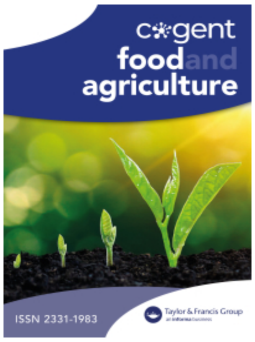

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Leaf reflectance and physiological attributes monitoring differentiate rice cultivars under drought-stress and non-stress conditions

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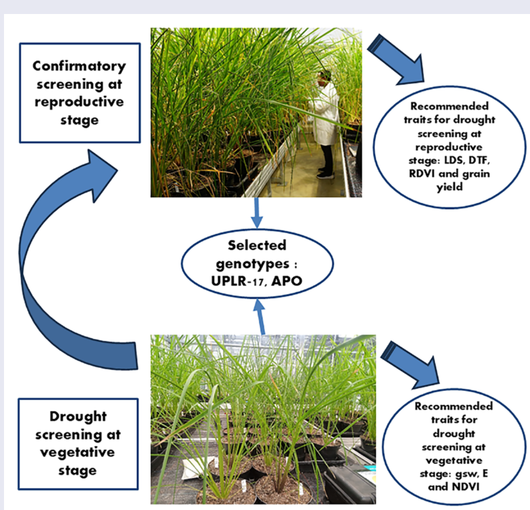
ABSTRACT

Rice production in Africa is unambiguously hampered by drought. This study aimed to monitor the efficiency of physiological traits (stomatal conductance (gsw), transpiration rate (E)), and leaf-reflectance (NDVI and RDVI) at vegetative (VS) and reproductive (RS) stages for selection of drought-tolerant genotypes. To achieve these objectives, we screened 14 rice genotypes under drought-stress and non-stress conditions in the greenhouse. At VS-drought-stress, the relative-gsw and relative-E consistently showed efficiency in differentiating drought-tolerant genotypes APO and UPLR-17 from the drought-sensitive ones at 11-, 18- and 27-days during VS-drought-stress, while NDVI, CRI1 and CRI2 at 18- and 27-days. At RS-drought-stress, genotypes APO and UPLR-17 were selected as drought-tolerant genotypes based on the multi-trait-genotype-ideotype-distance-index (MGIDI) confirming the selection at 11-, 18- and 27-days during VS-drought-stress. This consistency in selecting APO and UPLR-17 as drought-tolerant genotypes at both VS and RS proved the efficiency of gsw, E, NDVI, RDVI, CRI1 and CRI2 in selecting for drought-tolerant varieties at VS. Genotypes UPLR-17 and APO consistently showed homozygosity status for the favorable alleles *G, A, G* and *C* for drought-tolerant QTLs *DTY1.1 (snpOS00400), DTY1.1 (snpOS00402), DTY1.1 (snpOS00408)* and *DTY12.1 (snpOS00483)*, respectively, confirming their drought tolerance status. At RS, with GYP recorded positive and significant correlation with RDVI, while regression analysis revealed that 34% of the variability in GYP is explained by RDVI. The regression analysis coupled with correlation analysis between LDS, DTF, RDVI and GYP implied that these traits can be used as predictors of GYP at RS-drought-stress. While gsw, E and NDVI are recommended for monitoring during VS-drought-stress screening.

GRAPHICAL ABSTRACT



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1. Introduction

Rice is unambiguously one of the most important stable food crops in the world, but its production is hampered by drought (Reynolds et al., 2015), which is the most important abiotic stress in Africa affecting 33% of production area (van Oort, 2018). The increasing water deficits associated with the current climate change scenarios have led rice breeding programs to invest considerable efforts in the production of climate-smart rice varieties suited to grow under water deficit environments (Sandhu et al., 2013; Sellamuthu et al., 2015).

Targeted secondary traits have been used for effective breeding for drought tolerance in rice amongst which stomatal conductance (Price et al., 2002; Tiwari et al., 2021), and delayed flowering (Afiukwa et al., 2016; Lafitte et al., 2003; Pantuwan et al., 2002). Even though breeding for stomatal conductance is important because of its association with yield reduction, however, this trait is reported to be effective during the early drought-stress stage (Price et al., 2002).

Plant responses to droughts are complex processes involving several changes at the physiological, biochemical and molecular levels (Adnane et al., 2015; Atkinson & Urwin, 2012). Grain yield reduction is a result of leaf gas exchange parameters disruption (Farooq et al., 2009) by limiting the photosynthetic rate which leads to the carbon flux reduction in the reproductive organs, resulting in yield reduction (Centritto et al., 2009). The impairing effect of drought-stress on the leaf gas exchange attributes are reported by some previous works. Some workers reported that four-week drought-stress induced a significant reduction in transpiration rate, photosynthetic rate and stomatal conductance amongst 11 genotypes evaluated under field conditions. Genotype KS-282 displayed a higher transpiration rate, photosynthetic rate and stomatal conductance under drought-stress than the other genotypes (Mumtaz et al., 2020). Other authors reported that the physiological performance conducted on two contrasting rice varieties Heena (drought-tolerant) and Kiran (drought-sensitive) showed that the photosynthesis rate decreased by 70% in Kiran and 50% in Heena after seven days of drought-stress with a net prominent decrease in Kiran than Heena. While comparing these two varieties to their corresponding non-droughted plants, both exhibited decreased transpiration rate and stomatal conductance under the stressed and non-stressed conditions. Moreover, a significant decrease in Water use efficiency (WUE) was noticed under drought condition in both varieties with a 41% higher reduction in Kiran than Heena (Tiwari et al., 2021).

Leaf reflectance is generally estimated through several vegetative indices. Amongst these vegetative indices, Normalized Difference Vegetation Index known as NDVI (Rouse et al., 1974), Renormalized Difference Vegetation Index known as RDVI (Roujean & Breon, 1995) and Carotenoid Reflectance Indices known as CRI1 and CRI2 (Gitelson et al., 2002) are often used to assess the leaf reflectance. The NDVI measures the greenness and health of a vegetation. It is the most used index in stress studies. It is a robust index over a large range of conditions because it combines normalized difference formulation, the use of the highest absorption and reflectance regions of chlorophyll. Under moderate drought-stress a significant positive correlation was observed between NDVI and grain yield, while a relatively weak correlation with grain yield was noted under severe drought-stress. Under severe drought-stress, genotypes with high NDVI at early growth stages finished up with lower yield at the later growth stages of the plant. This study concluded that NDVI cannot be suggested in screening genotypes for yield under severe drought-stress (Thapa et al., 2019). A study conducted on rice under normal growing conditions has revealed that NDVI has a significant correlation with the yield at the flowering and grain-filling stages during the wet season, whereas during the dry season it was between panicle initiation and the booting stage. It was shown that NDVI can be used in selecting high-yielding cultivars in rice breeding programs under the tropic. By using portable equipment such as PolyPen RP 410 Photon Systems Instruments, NDVI could be adopted in breeding programs (Phyu et al., 2020). Unlike the NDVI, relatively few studies have been reported on RDVI in assessment of rice under drought-stress. The RDVI is a new vegetation index to measure the plant health, which is the index of interest in this study.

Most traits used in selecting for drought-tolerant genotypes are spikelet fertility score, biomass, grain yield, plant height, grain length, leaf rolling score, leaf drying score, recovery from drought, chlorophyll content index, days to flowering, tiller number, antioxidant and phytohormonal activities etc (Melandri et al., 2021) and oftentimes the combination of these traits. Even though these traits were efficient in selecting the drought-tolerant genotypes, majority of them were collected at full physiological maturity or post-harvest phase of the rice cycle. This could be a disadvantage in a sense that the breeding cycle became lengthy. The average breeding cycle in sub-Saharan African countries is 8 years, this can be halved when using omics tools such as genomic selection, but most breeding programs in Africa, particularly national agricultural research and evaluation system (NARES) are yet to have easy and full access to the omics technologies. In addition, breeding rice varieties for tolerance to water deficit is a tedious task since this trait is a complex trait governed by plethora of genes. Moreover, breeding for drought tolerance requires screening of large germplasm making the exercise more troublesome. This requires the need to establish clear and easy to measure traits for rapid assessment for drought tolerance especially at vegetative stage of the rice growing cycle. Having such traits can help save time in selecting potential tolerant genotypes at early stage of the rice growing cycle. Thus, the measurement of stomatal conductance, transpiration, NDVI and RDVI using specialized devices (e.g. LI-COR devices) can be one of the ideal solutions for rapid assessment of drought tolerance in rice.

A couple of studies used stomatal conductance and transpiration rate in screening for rice under drought-stress, but only few works have been reported on monitoring the variation of stomatal conductance, transpiration rate, NDVI and RDVI at vegetative stage drought-stress. The knowledge of this data will be helpful in targeting the growth stage of the rice plant for effective selection of the drought-tolerant genotypes. This study aimed to monitor the efficiency of stomatal conductance, transpiration rate, NDVI and RDVI at vegetative stage for potential selection of drought-tolerant genotypes and recommend the efficient traits to be used for rapid assessment of rice genotypes for their tolerance to drought-stress. We also assessed the efficiency of stomatal conductance, transpiration rate, NDVI and RDVI in differentiating the drought-tolerant from the drought-sensitive genotypes at the reproductive stage drought-stress.

2. Materials and methods

2.1. Plant materials

Fourteen diverse West Africa rice genotypes sourced from Crops Research Institute (CSIR-CRI), Kumasi, Ghana and AfricaRice rice genebank was used for this experiment. The genotypes consisted of Togo Marshall (G6), KE40 (62), SR35266-2–12-1-1 (G73), UPLR-17 (G100), APO (G99), GR18-SARI (G65) and 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). In this study, APO, a well-known drought-tolerant check is used as a tolerant check. 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). This experiment was conducted at the Institute for Agronomy and Plant Breeding of Justus-Liebig-Universität, Giessen, Germany.

2.2. Drought-stress treatment at vegetative stage and reproductive phases

Twelve-day-old seedlings were transplanted into pots filled with ready topsoil for potting on 24/Aug/2022. The topsoil is prepared following the mixture of 30 litters of organic soil plus 10 litters of ceramic soil plus 160 grams of slow-release fertilizer (15-9-11 + 2MgO + TE) following Wu et al. (2021). The humidity of the soil in the pots was measured every two days during the drought-stress phases and once a week during the whole experiment using the soil moisture meter (TRIME-PICO TDR, IMKO, Ettlingen, Germany). Thirty-three days after transplanting (vegetative stage), drought-stress was applied on 26/Sept/2022 by withholding the water until the plants showed clear symptoms of drought-stress on 22/Oct/2022 (27-days since watering was withheld). At the reproductive stage (when the plants started flowering), drought-stress was applied on 01/Dec/2022 by withholding the water until the plants showed clear symptoms of drought-stress on 16/Dec/2022 (16-days since watering was withheld). Non-stressed plants were kept under well-watered condition at 100% field capacity (FC). Throughout the experiment the soil moisture content was kept equal or above the field capacity (FC) of the soil (57.75 vol/vol) except during the drought-stress phases in the stressed pots. In all the stressed pots, it was ensured that the soil moisture content is not below the wilting point (10.66 vol/vol) before re-watering. All the rice genotypes were grown in the greenhouse with an average temperature between 25 to 30 °C with 70% relative humidity with the light on from 8 a.m. to 4 p.m. A completely randomized block design (RCRD) with 6 replications is used. Each replication contained both the non-stress and the drought-stress plates. Each plate contained four of five litre pots with one plant per genotype per pot. Pots were watered every three days with approximately one litre of water per pot at vegetative and reproductive stages except the drought-stress pots during the stress periods.

