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Analysis of agro-morphological, physio-biochemical, and genetic diversity in some selected landraces of rice during early drought stress

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Abstract: Drought severely threatens global rice production, particularly in West Bengal's red-lateritic zone, where early-stage physiological drought from erratic rainfall persists. In this study, twenty-two landraces from West Bengal and seven check varieties were subjected to drought stress at seedling stage for fifteen days under field condition. The landraces were chosen collected according to their better ability and response to *in vitro* screening. The seedlings were planted in 30 cm plastic pots and placed under a temporary plastic shade to allow controlled water supply. Root and shoot length (RL, SL), seedling-vigor-index (SVI), root fresh and dry weight (RFW, RDW), leaf rolling (LR), leaf drying (LD), proline (PRO), total carbohydrate (TCC), chlorophyll (CHL) and relative water content (RWC) were recorded. After 15 days of stress, re-watering was done and subsequently survivability percentage (SP), days to 50% flowering (DF) and maturity (DM), 100-seed-weight (SW), seed yield/plant (SY/P) were noted. Significant positive correlation was found between SP, SW, DM, DF, RFW, SL, PRO, TCC, CHL, RWC, and SY/P whereas LD, LR were found to be negatively correlated with SY/P. T-test revealed that sixteen germplasms did not vary significantly in SY/P. Landrace Morogjhota outperformed the check varieties in yield. Genetic diversity analysis with twenty drought-associated SSR markers divided the germplasms into two main clusters with a PIC value of 0.544 – 0.907. From this study, sixteen lines were identified among which five landraces showed high Drought Resistance Index which can be used as parent material in developing climate-resilient varieties and can also be used to detect major QTLs associated with drought tolerance.

Keywords: Agro-morphological, physio-biochemical characters, Drought resistance, Rice Landrace, SSR markers.

Introduction

Rice, sustaining over half the world's population, is vital in South and Southeast Asia (Panda et al., 2021), with India ranking second globally in production. West Bengal (WB) is known as the 'bowl of rice' in India because maximum agricultural land in this state is under rice cultivation (Sinha and Mishra, 2012). Purulia, the western-most district of WB, which comes under red lateritic plateau area, where around 2.5 lakh hectares (ha) of land is used for rice cultivation during the Kharif season (NABARD, GOI). However, in 2014, drought-affected 52.84% of the area, impacting over 95 thousand farmers due to erratic rainfall patterns. The lateritic soil's low water retention capacity intensifies moisture stress, hindering crop growth (Parab et al., 2021). De et al. (2023) noted data on rainfall and temperature patterns in Purulia district from 2014 to 2022, focusing on 2021 and 2022. The data, obtained from the Bureau of Applied Economics and Statistics, Government of West Bengal, highlighted the erratic rainfall pattern and its role in causing water stress during the seedling stage of Kharif rice growth. The main focus of this study is to identify germplasms resilient to early rainfall cessation, ensuring robust yields comparable to irrigated conditions.

Till date, no major gene or QTL (Quantitative Trait Loci) associated with drought tolerance has been discovered (Panda et al., 2021; Rahman and Zhang, 2016), though several minor genes/QTLs have been found to express themselves when drought stress is induced. There is also a lack of true drought-tolerant varieties (Rahman and Zhang, 2016). Different studies suggest yield to be the ultimate selection criterion in screening for drought-tolerant lines (Kumar et al., 2009; Dixit et al., 2014; Pandey and Shukla, 2015). Several root characters, leaf traits, physiological and biochemical factors also happen to play a major role in increasing the overall production during drought stress. In addition, root length and root weight are found to be important in drought-tolerant germplasm (Feng et al., 2012; Nahar et al., 2018). Likewise, leaf rolling and drying

Table 1. Leaf rolling, Leaf drying score, Survivability percentage under drought stress, Days to 50% flowering, and Days to maturity under stress and control condition.

