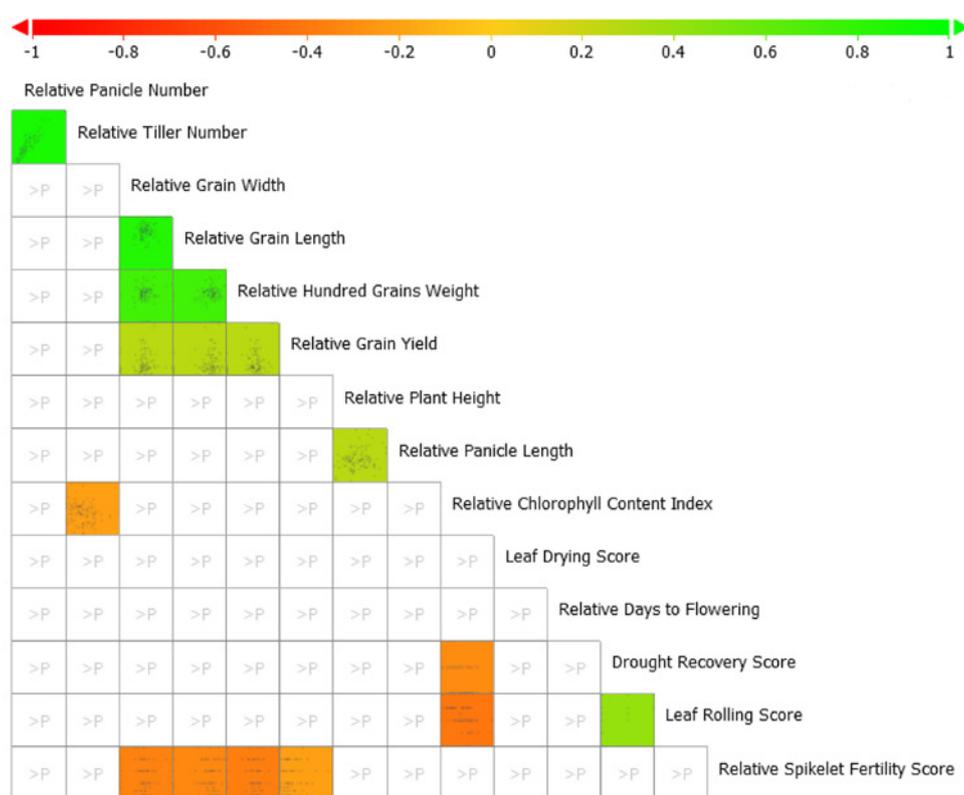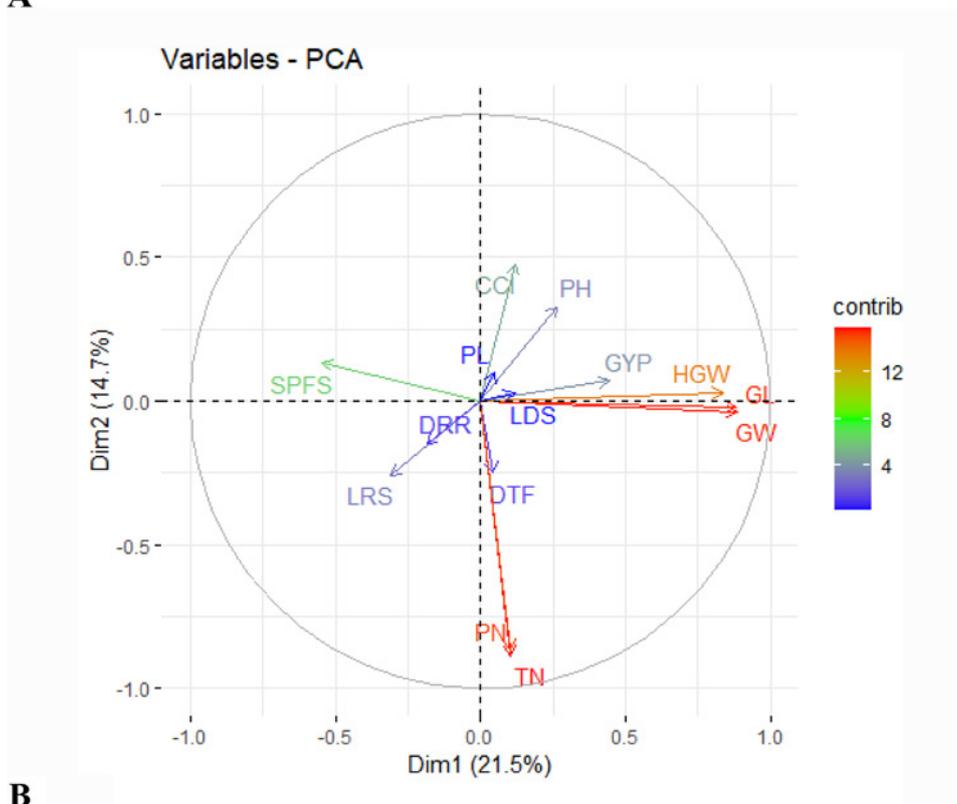
Fig. 2. Estimate of Pearson correlations of 100 rice genotypes evaluated under drought stress using the drought risk index or relative value of each trait at Fumesua-Kumasi, Ghana, in 2021–2022 (A). >P–Nonsignificant correlation. The light green and green colours indicate positive significant correlation. The orange colour indicates negative significant correlation. Principal component analysis (PCA) showing the contribution of each trait in the variation among 100 rice genotypes evaluated under drought stress using the drought risk index or relative value of each trait at Fumesua-Kumasi, Ghana, in 2021–2022 (B). The highest contributing trait is represented in red and the lowest contributing traits in blue. CCI: chlorophyll content index, SPFS: spikelet fertility score, GYP: grain yield per plant in g, DTF: days to flowering, TN: tiller number per plant, PN: panicle number per plant, PH: Plant Height in cm, PL: panicle length in cm, GL: grain length in mm, GW: grain width in mm, HGW: hundred grains weight in g, LRS: leaf rolling score, LDS: leaf drying score, DRR: drought recovery
