



RESEARCH ARTICLE

Genetic variability assessment of rice genotypes for drought stress tolerance under controlled conditions

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Abstract

Drought is the major abiotic stress that limits rice productivity in rainfed lowland and upland ecosystems. Rice yields in drought-prone rainfed systems remain low and tend to be unstable. We investigated the response of fifteen rice genotypes under control and drought conditions. Irrigation was given regularly for both the control and drought treatments up to flowering according to their duration and then irrigation was withdrawn for a period of 14 days to create drought conditions. Observations on physiological parameters were taken at this stage. Results revealed that total biomass plant⁻¹, total grain weight plant⁻¹, number of panicles, relative water content, membrane stability index, chlorophyll a, total chlorophyll content and plant height decreased significantly under drought conditions compared to the control condition. Analysis of genotype and genotype × trait exhibited that PC1 and PC2 governed 56.20 % variability under control conditions, while under drought conditions, they accounted for 54.6 % of variability. In drought conditions, the most contributing traits were found to be chlorophyll a content (35.3 %), total grain weight (33.5 %), total chlorophyll content (30 %), plant height (26.6 %), total biomass (24.7 %) and number of panicles (20 %). Since total grain weight governed maximum genetic variability, clustering of genotypes based on relative grain weight resulted in four distinct clusters. Out of 15 rice genotypes, 6 belonged to the efficient cluster, 5 to the moderately efficient cluster, 3 to the moderately inefficient cluster and one was included in the inefficient cluster. The genotypes identified in this study may be utilised as ‘donors’ by the breeders to develop drought-tolerant cultivars in rice.

Keywords: chlorophyll content; drought stress; relative water content; rice; yield

Introduction

Rice is a major staple food crop that feeds nearly half of the world's population and grows best under irrigated conditions. Rice crop mainly depends on rainfall for its water supply under rainfed conditions; drought is the major abiotic stress that limits rice productivity in rainfed lowland and upland ecosystems (1, 2). Water-deficient conditions caused by drought adversely affect the growth and development of rice, disrupting key physiological processes and ultimately leading to a significant decline in its productivity (3). Drought stress was shown to lower rice yield by 25.4 % (4). Ongoing climatic change is expected to further intensify the scenario in rice-growing areas (5, 6). Given the unpredictable rainfall patterns and ongoing climatic irregularities occurring globally, identifying stable and drought-tolerant rice varieties has become highly important (7).

Rapid and specific utilisation of the rich genetic diversity available within rice germplasm is highly critical to guarantee sustainable rice production in the ever-changing climatic conditions (8, 9). Many of the indigenous varieties have the genetic potential to tolerate drought conditions. Rice plants respond to drought through variation in morphological and physiological traits (10, 11). Drought

stress in rice leads to decreased leaf water potential, loss of turgor pressure, stomatal closure and reduced cell growth and division. As a result, vital physiological activities, including photosynthesis, respiration, nutrient uptake, carbohydrate production and translocation, are impaired, causing an overall reduction in plant growth (12). Utilising genetic variability for drought-associated traits can facilitate the development of rice genotypes with enhanced drought tolerance (13, 14).

For the efficacious selection of high-yielding rice genotypes under drought stress, it is necessary to do a proper screening of local indigenous rice genotypes to identify drought-tolerant genotypes. Developing drought-tolerant, high-yielding varieties suited to water-limited environments will be essential for ensuring stable rice production. Thus, the present study aimed to screen the rice genotypes for drought tolerance based on morpho-physiological traits associated with drought tolerance. The variability in morpho-physiological traits among the selected rice genotypes was hypothesised to provide a potential pre-breeding resource for enhancing drought resilience.

Materials and Methods

The study was conducted as a pot culture experiment at the Regional Agricultural Research Station, Ambalavayal, Wayanad, Kerala Agricultural University, during 2023. Fifteen rice genotypes used in the present study consist of land races and improved local strains of Kerala (Table 1). Rice genotypes were raised in 12-inch pots containing laterite soil and maintained inside a net house. All agronomic recommendations of KAU were followed to grow experimental plants. Rice genotypes were evaluated under control and drought conditions using a completely randomized design with three replications. Control plants were irrigated throughout the crop period. In the second set of plants, irrigation was maintained regularly up to flowering, based on crop duration and subsequently withheld for 14 days to induce drought stress. All physiological parameters were recorded at this stage in both control and drought-stressed plants. Re-watering was done after 14 days of drought imposition when the leaves were completely rolled and started drying at tips, margins and irrigation was continued till harvest. At the time of harvest, morphological and yield parameters were recorded.