Sl no.	Germplasm	LR	LD	SP (%)	DF (days)		DM (days)	
					Stress	Control	Stress	Control
1	Sahabhagidhan	7.00	3.00	76.92	80.33	75.33	107.67	101.00
2	DRR 44	5.67	1.67	80.00	100.33	95.67	127.00	120.67
3	Lalat	5.67	1.00	70.83	119.33	104.67	140.67	125.33
4	DRR 42	3.67	3.67	78.38	96.33	88.67	131.67	123.33
5	Nagina 22	5.33	3.67	82.35	91.33	86.33	118.00	112.67
6	Kerala Sundari	4.33	7.00	65.12	114.67	100.67	145.00	126.33
7	Vandana	5.33	1.00	80.95	67.33	64.33	95.67	90.33
8	Bhuri	8.33	8.33	0.00	0.00	104.33	0.00	135.33
9	Maniksal	3.00	3.67	83.33	75.33	67.67	100.33	95.33
10	Chhotodidi	3.33	3.00	84.21	110.00	103.33	132.67	126.33
11	Lohasal	5.67	4.33	77.10	71.67	64.67	105.67	94.67
12	Aswinsal	3.67	3.67	85.18	104.00	96.00	139.67	121.67
13	Morogjhota	5.00	1.67	83.10	109.67	98.67	134.33	127.67
14	Sonagori	8.33	3.67	83.94	74.67	68.67	97.67	95.67
15	Tulsikamal	3.00	1.00	89.47	116.33	109.67	144.33	136.00
16	Bhundi	7.67	4.33	80.10	120.67	106.33	142.33	132.33
17	Bhadoi	8.67	8.33	77.10	62.00	56.33	97.33	88.00
18	Kelesh	6.33	7.67	84.33	79.33	71.67	113.67	97.67
19	Vasamanik-i	8.33	7.67	0.00	0.00	106.67	0.00	129.67
20	Velchi-i	5.67	3.67	82.87	78.33	69.67	109.00	101.67
21	Langalmathi	9.00	7.00	0.00	0.00	105.00	0.00	129.67
22	Bhramarmali	8.33	5.67	72.98	103.67	90.67	141.00	126.00
23	Chandrakanti	7.67	5.00	73.12	100.67	90.33	133.33	115.67
24	Kalpana	9.00	8.33	0.00	0.00	100.67	0.00	124.67
25	Neta	5.33	5.67	80.56	105.33	92.00	131.33	120.67
26	Lakkansal	5.33	3.67	82.10	103.00	92.67	138.33	122.67
27	Vutmuri	3.00	1.00	83.45	94.00	72.33	128.33	99.33
28	Kashiphool	8.33	7.00	0.00	0.00	92.00	0.00	117.67
29	Swarna	8.67	8.33	43.75	142.00	122.67	167.67	141.33

are also associated with water stress conditions (Pandey and Shukla, 2015; Beena et al., 2012). Several other agro-morphological characters like days to flowering, days to maturity are also considered to assess the drought tolerance of a germplasm (Pantuwanet al., 2002; Kumar and Kujur, 2003). Biochemical factors like accumulation of proline (Nasrin et al., 2020), change in carbohydrate content (Diennet al., 2019) and physiological factors like reduction in chlorophyll (Nahakpam, 2017) and relative water content (Ahmadikhah and Marufinia, 2016) under drought stress has also been previously mentioned.

In India, the release of drought-tolerant varieties is limited. Landraces, with a broader genetic base, serve as a valuable gene bank for stress tolerance. This study aims to identify highly drought-tolerant germplasms, crucial for developing climate-resilient, stress-tolerant, and high-yielding rice varieties. Beyond regional impact, this effort could contribute globally to areas facing increasing drought challenges, preserving endangered landraces and uncovering key genes for drought tolerance.

Results

Among the landraces, Vasamanik, Kalpana, Langalmathi, Kashiphool, and Bhuri, did not survive after the removal of stress conditions, indicating their vulnerability to water stress. Consequently, assessments of post-rehydration parameters including SP, DF, DM, 100 SW, SY/P were not carried out for these varieties.

Roots were collected carefully (Supplementary Fig. 1) and it was noticed that application of stress significantly increased root length when compared with that of controlled conditions for each germplasm (Supplementary Fig. 2). Conversely, shoot length was reduced under stressed condition (Supplementary Fig. 2). Key indicators including Seedling Vigour Index (SVI), Root Fresh Weight (RFW), and Root Dry Weight (RDW) also found to be diminished under stress for majority of the germplasms. In addition, over accumulation of Proline (PRO), decrease in Relative Water Content (RWC) and Chlorophyll content (CHL) was noticed under stress condition. However, Total Carbohydrate Content (TCC) varied greatly among the germplasms.

Noteworthy, resilience was recorded in landraces like Tulsikamal, Aswinsal, Kelesh, Chhotodidi, Sonagori, Vutmuri, Maniksal, and Morogjhota with SP peaking between 83-89%. Drought-tolerant established varieties exhibited Survivability Percentage (SP) of 77-83%, while the drought-susceptible Swarna showed a 43.75% rate. Furthermore, it was revealed that values of Days to Flowering (DF) and Days to Maturity (DM) were increased under stress (Table 1). However, weight of 100 seeds was found to be unaffected.