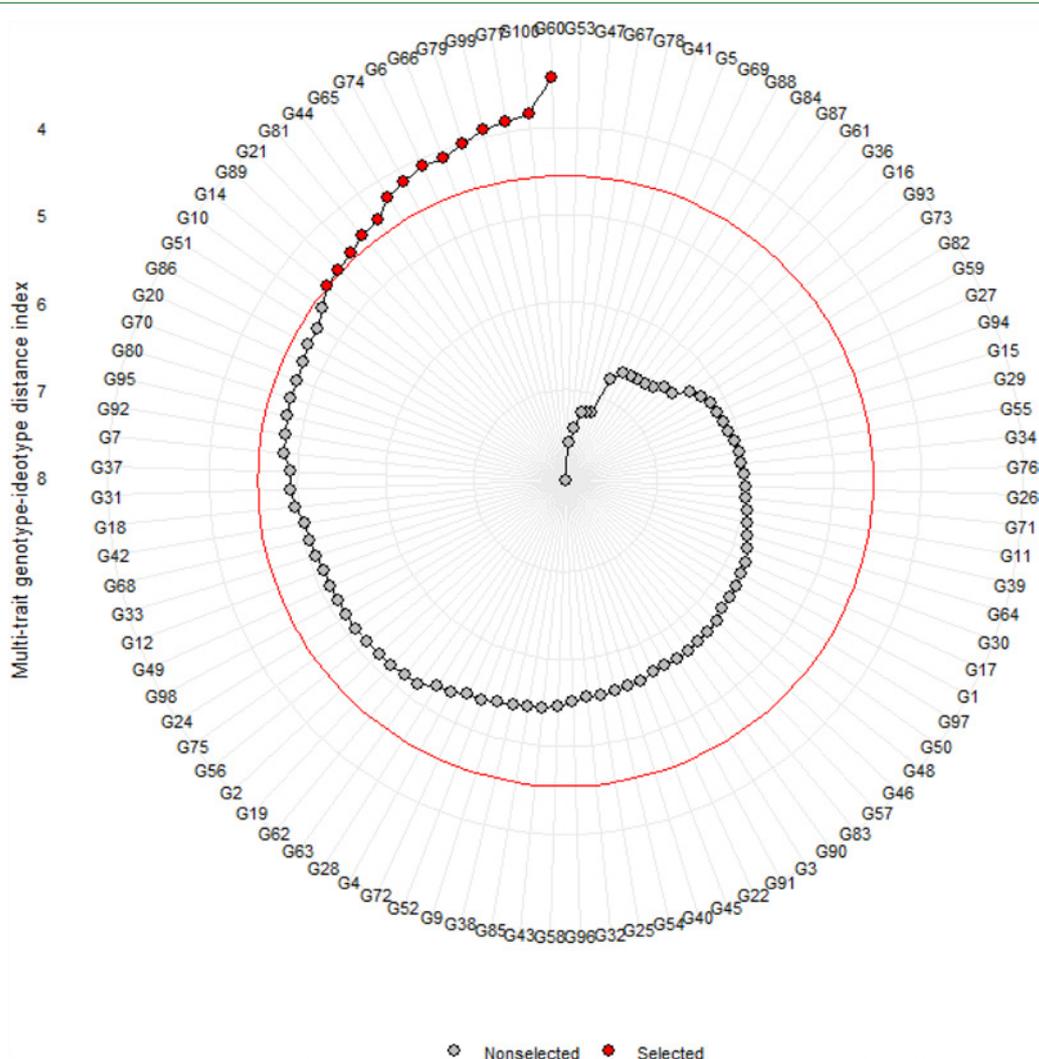
Fig. 3. Ranking of the 100 genotypes in ascending order for the MGIDI index under field conditions. The selected genotypes are shown in red. The circle represents the cut-point according to the selection pressure, and the selection intensity is 15. The full name of the genotypes with their corresponding ID are presented in the Additional file 2. The following genotypes were selected as drought-tolerant genotypes in chronological order of tolerance level: Viwonor short (G60), UPLR-17 (G100), SR35250-2-4-2-3 (G77), APO (G99), SA69-SARI (G79), UPL 32 (G66), Togo Marshall (G6), SR35266-2-12-4-1-SARI (G74), GR18-SARI (G65), ART75-33-1-1-B-B (G44), SR34590-HB3433-6-2-1 (G81), SR35266-2-12-4-1 (G21), SR35311-HB3497-87 (G89), AGRA-CRI-LOL-1-11 (G14), CRI-Mpuntuo (G10), and APO (G99) is the drought-tolerant check used in this study.