2.3. Data collection

- **Number of tillers** was counted on all the 14 genotypes at the end of the vegetative stressed phase on each plant per treatment (non-stress and drought-stress) per genotype and per replicate.
- **Days to flowering (DTF)** was recorded when the first panicle on each plant per pot has shown flowering.
- **Leaf drying score (LDS)** was recorded on all 14 genotypes at the end of the reproductive stressed phase on each plant per treatment (non-stress and drought-stress) per genotype and per replicate. Leaf drying is scored as follows: 0 (no symptoms), 1 (slight tip drying), 3 (tip drying extended up to ¼), 5 (one-fourth 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) according to SES (2002).
- **Aboveground biomass yield (Bio):** The aboveground biomass (grains + stover) was harvested and weighed to record the biomass weight on all the 14 genotypes at the maturity stage on each plant per treatment (non-stress and drought-stress) per genotype per replicate. The average of the six replications was recorded as aboveground biomass yield.
- **Grain yield per plant (GYP):** The panicle was harvested at full maturity stage, manually threshed and weighed to record the grain weight on all the 14 genotypes at the maturity stage on each plant per treatment (non-stress and drought-stress) per genotype per replicate. The average of the six replications was recorded as grain yield per plant.
- **Leaf gas exchange parameters:** Throughout the drought-stress period, leaf gas exchange attributes were measured using the LI-600 portable photosynthesis meter (LI-COR Inc., Lincoln, NE, USA). The medium portion of the 2nd fully expanded leaf of each genotype per treatment (non-stress and drought-stress) per replicate was used to measure the following physiological parameters between 9 AM and 1 PM: stomatal conductance (gsw) in mol m^{-2} s^{-1}, transpiration rate (E) in mol m^{-2} s^{-1}, electron transport rate (ETR) in μmol m^{-2} s^{-1} and the quantum yield of fluorescence (PhiPS II). The leaf gas exchange parameters were measured 4 times respectively on 5-, 11-, 18- and 27-days on the same rice plant per genotype during the vegetative drought-stress period noted as 5D, 11D, 18D and 27D, respectively. While, during the reproductive drought-stress period, the leaf gas exchange parameters were measured on 14th day after the stress initiation on the same plant used at vegetative stages measurements.
- **Leaf reflectance parameters:** Throughout the drought-stress period, leaf reflectance parameters were measured using the PolyPen RP 410 Photon Systems Instruments (Czech Republic). The medium portion of the 2nd fully expanded leaf of each genotype per treatment per replicate under both non-stress and drought-stress conditions were used to measure the following parameters between 1 PM and 4 PM: normalized difference vegetation index (NDVI), (Rouse et al., 1974), renormalized difference vegetation index (RDVI), (Roujean & Breon, 1995) and carotenoid reflectance indices (CRI1 and CRI2), (Gitelson et al., 2002). The CRI 1 and 2 were calculated to estimate carotenoid concentration in relation to chlorophyll concentration. The leaf reflectance parameters were measured 4 times respectively on 5-, 11-, 18- and 27-days on the same rice plant for each genotype during the vegetative drought-stress period noted as 5D, 11D, 18D and 27D, respectively. While, during reproductive drought-stress period, the leaf reflectance parameters were measured on 14th days after the stress initiation on the same plant used for measurements at vegetative stage.

The leaf reflectance parameters were estimated using the following parameters:

$$NDVI = \frac{RNIR - RRED}{RNIR + RRED}$$

$$RDVI = \frac{R780 - R670}{\sqrt{R780 + R670}}$$

$$CRI1 = \frac{1}{R510} - \frac{1}{R550}$$

$$CRI2 = \frac{1}{R510} - \frac{1}{R700}$$

Where, R stands for a given wavelength reflectance; NDVI stands for normalized difference vegetation index; RDVI stands for renormalized difference vegetation index; CRI1 and CRI2 stand for carotenoid reflectance indices 1 and 2; RNIR stands for a wavelength reflectance in near infrared; RRED stands for wavelength reflectance in red.

2.4. Effectiveness of KASP-SNP markers in selecting for drought-tolerant genotypes

To confirm the tolerance of the selected drought-tolerant genotypes, QTL profile using known drought tolerance yield QTL (*qDTY1.1, qDTY12.1*) was used. The QTL profile of each genotype was retrieved from the QTL profiling data obtained from the KASP genotyping of 300 genotypes of the core breeding germplasm of CSIR-CRI, Ghana using KASP-SNP markers according to Asante et al. (2024). The QTL profiling was done using the service of INTERTEK (ScanBi Diagnostics AB, Alnarp-Sweden), Sweden. The samples collection and preparation were done in the Lab at CSIR-CRI, Ghana. Leaves that were not too young or too old were collected on three biological replicates at the reproductive stage. Three leaf disks (6 mm in diameter) of samples were collected in 96-well plates and oven-dried for 24 hours at 50 °C. The samples were then packaged and shipped to INTERTEK, Sweden, for KASP genotyping and analysis. The QTL profile data were then sent back to the Lab at CSIR-CRI, Ghana, where this information was used to assist in the selection of the genotypes with tolerance to drought. The information on QTLs (*qDTY1, qDTY12.1*) and SNPs used are summarized in Table 1. To confirm the effectiveness of KASP-SNP markers in selecting for drought-tolerant genotypes, we conducted a hierarchical cluster from KASP genotyping by using Euclidean distance with average UPGMA method, with the aim to classify the genotypes into their tolerance clusters.

2.5. Statistical analysis

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 were transformed using z-score standardization in Microsoft Excel. Duncan's multiple rank test was used to separate the means among the genotypes screened in the drought-stress and non-stress experiments after the significance of the ANOVA. Graphics were made using R and Microsoft Excel. Pearson correlation and multiple linear regression analysis (R software) were done to understand variations and relationships among various traits and treatments.

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. The relative trait value is calculated as trait value under drought-stress over trait value under non-stress conditions.

If the relative trait value is equal to 1, that trait is not affected by drought-stress, and if it is equal to 0, complete failure of that trait due to drought-stress. The multi-trait genotype-ideotype distance index (MGIDI) implemented in R, was also used to select the genotypes showing tolerance to drought (Olivoto & Nardino, 2021). To compute the MGIDI index, the trait relative value (drought risk index) was used as input data. The following parameters LDS, DTF, gsw, Et, PhiPS2, ETR, NDVI, CRI1, CRI2, RDVI, TN, Biomass and GYP were inputted in the computation of the MGIDI index. The selection intensity of 15 and REML/BLUP method were used. Genotypes were considered as random effects and repetitions as fixed effects. Genotypes and repetitions were used as factors.

Heritability in the broad-sense (h^2_{bs}) was computed following Allard (1960):

$$h^2_{bs} = \frac{\sigma^2_g}{\sigma^2_g + \frac{\sigma^2_e}{nreps}}$$

Where, σ^2_g and σ^2_g are the genotypic and error variances respectively; and nreps is the number of replications. Broad-sense Heritability (σ^2_g) estimates were categorized as: Low (0–30%), moderate (30–60%) and High (above 60%) according to Johnson (1955).

3. Results

3.1. Changes in moisture content of the soil under drought-stress at vegetative and reproductive stages

Under vegetative stage drought, there was a progressive decrease in the soil moisture with an

Table 1. List of rice KASP-SNP markers used for genotyping known drought-tolerant QTLs for grain yield.

Target QTL	Intertek SNP ID	Chromosome number	Position (bp)	Favorable allele	Unfavorable allele
DTY1.1	snpOS00400	1	38081544	G	C
DTY1.1	snpOS00402	1	39014751	A	G
DTY1.1	snpOS00408	1	39610271	G	T
DTY12.1	snpOS00483	12	17393569	G	C
DTY12.1	snpOS00484	12	17396363	A	G

average soil water loss of 6.09 vol/vol, while under reproductive stage drought, a rapid soil moisture loss is observed with an average soil water loss of 9.99 vol/vol every three to four days. The rice plants started to show severe drought-stress symptoms when the soil moisture reached 18.14 vol/vol at the vegetative stage and 18.33 vol/vol at the reproductive stage on average.

3.2. Weekly changes in physiological and leaf reflectance parameters of the genotypes during the vegetative drought stage

The data were collected on the different genotypes at a time interval of 5-, 11-, 18- and 27-days after the stress initiation (5D, 11D, 18D and 27D, respectively) on drought-stressed rice plants to determine the changes in the physiological and leaf reflectance parameters. For the physiological parameters, stomatal conductance (gsw) in mol m^{-2} s^{-1} recorded a progressive decrease from 5D to 27D on all the genotypes except G100 and G99. Genotype (G100) recorded an increase of 29.28% from the 18D (0.158 mol m^{-2} s^{-1}) to 27D (0.205 mol m^{-2} s^{-1}) while G99 recorded the lowest reduction of 17.89% from the 18D (0.251 mol m^{-2} s^{-1}) to 27D (0.206 mol m^{-2} s^{-1}), (Additional file 1_S1). For the quantum yield of fluorescence (PhiPS II), a progressive reduction was equally observed from 5D to 27D on all the genotypes except G100 and G99, and G63 which recorded a slight increase over the time interval of 5D to 27D (Additional file 1_S1). Transpiration rate (E) in mol m^{-2} s^{-1} recorded the similar pattern as stomatal conductance. A progressive and rapid decrease was recorded from 5D to 27D on all the genotypes except G100 and G99. Genotype G100 recorded an increase of 89.97% of transpiration rate from 18D (1.726 mol m^{-2} s^{-1}) to 27D (3.279 mol m^{-2} s^{-1}) while G99 recorded an increase of 20.33% from 18D (2.447 mol m^{-2} s^{-1}) to 27D (2.944 mol m^{-2} s^{-1}), (Additional file 1_S1). Electron transport rate (ETR) in μmol m^{-2} s^{-1} recorded high ETR values at 5D and 27D for all the genotypes except G100 which recorded a progressive decrease in ETR from 5D to 27D (Additional file 1_S1). For leaf reflectance parameters, NDVI recorded high values at 5D followed by a progressive decrease from 11D to 27D in all the rice genotypes expect G99 and G78 which recorded a slight and progressive increase at 11D to 27D (Additional file 1_S2). Renormalized difference vegetative index (RDVI) recorded higher values on 5D and 27D while the lowest values were obtained on 11D (Additional file 1_S2). Carotenoid reflectance index 1 and 2 (CRI1 and CRI2) displayed similar pattern. Both indexes (CRI1 and CRI2) scored highest at 5D followed by a rapid decrease at 11D, 18D and 27D. The previously selected two drought-tolerant genotypes, namely G99 and G100, and G78 maintained the highest CRI1 and CRI2 values at 27D (Additional file 1_S2). In this study, CRI1 and CRI2, stomatal conductance, transpiration rate, Electron transport rate and PhiPS II exhibited enough variation and pattern for tolerance to drought among the genotypes and therefore can be used as a selection criterion at the vegetative stage drought-stress.

3.3. Mean performances of the rice genotypes under vegetative stage drought-stress and non-stress conditions

Overall genotypes mean for each trait, coefficient of variations (C.V.%) and broad-sense heritability (h^2_{bs}) from the analyses of variances of 14 rice genotypes evaluated for eight physiological and leaf reflectance traits under non-stress and drought-stress conditions at 5-, 11-, 18- and 27-days after the drought-stress initiation is presented in Table 2.

3.3.1. Five days (5D) after the drought-stress initiation

The analysis of variance (ANOVA) revealed the presence of significant differences among the rice genotypes for NDVI, CRI1 and CRI2 under non-stress conditions, while under drought-stress, significant differences were observed on stomatal conductance, NDVI and RDVI at 5D (Additional file 1_S3). Under both non-stress and drought-stress conditions, NDVI and RDVI recorded low C.V. and moderate to high broad-sense heritability. Low to moderate C.V. were obtained under non-stress conditions (NDVI = 1.87 and RDVI = 3.95) and drought-stress (NDVI = 2.01 and RDVI = 4.48). Moderate to high C.V. were obtained under non-stress conditions (CRI1 = 13.43 and CRI2 = 13.63) and drought-stress (CRI1 = 13.09 and CRI2 = 12.97) combined with moderate to heritability values (Additional file 1_S3). High C.V. and moderate heritability were also recorded for stomatal conductance under both drought-stress and non-stress conditions.

The means performance with the ranking of the genotypes using the Duncan multiple rank test is presented in Table 3. Genotypes G63 recorded the highest stomatal conductance value of 0.763 mol m^{-2} s^{-1} under drought-stress and 0.749 mol m^{-2} s^{-1} under non-stress conditions. Genotype G63 is followed by G65 under both water regimes and both genotypes

Table 2. Overall genotypes mean for each trait, coefficient of variations (C.V.%) and broad-sense heritability (h^2_{bs}) among 14 rice genotypes evaluated for eight physiological and leaf reflectance traits under non-stress and drought-stress conditions at University of Giessen, Germany in 2022, at 5-, 11-, 18- and 27-days after the start of the drought treatment.