Table 2. Correlation study between agro-morphological characters under stress condition.

	CORRELATION MATRIX: SEEDLING STAGE															
Pearson's correlation	**SL**	**RL**	**SVI**	**RFW**	**RDW**	**LR**	**LD**	**SP**	**DF**	**DM**	**SW**	**PRO**	**RWC**	**CHL**	**TCC**	**SY/P**
SL	1															
RL	.197	1														
SVI	.774**	.739**	1													
RFW	.481**	.184	.458*	1												
RDW	-.019	.389*	.244	.636*	1											
LR	.551**	-.197	-.504**	-.690**	-.268	1										
LD	-.629**	-.063	-.413*	-.656**	-.116	-.681**	1									
SP	.432*	.107	.360	.732**	.410*	-.640**	-.661**	1								
DF	.198	-.067	.094	.495**	.322	-.521**	-.498**	.818**	1							
DM	.206	-.016	.136	.527**	.363*	-.540**	-.505**	.869**	.990**	1						
SW	.400*	.011	.277	.668**	.426*	-.547**	-.538**	.895**	.819**	.848**	1					
PRO	.418*	.045	.322	.658**	.361	-.705**	-.674**	.787**	.626**	.649**	.707**	1				
RWC	.510**	.140	.442*	.703**	.416*	-.506**	-.622**	.859**	.659**	.700**	.766**	.720**	1			
CHL	.278	.096	.247	.595**	.396*	-.545**	-.533**	.662**	.546**	.573**	.533**	.561**	.520**	1		
TCC	.298	.048	.212	.643*	.421*	-.417*	-.423**	.787**	.598**	.635**	.676**	.675**	.770**	.570**	1	
SY/P	.419*	.047	.317	.719**	.331	-.633	-.745**	.965**	.799**	.839**	.859**	.813**	.869**	.667**	.735**	1

Note: *significant at 5% level, ** significant at 1% level.

Table 3. Drought Resistance Index of selected 17 germplasms.

Sl no.	Germplasm	DRI
1	DRR 44	1.23
2	DRR 42	1.19
3	Vandana	1.26
4	Sahabhagidhan	1.19
5	Nagina 22	1.28
6	Morogjhota	1.36
7	Lakkansal	1.38
8	Tulsikamal	1.34
9	Kelesh	1.31
10	Bhundi	1.28
11	Velchi	1.32
12	Vutmuri	1.37
13	Chhotodidi	1.34
14	Maniksal	1.32
15	Sonagori	1.37
16	Aswinsal	1.38
17	Lohasal	1.09

Table 4. No. of alleles, PIC Value, gene diversity, and No. of effective alleles of 20 SSR primers.

Sl no.	Primer name	No of alleles	PIC	Gene diversity	No of effective alleles
1	RM279	9	0.847	0.847	6.519
2	RM555	10	0.868	0.868	7.577
3	RM263	10	0.863	0.863	7.313
4	RM431	6	0.809	0.809	5.224
5	RM324	9	0.857	0.867	7.509
6	RM3549	10	0.857	0.867	7.509
7	RM250	11	0.844	0.844	6.420
8	RM3212	7	0.770	0.786	4.672
9	RM416	7	0.754	0.754	4.063
10	RM16030	6	0.797	0.797	4.918
11	RM19367	5	0.702	0.702	3.351
12	RM3805	6	0.544	0.713	3.490
13	RM589	9	0.802	0.864	7.377
14	RM204	8	0.804	0.804	5.097
15	RM28166	8	0.805	0.843	6.371
16	RM28052	13	0.907	0.964	28.033
17	RM28048	4	0.590	0.590	2.438
18	RM28199	8	0.823	0.847	6.519
19	RM28076	8	0.804	0.804	5.097
20	RM1261	14	0.844	0.844	6.420