GREENHOUSE SCREENING

Changes in soil moisture content and performance of the rice genotypes under vegetative-stage drought stress

Under the vegetative-stage drought stress, there was a progressive decrease in the soil moisture with an average soil water loss of 6.09 vol/vol every 3–4 days. The rice plants started to show severe drought stress symptoms when the soil moisture reached 18.14 vol/vol at the vegetative-stage, after 27 days of water deficit.

At 27 days after drought stress initiation (27D), ANOVA revealed the presence of significant differences among the rice genotypes for MDA concentration and proline content under drought stress (Additional file 1-S3 and 1-S4), while under non-stress condition, no significant differences were observed in MDA, proline content, or RWC (%) (Additional file 1-S5). Under both non-stress and drought stress, MDA and proline content recorded high CVs. It was not the case for RWC (%), which recorded moderately low CV under non-stress condition. High heritability was obtained for MDA (79.60%) and proline content (86.75%), whereas RWC (43.48%) recorded moderate heritability under drought stress, indicating the presence of large variability among the genotypes for these traits (Additional file 1-S3, 1-S4, and 1-S5).

In general, the mean performance of all the genotypes was reduced notably by more than 14% under drought stress compared to the non-stress for RWC (%) for all the genotypes except G11 (1.58%), G99 (8.43%) and G100 (5.43%), which were previously identified as drought-tolerant based on the field screening. The MDA concentration was increased by more than 45% under drought stress compared to the non-stress, for all the genotypes except G11 (25.15%), G99 (39.37%), and G100 (43.65%). The proline content analysis also revealed that the mean performance of G78 (94.69%) and G100 (93.24%), notably increased under drought stress compared with that of G22 (81.73%), which is considered a drought-sensitive variety based on the field screening.