Soil moisture estimation

During the imposed drought stress, soil moisture was recorded periodically by using the gravimetric method. Samples of soil were collected from the root zone using augur. For each sample, 25 g fresh weight (FW) of soil was taken. After that, soil samples were put at 105 °C and thoroughly dried until a stable dry weight (DW) was obtained. Soil moisture content (%) was determined by using the following formula (15).

Soil moisture estimation

$$= [(Fresh\ weight - Dry\ weight) / Dry\ weight] \times 100 \quad (Eqn. 1)$$

Relative water content (RWC)

Leaf RWC was estimated by recording the turgid weight of 0.5 g fresh leaf samples by keeping them in water for 4 hr, followed by drying in a hot air oven at 70 °C till constant weight was achieved. Relative water content was determined by using the following formula (16).

RWC (%)

$$= [(Fresh\ weight - Dry\ weight) / (Turgid\ weight - Dry\ weight)] \times 100 \quad (Eqn.2)$$

Membrane stability index (MSI)

Fresh leaf green tissue 0.1 g was collected in test tubes having 10 mL DH_2O . Then, in a water bath, these test tubes were placed where 40 and 100 °C temperatures were maintained for 30 and

10 min, respectively. After cooling at room temperature, their individual conductivities (C_1) and (C_2) were recorded with the help of a conductivity meter. The MSI (%) was estimated using the following formula (17).

$$MSI = 1 - (C_1/C_2) \times 100 \quad (Eqn.3)$$

Photosynthetic pigments

For chlorophyll extraction, 25 mg of leaf tissue was put in test tubes containing 4 mL dimethyl sulfoxide (DMSO). Then, these tubes were placed for 4–5 hr at 65 °C in the oven for complete extraction. Thereafter, absorbance values of extracts were determined at 663 and 645 and DMSO was used as a blank. Chlorophyll content was worked out as described earlier (18).

Post-harvest observations

At harvest observations on plant height, total biomass (TBM) per plant, number of panicles per plant (NP) and total grain weight (TGW) per plant were assessed. Relative grain weight of genotypes was calculated as the ratio of total grain weight at drought conditions to the total grain weight at control conditions.

Statistical analysis

The experiment was conducted in a completely randomized design with two factors, water level and genotypes. Data analysis was carried out by two-way analysis of variance (ANOVA). Principal component analysis (PCA) and hierarchical cluster analysis were carried out using statistical software R version 3.5.1 (R Foundation for Statistical Computing, Vienna). Principal component analysis was conducted based on 9 quantitative traits and the parameters that differentiated genotypes for drought tolerance were identified by the components of the eigen vectors. Genotype and genotype $\times$ trait analysis were carried out in R using the package GGE Biplot GUI version 1.0–9. Clustering based on Wards' method was executed using the squared Euclidean distance matrix of genotypic relative grain weight. Graphs were plotted using GraphPad Prism version 5.01 (GraphPad Software, La Jolla, CA, USA).

Results and Discussion

Traits were recorded and data were subjected to analysis of variance. The genotype (G) and water (W) levels significantly ($p < 0.001$) influenced TBM, TGW, NP, chlorophyll a (CHLA), chlorophyll b (CHLB), total chlorophyll content (TCHL) and plant height (PH) in rice genotypes. No significant $W \times G$ interactions were observed for any of the traits except CHLA, CHLB and PH (Table 2). The descriptive statistics show the mean, range and standard

Table 1. List of rice genotypes used for phenotyping under control and drought conditions

Sl. No.	Genotypes	Pedigree
1	Gandhakashala	Landrace
2	Aathira (Ptb 51)	Hybrid derivative-BR51-46-1 $\times$ Cul-23332-2
3	Vadakkan Chitteni	Landrace
4	Vaisakh (Ptb 60)	Re-selection from Ptb-43
5	Kavungin poothala	Landrace
6	Deepthi	Pureline selection from the local variety Edavaka
7	Veliyan	Landrace
8	Parambuvettan	Landrace
9	Sabari (Ptb 40)	Hybrid derivative-Ptb35 $\times$ IR8/2
10	Jyothi (Ptb 39)	Hybrid derivative-Ptb10 $\times$ IR8
11	Bharathi (Ptb 41)	Hybrid derivative-Ptb10 $\times$ IR8
12	Karutha modan	Landrace
13	Kanali	Landrace
14	Aswathy (Ptb 37)	Hybrid derivative-Ptb10 $\times$ Dee-Geo-Woo-Gen
15	Thavalakkannan	Landrace