	Water regimes																							
	5-days after drought-stress initiation						11-days after drought-stress initiation						18-days after drought-stress initiation						27-days after drought-stress initiation					
	Non-stress			Drought-stress			Non-stress			Drought-stress			Non-stress			Drought-stress			Non-stress			Drought-stress		
TRAITS	GM	C.V.	h^2_{bs}	GM	C.V.	h^2_{bs}	GM	C.V.	h^2_{bs}	GM	C.V.	h^2_{bs}	GM	C.V.	h^2_{bs}	GM	C.V.	h^2_{bs}	GM	C.V.	h^2_{bs}	GM	C.V.	h^2_{bs}
TN	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	11.92	30.72	76.48	8.30	30.59	62.93
gsw	0.47	56.52	43.62	0.45	46.08	53.69	0.35	61.55	35.79	0.22	60.32	35.5	0.53	49.43	63.61	0.16	55.08	48.65	0.51	43.22	42.9	0.07	84.10	70.98
E	3.98	41.63	25.51	4.01	34.99	24.59	4.14	42.38	39.02	3.06	44.91	44.27	3.46	25.10	70.16	1.65	39.50	57.19	3.98	32.59	–	1.18	73.23	71.51
PhiPS II	0.69	4.70	–	0.69	5.77	0.33	0.70	5.74	47.94	0.71	3.65	36.9	0.69	6.65	58.29	0.69	6.60	35.67	0.68	6.57	–	0.68	8.80	34.26
ETR	35.22	22.16	13.08	34.23	29.43	–	33.39	88.59	17.2	26.60	30.45	–	23.84	26.44	–	24.32	34.14	–	28.35	22.73	29.44	28.41	30.98	–
NDVI	0.76	1.87	80.36	0.76	2.01	58.69	0.68	1.62	74.55	0.69	1.51	45.94	0.69	1.49	45.33	0.69	1.63	2.83	0.69	1.83	24.43	0.68	4.07	23.63
CRI1	8.49	13.43	54.21	8.76	13.09	48.07	5.02	7.37	42.24	5.02	5.96	68.77	4.85	7.65	51.63	4.84	6.67	61.74	4.85	6.47	78	4.42	16.11	20.30
CRI2	7.22	13.63	58.67	7.46	12.97	48.41	4.76	7.62	44.66	4.77	6.63	64.34	4.63	8.34	49.18	4.61	7.24	60.54	4.64	6.76	79.28	4.24	12.02	35.86
RDVI	0.57	3.95	41.84	0.56	4.48	69.18	0.53	3.94	–	0.53	3.94	34.2	0.54	4.11	29.87	0.54	3.45	53.46	0.55	3.72	67.93	0.56	3.45	39.89

GM-Overall mean of the genotypes for each trait; TN-Tillers number; gsw-Stomatal conductance; E-transpiration rate; PhiPS II-Quantum yield of fluorescence; ETR-Electron transport rate; NDVI-Normalized difference vegetative index; CRI1-Carotenoid reflectance index 1; CRI2-Carotenoid reflectance index 2; RDVI-Renormalized difference vegetative index; C.V.- Experimental coefficient of variation in %, h^2_{bs}-Broad-sense heritability.

Table 3. Mean performance of 14 rice genotypes evaluated for eight physiological and leaf reflectance traits under 5-days drought-stress and non-stress conditions at the Institute for Agronomy and Plant Breeding of Justus-Liebig-University of Giessen, Germany in 2022.

		gsw		E		PhiPS2		ETR		NDVI		CRI1		CRI2		RDVI	
ID	Genotype	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS
G6	Togo Marshall	0.287a	0.255c	3.001a	2.625a	0.680a	0.680a	33.16abc	35.83	0.780b	0.780b	8.757abc	9.459a	7.312abc	7.904a	0.577a	0.565acd
G62	KE40	0.524a	0.400abc	4.037a	3.870a	0.713a	0.721a	34.50	35.71	0.744d	0.759abc	8.317abc	9.379a	7.171abc	8.064a	0.549a	0.535bd
G73	SR35266-2–12-1-1	0.454a	0.463abc	3.858a	4.151a	0.711a	0.684a	36.48	34.01	0.759ac	0.767abc	7.710ac	8.285a	6.570ac	7.092a	0.571a	0.578c
G100	UPLR-17	0.290a	0.318ac	3.159a	3.393a	0.700a	0.684a	39.26	33.41	0.760ac	0.754ac	9.787b	9.077a	8.420b	7.789a	0.535a	0.542abcd
G99	APO	0.522a	0.385abc	4.339a	3.976a	0.715a	0.717a	39.05	32.89	0.758ac	0.750ac	8.771abc	9.667a	7.458abc	8.328a	0.566a	0.517bd
G65	GR18-SARI	0.655a	0.608ab	4.749a	4.834a	0.709a	0.684a	30.17	38.44	0.766ab	0.769ab	8.966abc	9.003a	7.673ab	7.531a	0.571a	0.561acd
G5	ARICA 3	0.549a	0.571ab	4.344a	4.334a	0.682a	0.682a	37.84	31.07	0.767ab	0.765abc	8.758abc	9.354a	7.415abc	7.861a	0.571a	0.563acd
G63	ARICA 2	0.749a	0.763b	5.230a	5.435a	0.678a	0.684a	33.73	34.99	0.753acd	0.747c	7.418c	7.266a	6.149c	6.248a	0.567a	0.577c
G2	CRI-Agrarice	0.367a	0.495abc	3.362a	4.363a	0.692a	0.717a	36.51	33.17	0.773ab	0.771ab	8.814abc	8.549a	7.404abc	7.175a	0.567a	0.567acd
G11	CRI-Enapa	0.398a	0.512abc	3.577a	4.502a	0.715a	0.707a	32.57	25.64	0.736d	0.754ac	7.366c	8.380a	6.273c	7.321a	0.571a	0.560acd
G22	Jasmine 85	0.395a	0.497abc	3.700a	4.471a	0.694a	0.695a	33.35	35.73	0.757ac	0.765abc	7.734ac	8.214a	6.610ac	6.823a	0.555a	0.572ac
G53	WAB 2085-TGR2-WAT4-1-1	0.387a	0.254c	3.659a	2.871a	0.685a	0.678a	40.18	36.80	0.772ab	0.763abc	9.104ab	8.490a	7.860ab	7.517a	0.567a	0.566acd
G36	ART132-35-1-1-B-B	0.494a	0.354abc	4.150a	3.369a	0.669a	0.702a	30.08	44.15	0.758ac	0.752ac	8.549abc	9.148a	7.175abc	7.661a	0.570a	0.537abd
G78	SA68-SARI	0.566a	0.446abc	4.607a	3.872a	0.698a	0.716a	36.21	27.38	0.783b	0.771ab	8.799abc	8.405a	7.542abc	7.173a	0.579a	0.573c

gsw-Stomatal conductance; E-transpiration rate; PhiPS II-Quantum yield of fluorescence; ETR-Electron transport rate; NDVI-Normalized difference vegetative index; CRI1-Carotenoid reflectance index 1; CRI2-Carotenoid reflectance index 2; RDVI-Renormalized difference vegetative index; abcd- letter used to rank the genotypes based on the Duncan multiple rank test, the mean value with the same letter for a particular trait under the drought-stress or non-stress means these genotypes are not statistically different in their mean performance for that trait under weather the drought-stress or non-stress conditions; ID-Genotype Identity; NS-Non-stress; DS-Drought-stress.

are not statistically different in their mean performance at 5-days after the drought-stress initiation (5D). The lowest value of 0.254 mol m^{-2} s^{-1} of stomatal conductance under 5-days drought-stress was occupied by G53, while Togo Marshall recorded the lowest value (0.287 mol m^{-2} s^{-1}) under non-stress conditions. Statistically, G53 showed a difference in the mean performance with G63 and G65. Genotype G6 recorded the highest NDVI of 0.780 under both drought-stress and non-stress conditions. Genotype G6 is followed by G2 and G78 under drought-stress. Under non-stress conditions, SA68-SARI topped first (0.783) followed by G6 and G2. Under both water regimes the three genotypes are not statistically different in their mean performance at 5D. The lowest value of 0.747 of NDVI under 5-days drought-stress was occupied by G63, while G11 recorded the lowest value (0.736) under non-stress conditions. Statistically, G63 showed a difference in the mean performance with G6 but not with G2 and G78 under 5-days drought-stress. On the other hand, under the non-stress conditions G11 showed a statistical difference in the mean performance with G6, G2 and G78.

3.3.2. Eleven days (11D) after the drought-stress initiation

At 11-days after the drought-stress initiation (11D), ANOVA revealed the presence of significant differences among the rice genotypes for NDVI under non-stress conditions (Additional file 1_S4). Under drought-stress conditions, ANOVA showed significant differences for CRI1 and CRI2 (Additional file 1_S4). Under both non-stress and drought-stress conditions all the leaf reflectance parameters namely NDVI, RDVI, CRI1 and CRI2 recorded low to moderate C.V. and moderate to high heritability. Low to moderate C.V. obtained under non-stress conditions (NDVI = 1.62, RDVI = 3.94, CRI1 = 7.37 and CRI2 = 7.62) and drought-stress (NDVI = 1.51, RDVI = 3.94, CRI1 = 5.96 and CRI2 = 6.63) were obtained at 11D. Moderate heritability under non-stress conditions (CRI1 = 42.24 and CRI2 = 44.66) and high heritability under drought-stress (CRI1 = 68.77 and CRI2 = 64.34) were recorded at 11D in this study (Additional file 1_S4). High C.V. and moderate heritability were also recorded for stomatal conductance and transpiration rate under both drought-stress and non-stress conditions. The means performance with the ranking of the genotypes using the Duncan multiple rank test is presented in Table 4.

In general, the mean performance of all the genotypes has been decreased notably under drought-stress compared to the non-stress conditions for

Table 4. Mean performance of 14 rice genotypes evaluated for eight physiological and leaf reflectance traits under 11-days drought-stress and non-stress conditions at the Institute for Agronomy and Plant Breeding of Justus-Liebig-University of Giessen, Germany in 2022.

ID	Genotype	gsw		E		PhiPS2		ETR		NDVI		CRI1		CRI2		RDVI	
		NS	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS
G6	Togo Marshall	0.184a	0.273a	2.659a	3.429a	0.700a	0.719a	29.44a	24.44a	0.692ace	0.694a	5.185a	4.995abcd	4.828a	4.642abd	0.525a	0.537a
G62	KE40	0.242a	0.125a	3.073a	2.140a	0.727a	0.731a	23.65a	24.47a	0.674bd	0.686a	5.015a	5.009abcd	4.803a	4.821abcd	0.519a	0.527a
G73	SR35266–2–12-1-1	0.365a	0.211a	3.936a	3.070a	0.705a	0.709a	24.72a	24.03a	0.687abce	0.682a	4.937a	4.643b	4.723a	4.440b	0.541a	0.539a
G100	UPLR-17	0.249	0.275a	3.550a	3.703a	0.699a	0.728a	63.08a	27.95a	0.678be	0.682a	5.099a	5.139acd	4.917a	4.941acd	0.523a	0.525a
G99	APO	0.427a	0.256a	5.025a	3.672a	0.730a	0.727a	30.87a	26.00a	0.685abce	0.678a	5.421a	5.382c	5.185a	5.173c	0.519a	0.508a
G65	GR18-SARI	0.475a	0.190a	4.891a	2.554a	0.688a	0.710a	31.01a	21.66a	0.691ace	0.694a	5.182a	5.159acd	4.860a	4.860abcd	0.534a	0.542a
G5	ARICA 3	0.321a	0.264a	3.815a	3.422a	0.686a	0.710a	24.73a	25.49a	0.688abce	0.694a	5.173a	5.259ac	4.858a	4.910acd	0.534a	0.538a
G63	ARICA 2	0.533a	0.241a	5.284a	3.428a	0.684a	0.684a	29.61a	28.48a	0.688abce	0.683a	4.682a	4.681b	4.404a	4.443b	0.546a	0.540a
G2	CRI-Agrarice	0.291a	0.214a	3.621a	2.844a	0.698a	0.727a	34.16a	23.29a	0.693ace	0.685a	5.052a	4.965abcd	4.783a	4.763abcd	0.538a	0.520a
G11	CRI-Enapa	0.274a	0.200a	3.494a	2.883a	0.723a	0.683a	23.98a	31.36a	0.662d	0.681a	4.789a	4.926abd	4.504a	4.618abd	0.525a	0.538a
G22	Jasmine 85	0.557a	0.340a	6.504a	4.593a	0.714a	0.707a	26.39a	32.08a	0.684abce	0.692a	4.982a	4.773bd	4.711a	4.548bd	0.523a	0.544a
G53	WAB 2085-TGR2-WAT4-1-1	0.200a	0.116a	2.990a	1.875a	0.651a	0.719a	61.02a	21.35a	0.695ac	0.695a	5.245a	5.173acd	5.029a	5.016ac	0.532a	0.528a
G36	ART132-35-1-1-B-B	0.444a	0.138a	4.228a	2.125a	0.686a	0.711a	32.59a	32.19a	0.680abe	0.689a	4.769a	5.185acd	4.499a	4.837abcd	0.528a	0.524a
G78	SA68-SARI	0.405a	0.234a	4.873a	3.083a	0.674a	0.714a	32.29a	29.60a	0.698c	0.683a	4.778a	4.921abd	4.548a	4.711abd	0.539a	0.524a

gsw-Stomatal conductance; E-transpiration rate; PhiPS II-Quantum yield of fluorescence; ETR-Electron transport rate; NDVI-Normalized difference vegetative index; CRI1-Carotenoid reflectance index 1; CRI2-Carotenoid reflectance index 2; RDVI-Renormalized difference vegetative index; abcde- letter used to rank the genotypes based on the Duncan multiple rank test, the mean value with the same letter for a particular trait under the drought-stress or non-stress means these genotypes are not statistically different in their mean performance for that trait under weather the drought-stress or non-stress conditions; ID-Genotype identity; NS-Non-stress; DS-Drought-stress.