The mean performance with the ranking of the genotypes using the Duncan multiple rank test is presented in Fig. 4 for RWC, Fig. 5 for MDA and Fig. 6 for proline concentration after 27 days of drought stress. In the current study, the genotype G22 recorded the highest MDA (15.08 nmol ml^{-1}g^{-1}), followed by G53 (12.90 nmol ml^{-1}g^{-1}), while G11 recorded the lowest value of 6.56 nmol ml^{-1}g^{-1}. Genotype G78 had the highest proline content (39.87 mg L^{-1}), followed by G53 (21.69 mg L^{-1}), while G11 had the lowest value of 3.16 mg L^{-1}. Genotype G11 recorded the highest RWC (86.55%), followed by G100 (83.78%) and G99 (81.79%), under
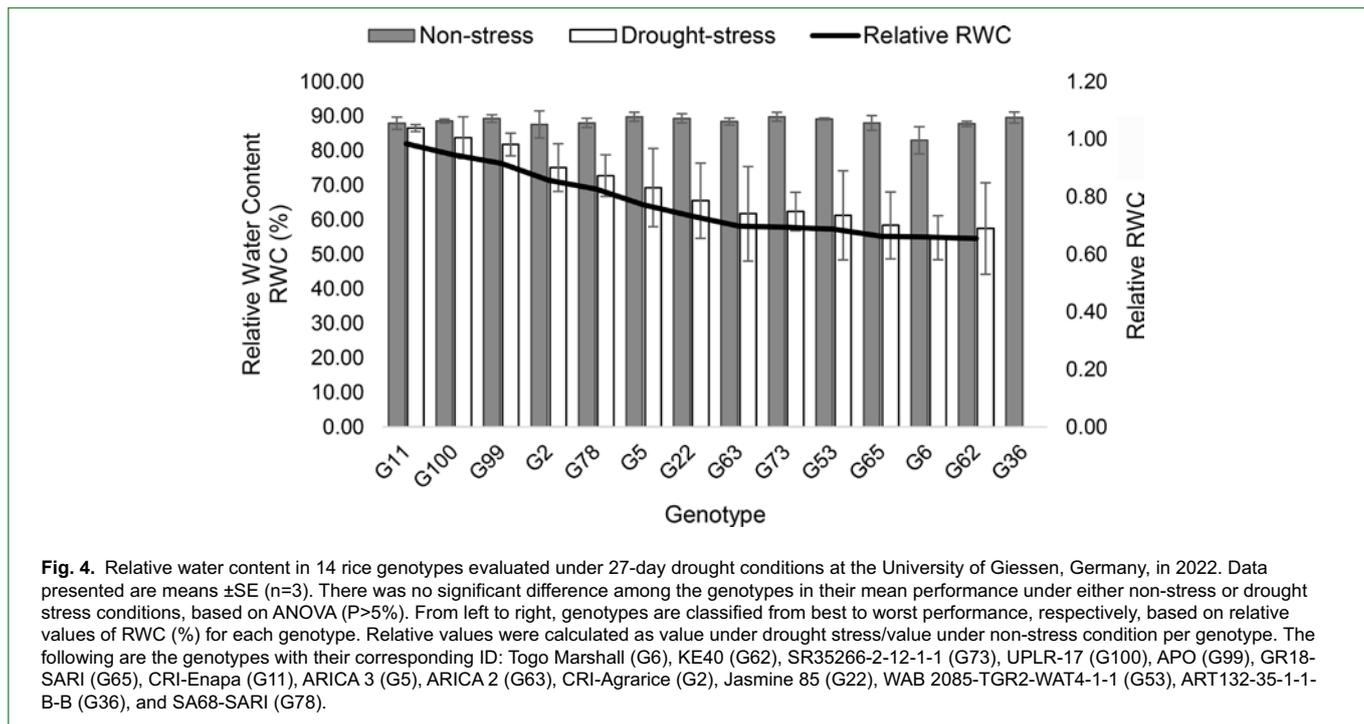
Fig. 4. Relative water content in 14 rice genotypes evaluated under 27-day drought conditions at the University of Giessen, Germany, in 2022. Data