Table 2. Analysis of variance (ANOVA) for different traits obtained from fifteen rice genotypes grown under control and drought conditions

Traits	F-value		
	W	G	WXG
Total biomass (g plant ⁻¹)	15.73***	41.17***	0.72
Total grain weight (g plant ⁻¹)	34.40***	12***	0.87
Number of panicles	21.81***	19.80***	1.83
Relative water content (%)	273.43 ***	0.53	0.31
Membrane stability index (%)	178.65***	1.31	0.86
Total chlorophyll content (mg g ⁻¹ FW)	96.26***	51.05***	2.31*
Chlorophyll a content (mg g ⁻¹ FW)	254.49***	57.13***	10.12***
Chlorophyll b content (mg g ⁻¹ FW)	15.82***	30 ***	11.27***
Plant height (cm)	110.08***	72.70***	12.38***

* $p < 0.05$; ** $p < 0.01$, *** $p < 0.001$; W: water level; G: accession; WXG: interaction between W and G.

deviation (SD) for each trait under control and drought conditions (Table 3). Results revealed that TBM, TGW, NP, RWC, MSI, CHLA, TCHL and PH were decreased significantly in the drought compared to the control condition.

Significant reduction of TBM (8.3 %) was observed under drought compared to the control condition (Table 3). Research has demonstrated that drought stress leads to a significant reduction in total dry weight (19–21). Total biomass is closely linked to water stress and may be used as a reliable measure of drought tolerance (22). The reduction in biomass under drought conditions may be attributed to the plants allocating less photosynthates to culm and leaf growth under limited soil moisture, which helps minimise water loss through transpiration and enhances their chances of survival (19). Under drought conditions, rice genotype Parambuvettan (84.17 g) recorded higher TBM followed by Vaisakh (67.92 g) and Vadakkan Chitteni (57.22 g), whereas under control conditions rice genotype Parambuvettan (95.83 g) recorded higher TBM followed by Vaisakh (72.92 g) and Thavalakkannan (66.94 g). The minimum value of TBM was recorded by rice genotype Gandhakashala under both drought (28.44 g) and control (34.44 g) conditions.

Total grain weight decreased significantly by 12.8 % under drought conditions compared to the control (Table 3). Reports indicate that grain yield declined due to a reduced supply of water (19, 23, 24). Water deficit occurring just before and during the early flowering stage reduced the number of fertile spikelets, leading to a decline in the final grain yield (25). Among the rice genotypes evaluated, Parambuvettan (19.50 g) recorded the highest TGW under drought, followed by Kanali (17.50 g) and Kavungin Poothala (17.22 g). Under control conditions, Parambuvettan (20.00 g) again

exhibited the highest TGW, followed by Jyothi (19.72 g) and Kanali (18.75 g). The lowest TGW was observed in Thavalakkannan (10.25 g) under drought and Karutha modan (11.75 g) under control conditions. The extent of grain yield reduction is influenced by the duration and intensity of drought stress, as well as the growth stage of the crop (26, 27). Similarly, the number of panicles decreased by 11.8 % under drought conditions compared to the control (Table 3). Research has demonstrated that rice plants exposed to drought stress tend to have reduced NP compared to those under normal conditions (20, 28, 29). Research indicates that it is one of the traits known to be sensitive to drought during the anthesis stage (30, 31). Under drought, the genotype Parambuvettan (6.0) recorded the highest NP, whereas under control conditions, Jyothi (7.0) exhibited the highest NP.

Relative water content serves as a crucial measure of cell and tissue hydration, which is essential for maintaining optimal physiological activities and growth processes (32). A significant reduction of 19.8 % in RWC was recorded under drought conditions compared to the control (Table 3). This is in agreement with the results that drought-stressed rice plants exhibit a lower RWC than non-stressed rice plants (21, 29, 33). Studies have shown that genotypes exhibiting a smaller decline in RWC possess greater tissue tolerance potential under drought conditions (34, 35). Under drought, the rice genotype Karutha modan (73.69 %) exhibited the highest RWC, followed by Kanali (72.47 %). The lowest RWC was observed in Gandhakashala (65.92 %) under drought conditions. Research has demonstrated that varieties capable of maintaining higher RWC are likely to preserve protoplast hydration for a longer duration under drought stress, thereby supporting sustained productivity (36). Reports indicated that RWC contributed most positively and directly to yield performance (37). Rice genotypes with higher RWC have developed adaptive traits that enhance their tolerance to moisture stress conditions (23).