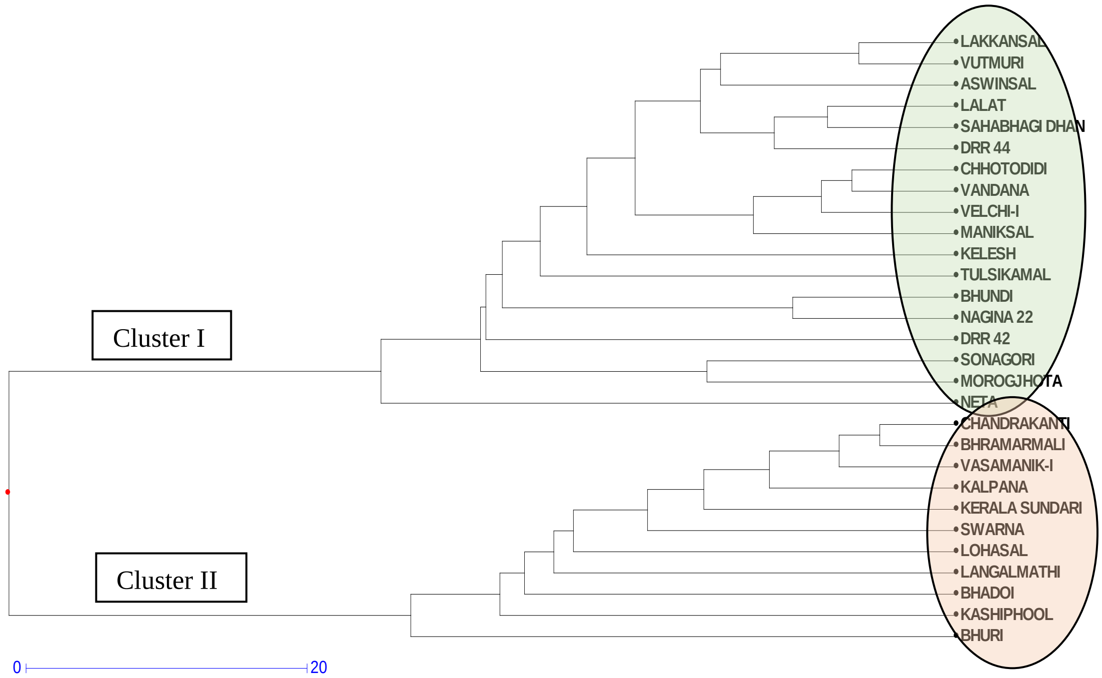

Fig. 1 Dendrogram generated from UPGMA cluster analysis of 29 germplasms based on Jaccard's dissimilarity coefficient using SSR marker data distributed the germplasms within 2 clusters, where Cluster I represents the tolerant ones and Cluster II comprises of the susceptible germplasms.

Variability study

ANOVA, assessing variations in traits across individuals, revealed non-significant differences within replications (Supplementary Table 3). To estimate variation among the 29 germplasms, grand mean, Coefficient of Variation (CV), and Critical Difference (CD) at a 5% significance level were recorded (Supplementary Table 4). Highest CV was observed in Leaf Drying (LD), followed by Root length (RL), Shoot Length (SL), RFW, SVI, TCC, RDW, Leaf Rolling (LR), PRO, Seed Yield/ Plant (SY/P), and RWC. Whereas the lowest was observed in CHL, DM, 100 Seed Weight (100 SW), DF, and SP. Morogjhota outshone the checks in seed yield, while 10 others (Lakkansal, Tulsikamal, Kelesh, Bhundi, Velchi, Vutmuri, Chhotodidi, Maniksal, Sonagori, Aswinsal) matched up under stress. Based on the CD value for seed yield/plant (2.81) those germplasms were grouped in two classes (Supplementary Table 5). For better understanding a t-test was done to compare the means of seed yield/plant for all the germplasms in treated and control condition (Supplementary Table 6). Lines those did not significantly differ in seed yield/plant in treated and control condition were Morogjhota, Lakkansal, Tulsikamal, Kelesh, Bhundi, Velchi, Vutmuri, Chhotodidi, Maniksal, Sonagori, Aswinsal and the check varieties Sahabhagidhan, DRR 44, Nagina 22, Vandana. SY/P for tolerant check DRR 42 and landrace Lohasal varied significantly at 5% level of significance.

Correlation study

Correlation study showed that seed yield/plant showed a high positive significant correlation with SP, 100 SW, DM, DF, RFW, PRO, RWC, CHL and TCC, moderate positive significant correlation with SL, and high negative correlation with leaf drying and leaf rolling (Table 2).

Drought resistance index

The DRI was estimated for the selected germplasms following the formula led by Fisher and Maurer, 1978. (Table 3). Highest DRI was observed in Aswinsal and Lakkansal (1.38), followed by Vutmuri and Sonagori (1.37). Morogjhota (1.36), Chhotodidi and Tulsikamal (1.34), Velchi and Maniksal (1.32), Kelesh (1.31), Bhundi and Nagina 22 (1.28), Vandana (1.26), DRR 44 (1.23), Sahabhagidhan and DRR 42 (1.19) and Lohasal (1.09). In case of DRI, the landraces had a higher value than almost all the check varieties.