presented are means ±SE (n=3). There was no significant difference among the genotypes in their mean performance under either non-stress or drought stress conditions, based on ANOVA (P>5%). From left to right, genotypes are classified from best to worst performance, respectively, based on relative values of RWC (%) for each genotype. Relative values were calculated as value under drought stress/value under non-stress condition per genotype. The following are the genotypes with their corresponding ID: Togo Marshall (G6), KE40 (G62), SR35266-2-12-1-1 (G73), UPLR-17 (G100), APO (G99), GR18-SARI (G65), CRI-Enapa (G11), ARICA 3 (G5), ARICA 2 (G63), CRI-Agrarice (G2), Jasmine 85 (G22), WAB 2085-TGR2-WAT4-1-1 (G53), ART132-35-1-1-B-B (G36), and SA68-SARI (G78).
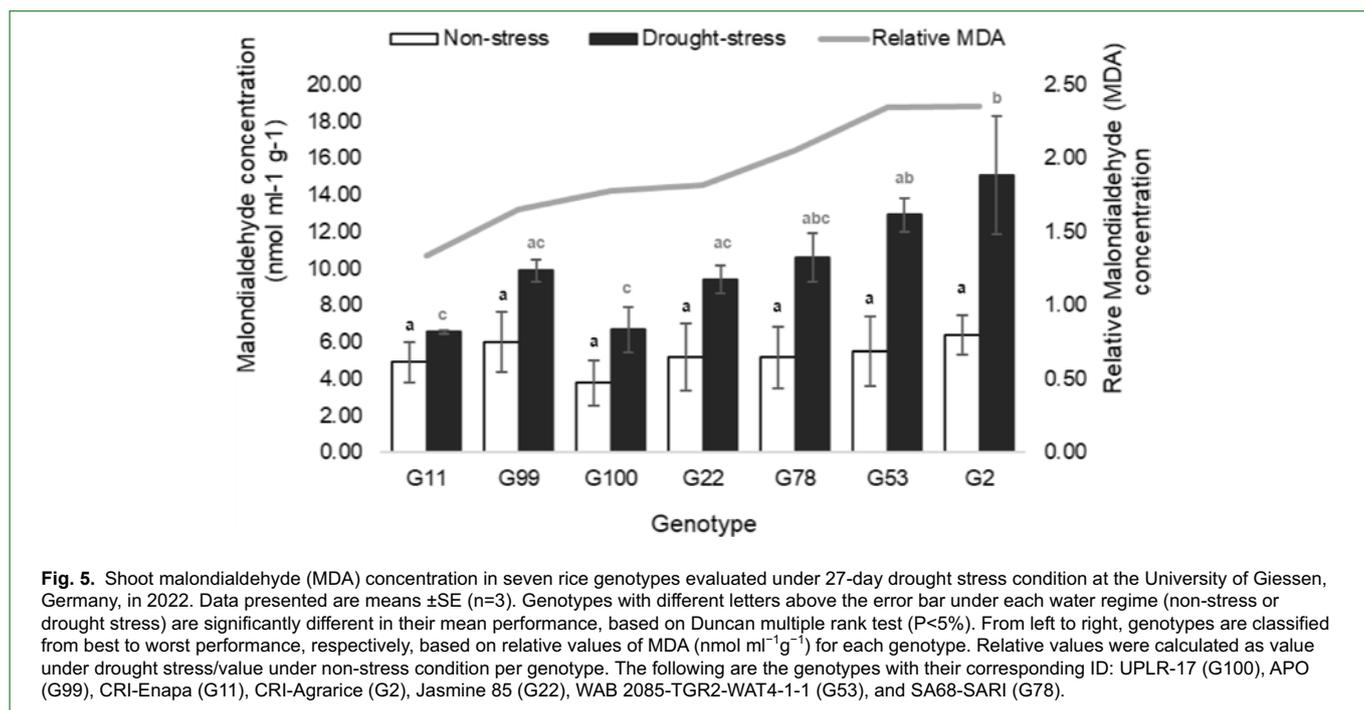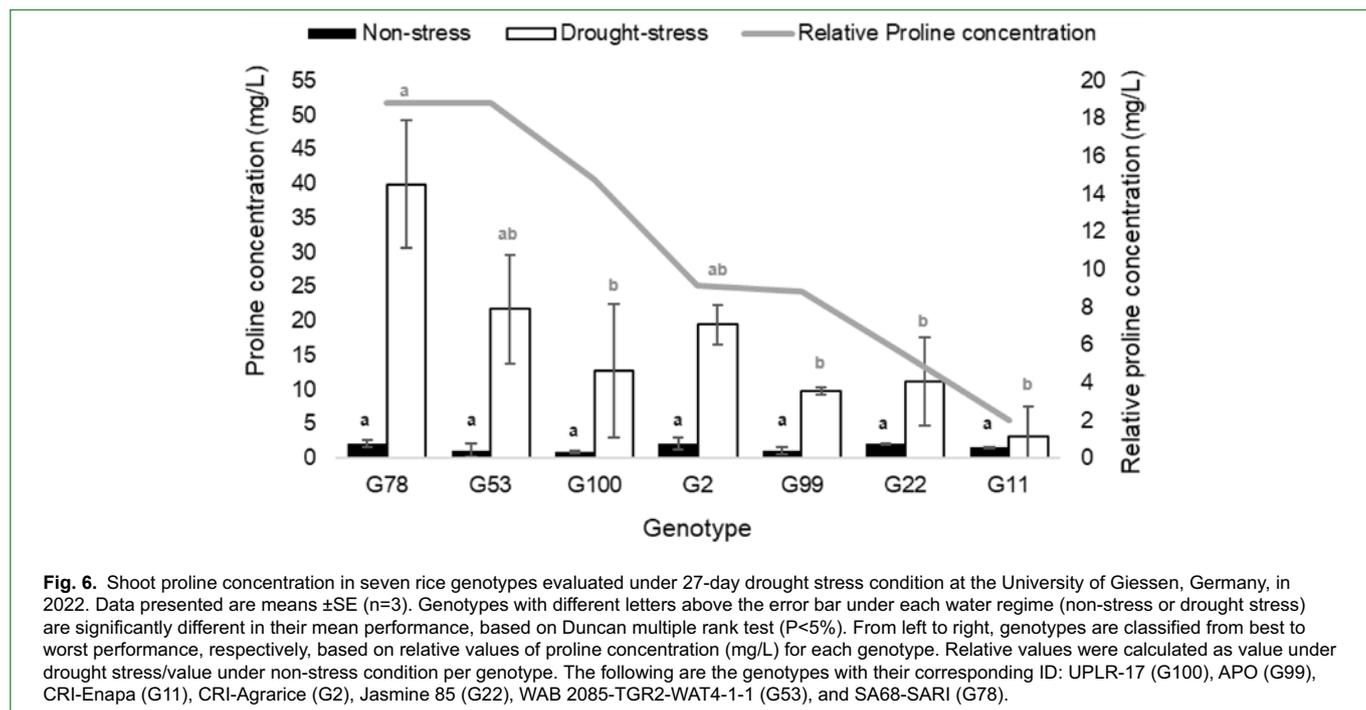