Reduction in MSI (15.8 %) was recorded under drought conditions when compared to the control condition (Table 3). Evidence suggests that MSI showed significant variation under drought stress (38). The maximum value of MSI was recorded by rice genotype Parambuvettan under both drought (78.32 %) and control (88.49 %) conditions. These results concur with previous findings that indigenous landraces exhibited significantly higher RWC and MSI than the susceptible check under drought stress, suggesting that they may possess mechanisms to prevent membrane damage and maintain leaf turgor pressure during water deficit conditions (38). Studies have shown that mung bean genotypes exhibiting higher MSI possess acquired traits that confer tolerance to moisture stress conditions (39). The association between MSI and rice yield under drought conditions has been investigated (40).

Table 3. Descriptive statistical results for different traits obtained from fifteen rice genotypes grown under control and drought conditions

Traits	Control			Drought		
	Mean	Range (Min–Max)	SD	Mean	Range (Min–Max)	SD
Total biomass (g plant ⁻¹)	54.88	34.44–95.83	15.25	50.35	28.44–84.17	13.34
Total grain weight (g plant ⁻¹)	16.53	11.75–20.00	2.39	14.42	10.25–19.50	2.59
Number of panicles	4.49	3.00–7.00	1.21	4.0	2.00–6.00	0.99
Relative water content (%)	87.05	84.26–88.65	1.10	69.80	65.92–73.69	2.36
Membrane stability index (%)	86.76	84.46–88.49	1.17	73.02	66.51–78.32	3.98
Total chlorophyll content (mg g ⁻¹ FW)	5.31	3.73–6.58	0.98	4.67	3.56–6.14	0.87
Chlorophyll a content (mg g ⁻¹ FW)	3.74	2.37–4.83	0.70	2.92	1.84–4.41	0.91
Chlorophyll b content (mg g ⁻¹ FW)	1.57	0.85–2.33	0.50	1.75	0.58–2.52	0.58
Plant height (cm)	97.42	76.67–126.67	17.47	87.53	59.33–114.00	16.17

Chlorophyll is essential for photosynthesis in rice and serves as a reliable indicator of photosynthetic efficiency. Significant influence of both environment and genotype was observed on leaf chlorophyll content, aligning with earlier studies that reported alterations in leaf pigments under drought conditions (19, 21). Findings indicate that rice plants subjected to drought conditions experience accelerated chlorophyll degradation and impaired photosynthesis, culminating in a pronounced reduction in chlorophyll content (34). The decline in relative chlorophyll content was likely linked to oxidative damage in the chloroplasts, which led to lipid membrane peroxidation in the thylakoids and consequently promoted chlorophyll degradation (41). A reduction of 12.0 % in TCHL was observed under drought compared to the control (Table 3). Among genotypes, Aathira recorded higher TCHL under both drought (6.14 mg g⁻¹ FW) and control (6.58 mg g⁻¹ FW) conditions. The lowest values were observed in Kavungin Poothala (3.56 mg g⁻¹ FW) under drought and Vaisakh (3.73 mg g⁻¹ FW) under control conditions.

Chlorophyll a content decreased by 21.9 % under drought (Table 3). These results are in agreement with earlier reports in rice (29, 33, 42), where a significant decrease in the CHLA and TCHL was observed in plants exposed to drought conditions. Aathira (4.41 mg g⁻¹ FW) and Deepthi (4.36 mg g⁻¹ FW) exhibited higher values under drought, whereas Gandhakashala (4.83 mg g⁻¹ FW) and Aathira (4.56 mg g⁻¹ FW) showed higher values under control. The minimum CHLA content was recorded in Sabari (1.84 mg g⁻¹ FW) under drought and Jyothi (2.37 mg g⁻¹ FW) under control. In contrast, CHLB content increased significantly by 11.5 % under drought (Table 3). Veliyan (2.52 mg g⁻¹ FW) and Thavalakkannan (2.44 mg g⁻¹ FW) exhibited higher values under drought, while Jyothi (2.33 mg g⁻¹ FW) and Vadakkan Chitteni (2.24 mg g⁻¹ FW) recorded higher values under control. Vaisakh showed the lowest CHLB content under both drought (0.58 mg g⁻¹ FW) and control (0.85 mg g⁻¹ FW) conditions.