stomatal conductance and transpiration rate for all the genotypes except G100 and G6. Genotype G22 recorded the highest stomatal conductance (0.340 mol m^{-2} s^{-1}) and transpiration rate (4.593 mol m^{-2} s^{-1}) under drought-stress and 0.557 mol m^{-2} s^{-1} and 6.504 mol m^{-2} s^{-1} respectively for stomatal conductance and transpiration rate under non-stress conditions. Genotype G22 was followed by G100 and G6 under drought-stress for stomatal conductance while G100 and G99 for transpiration rate. Under non-stress conditions, G22 was followed by G63 and G65 for stomatal conductance and G63 and G99 for transpiration rate. The lowest stomatal conductance (0.116 mol m^{-2} s^{-1}) and transpiration (1.875 mol m^{-2} s^{-1}) under 11-days drought-stress was occupied by G53, while G6 recorded lowest value for stomatal conductance (0.184 mol m^{-2} s^{-1}) and transpiration rate (2.659 mol m − 2 s − 1) under non-stress conditions. Statistically, there was no difference in the mean performance among all the genotypes at 11D under each water regime for the stomatal conductance and transpiration rate. Genotype G53 recorded the highest NDVI of 0.695 under drought-stress. Genotype G53 was followed by G6, G5 and G65 recording NDVI of 0.694 each under drought-stress. Under non-stress conditions, G78 topped first (0.698) followed by G53, G2 and G6.

Under non-stress conditions, these four genotypes showed statistical differences in their mean performance at 11D, while no statistical difference was seen under drought-stress conditions. The lowest value of 0.678 of NDVI under 11-days drought-stress was occupied by G99, while G11 recorded the lowest value (0.662) under non-stress conditions. Statistically, G99 showed no significant difference in the mean performance with other genotypes under 11-days drought-stress. On the other hand, under non-stress conditions G11 showed a statistical difference in the mean performance with G53, G6, G2 and G78. Genotype G99 recorded the highest CRI1 (5.382) and CRI2 (5.173) under drought-stress. Genotype G99 is followed by G5 recording CRI1 of 5.259 and by G53 recording CRI2 of 5.016 under drought-stress. Under non-stress conditions, G99 topped first for both CRI1 (5.421) and CRI2 (5.185) followed by G53. Under both water regimes, these genotypes showed no statistical differences among themselves in their mean performance at 11D. The lowest value of CRI1(4.682) and CRI2 (4.404) under non-stress conditions was occupied by G63, while G73 recorded the lowest value of CRI1 (4.643) and CRI2 (4.440) under non-stress conditions. Statistically, G73 showed a significant difference in the mean performance with other genotypes under 11-days drought-stress. On the other hand, under non-stress conditions, G73 showed no statistical difference in the mean performance with other genotypes.

3.3.3. Eighteen days (18D) after the drought-stress initiation

At 18-days after the drought-stress initiation (18D), ANOVA revealed the presence of significant differences among the rice genotypes for transpiration rate, CRI1, CRI2 and RDVI under non-stress conditions. Under drought-stress, ANOVA revealed significant differences for PhiPS II, NDVI, RDVI, CRI1 and CRI2 (Additional file 1_S5). Under both non-stress and drought-stress conditions all the leaf reflectance parameters namely NDVI, RDVI, CRI1 and CRI2 recorded moderate C.V. and moderate to high heritability. Moderate C.V. was obtained under non-stress conditions (CRI1 = 7.65 and CRI2 = 8.34) and drought-stress (CRI1 = 6.67 and CRI2 = 7.24), and moderate heritability under non-stress conditions (CRI1 = 51.63 and CRI2 = 49.18) and high heritability under drought-stress (CRI1 = 61.74 and CRI2 = 60.54) was obtained at 18-days drought-stress in this study. High C.V. and moderate to high heritability were also recorded for stomatal conductance and transpiration rate under both drought-stress and non-stress conditions. The means performance with the ranking of the genotypes using the Duncan multiple rank test is presented in Table 5 at 18D.

In general, the mean performance of all the genotypes has been reduced notably under drought-stress compared to the non-stress conditions for stomatal conductance and transpiration rate for all the genotypes. Genotype G99 recorded the highest stomatal conductance (0.251 mol m^{-2} s^{-1}) and transpiration rate (2.447 mol m^{-2} s^{-1}) and PhiPS2 (0.726) under drought-stress. Genotype G99 was followed by G2 under drought-stress for stomatal conductance, transpiration rate while G62 and G100 for PhiPS2. Under non-stress conditions, the genotypes G63 recorded the highest stomatal conductance (1.003 mol m^{-2} s^{-1}) and transpiration rate (4.929 mol m^{-2} s^{-1}) and G99 for PhiPS2 (0.732). Genotype G63 is followed by G78 for stomatal conductance and G5 for transpiration rate and G99 is followed by G62 and G100 for PhiPS2. The lowest stomatal conductance (0.086 mol m^{-2} s^{-1}) and transpiration rate (0.981 mol m^{-2} s^{-1}) under 18-days drought-stress was occupied by G53 and G65 (0.652) for PhiPS2, while G53 recorded lowest value for stomatal conductance (0.295 mol m^{-2} s^{-1}) and transpiration rate (2.459 mol m^{-2} s^{-1}) and G63 (0.626) for PhiPS2

Table 5. Mean performance of 14 rice genotypes evaluated for eight physiological and leaf reflectance traits under 18-days drought-stress and non-stress conditions at the Institute for Agronomy and Plant Breeding of Justus-Liebig-University of Giessen, Germany in 2022.

		gsw		E		PhiPS2		ETR		NDVI		CRI1		CRI2		RDVI	
ID	Genotype	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS
G6	Togo Marshall	0.379a	0.120a	2.845ac	1.350a	0.646a	0.701bd	21.60a	18.09a	0.697a	0.693c	4.831ab	4.967abce	4.594bcde	4.697acd	0.553ab	0.541b
G62	KE40	0.509a	0.115a	3.281ac	1.310a	0.722a	0.717ab	25.95a	21.27a	0.683a	0.686abcd	4.907abc	4.601abde	4.733ace	4.408abcd	0.528ab	0.548abc
G73	SR35266-2–12-1-1	0.525a	0.117a	3.500ac	1.249a	0.678a	0.675abd	25.39a	25.84a	0.684a	0.684abcd	4.571ac	4.377d	4.454a	4.165bd	0.547b	0.563ab
G100	UPLR-17	0.363a	0.158a	2.799abc	1.726a	0.718a	0.709abd	26.19a	27.00a	0.686a	0.687abcd	5.091b	5.149c	4.894d	4.944c	0.527ac	0.533ac
G99	APO	0.499a	0.251a	3.575b	2.447a	0.732a	0.726b	25.36a	27.05a	0.675a	0.680b	5.069ab	5.108ac	4.834bde	4.880ac	0.517ac	0.529c
G65	GR18-SARI	0.487a	0.150a	3.255abc	1.559a	0.693a	0.652bd	21.83a	23.65a	0.689a	0.694acd	5.079ab	4.927ace	4.821abcde	4.622ac	0.536ab	0.554ab
G5	ARICA 3	0.677a	0.164a	4.232abc	1.679a	0.671a	0.659b	26.20a	26.43a	0.691a	0.697ac	5.196ab	4.893ace	4.909abcde	4.567ac	0.537b	0.563ab
G63	ARICA 2	1.003a	0.151a	4.929ac	1.504a	0.626a	0.688b	21.61a	22.03a	0.683a	0.686abcd	4.594ac	4.612bd	4.301ac	4.350bd	0.538ab	0.548ab
G2	CRI-Agrarice	0.611a	0.242a	3.953ab	2.176a	0.702a	0.699abcd	21.07a	26.14a	0.691a	0.691acd	4.941ab	4.882abce	4.723bcde	4.692ac	0.530abc	0.537ac
G11	CRI-Enapa	0.468a	0.190a	3.474ab	1.958a	0.662a	0.658b	25.98a	24.44a	0.682a	0.685bd	4.442abc	4.754bd	4.129abce	4.444b	0.551ab	0.541ab
G22	Jasmine 85	0.520a	0.217a	3.517ab	2.010a	0.695a	0.662abd	24.82a	23.75a	0.692a	0.688acd	4.686abc	4.743bde	4.465abcde	4.568abd	0.549ab	0.552ab
G53	WAB 2085-TGR2-WAT4-1-1	0.295a	0.086a	2.459c	0.981a	0.700a	0.689abcd	23.34a	20.32a	0.688a	0.691acd	4.795ab	4.863abce	4.662bcde	4.717ac	0.547ab	0.543ab
G36	ART132-35-1-1-B-B	0.357a	0.147a	2.665ac	1.497a	0.703a	0.706abcd	21.00a	33.69a	0.683a	0.681bd	4.800ab	5.112abce	4.535bd	4.911ac	0.528c	0.514c
G78	SA68-SARI	0.723a	0.151a	3.942abc	1.618a	0.691a	0.696abcd	23.49a	20.81a	0.695a	0.689acd	4.871abc	4.778abce	4.709abcde	4.531abcd	0.534ab	0.547abc

gsw-Stomatal conductance; E-transpiration rate; PhiPS II-Quantum yield of fluorescence; ETR-Electron transport rate; NDVI-Normalized difference vegetative index; CRI1-Carotenoid reflectance index 1; CRI2-Carotenoid reflectance index 2; RDVI-Renormalized difference vegetative index; abcde- letter used to rank the genotypes based on the Duncan multiple rank test, the mean value with the same letter for a particular trait under the drought-stress or non-stress means these genotypes are not statistically different in their mean performance for that trait under weather the drought-stress or non-stress conditions; ID-Genotype identity; NS-Non-stress; DS-Drought-stress.

under non-stress conditions. Statistically, there were differences in the mean performance at 18D between G99 and G53 for transpiration rate under non-stress conditions and PhiPS2 under drought-stress. The genotype G5 recorded the highest NDVI of 0.697 under drought-stress. Genotype G5 was followed by G65 and G6 recording NDVI of 0.694 and 0.693, respectively under drought-stress. Under non-stress conditions, G6 topped first (0.697) for NDVI followed by G78. Under drought-stress conditions, these genotypes showed statistical differences in their means performance at 18D, while no statistical difference was seen under non-stress conditions. The lowest value of 0.675 and 0.680 of NDVI was occupied by G99, under non-stress and 18-days drought-stress, respectively. Statistically, G99 showed a significant difference in the mean performance with other genotypes under 18-days drought-stress. On the other hand, under non-stress, no statistical difference was observed among the genotypes. Genotype G100 recorded the highest value of CRI1 (5.149) and CRI2 (4.944), while G73 and G5 recorded the highest value for RDVI (0.563) under drought-stress. Genotype G100 was followed by G36 (CRI1 = 5.112 and CRI2 = 4.911). Under non-stress, G5 topped first for both CRI1(5.196) and CRI2(4.909) followed by G100, while G6 topped first for RDVI (0.553) followed by G11. Under both water regimes, these genotypes showed no statistical differences among themselves in their means performance at 18D. The lowest value of CRI1(4.442) and CRI2 (4.129) under non-stress was occupied by G11, while G99 showed the lowest value for RDVI (0.517). Genotype G73 recorded lowest value of CRI1(4.377) and CRI2 (4.165) under drought-stress, while G36 showed the lowest value for RDVI (0.514).