Fig. 5. Shoot malondialdehyde (MDA) concentration in seven rice genotypes evaluated under 27-day drought stress condition at the University of Giessen, Germany, in 2022. Data presented are means ±SE (n=3). Genotypes with different letters above the error bar under each water regime (non-stress or drought stress) are significantly different in their mean performance, based on Duncan multiple rank test (P<5%). From left to right, genotypes are classified from best to worst performance, respectively, based on relative values of MDA (nmol ml^{-1}g^{-1}) for each genotype. Relative values were calculated as value under drought stress/value under non-stress condition per genotype. The following are the genotypes with their corresponding ID: UPLR-17 (G100), APO (G99), CRI-Enapa (G11), CRI-Agrarice (G2), Jasmine 85 (G22), WAB 2085-TGR2-WAT4-1-1 (G53), and SA68-SARI (G78).

drought stress, while G6 recorded the lowest value of 54.76%. Genotypes G11, G100 and G99, topped first for relative RWC of 0.98, 0.95, and 0.92, respectively. Based on the above results from MDA, proline content, and RWC (%) analysis, G11, G100, and G99, were selected as showing tolerance to drought stress, confirming the results from field screening.

Discussion

Under the field screening, the 2.05% volumetric soil moisture content observed in this study at a 10 cm soil depth indicates that the drought stress imposed was extremely severe, consistent with data observed in drought experiments conducted by (Zu *et al.*,

2017; Huang *et al.*, 2019; Ahmad *et al.*, 2020). The significance of the Mann-Whitney U test observed for chlorophyll content index, spikelet fertility score, grain yield per plant (g), days to flowering, tiller number per plant, panicle number per plant, plant height (cm), panicle length (cm), and hundred grains weight (g) in this study indicates that a complete difference in performance under both water regimes and genotype by environment (G×E) effects will play a large role in the expression of these traits across both water regimes. This is also reflected by the huge variation recorded in the broad-sense heritability, σ_e^2 and ECV of these traits under drought stress and non-stress conditions. For all the investigated traits in this study, a general reduction was observed among the genotypes under drought stress compared to the non-stress

Fig. 6. Shoot proline concentration in seven rice genotypes evaluated under 27-day drought stress condition at the University of Giessen, Germany, in 2022. Data presented are means ±SE (n=3). Genotypes with different letters above the error bar under each water regime (non-stress or drought stress) are significantly different in their mean performance, based on Duncan multiple rank test (P<5%). From left to right, genotypes are classified from best to worst performance, respectively, based on relative values of proline concentration (mg/L) for each genotype. Relative values were calculated as value under drought stress/value under non-stress condition per genotype. The following are the genotypes with their corresponding ID: UPLR-17 (G100), APO (G99), CRI-Enapa (G11), CRI-Agrarice (G2), Jasmine 85 (G22), WAB 2085-TGR2-WAT4-1-1 (G53), and SA68-SARI (G78).