Plant height showed a 10.2 % reduction under drought conditions relative to the control (Table 3). This observation aligns with findings that drought-stressed rice plants generally display lower PH compared to non-stressed plants (19, 20, 28). Reduction in height is mainly due to restricted cell elongation and turgor loss (43). Under drought conditions, Vaisakh (114.00 cm) and Kavungin Poothala (110.33 cm) recorded the highest PH, while under control conditions, Thavalakkannan (126.67 cm) and Karutha modan (121.67 cm) exhibited superior performance. The growth response of rice genotypes at control and drought conditions was analysed by PCA, taking into consideration the variables as mentioned in Table 3. Analysis of genotype and genotype × trait exhibited that PC1 and PC2 governed 56.20 % variability at control condition while at drought condition, it accounted for 54.6 % of variability (Fig. 1). At drought condition, the most contributing traits were found to be CHLA (35.3 %), TGW (33.5 %), TCHL (30 %), PH (26.6 %), TBM (24.7 %) and NP (20 %). In the control condition, the most contributing traits were found to be TGW (36.2 %), TCHL (27.7 %), CHLA (26 %), NP (24.8 %), PH (22.5 %) and CHLB content (22 %).

Since total grain weight governed maximum genetic variability, clustering of genotypes based on relative grain weight resulted in four distinct clusters. The four clusters were as efficient (average values for relative grain weight 95.05), moderately efficient (average values for relative grain weight 86.03), moderately inefficient (average values for relative grain weight 79.09) and inefficient (average values for relative grain weight 70.01). Out of

15 rice genotypes, 6 belonged to the efficient cluster, 5 to the moderately efficient cluster, 3 to the moderately inefficient cluster and one to the inefficient cluster (Fig. 2). Significant variation was observed among these clusters. In genotypes belonging to the efficient cluster, the reduction in TBM was 6.60 %, whereas in the inefficient cluster, it was 19.71 %. Similarly, efficient, moderately efficient, moderately inefficient and inefficient clusters showed a reduction in TGW at drought as compared to the control condition by 4.93, 13.94, 20.87 and 29.98 %, respectively. Drought resulted in a reduction in NP, which was 29.03 % in the inefficient cluster against 5.64 % in the efficient cluster (Fig. 3). An efficient cluster consists of 6 rice genotypes: Karutha modan, Kanali, Parambuvettan, Vadakkan Chitteni, Kavungin poothala and Vaisakh. According to the phenotypic data, the MSI of this subpopulation is found to range between 72.50–78.32 % under drought conditions, RWC ranged between 68.51–73.69 %, TCHL ranged between 3.63–5.32 mg g⁻¹ FW, TBM ranged between 34.83–84.17 g plant⁻¹ and TGW ranged between 11.08–19.50 g plant⁻¹ (Fig. 3). Studies focusing on root traits associated with drought tolerance in rice have identified Karutha modan, Parambuvettan, Kavungin poothala and Vaisakh as drought-tolerant genotypes (44). Research indicates that the Kavungin poothala genotype develops deeper and thicker root systems under drought stress (45).

A moderately efficient cluster consists of 5 rice genotypes: Aathira, Bharathi, Deepthi, Veliyan and Sabari. According to the phenotypic data, the MSI of this subpopulation is found to range between 70.00–76.34 % under drought conditions, RWC ranged between 67.66–72.02 %, TCHL ranged between 3.79–6.14 mg g⁻¹ FW, TBM ranged between 40.14–52.78 g plant⁻¹ and TGW ranged between 12.11–16.06 g plant⁻¹ (Fig. 3). The moderately inefficient cluster consists of 3 rice genotypes: Gandhakashala, Jyothi and Aswathi. According to the phenotypic data the MSI of this subpopulation is found to range between 66.60–71.00 % under drought condition, RWC ranged between 65.92–67.35 %, TCHL range between 4.00–5.80 mg g⁻¹ FW, TBM ranged between 28.44–49.17 g plant⁻¹ and TGW ranged between 11.89–15.69 g plant⁻¹ (Fig. 3). Findings indicate that both Jyothi and Aswathi genotypes exhibited low water use efficiency (45).