3.3.4. Twenty-seven days (27D) after the drought-stress initiation

At 27-days after the drought-stress initiation (27D), ANOVA revealed the presence of significant differences among the rice genotypes for TN, CRI1, CRI2 and RDVI under non-stress conditions. Under drought-stress, ANOVA showed significant differences among the rice genotypes for TN, stomatal conductance, and transpiration rate (Additional file 1_S6). Under both non-stress and drought-stress, all the leaf reflectance parameters namely NDVI, RDVI, CRI1 and CRI2 recorded low to moderate C.V. and moderate to high heritability. Moderate C.V. was obtained under non-stress condition (CRI1 = 6.47 and CRI2 = 6.76) and drought-stress (CRI1 = 16.11 and CRI2 = 12.02), while high heritability under non-stress condition (CRI1 = 78 and CRI2 = 79.28) and moderate heritability under drought-stress (CRI2 = 35.86) were obtained (Additional file 1_S6). High C.V. and moderate to high heritability were also recorded for tiller number, stomatal conductance and transpiration rate under both drought-stress and non-stress conditions. The means performance with the ranking of the genotypes using the Duncan multiple rank test is presented in Table 6 under 27-days drought-stress. In general, the mean performance of all the genotypes has been reduced notably by more than 60% under drought-stress compared to the non-stress conditions for stomatal conductance and transpiration rate for all the genotypes except G99 and G100. Genotype G99 recorded the highest stomatal conductance (0.206 mol m^{-2} s^{-1}) followed by G100 (0.205 mol m^{-2} s^{-1}) and G78 (0.080 mol m^{-2} s^{-1}), while G22 recorded the lowest value of 0.031 mol m^{-2} s^{-1} under the 27-days drought-stress. Genotype G100 recorded the highest transpiration rate (3.279 mol m^{-2} s^{-1}) followed by G99, G78 and G6 under drought-stress, while G22 recorded the lowest value of 0.599 mol m^{-2} s^{-1}. Genotype G100 recorded the highest PhiPS2 (0.738) followed by G99, G11 and G62 under drought-stress, while G73 recorded the lowest value of 0.609. Under non-stress conditions, the genotype G22 recorded the highest stomatal conductance (0.869 mol m^{-2} s^{-1}) followed by G63 and G78, while G73 recorded the lowest value of 0.314 mol m^{-2} s^{-1}. Genotype G22 recorded the highest transpiration rate (5.705 mol m^{-2} s^{-1}) followed by G78, G6 and G100 under non-stress conditions, while G11 recorded the lowest value of 2.112 mol m^{-2} s^{-1}. Genotype G36 recorded the highest PhiPS2 (0.711) followed by G100, G6 and G62 under non-stress conditions, while G11 recorded the lowest value of 0.556. Statistically, there was a significative difference in the mean performance at 27D between G99 and G22, and between G100 and G22 for stomatal conductance and transpiration rate under drought-stress, respectively.

Genotype G53 recorded the highest NDVI of 0.701 under drought-stress. Genotype G53 was followed by G5 and G99 recording NDVI of 0.697 and 0.694, respectively under drought-stress conditions. Under non-stress conditions, SA68-SARI topped first (0.704) followed by G53 for NDVI. The lowest value of 0.678 and 0.649 of NDVI was occupied by G11 and G63, under non-stress conditions and 27-days drought-stress, respectively.

Under both water regimes no statistical difference was observed between G11 and G63 with other genotypes. Genotype G99 recorded the highest value of CRI1 (4.902) followed by G78 and G100, while G63 recorded the lowest value of CRI1 (3.705) under 27-days drought-stress. Genotype G78 recorded the highest value of CRI2 (4.689) followed by G100 and G99 while G63 recorded the lowest value CRI2 (3.706) under 27-days drought-stress. Genotype G73 recorded the highest value of RDVI (0.573) followed by G5 and G53, while G2 recorded the lowest value of RDVI (0.536) under 27-days drought-stress. Under non-stress conditions, G99 recorded the highest value of CRI1 (5.652) followed by G65 and G53, while G63 recorded the lowest value of CRI1 (4.389) under non-stress conditions. Genotype G99 recorded the highest value of CRI2 (5.492) followed by G100 and G53, while G63 recorded the lowest value of CRI2 (4.128) under non-stress conditions. Genotype G78 recorded the highest value of RDVI (0.566) followed by Togo Marshall and G2, while G99 recorded the lowest value of RDVI (0.492) under non-stress conditions.

Based on the relative value of the leaf gas exchange attributes and leaf reflectance parameters (Additional file 1_S7), G11 and G73 were best performing under 5-days drought-stress based on high relative NDVI, relative CRI, relative CRI2, relative stomatal conductance and relative transpiration rate. Genotypes G6 and G100 were best performing under 11-days drought-stress based on high relative RDVI, relative stomatal conductance and relative transpiration rate. Genotypes G99 and G100 were best performing to 18-days drought-stress based on high relative NDVI, relative CRI, relative CRI2, relative stomatal conductance and relative transpiration rate. Genotypes G99 and G100 were best performing under 27-days drought-stress based on high relative NDVI, relative CRI, relative CRI2, relative stomatal conductance and relative transpiration rate. Genotypes G11 and G78 were also considered among the best performing under 27-days drought-stress based on high relative PhiPS2, relative CRI, relative CRI2, relative stomatal conductance and relative transpiration rate.

3.4. Mean performance of the rice genotypes under both reproductive stage drought-stress and non-stress conditions

During the reproductive stage of drought-stress, ANOVA revealed the presence of significant differences among the rice genotypes for ETR and RDVI under non-stress conditions. Under drought-stress conditions, ANOVA revealed no significant differences for all the traits among the genotypes. Under both non-stress and drought-stress conditions, all the physiological and leaf reflectance parameters recorded moderate to high C.V. and low to moderate heritability (Additional file 1_S8).

Table 6. Mean performance of 14 rice genotypes evaluated for eight physiological and leaf reflectance traits under 27-days drought-stress and non-stress conditions at the Institute for Agronomy and Plant Breeding of Justus-Liebig-University of Giessen, Germany in 2022.

		gsw		E		PhiPS2		ETR		NDVI		CRI1		CRI2		RDVI		TN	
ID	Genotype	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS
G6	Togo Marshall	0.581a	0.074b	4.711a	1.120c	0.709a	0.631a	24.80a	21.62a	0.695a	0.684a	4.684ac	4.483a	4.395ac	4.424a	0.562a	0.567a	19.00c	11.50b
G62	KE40	0.380a	0.038b	3.496a	0.707c	0.706a	0.703a	30.12a	28.50a	0.683a	0.663a	4.687ac	3.949a	4.464ac	4.027a	0.546a	0.547a	10.17abde	9.50ab
G73	SR35266-2–12-1-1	0.314a	0.035b	2.739a	0.691c	0.686a	0.609a	28.01a	29.34a	0.690a	0.676a	4.602ac	4.195a	4.440ac	3.938a	0.555a	0.573a	11.83abde	10.17ab
G100	UPLR-17	0.510a	0.205a	4.525a	3.279b	0.710a	0.738a	32.79a	26.03a	0.690a	0.674a	5.083c	4.764a	4.910c	4.641a	0.542a	0.544a	6.80bd	5.50c
G99	APO	0.468a	0.206a	4.214a	2.944ab	0.689a	0.732a	35.91a	34.58a	0.690a	0.694a	5.652b	4.902a	5.492b	4.543a	0.492b	0.557a	8.17bde	5.67c
G65	GR18-SARI	0.580a	0.040b	4.174a	0.701c	0.662a	0.654a	35.77a	35.29a	0.695a	0.684a	5.097c	4.588a	4.831c	4.370a	0.546a	0.564a	12.00ade	8.83abc
G5	ARICA 3	0.443a	0.031b	3.745a	0.613c	0.690a	0.633a	31.14a	22.16a	0.692a	0.697a	5.028c	4.683a	4.732c	4.368a	0.543a	0.571a	13.17ade	8.83abc
G63	ARICA 2	0.690a	0.057b	4.085a	0.955c	0.683a	0.701a	27.47a	25.08a	0.679a	0.649a	4.389a	3.705a	4.128a	3.706a	0.555a	0.546a	10.60abde	7.50ac
G2	CRI-Agrarice	0.407a	0.074b	3.499a	1.065c	0.687a	0.634a	22.72a	27.26a	0.699a	0.665a	4.688ac	3.990a	4.503ac	4.010a	0.560a	0.536a	8.83bde	7.17ac
G11	CRI-Enapa	0.453a	0.056b	2.112a	0.692c	0.556a	0.721a	25.06a	31.36a	0.678a	0.657a	4.849ac	4.281a	4.620ac	4.128a	0.543a	0.557a	12.00ade	9.50ab
G22	Jasmine 85	0.869a	0.031b	5.705a	0.599c	0.686a	0.676a	25.43a	31.10a	0.698a	0.693a	4.890ac	4.429a	4.687ac	4.159a	0.550a	0.561a	13.40ad	7.50ac
G53	WAB 2085-TGR2-WAT4-1-1	0.389a	0.040b	3.922a	0.714c	0.676a	0.652a	25.62a	25.54a	0.704a	0.701a	5.096c	4.703a	4.903c	4.482a	0.543a	0.569a	13.83ad	9.33ab
G36	ART132-35-1-1-B-B	0.390a	0.055b	3.653a	0.988c	0.711a	0.682a	27.14a	35.06a	0.684a	0.676a	4.584ac	4.267a	4.423ac	3.938a	0.538a	0.553a	11.50abde	8.67abc
G78	SA68-SARI	0.681a	0.080b	5.109a	1.471ac	0.687a	0.691a	24.90a	24.86a	0.704a	0.689a	4.541ac	4.892a	4.370ac	4.689a	0.566a	0.541a	14.67ac	7.17ac

TN-tiller number; gsw-Stomatal conductance; E-transpiration rate; PhiPS II-Quantum yield of fluorescence; ETR-Electron transport rate; NDVI-Normalized difference vegetative index; CRI1-Carotenoid reflectance index 1; CRI2-Carotenoid reflectance index 2; RDVI-Renormalized difference vegetative index; abcde- letter used to rank the genotypes based on the Duncan multiple rank test, the mean value with the same letter for a particular trait under the drought-stress or non-stress means these genotypes are not statistically different in their mean performance for that trait under weather the drought-stress or non-stress conditions; ID-Genotype Identity; NS-Non-stress; DS-Drought-stress.

The means performance with the ranking of the genotypes using the Duncan multiple rank test is presented in Table 7 under reproductive drought-stress stage. In general, the mean performance of all the genotypes has been reduced notably under drought-stress compared to the non-stress conditions for stomatal conductance, transpiration rate and PhiPS2 for all the genotypes except G11 for stomatal conductance and PhiPS2, and G100 for transpiration rate. Genotype G100 recorded the highest stomatal conductance (0.353 mol m^{-2} s^{-1}), while G53 recorded the lowest value of 0.090 mol m^{-2} s^{-1} under reproductive drought-stress. Genotype G100 recorded the highest transpiration rate (2.948 mol m^{-2} s^{-1}), while G5 recorded the lowest value of 0.919 mol m^{-2} s^{-1} under reproductive drought-stress. Genotype G11 recorded the highest PhiPS2 (0.689) followed by G100 (0.645) under reproductive drought-stress, while G36 recorded the lowest value of 0.379. Genotype G100 recorded the highest NDVI of 0.693 under drought-stress followed by G78, G99 and G11 with the lowest value of 0.628 for G36. Under drought-stress conditions, G11 topped first for RDVI (0.571) followed by G62 and G99 with the lowest value of 0.513 for G2. Genotype G11 recorded the highest drought risk index for stomatal conductance or relative stomatal conductance (1.135), relative PhiPS2 (1.172), relative CRI1 (1.104), relative CRI2 (1.063) and topped second for relative transpiration rate (0.980), third for relative RDVI (1.035). Genotype G100 recorded the highest relative transpiration rate (1.020), relative ETR (1.553), relative NDVI (1.035), and topped second for relative stomatal conductance (0.754), relative PhiPS2 (0.950), relative CRI1 (1.051), relative CRI1 (1.039) with a high value of relative RDVI (1.001). Based on the above results from the physiological and leaf reflectance parameters analysis using the relative value of the traits, the following genotypes namely, CRI-Enapa (G11), UPLR-17 (G100), APO (G99) and SA68-SARI (78) were the best performing under the reproductive stage drought-stress.