conditions. Several studies have recorded grain yield reduction of rice genotypes under drought stress compared to non-stress conditions, where the drought-tolerant checks yielded better results than most of the landraces and the susceptible checks under the reproductive stage drought stress (Pantuwan et al., 2002; Yue et al., 2006; Luo, 2010; Iseki et al., 2014; Anyaoha et al., 2018). In this study under the field conditions, nineteen percent of the genotypes showed a low relative spikelet fertility score of 1.00, implying that 81% of the genotypes used in this study are severely affected by drought stress regarding to spikelet fertility and, by extension, to grain yield, since grain yield and spikelet fertility showed positive correlation. Similar performance for spikelet fertility was reported by Afiukwa et al. (2016), who obtained up to a 40.1% reduction in spikelet fertility in more than 78% of the 30 genotypes under drought stress compared to the non-stress conditions during an 8-day drought treatment. On the other hand, the low relative grain yield recorded by G11 in this study despite its best performance in the secondary traits can be explained by the fact that this genotype is known to be a short-term drought-tolerant genotype. In fact, the yield of this genotype might be harshly affected after 15 days of severe drought stress. Even though delays in 50% flowering date was observed in almost all the genotypes, 5% of the genotypes in the present study flowered 1 to 17 days earlier under drought stress compared to non-stress conditions. Drought escape is the ability of a plant to complete its life cycle before severe drought stress occurs (Yadav and Sharma, 2016). These genotypes that flowered earlier under drought stress might apply this mechanism to escape the upcoming severe drought stress and therefore can be suitable candidate genetic resources to be used in the development of drought-escaping rice genotypes (Ahmad et al., 2020). The effectiveness of a delay in DTF in breeding for drought tolerance is proven only during a short drought stress period (Lamo, 2010; Afiukwa et al., 2016).

Based on the drought risk index, the relative grain yield was correlated with the relative grain length, grain width (Haider et al., 2015), hundred-grain weight, and relative spikelet fertility score (Gaballah et al., 2022) making these traits desirable in prediction and selection of drought-tolerant rice genotypes under drought stress condition. The positive correlation among relative grain yield, hundred-grain weight, and grain length implied that the selection of varieties with long-slender grains (which is preferred by West Africa consumers)

will result into high-yielding varieties. The low spikelet sterility score implied a high spikelet fertility percentage. The negative correlation observed by Gaballah et al. (2022) between spikelet sterility score and grain yield, and leaf rolling confirms our findings that the increase in grain yield is directly attributable to high spikelet fertility with low reduced leaf rolling and high recovery from drought. In this study, three of the genotypes performed better than the drought-tolerant check G99 based on the MGIDI index ranking, agreeing with the results of Kumar et al. (2008), where breeding lines were found to be superior to the tolerant parental by 2–2.5 times.

Effective selection requires the association of secondary traits. A couple of secondary traits have been used in breeding and as selection criteria for the selection of drought-tolerant cultivars, especially traits (SPF, GYP, HGW, LRS, and LDS) which combined high broad-sense heritability with high genetic advance as percent of mean. Some of these traits are leaf rolling and leaf drying at the vegetative growth drought stage (Thanh et al., 2006; Farooq et al., 2010; Umego et al., 2020) and spikelet fertility at the reproductive drought stress stage, which is the most important determinant of yield under drought stress conditions (Lafitte et al., 2003; Yue et al., 2006). Root architecture such as deep rooting with many branches and high root to shoot ration plays an outstanding role in the overall grain yield production. Cultivars with such root system yielded better under drought conditions (Kim et al., 2020). The DRO1 gene has been reported to induce deep rooting through root elongation (Uga et al., 2013), EcNAC67 induces higher root and shoot biomass (Rahman et al., 2016), OsDREB2B induces root elongation and increment in root number (Matsukura et al., 2010), and OsNAC5 enhances diameter of the root (Jeong et al., 2013). A couple of breeding efforts for drought tolerance has been reported by monitoring chlorophyll content (Monteoliva et al., 2021). On the other hand, grain yield has been used as the main criteria for the successful development of high-yielding drought-tolerant rice varieties in Asia and Africa (Dixit et al., 2014; Kumar et al., 2014). It was derived from this current study that relative spikelet fertility score, relative grain yield, relative plant height, relative grain length, relative leaf rolling, relative leaf drying, relative recovery from drought, relative chlorophyll content index, relative days to flowering, and relative tiller number were used to compute the MGIDI index, which was used to select 15 potential genotypes with tolerance to drought.