An inefficient cluster consists of one rice genotype, Thavalakkannan. According to the phenotypic data, the MSI of this subpopulation is found to be 66.51 % under drought conditions, RWC is 68.43 %, TCHL is 4.66 mg g⁻¹ FW, TBM is 53.75 g plant⁻¹ and TGW is 10.25 g plant⁻¹ (Fig. 3). This genotype is reported to be drought-susceptible (44). The Thavalakkannan genotype also possesses shallow root systems under drought stress, which may be associated with its drought-susceptible nature (45). Among fifteen genotypes, Parambuvettan consistently exhibited superior performance under drought, maintaining higher biomass, grain weight, panicles, MSI values and can be considered a drought-tolerant genotype. Parambuvettan maintained the highest grain yield under water-stressed conditions and can therefore be recommended for detailed investigation to elucidate the mechanisms that enable plants to withstand the adverse effects of drought. The Parambuvettan genotype was reported to exhibit high water-use efficiency (45). Parambuvettan genotype also possess high root to shoot ratio under drought stress (46). By exploring the genetic basis and variation of these traits under stress conditions, breeders can more efficiently develop resilient rice varieties that maintain productivity in unfavourable environments.

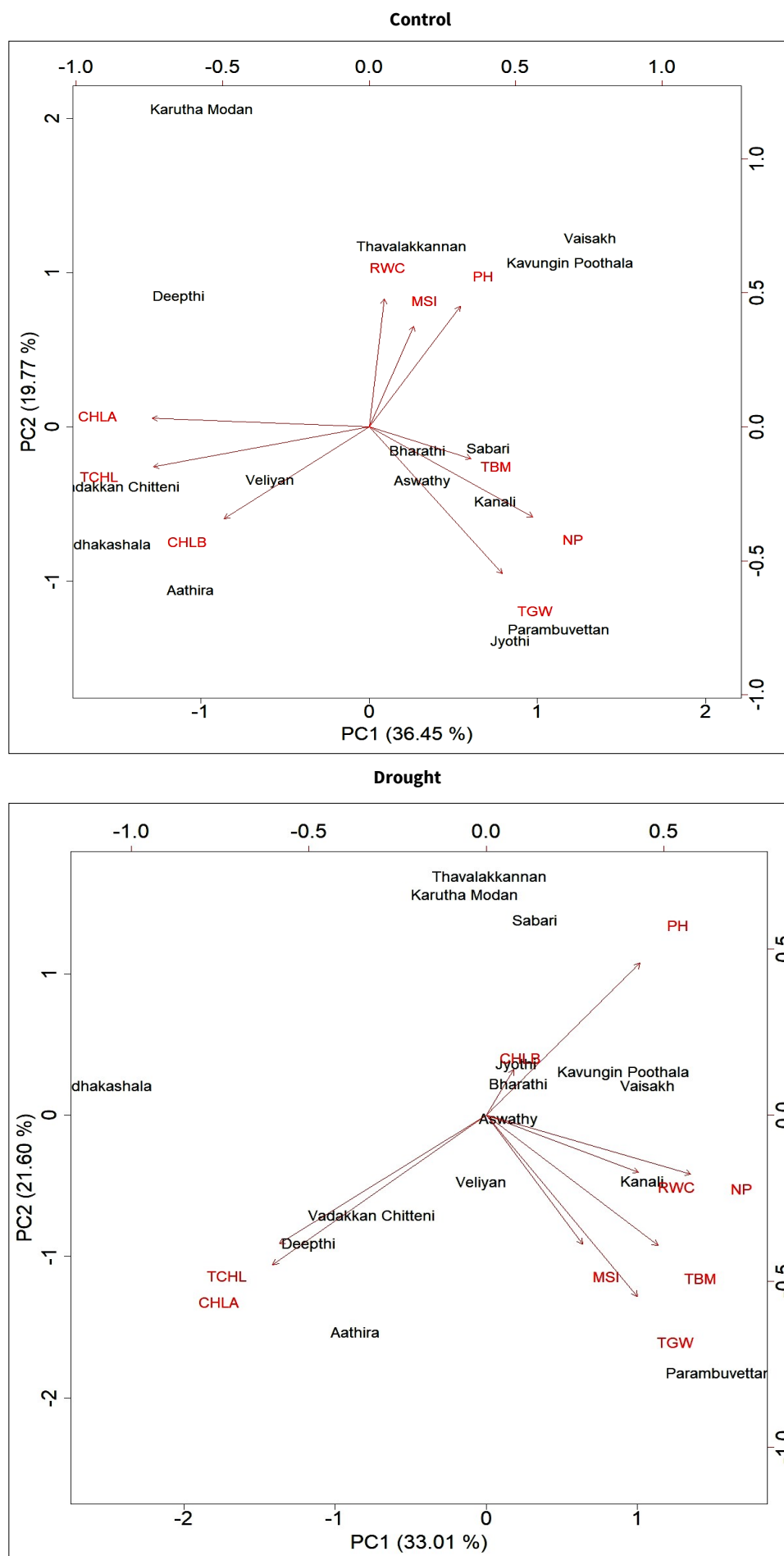


Fig. 1. Genotype and genotype $\times$ trait interaction biplot at control and drought conditions. TBM: total biomass per plant; TGW: total grain weight per plant; NP: number of panicles; RWC: relative water content; MSI: membrane stability index; CHLA: chlorophyll a; CHLB: chlorophyll b; TCHL: total chlorophyll content; PH: plant height.