3.5. Mean performance of the rice genotypes under drought-stress and non-stress based on grain yield and yield-related traits

ANOVA revealed the presence of significant differences among the rice genotypes for all the grain yield and yield-related traits namely days to flowering (DTF), leaf drying score (LDS), aboveground biomass (grain + stover) and grain yield per plant under both drought-stress and non-stress conditions except DTF under non-stress conditions. Under both non-stress and drought-stress

Table 7. Mean performance of 14 rice genotypes evaluated for eight physiological and leaf reflectance traits under reproductive drought-stress and non-stress conditions at the Institute for Agronomy and Plant Breeding of Justus-Liebig-University of Giessen, Germany in 2022.

		gsw		E		PhiPS2		ETR		NDVI		CRI1		CRI2		RDVI	
ID	Genotype	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS
G6	Togo Marshall	0.638a	0.260a	3.367a	1.865a	0.652a	0.539a	12.57c	19.28a	0.676a	0.660a	4.160a	3.827a	3.962a	3.712a	0.543acd	0.536a
G62	KE40	0.568a	0.278a	3.204a	2.289a	0.683a	0.615a	20.83abc	27.26a	0.660a	0.638a	4.243a	3.232a	4.068a	3.227a	0.513abd	0.556a
G73	SR35266-2–12-1-1	0.608a	0.116a	3.074a	1.230a	0.617a	0.460a	17.19ac	19.27a	0.681a	0.664a	4.255a	3.744a	4.083a	3.662a	0.552ac	0.542a
G100	UPLR-17	0.468a	0.353a	2.889a	2.948a	0.679a	0.645a	18.94ac	29.40a	0.669a	0.693a	4.242a	4.460a	4.073a	4.234a	0.541acd	0.542a
G99	APO	0.390a	0.160a	2.426a	1.162a	0.652a	0.582a	19.33ac	23.60a	0.687a	0.674a	4.269a	3.897a	3.981a	3.664a	0.548acd	0.551a
G65	GR18-SARI	0.591a	0.225a	3.041a	1.901a	0.683a	0.601a	19.36ac	22.79a	0.680a	0.655a	4.460a	3.854a	4.297a	3.643a	0.527abcd	0.532a
G5	ARICA 3	0.895a	0.100a	4.111a	0.919a	0.644a	0.589a	22.01abc	22.36a	0.685a	0.670a	4.571a	4.204a	4.371a	4.052a	0.532abcd	0.516a
G65	ARICA 2	0.743a	0.227a	3.945a	1.673a	0.648a	0.390a	27.54ab	16.96a	0.651a	0.659a	3.854a	3.673a	3.682a	3.555a	0.510bd	0.544a
G2	CRI-Agrarice	0.532a	0.159a	3.705a	1.235a	0.676a	0.618a	31.27b	26.96a	0.679a	0.652a	4.060a	3.948a	3.881a	3.868a	0.539acd	0.513a
G11	CRI-Enapa	0.276a	0.314a	1.835a	1.797a	0.588a	0.689a	20.95abc	23.47a	0.666a	0.673a	3.875a	4.277a	3.686a	3.919a	0.552ac	0.571a
G22	Jasmine 85	0.521a	0.242a	3.345a	1.836a	0.587a	0.554a	21.76abc	30.55a	0.682a	0.659a	4.011a	4.018a	3.795a	3.902a	0.556c	0.541a
G53	WAB 2085-TGR2-WAT4-1-1	0.662a	0.090a	3.806a	0.927a	0.677a	0.486a	21.99abc	24.52a	0.685a	0.664a	4.599a	3.921a	4.403a	3.828a	0.526abcd	0.536a
G36	ART132-35-1-1-B-B	0.620a	0.350a	3.473a	2.347a	0.754a	0.379a	20.55ac	7.77a	0.663a	0.628a	4.788a	3.059a	4.513a	3.355a	0.496b	0.526a
G78	SA68-SARI	0.688a	0.113a	3.886a	1.216a	0.697a	0.497a	19.49ac	25.66a	0.688a	0.677a	4.399a	4.386a	4.211a	4.116a	0.542acd	0.526a

gsw-Stomatal conductance; E-transpiration rate; PhiPS II-Quantum yield of fluorescence; ETR-Electron transport rate; NDVI-Normalized difference vegetative index; CRI1-Carotenoid reflectance index 1; CRI2-Carotenoid reflectance index 2; RDVI-Renormalized difference vegetative index; abcde- letter used to rank the genotypes based on the Duncan multiple rank test, the mean value with the same letter for a particular trait under the drought-stress or non-stress means these genotypes are not statistically different in their mean performance for that trait under weather the drought-stress or non-stress conditions; ID-Genotype Identity; NS-Non-stress; DS-Drought-stress.

conditions for all the grain yield and yield-related traits namely LDS, aboveground biomass and grain yield per plant recorded high C.V. except DTF which had moderate C.V. High heritability was obtained for all the grain yield and yield related traits namely DTF, LDS, aboveground biomass and grain yield per plant under both non-stress and drought-stress except DTF which recorded moderate heritability under non-stress conditions indicating that selection will be effective for these traits (Additional file 1_S9&10).

In general, the flowering date of all the genotypes has been delayed at least by more than 5-days under drought-stress compared to the non-stress conditions except G99, G100 and G11 which flowered earlier under drought-stress compared to the non-stress conditions with 1.17 days, 3.37 days and 6.00 days, respectively. Genotype G5 recorded the highest delay in DFT (14.67 days) followed by G36 (13.50 days), while G2 recorded the lowest value of 5.67 days under drought-stress. On the other hand, the highest LDS were obtained on G36 (7.00) and G53 (5.67), while G78, G100 and G11 ranked lowest with LDS of 1.17, zero and zero, respectively. Genotype G99 recorded an LDS of 2.50, while G6 scored 3.00. The mean performance of all the genotypes has been reduced notably under drought-stress compared to non-stress conditions for the aboveground biomass except G100 which recorded a similar aboveground biomass under non-stress (109.16 g) and drought-stress (108.71 g) conditions with a relative biomass of 1.00. Second to G100, G11 recorded a relative biomass of 0.87 with aboveground biomass of 239.52 g under drought-stress and 275.53 g under non-stress conditions. Genotype G53 ranked lowest for a relative biomass value of 0.43 with aboveground biomass of 279.65 g under drought-stress and 643.32 g under non-stress conditions, implying its high sensitivity to drought-stress. The mean performance of all the genotypes has been notably reduced under drought-stress compared to the non-stress conditions for grain yield. Genotype G100 recorded the highest relative grain yield value of 0.62 with the grain yield of 8.18 g per plant under drought-stress and 13.26 g per plant under non-stress conditions, confirming its tolerance to drought-stress.

Genotype G73 ranked second to G100 with a relative grain yield of 0.34, a grain yield per plant of 11.83 g under drought-stress and 34.53 g under non-stress conditions. Next to G73, was G99 which scored 0.32 of relative grain yield with the grain yield per plant of 9.33 g under drought-stress and 29.52 g under non-stress conditions. The lowest relative grain yield of 0.03 was recorded by G5 with a grain yield per plant of 0.29 g under drought-stress and 9.60 g under non-stress conditions. The genotypes performances in the greenhouse under drought-stress and non-stress conditions after two weeks of drought-stress at the reproductive stage are presented in Figure 1. The means performance with the ranking of the genotypes under drought-stress using the Duncan multiple rank test is presented in Figure 2. Based on the above results from grain yield and relative yield-related traits analysis, UPLR-17 (G100), APO (G99), SR35266-2–12-1-1 (G73) and CRI-Enapa (G11) were the best performing genotypes.

On the other hand, MGIDI index using the relative values of all the traits (LDS, DTF, gsw, Et, PhiPS2, ETR, NDVI, CRI1, CRI2, RDVI, TN, Biomass and GYP) was employed to select for the drought-tolerant genotypes at reproductive stage drought-stress (Figure 3). The following genotypes were selected in the chronological order: APO (G99) and UPLR-17 (G100).

3.6. Relatedness and regression analysis among the traits under reproductive stage drought-stress and non-stress

The Pearson correlation analysis conducted at reproductive stage drought-stress revealed that under drought-stress, grain yield has a negative significant correlation with delay in flowering and DTF, and positively significantly associated with RDVI, while negatively with no significance related to LDS. This implies that the genotypes with early flowering under drought-stress and low leaf drying score tended to have a high relative grain yield and high aboveground relative biomass values, therefore showing more tolerance to drought. This confirms the consistent tolerance shown by G100, G11 and G99 throughout the analysis of the various traits and parameters under various numbers of days after the drought-stress initiation (5D, 11D, 18D & 27D) at vegetative stage and reproductive stage drought. The LDS, DTF and delay in flowering recorded a positive significant correlation among themselves. Pearson correlation analysis revealed under both water regimes, a strong positive significant correlation between stomatal conductance and transpiration rate (Figure 4). Both indexes (CRI1 and CRI2) had a strong positive significant correlation with each other, and both were negatively correlated with RDVI and positively correlated with NDVI, whereas NDVI and RDVI recorded a positive association with each other and with grain yield under both water regimes.

The regression analysis using grain yield as dependent variable and RDVI as explanatory variable recorded $R^2 = 0.3366$ with the model significance, implying that close to 34% of the variability of the dependent variable grain yield was explained by the explanatory variable RDVI (Figure 5). This confirmed the significant positive correlation ($r = 0.555$) recorded between grain yield and RDVI under drought-stress (Figure 4). Given the $R^2 = 0.3140$, 31% of the variability of the dependent variable grain yield was explained by the explanatory variable delay in flowering, but the model underlying this relationship between them was not significant (Additional file 1_S11). However, this confirmed the negative significant correlation of $r = -0.559$ recorded between grain yield and delay in flowering under drought-stress. Taken together, delay in flowering and RDVI explained 43% of the variability of the dependent variable grain yield confirming the correlation pattern depicted between grain yield, delay in flowering and RDVI under drought-stress (Additional file 1_S11). On the other hand, 44% of the variability of the dependent variable grain yield was explained by the two explanatory variables aboveground biomass and delay in flowering, while the leaf drying score explained close to 15% of the variability of the dependent variable grain yield under drought-stress (Additional file 1_S11), implying that the ability of the genotype to maintain its water status and prevent leaf drying under drought-stress contributed up to 15% to the final yield performance of the genotype.

3.7. Effectiveness of KASP-SNP markers in selecting drought-tolerant genotypes

Genotypes G100 and G99 consistently showed homozygosity status for the favorable alleles *G, A, G* and *C* for *DTY1.1 (snpOS00400), DTY1.1 (snpOS00402), DTY1.1 (snpOS00408)* and *DTY12.1 (snpOS00483)*, respectively (Table 8). None of the genotypes showed homozygosity for favorable allele *T* for *DTY12.1 (snpOS00484)*. Based on the cluster analysis, the genotypes were classified into three clusters where the drought-tolerant genotypes G100 and G99 were grouped in cluster I, G53 and G6 in Cluster II and while the remaining genotypes in Cluster III, confirming the greenhouse screening results (Figure 6).