Genetic diversity study

Twenty SSR markers were used for understanding the genetic diversity between the 29 germplasms (Supplementary Fig 3 and 4). PIC value ranged from 0.544 – 0.907 with an average of 0.795 (Table 4). Highest PIC value was observed in RM28052, followed by RM555, RM263, RM324, and RM3549 and the lowest value was of RM3805. The dendrogram generated from the matrix data (Fig. 1) divided the germplasms into 2 clusters. Bhundi, Neta, Chhotodidi, Velchi, Maniksal, Morogjhota, Sonagori, Vutmuri, Lakkansal, Aswinsal, Kelesh, Tulsikamal were clustered along with the established drought-tolerant varieties.

Discussion

Agro-morphological and physio-biochemical study

Five landraces including Vasamanik, Kalpana, Langalmathi, Kashiphool, and Bhuri could not withstand drought stress at seedling stage. These landraces might be appropriate for the then environment where the abundance of water was present but presently, in physiological drought condition these landraces showed poor performance and can be labelled as susceptible variety.

Seed yield/plant is the most important parameter to screen for drought-tolerant genotypes. It was observed that 16 germplasms (12 landraces and 4 established varieties) did not differ significantly in yield under stressed and control condition (Supplementary Table 6). Out of them, Morogjhota yielded highest 24.58 g, followed by check varieties DRR44 (24.56 g), Vandana (24.00 g). Based on yield, 12 landraces can be stated as drought-tolerant whereas 5 landraces that failed to survive the stress and 4 landraces had a huge reduction in yield can be stated as drought susceptible. While, Lohasal can be stated as moderately tolerant as its yield reduction was not significant.

Root length was increased for each germplasm under stress condition (Supplementary Fig 5 and 6). Increase in root length was highest in Maniksal, followed by Lohasal, Vandana (positive check), Bhundi, and Vutmuri. Fukai and Cooper (1995), Gowda et al.,(2011), and Kim et al.,(2020) also reported an increase in root length under stress in rice germplasms. Root length is increased under stress in search of more water and nutrients, it is also a good predictor of yield and stress tolerance.

Shoot length decreased in each germplasm under early drought stress (Supplementary Fig. 7). Similar observation was reported by Usmal et al.,(2013). Reduction in shoot length was lowest in Maniksal, followed by Chhotodidi, Lakkansal, Sahabhagidhan (positive check) (Supplementary Fig. 8). Lower reduction in shoot length might be an indicator of drought resistance.

Average value of Seedling Vigour Index was found to be higher in control condition in comparison with stress (Supplementary Fig. 9). Vibhuti et al.,(2015) also noticed reduction in vigour under drought stress. Exceptionally, Maniksal showed higher SVI (32.83% increase) and few other landraces including Chhotodidi, Bhadoi and Vutmuri (1.58, 0.84, 2.94% increased respectively) also showed slightly higher SVI under stress, which indicates resistance capability.

Root fresh weight and dry weight is an important morphological character related to drought resistance (Usmal et al., 2013; Verma et al., 2019). It was noticed that, RFW was increased under stress in all tolerant checks as well as in landraces like Maniksal, Chhotodidi, Aswinsal, Morogjhota, Sonagori, Tulsikamal, Bhundi, Velchi, Neta, Vutmuri (Supplementary Fig. 10). Whereas, landraces including Kerala Sundari, Bhadoi, Kelesh, Bhramarmali, Chandrakanti and susceptible variety Swarna showed lower root weight during water stress. This infers that increase in RFW under stress provides with higher yield. Root Dry Weight was decreased for most of the germplasms under stress excluding tolerant check Nagina 22 and landraces like Maniksal, Lohasal, Bhadoi, Velchi, Neta (Supplementary Fig. 11).

Leaf Rolling and drying score varied fom 0-9 for various genotypes. Most of the germplasms with lower scores of leaf rolling and drying revealed higher recovery and yield under stress (Table 1). Similiar observation was noticed by Manickavelu et al.,(2006).

Chlorophyll content was decreased in seedling stage under stress (Supplementary Fig. 12). Reduced chlorophyll content in this study may be associated with stress-induced disruptions in biosynthesis or degradation of pigment, chloroplast membrane loss, leading to generation of reactive oxygen species (Nahakpam, 2017; Reddy et al., 2004).