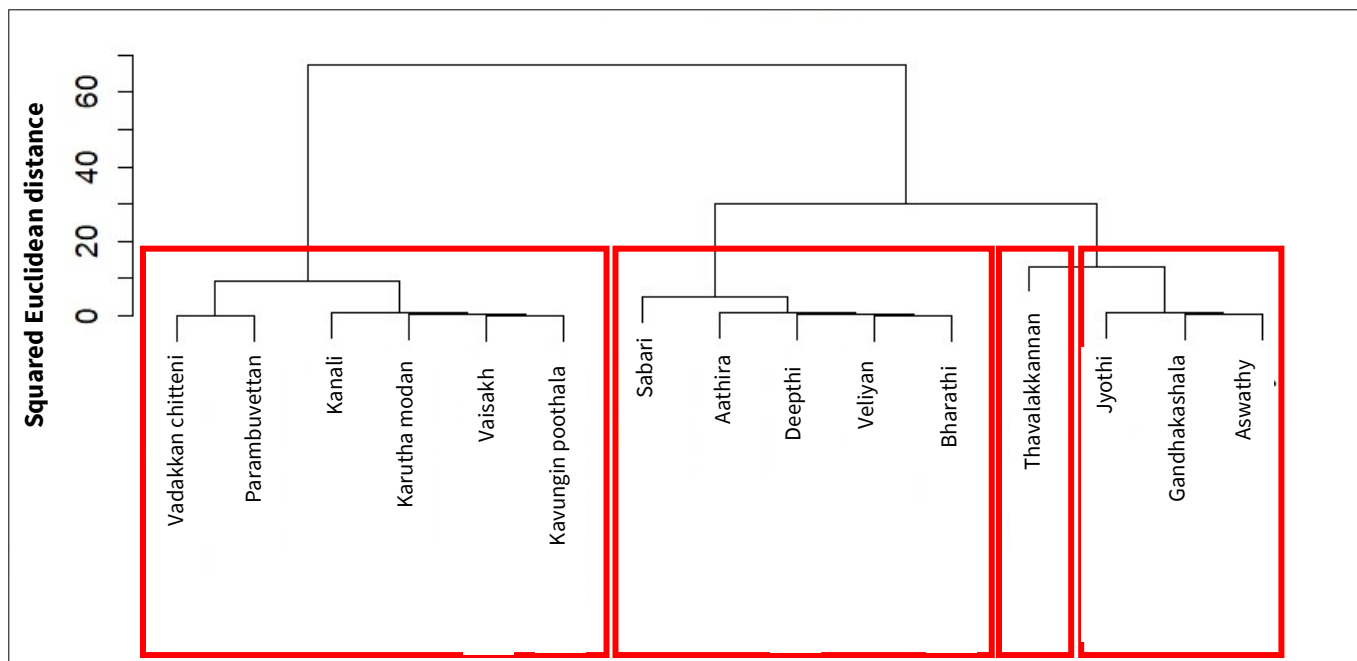


Fig. 2. Clustering of 15 rice genotypes by Wards' method using the squared Euclidean distance matrix of relative grain weight. Plants were grown in control and drought conditions.