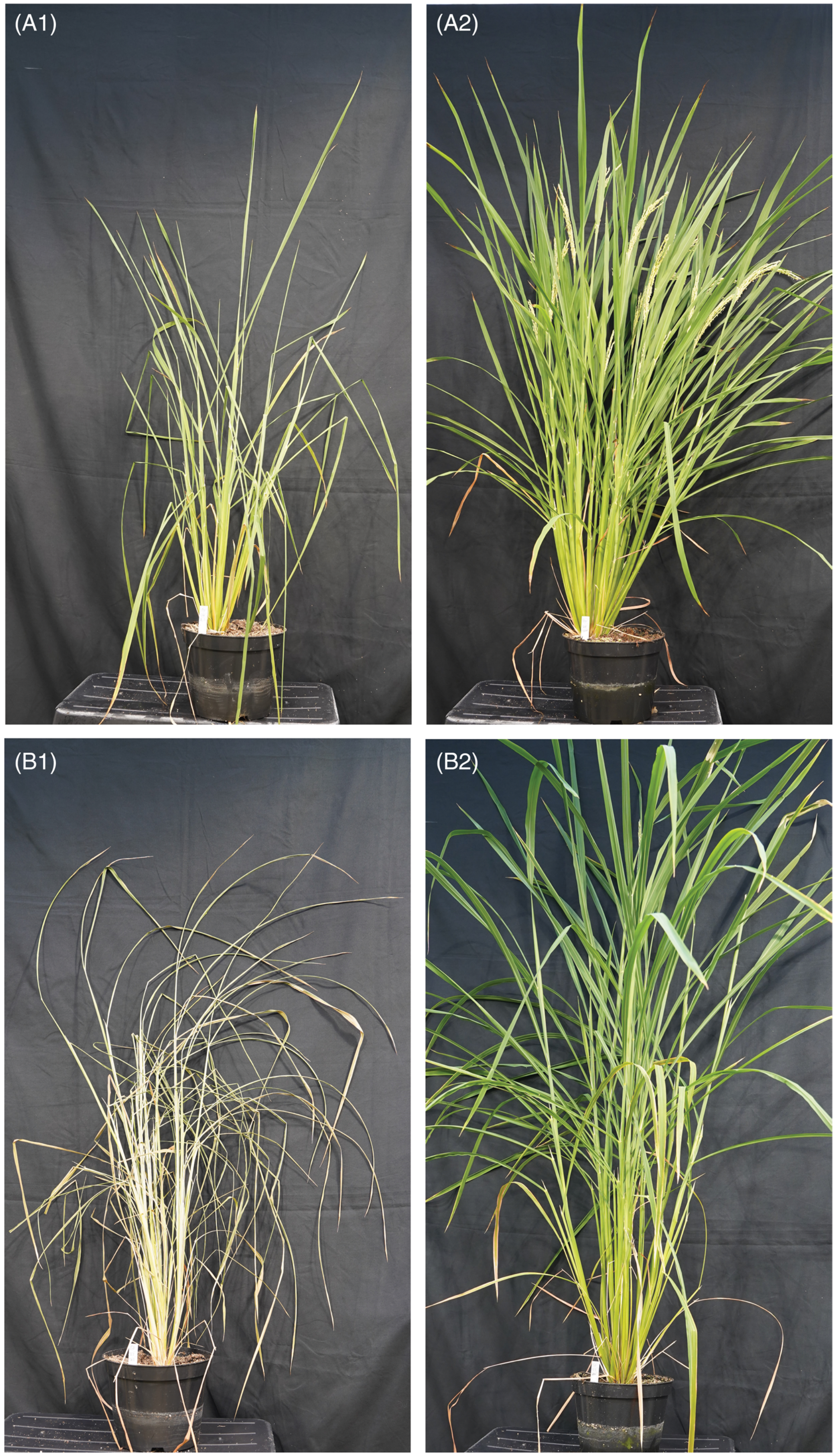

Figure 1. Performance of genotypes UPLR-17 (G100) and WAB 2085-TGR2-WAT4-1-1 (G53) under drought-stress and non-stress conditions evaluated for physiological and leaf reflectance parameters at the reproductive stage at 14 days of drought-stress at the Institute for Agronomy and Plant Breeding of Justus-Liebig-University of Giessen, Germany in 2022. Genotype UPLR-17 (G100) under drought-stress (A1), UPLR-17 (G100) under non-drought (A2), WAB 2085-TGR2-WAT4-1-1 (G53) under drought-stress (B1), WAB 2085-TGR2-WAT4-1-1 (G53) under non-drought (B2). In this study UPLR-17 (G100) was selected as drought-tolerant genotype while WAB 2085-TGR2-WAT4-1-1 (G53) was among the drought-sensitive ones.

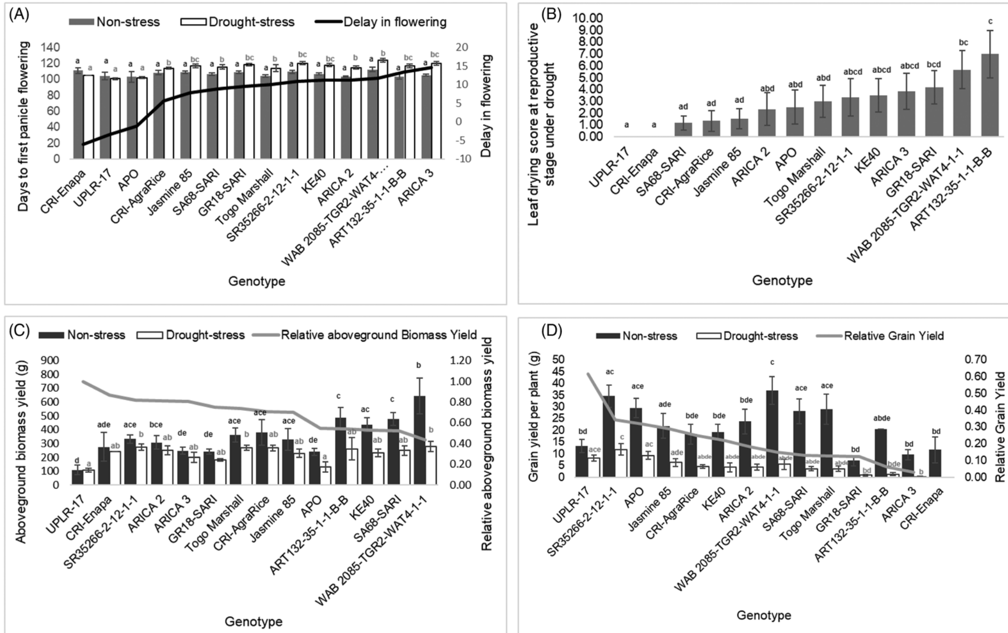

Figure 2. Mean performance of 14 rice genotypes evaluated for days to flowering (A), leaf drying score (B), aboveground biomass (C) and grain yield (D) at the reproductive stage under drought-stress and non-stress at the University of Giessen, Germany, in 2022. Data presented are means ± SE (n = 6). Genotypes with different letters above the error bar under each water regime (non-stress or drought-stress conditions) 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 delay in flowering, leaf drying score, relative aboveground biomass and relative grain yield for each genotype. Delay in flowering is calculated as number of days to flowering under drought-stress - number of days to flowering under non-stress conditions per genotype. Wherever, the delay in flowering is negative, indicating that the genotype flowered early under drought-stress than under non-stress conditions. For the leaf drying score, the lower the score, the better the performance of the genotype under drought-stress. Relative values were calculated as value under drought-stress/value under non-stress conditions per genotype. The genotypes with their corresponding ID: Togo Marshall (G6), KE40 (62), 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).

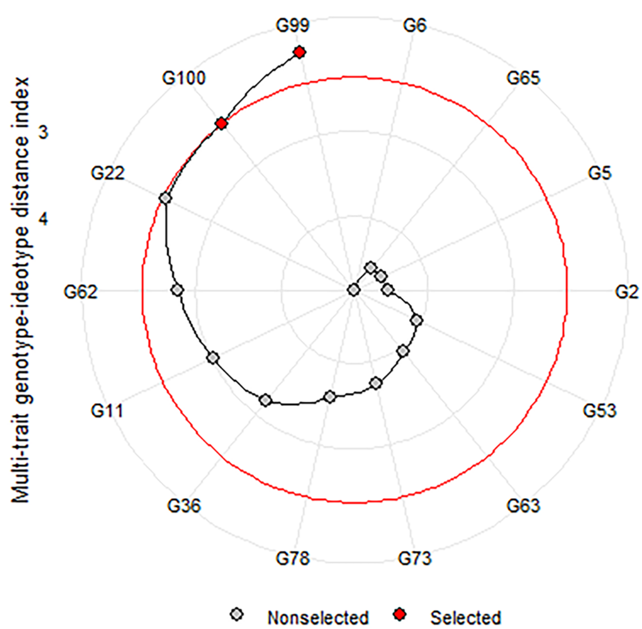

Figure 3. Ranking of the 14 genotypes in ascending order based on the multi-trait genotype-ideotype distance index (MGIDI). The genotypes were evaluated under both drought-stress and non-stress conditions at the Institute for Agronomy and Plant Breeding of Justus-Liebig-University of Giessen, Germany in 2022. 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 following genotypes were selected as drought-tolerant genotypes in chronological order of tolerance level: APO (G99) and UPLR-17 (G100).

4. Discussions

This study not only gave information about monitoring variation of leaf reflectance (gsw, E, PhiPS2, ETR) and physiological (NDVI, CRI1, CRI2, RDVI) parameters under vegetative stage drought stress but also gave the predictors used in selecting drought-tolerant genotypes.

Under both non-stress and drought-stress conditions, all the leaf reflectance parameters (NDVI, RDVI, CRI1 and CRI2) and PhiPS II recorded low to moderate C.V. and moderate to high heritability at 5D, 11D, 18D and 27D. High C.V. and moderate to high heritability were recorded for stomatal conductance, transpiration rate, ETR and tiller number under both drought-stress and non-stress conditions at 5D, 11D, 18D and 27D. Moderate to high C.V. coupled with high heritability indicate the presence of enough variability among the genotypes for these traits (Asante et al., 2019) and therefore, suitable for selection. But the combination of low C.V. with low heritability like in case of NDVI at 27D doesn't give room for selection in population improvement. Furthermore, no consistent reductions in heritability under drought-stress were visible across the entire stress period, in some cases, the heritability increased under drought-stress. Proximal heritability estimates for stomatal conductance, transpiration rate, ETR and tiller number under drought-stress and non-stress conditions indicate that selection for these traits under drought-stress at the vegetative stage in rice will be rewarding with the same level of accuracy as that under non-stress conditions, as suggested by Kumar et al. (2008) for grain yield.

A progressive and rapid decrease in transpiration rate (E) and stomatal conductance was recorded from 5D to 27D on all the genotypes except on the two drought-tolerant genotypes G100 and G99 implying increase in water use efficiency in these genotypes as observed by Khan et al. (2017) in evaluating two contrasting rice cultivars for their tolerance to drought where the water use efficiency increased in drought-tolerant PR-115, while it rapidly decreased in a drought-sensitive Super-7 at 4-, 7- and 10-days of drought-stress. While other genotypes continued to decrease 18-days after the stress initiation, genotypes G100 and G99 recorded an increase of 89.97% and 20.33% for transpiration rate, from the 18D to 27D, respectively. For stomatal conductance genotype G100 recorded an increase of 29.28%, while genotype G99 recorded the lowest reduction of 17.89% from the 18D to 27D. These results indicate that after 18-days of drought-stress where the average moisture content of the soil dropped from 60.78 vol/vol to 32.80 vol/vol, rice plant started to feel the severity of the imposed drought-stress. At these points, tolerant genotypes like G100 and G99 could start producing phytohormones and compounds such as proline, ABA to trigger their tolerance mechanisms and regulate their photosynthesis rate and stomatal conductance to improve their water use efficiency as reported by previous studies (Khan et al., 2017). In this study, CRI1 and CRI2, physiological parameters such as stomatal

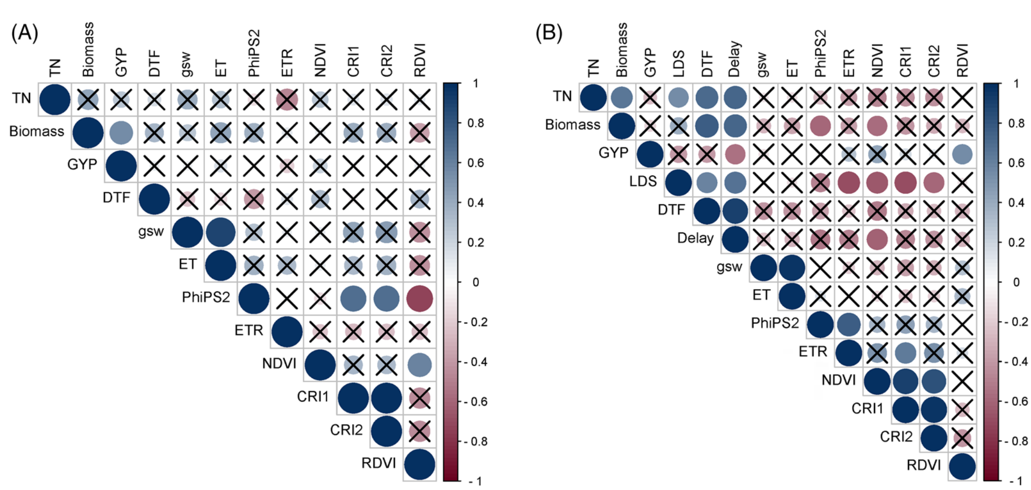

Figure 4. Pearson correlations among 14 rice genotypes evaluated for 13 grain yield and its related traits, physiological and leaf reflectance parameters and biochemical traits under non-stress (A) and drought-stress (B) conditions at the Institute for Agronomy and Plant Breeding of Justus-Liebig-University of Giessen, Germany in 2022. X-Insignificant labeled with X; * Significance at 5% level; gsw-Stomatal conductance; E-transpiration rate; PhiPS II-Quantum yield of fluorescence; ETR-Electron transport rate; NDVI-Normalized difference vegetative index; CRI1-Carotenoid reflectance index 1; CRI2-Carotenoid reflectance index 2; RDVI-Renormalized difference vegetative index; GYP- Grain yield per plant in gram; DTF- Days to flowering; Biomass- Aboveground biomass yield in gram; Delay-Delay in flowering; TN-Tiller number; LDS-Leaf drying score; NS-Non-stress; DS-Drought-stress.