Drought stress led to a decrease in relative water content (RWC) in all germplasms (Supplementary Fig. 13). Similar result was also reported by Ahmadikhah and Marufinia (2016), where prolonged drought also decreased RWC in rice germplasms. Conversely, in this study Bhramarmali, Chandrakanti, Vutmuri, Sonagori, and Velchi showed relatively lower reduction in RWC, which could be a selection criterion for drought resistance, as suggested by Manickavelu et al., 2006.

The accumulation of proline serves as a crucial adaptive response of plants to drought stress. Kelesh exhibited the maximum increase in proline accumulation (2.11 times) followed by Chhotodidi (1.96 times) and Chandrakanti (1.33 times) (Supplementary Fig. 14). Such an increase in proline content under drought stress was also repoted by Nasrin et al., (2020) and Hanif et al., (2021). The total carbohydrate content (TCC) exhibited significant variation among each germplasm under seedling stage stress conditions (Supplementary Fig. 15). Fluctuations in total starch levels were influenced by the severity of drought stress, environmental factors, and specific rice varieties (Dien et al., 2019). Tolerant check varieties (Sahabhagidhan, DRR44, N22, Vandana) and landraces including Maniksal, Aswinsal, Morogjhota, Velchi, and Vutmuri maintained higher TCC levels under stress, preserving carbohydrates for later stages through tolerance mechanisms. In addition, slight decrease inTCC under stress compared to control in germplasms like DRR42, Kerala Sundari, Chhotodidi, Sonagori, Tulsikamal, Bhundi, Bhadoi, Neta, Bhramarmali, Chandrakanti, Kelesh, and Swarna, indicating starch utilization under stress. Conversely, significant decrease of TCC under stress in Lalat, Bhuri, Vasamanik, Kalpana, Langalmathi, Kashiphool, Lohasal, and Lakkansal, might be connected with failure to survive stress or severe yield reduction.
Furthermore, it was noted that days to 50% flowering and days to maturity were delayed for all the germplasms. Lowest delay in DF was observed in tolerant checks Vandana (3 days), followed by DRR44 (4.67 days), Sahabhagidhan N22 (5 days) and DRR42 (7.67 days). Whereas, in landraces delay in DF varied from 5.67 – 21.67 days in all germplasms indicating poor to high drought resistance. Likewise, DM was delayed by 5.33-8.33 days and 5-29.33 days for the tolerant checks and landraces respectively. It was observed that the delay in DM was lower than delay in DF for landraces like Maniksal, Chhotodidi, Morogjhota, Sonagori, Bhundi, Velchi and Neta. This might be associated with the tendency to shorten their grain-filling stage to reach maturity phase faster than others. Similar observations were also made by Pantuwan et al.,(2002) and Kang and Futakuchi. (2019). However, in this study DF and DM cannot be used as a parameter to differentiate drought resistant and susceptible genotypes because few landraces like Vutmuri showed higher drought resistance even having a delay of 21 and 29 days in DF and DM respectively. In additiion, 100 seed weight was reduced but not significantly under stress as repoted by Bhutta et al.,(2019).

Variability study

It was noticed that, variation of different characters among the germplasms were significant at 1% and 5% level. Highest Coefficient of Variation (Supplementary Table 4) was observed in Leaf Drying (22.13), followed by Root Length (20.94), Shoot Length (20.75), Root Fresh Weight (16.66), Seedling Vigour Index (14.82), Total carbohydrate content (14.63), Root Dry Weight (13.99), Leaf Rolling (13.91), Proline content (11.26) and Seed yield/ plant (10.62). Critical Difference value of seed yield/plant divided the 16 germplasms in two categories (Supplementary Table 5). However, it was noted that germplasms did not significantly differ in yield under stress and control condition. Variations among germplasms for characters like shoot and root growth, relative water content, proline content, chlorophyll content, peroxidation rate in leaves were reported by Miftahudin et al., (2020). Analysis of variance indicated highly significant differences among genotypes for traits like root volume, root length, root:shoot ratio, leaf rolling, plant height, 100 grain weight, relative water content, grain yield/plant (Gaballah et al., 2020). Moreover, drought resistance index (DRI) values were also found to be higher in landraces when compared to that of tolerant check varieties (Table 3).