Under the greenhouse screening, at 27 days after drought stress initiation, the results revealed significant variability among the rice genotypes for MDA and proline content under drought stress condition, confirming the existence of large segregation among the genotypes for these traits (Asante *et al.*, 2019), which can be rewarding in selection. The MDA is used as a drought indicator to evaluate the degree of lipid peroxide in plasma membrane (Zhang *et al.*, 2021), implying that higher MDA concentration under drought stress more sensitive the genotype is. On the other hand, the accumulation of proline in plants is considered in several species as a physiological response to withstand drought stress (Mafakheri *et al.*, 2010). In this study, proline content of all the seven genotypes notably increased by more than 50% under drought stress compared to non-stress conditions. A non-increase in MDA concentration and an increase in proline content is associated with a protective role in tolerant rice cultivars against drought stress (Nahar *et al.*, 2018), implying that the genotypes G99, G100, G11, and G78 exhibited good tolerance to drought stress. Other studies reported that drought stress induced an increase in proline accumulation in leaf samples by 1.3–10.2 times from 9 to 18 days after drought stress initiation in the rice variety BRRI Dhan-24 (Nasrin *et al.*, 2020). The mean performance of all the genotypes was reduced notably under drought stress compared to the non-stress conditions for RWC (%), except G11, G99, and G100. Similar variations in RWC (%) were reported by several studies, confirming the results of this study (Bunnag *et al.*, 2013; Afiukwa *et al.*, 2016; Sahoo *et al.*, 2020; Umego *et al.*, 2020; Gaballah *et al.*, 2022).

CONCLUSION

Climate change, which brings about unprecedented seasonal droughts, is greatly affecting rice production in Africa thereby threatening food security on the continent. The effect of climate change on rice production can be mitigated through the development of drought-tolerant cultivars. In this study, in addition to enough genetic variability observed among the genotypes for grain yield and yield-related traits under drought stress, these traits allowed us to select genotypes with enhanced tolerance to drought stress at early reproductive stage under upland field conditions. Moreover, relative water content and MDA allowed us to confirm the tolerance level of these genotypes to drought stress under greenhouse conditions. These traits can, therefore, be used for rapid assessment of drought tolerance in rice breeding programs under tropical conditions. Genotypes Viwonor short (G60), UPLR-17 (G100), APO (G99), and CRI-Enapa (G11) were selected as genotypes with enhanced tolerance to drought and can be released for commercial purposes after multilocational trials, while G99 and G100 which are indica ecotypes and G60 which is glaberrima ecotype can be used as drought-tolerant donors in further drought tolerance rice breeding programs.

ABBREVIATIONS

RWC   relative water content

MDA   malondialdehyde

NS   Non-stress condition

RS   reproductive stage drought stress

CCI   chlorophyll content index

SPFS   spikelet fertility score

GYP   grain yield per plant in g

DTF   days to flowering

TN   tiller number per plant

PN   panicle number per plant

PH   plant height in cm

PL   panicle length in cm

GL   grain length in mm

GW   grain width in mm

HGW   hundred grains weight in g

LRS   leaf rolling score

LDS   leaf drying score

DRR   drought recovery

σ_g^2 genotypic variance

σ_e^2 environmental/error variance

GCV   genotypic coefficient of variation

ECV   phenotypic coefficient of variation

h_{bs}^2 broad-sense heritability

GA   genetic advance

GA%   genetic advance as percentage of mean

MGIDI   multi-trait genotype-ideotype distance index

G1 to G100   genotype 1 to 100

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

SUPPLEMENTARY MATERIAL

See online version of this article for supplementary material. Please note: the publisher is not responsible for the content or functionality of supplementary files provided by the authors and any queries should be directed to the corresponding author.

DATA AVAILABILITY

All data generated or analysed during this study are included in this published article and its Supplementary Materials.

ETHICS STATEMENT

The authors confirm that the research meets any required ethical guidelines, including adherence to the legal requirements of the study country.

AUTHOR CONTRIBUTIONS

Kossi Lorimpo Adjah: Writing – original draft, Writing – review and editing, Formal analysis, Data curation, Visualization, Methodology, Conceptualization, Project administration. Maxwell Darko Asante, Aboubacar Toure, Mawuli Aziadekey and Michael Frei: Writing – review and editing, Validation, Supervision, Methodology, Conceptualization. Linbo Wu, Andriele Wairich, Shailesh Yadav and Yavar Vaziritabar: Writing – review and editing, Validation. Daniel Dzorkpe Gamenyah and Yavar Vaziritabar: Data curation.

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