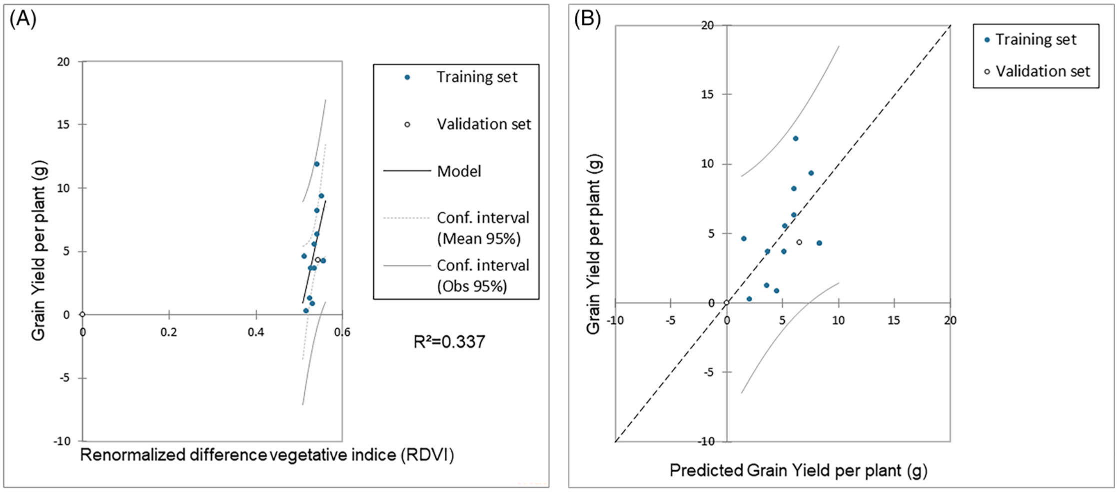

Figure 5. Regression analysis between grain yield (GYP) and renormalized difference vegetative index (RDVI) among 14 rice genotypes evaluated under drought-stress and non-stress at the Institute for Agronomy and Plant Breeding of Justus-Liebig-University of Giessen, Germany in 2022: Regression of grain yield (GYP) by RDVI (A); Predicted grain yield with the regression model (B). Equation of the model is $GYP = -77.938 + 155.082 \times RDVI$. This regression model is significant at 5% level.

Table 8. QTL results of the 14 genotypes retrieved from the QTL profiling data obtained from the KASP genotyping of 300 genotypes of the core breeding germplasm of CSIR-CRI, Ghana using KASP-SNP markers according to Asante et al. (2024), 2019.

	QTL ID	DTY1.1	DTY1.1	DTY1.1	DTY12.1	DTY12.1
	SNP ID	snpOS00400	snpOS00402	snpOS00408	snpOS00483	snpOS00484
Genotype	FAVORABLE ALLELE	G	A	G	C	T
G2	CRI-AgraRice	C:C	G:G	T:T	G:G	A:A
G5	ARICA 3	C:C	G:G	*G:G*	G:G	A:A
G6	TogoMarshall	C:C	G:G	T:T	*C:C*	G:A
G11	CRI-Enapa	*G:G*	G:G	T:T	G:G	G:G
G22	Jasmine 85- SARI	C:C	G:G	T:T	G:G	G:G
G36	ART132-35-1-1-B-B	*G:G*	G:G	T:T	G:G	A:A
G53	WAB 2085-TGR2-WAT4-1-1	*G:G*	G:G	T:T	*C:C*	G:G
G62	KE40	C:C	G:G	T:T	G:G	A:A
G63	ARICA 2	C:C	G:G	T:T	G:G	A:A
G65	GR18-SARI	C:C	G:G	T:T	G:G	A:A
G73	SR35266-2–12-1-1	C:C	G:G	T:T	G:G	G:G
G78	SA68-SARI	C:C	G:G	T:T	G:G	A:A
G99	APO	*G:G*	*A:A*	*G:G*	*C:C*	G:G
G100	UPL R17	*G:G*	*A:A*	*G:G*	*C:C*	G:G
Percentage of drought-tolerant favourable alleles per QTL, SNP and genotype		36%	14.29%	21.43%	28.57%	0%

conductance, transpiration rate, electron transport rate and PhiPS II exhibited enough variation and pattern for tolerance to drought among the genotypes as reported by previous studies (Tiwari et al., 2021) and therefore can be used as a selection criterion at vegetative stage drought-stress.

In general, the mean performance of all the genotypes has been reduced notably under 11-, 18- and 27-days drought-stress compared to the non-stress conditions for stomatal conductance and transpiration rate for all the genotypes except G99, G100 and G6. It has been reported that drought-stress has induced a significant decrease in photosynthetic rate, stomatal conductance, transpiration rate, and significant genotypic variations were observed among different rice genotypes for these leaf gas exchange parameters (Khan et al., 2017; Mumtaz et al., 2020). When the soil moisture content reached 18.14 vol/vol and the stress became severe at 27D, significative difference in the mean performance were observed between G99 (best performing) and G22 (waste performing), and between G100 (best performing) and G22 (waste performing) for stomatal conductance and transpiration rate under drought-stress, respectively. Gaballah et al. (2022) reported that cultivars ET1444, Egyptian Yasmine, and Giza177 exhibited similar performance under both non-stress and drought-stress conditions for stomatal conductance and transpiration rate implying their ability to tolerate drought-stress like genotypes G99 and G100 in this current study.

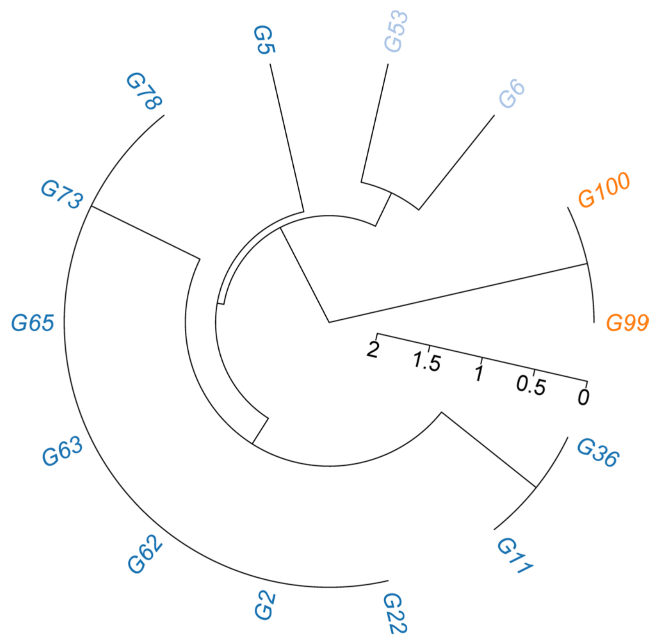

Figure 6. Cluster analysis among the 14 genotypes based on QTLs retrieved from the QTL profiling data obtained from the KASP genotyping of 300 genotypes of the core breeding germplasm of CSIR-CRI, Ghana using KASP-SNP markers according to Asante et al. (2024), 2019. Cluster I [APO (G99) and UPL R17 (G100)]; Cluster II [TogoMarshall (G6) and WAB 2085-TGR2-WAT4-1-1 (G53)]; Cluster III [CRI-AgraRice (G2), ARICA 3 (G5), CRI-Enapa (G11), Jasmine 85- SARI (G22), ART132-35-1-1-B-B (G36), KE40 (G62), ARICA 2 (G63), GR18-SARI (G65), SR35266-2–12-1-1 (G73) and SA68-SARI (G78)].

At 5D, the genotype G63 (waste performing) showed a difference in the mean performance with G6 (best performing) for NDVI. On the other hand, under non-stress conditions G11 (best performing) showed a statistical difference in the mean performance with G6, G2 and G78 (best performing). At 27D, under both water regimes no statistical difference was observed among the worst performing genotypes (G11 and G63) with the best performing genotypes (G53, G5 and G99). Other studies such as (Phyu et al., 2020) reported similar results for NDVI in evaluation of 36 genotypes under non-stress conditions and suggested that NDVI can be used in screening for high yield rice genotypes in tropical agriculture. Till date, few works have been reported about the screening of genotypes using NDVI under drought-stress in rice, however it has been demonstrated that NDVI can be used for high yield wheat genotypes selection under mild drought-stress (Naser et al., 2020), while it is not recommended under severe drought-stress (Thapa et al., 2019).

The results revealed significant variability among the rice genotypes for all the grain yield and yield-related traits under both drought-stress and non-stress conditions, indicating the presence of large variability among the genotypes (Asante et al., 2019) at the reproductive stage, which can be used for effective selection for tolerance to drought-stress among the genotypes used in this study. The approximate heritability values of biomass and grain yield obtained under drought-stress and non-stress conditions in this study show that selection for biomass and grain yield under drought-stress in rice will give outcomes with the same level of precision as under non-stress conditions (Kumar et al., 2008) provided that the screening process is well managed.

In general, the flowering date of all the genotypes was delayed for more than 5-days under drought-stress compared to that under non-stress conditions, except in G99, G100 and G11, which flowered earlier under drought-stress compared to non-stress conditions. These three genotypes that flowered earlier seem to have exhibited drought escape ability to produce grain before the drought became severe at the late maturing stage. Additionally, these three genotypes G99, G100 and G11 exhibited a typical characteristic of drought escape by maintaining high stomatal conductance and transpiration rates associated with effective photosynthesis under drought, resulting in rapid plant development to produce early flowers, as suggested by Kooyers (2015) and Shavrukov et al. (2017). Furthermore, similar observations were made by Sahoo et al. (2023), where at the reproductive stage, DTF were delayed in all genotypes, except

Anjali and N22, which flowered earlier. Moreover, the low leaf drying score obtained for these traits confirmed their predisposition to tolerate drought-stress. A notable reduction in the mean performance for all the genotypes was registered under drought-stress compared to the non-stress conditions for the aboveground biomass and grain yield, except G100, which recorded a similar biomass under non-stress and drought-stress. Similar results for grain yield and other yield-related traits were reported by previous studies (Gaballah et al., 2021, 2022; Huang et al., 2019; Sahoo et al., 2023; Yang et al., 2019).

The negative correlation obtained between grain yield and delay in DTF tended to prove that the genotypes with early flowering under drought-stress compared to the non-stress conditions manifested drought escape abilities rather than 100% tolerance capacities. The LDS, DTF and delay in DTF showed positive correlations among themselves, confirming that the genotypes with early flowering under drought-stress and low leaf drying are better in maintaining their leaf water status to withstand drought-stress by effectively fine adjusting their transpiration rate and stomatal conductance. Grain yield showed a positive correlation with NDVI and RDVI, as reported by Phyu et al. (2020) in a wet season drought-free trial and concluded that NDVI can be used as a screening criterion in varietal selection for high yield. However, in the present study, RDVI has shown more promising results to be used as a screening criterion for selecting high-yielding genotypes under drought-stress. This is confirmed by the regression analysis using grain yield as dependent variable and RDVI where close to 34% of the variability in grain yield is explained by RDVI. The correlation analysis between LDS, DTF, and RDVI implied that these traits can be used as predictors of grain yield and drought-tolerant genotypes.

5. Conclusion

One of the major challenges is to increase rice production under increasing drought as result of climate change, however, the use of drought-tolerant rice cultivars can be one of the solutions as they could secure high yield under drought. This study aimed to assess the impact of drought-stress on physiological and leaf reflectance traits among the rice genotypes and appraise the effectiveness of these traits in selecting the genotypes that showed enhanced tolerance to drought-stress. A progressive decrease in transpiration rate and stomatal conductance was recorded from 5D to 27D on all the genotypes except on G100 and G99 implying increase in water use efficiency in these genotypes. Transpiration rate and stomatal conductance have consistently separated drought-tolerant G99 and G100 out of the 14 genotypes during the 11D, 18D and 27D vegetative stage drought-stress. At reproductive stage drought-stress, regression analysis revealed that 34% of the variability in grain yield is explained by RDVI. The regression analysis coupled with correlation analysis between LDS, DTF, RDVI and grain yield implied that these traits can be used as predictors of grain yield in selecting for drought-tolerant genotypes. Genotypes G100 and G99 were selected as genotypes with enhanced tolerant to drought using MGIDI index based on the relative value of the leaf gas exchange attributes, leaf reflectance parameters, grain yield and yield-related traits under the reproductive stage drought.

Authors' contributions

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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Availability of data and materials

All data generated or analyzed during this study are included in this published article [and its supplementary information files]. Original data can be made available upon request from corresponding author.

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