Correlation study

Pearson's Correlation study was carried out to attain a complete knowledge on inter-relationship between characters (Table 2). Association between seed yield and other characters is of paramount importance to the breeder for making improvements in complex quantitative character like yield for which direct selection is not always effective. High positive significant correlation between SY/P and SP, 100 SW, DM, DF, RFW, PRO, RWC, CHL and TCC was noted in this study. However, moderate significant correlation with SL and a strong negative correlation between LD and LR were observed. Similar observation i.e. positive correlation between SY/P and RL, SL, and RFW were observed by Usman et al., (2013) and Verma et al., (2019). Manickavelu et al., (2006), reported negative correlation between SY/P and LD, LR score. Positive correlation between SY/P and SP, DF, DM, and 100SW was studied by Kang and Futakuchi, 2019. In a similar study by Nithya et al., (2020) under drought stress conditions, phenotypic and genotypic correlations revealed that grain yield was positively and significantly associated with 1000-grain weight, spikelet fertility percentage, and relative water content, indicating that an increase in these parameters enhanced grain yield. Similarly, Abarshahr et al., (2011) reported a positive and significant association of plant height, 1000-grain weight, and spikelet fertility percentage with grain yield under drought stress. In contrast, grain yield showed a negative and significant correlation with days to 50% flowering at both phenotypic and genotypic levels, while its association with tillers per plant was positive but non-significant at both levels. Comparable findings were observed for days to 50% flowering (Kole et al., 2008) and tillers per plant (Watto et al., 2010). Based on all the parameters as well as statistical analysis, it can be deduced that mostly the landraces performed better as compared to the tolerant check varieties under dry spells at seedling stage.

Genetic diversity study

Twenty SSR markers associated with grain yield under drought stress were used to assess the genetic diversity among the germplasms (Supplementary Table 7). PIC value (ranged from 0.544 – 0.907 with an average of 0.795) and no. of alleles (ranged from 4-18 with an average of 8.7) revealed that the primers were appropriate to be used for molecular characterization of rice germplasms (Table 4). Highest PIC value was observed in RM201 (0.92), followed by RM28052 (0.91), RM328, 316, 215 (0.88), RM566 (0.87) suggested their usefulness for characterizing this set of rice germplasms. Likewise, highest no of alleles in RM201 (18), followed by RM1261 (14), RM28052 (13), RM489 (12), RM 250, 72, 17, 215 (11) and RM302, 555, 549, 263, 566, 328, 316 (10) also indicated that these markers could be potentially used for molecular

characterization. Khan et al., (2022) studied the morpho-physiological variability along with genetic diversity through use of 19 SSR markers that generated a total of 64 alleles across 13 varieties, with 2 to 7 alleles per locus (average of 3 alleles). In addition, the polymorphic information content (PIC) values (ranged from 0.13 to 0.78, with an average of 0.45 ± 0.19) in this present study are in congruence with the reports of Gaballah et al. (2020), Sajib et al., (2012), Zeng et al., (2004) and Ram et al., (2007).
Furthermore, two clusters were generated that mostly dividing the germplasms in two categories (Fig.1). Cluster I comprised of germplasms having higher seed yield under stress that included the tolerant checks and Cluster II consists of germplasms that performed poorly under drought involving the susceptible check. Interestingly, high yielding variety Lalat that had significant reduction in yield during stress condition was grouped under Cluster I. Gaballah et al., (2020) demonstrated that SSR markers clustered japonica and indica cultivars into drought-resistant and drought-sensitive groups. Similarly, El-Malky et al., (2007) reported effectiveness of SSR markers in dividing varieties into two distinct groups: one comprising indica varieties and the other japonica varieties. Additionally, Zeng et al., (2004) also observed that genotypes clustered into two major branches in the dendrogram, with less than 10% similarity based on Jaccard's similarity index. One branch represented the japonica subspecies, while the other represented the indica subspecies or hybrids between japonica and indica rice.

Materials and methods

Selection of plant materials
More than 50 landraces were collected from ARW Society, Purulia and from various farmers of Purulia and Bankura district of West Bengal those were once cultivated for traditional purpose. Whereas seven check varieties, out of which five are recognized drought-tolerant varieties, one is drought-susceptible, and one is a high-yielding variety were collected from Zonal Drought Resistance Paddy Research Station (ZDRPRS), Hathwara, Purulia, Govt. of West Bengal. Artificial drought stress was imposed on all the collected germplasms under *in vitro* condition using Polyethylene Glycol. Twenty-two landraces that showed promising tolerance under *in vitro* condition were selected as experimental materials for field screening along with the seven check varieties (Supplementary Table 1).