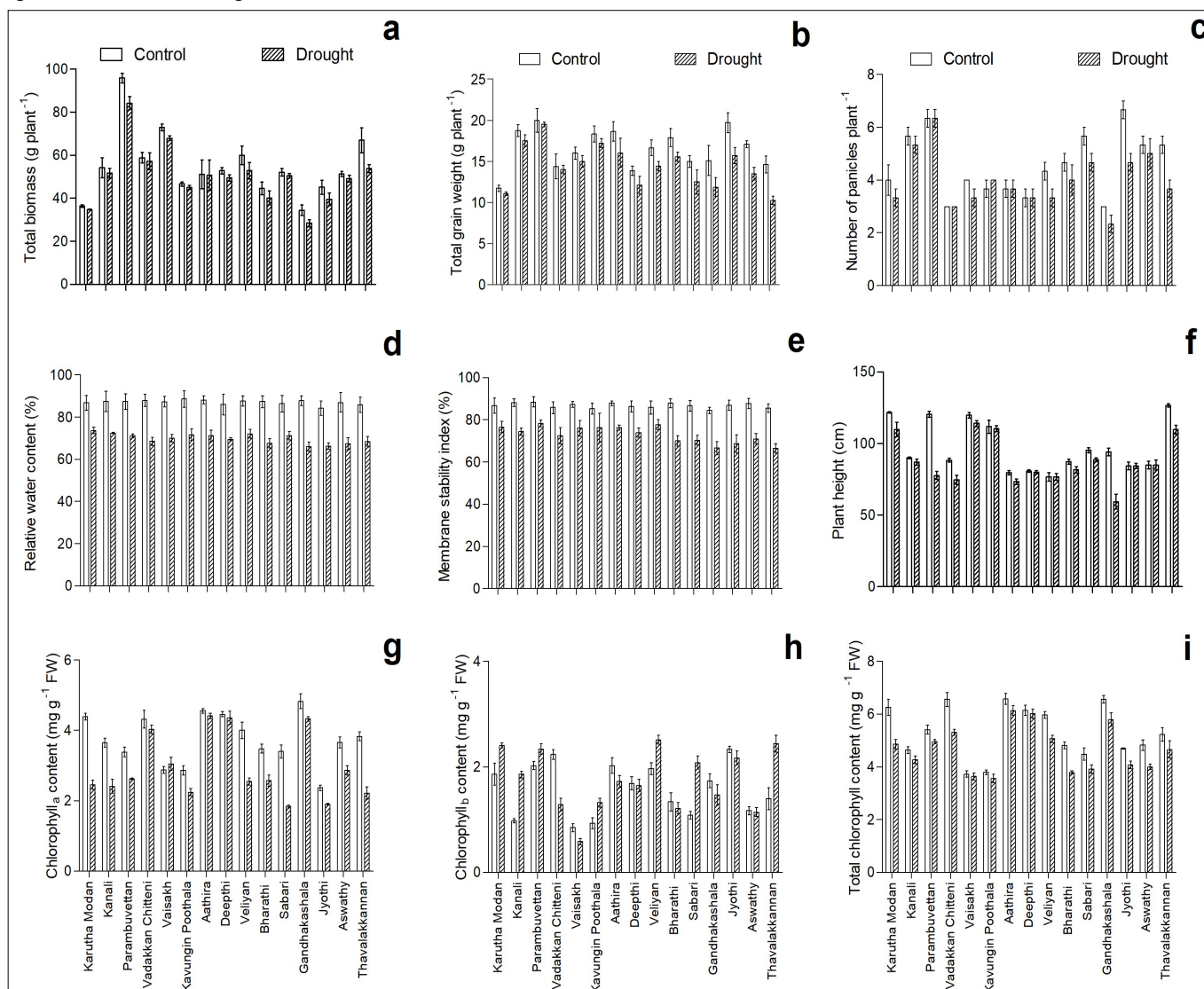


Fig. 3. Difference in rice genotypes with respect to (a) total biomass per plant; (b) total grain weight per plant; (c) number of panicles; (d) relative water content (RWC); (e) membrane stability index (MSI); (f) plant height; (g) chlorophyll a; (h) chlorophyll b; (i) total chlorophyll content.

Clustering of genotypes was performed based on Ward's method using squared Euclidean distance matrix of genotypic relative grain weight. C I: efficient; C II: moderately efficient; C III: moderately inefficient; C IV: inefficient.

Conclusion

The present study offers comprehensive insights into the mechanisms underlying drought tolerance in rice by thoroughly analysing physiological and morphological traits under contrasting water levels. Drought stress significantly reduced growth and yield-related traits in rice, but the magnitude of reduction varied widely among genotypes. Efficient genotypes (Karutha modan, Kanali, Parambuvettan, Vadakkan chitteni, Kavungin poothala and Vaisakh) showed minimal reductions in TBM, TGW and NP, demonstrating superior physiological stability under drought stress. The genotypes identified in this study may be utilised as 'donors' by the breeders to develop drought-tolerant cultivars in rice.

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Authors' contributions

PRS contributed to conceptualisation, funding acquisition, investigation, methodology, project administration, resources, supervision, writing - original draft and writing - review and editing. TM, MAR, SK and PSJ were involved in methodology and writing - review and editing. KAK and CKYV were responsible for project administration, resources and supervision. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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