Field screening by stress induction at seedling stage
The field experiment spanned two consecutive Kharif seasons at ZDRPRS, Hathwara, Purulia. The study employed a Randomised Block Design with three replications, consisting of a control set and a treated set. In Control set, germinated seeds of each variety were planted under normal field conditions with regular irrigation provided until harvesting. Whereas, in treated set seeds were placed in plastic bags with controlled water supply. A shade using plastic sheets prevented rainwater from reaching the seedlings. The treated set received normal watering for the first 15 days. Subsequently, water application was ceased for next 15 days. Soil moisture levels were measured at 5 cm and 30 cm soil depths for both control and treated plots (Supplementary Table 2).
On 15th day of stress, seedlings were carefully collected from each replication of the treated set and control set.

Parameters evaluated under drought stress
Shoot and root length (SL and RL), seedling vigour index (SVI), root fresh and dry weight (RFW and RDW), proline (PRO), total carbohydrate (TCC), chlorophyll (CHL) and leaf relative water content (RWC) were investigated. Estimation of proline, carbohydrate, chlorophyll and relative water content was done following the methods led by Bates et al., (1973), Sadasivam and Manickam (1996), Hiscox and Israelstam (1979), Barrs and Weatherley (1962) respectively. Leaf rolling (LR) and drying (LD) score, was assigned to the treated plots following the IRRI guidelines (irri.org).
After that, normal irrigation was provided to the treated set. After 7 days of re-watering, survivability percentage (SP) was observed. Plantlets were then allowed to grow till harvesting. Yield related attributes including seed yield/plant (SY/P), days to 50% flowering (DF), days to maturity (DM), 100 seed weight (100 SW) were noted. Drought Resistance Index was estimated for selected varieties (Fischer and Maurer 1978).

Genetic diversity study
Using twenty Simple Sequence Repeat (SSR) markers (Supplementary Table 7) linked to drought tolerance; genetic diversity was assessed among collected germplasms. Genomic DNA, extracted with Qiagen DNeasy Plant Mini Kit, was quantified using Nanodrop spectrophotometer (Thermo Fisher). Polymerase Chain Reaction (PCR) employed 10 µl template DNA (10-30 ng/µl concentration) using BIORAD T-100 thermal cycler following the modified method of Xiao et al., (1996). Amplified PCR products were separated on a 2% agarose gel, visualized in Gel-Doc, and bands were determined using ImageLab software. Polymorphism Information Content (PIC) was calculated according to the formula PIC (i) =1 – $\sum P2ij$ (Hwang et al., 2009).
Where, i = 1 to n and P_{ij} is the frequency of j^{th} allele for the i^{th} band scored for a particular marker. Also, marker-based population genetics study, including effective number of alleles (Ne) (Kimura and Crow. 1964) and Nei's gene diversity (Nei M. 1973) was carried out.

Statistical analysis
A Randomised Block Design with three replications was followed in the field condition for treated and control plots. Agro-morphological characters under drought stress were compared using Analysis of Variance (ANOVA), revealing mean, standard deviation, critical difference, and coefficient of variation. Pearson's Correlation, accessed via SPSS software ver.

18, to explore relationships between parameters and seed yield. A critical t-test compared the seed yield under control and treated plots for each variety was carried out. Moreover, a dendrogram was generated by means of SSR marker data using Unweighted Pair Group Method with Arithmetic Mean (UPGMA) based on Jaccard's dissimilarity coefficient in Darwin version 6.

Conclusion

Screening of germplasms having drought tolerance potential is very important. Parameters with significant variation in this study can be used for better selection from a breeder's point of view. From the correlation study it was evident that positive selection for survivability percentage, 100 Seed Weight, days to maturity, days to 50% flowering and root fresh weight will be helpful for screening lines to have higher seed yield in early drought condition. Positive correlation between seed yield and root fresh weight implies that coarser roots in water stress condition might lead to better grain production. Negative correlation between seed yield/plant and leaf rolling and leaf drying implied that genotypes with lower leaf drying and rolling score should be selected.

Out of the 22 landraces, Morogjhota is the only germplasm that surpassed the check varieties for seed yield. Morogjhota, Lakkansal, Tulsikamal, Kelesh, Bhundi, Velchi, Vutmuri, Chhotodidi, Maniksal, Sonagori, Aswinsal landraces had higher survivability percentage and drought resistance index, low yield reduction in drought stress and were also grouped in the same cluster in the phylogenetic tree with tolerant check varieties. These landraces can be further studied for their underlying drought mechanism and can be used in various breeding programs to develop climate-resilient paddy variety.

Statements and Declarations

We declare that no funding was received for conducting this study. Moreover, this is to state that the authors have no competing interests and all data generated or analyzed during this study are included in this manuscript.

Author contribution

All the authors contributed equally in conceptualization, research methodology, writing as well as preparation of the manuscript.

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