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RESEARCH ARTICLE

Screening of rice land races for drought tolerance (*Oryza sativa* L.)

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Abstract

Rice is the staple food for over half of the world population. The current unprecedented climate change scenario poses a severe threat to national food security. As a result, the development of climate-resilient genotypes is necessary to boost rice yield in India and alleviate food scarcity due to rising population demand. Though drought is one of the complex quantitative traits highly influenced by the environment, it is necessary to better understand the attributes and mechanisms of drought tolerance. Landraces with a broad genetic base serve as a hub of novel traits contributing to drought tolerance. In the present investigation, a total of 22 traditional rice landraces collected from different agro-climatic zones of Tamil Nadu were screened to observe their responses to induced moisture stress under *in vitro* and *in vivo* conditions. The traditional rice land races, along with drought-tolerant (Anna 4) and drought-susceptible (IR 64) checks, were screened for drought tolerance. The genotypes used were Vadan Samba, Orissa Kuttai, Thanga Samba, Kalapath, Kattuyanam, Boodhakali Karupam, Kangam Samba, Keerai Samba, Kollan Samba, Chinnar, Manam Kuruvai, Kasthuri Samba, Sivan Samba, Sinkar, Manisamba, Kaalanamak, Madumulungi, Karudan Samba, Athur kichili Samba, Chengalpattu Sirumani Samba, Karuthakar, Rathasali, Vasanal Seraka Samba, Sivappu Kavuni, along with Anna 4 and IR 64. The land races were evaluated under moisture stress using polyethene glycol (PEG) with three different concentrations, 5 %, 10 % and 20 % and a control. The land races that showed tolerance to PEG germination were Keerai Samba, Vadan Samba, Sinkar and Kollan Samba. The rice land races were subjected to proline estimation. The land-races Madumulungi, Garudan Samba, Kasturi Samba and Keerai Samba showed drought tolerance. The land races screened under field conditions exhibit drought and tip drying under induced moisture stress, adapting the drought avoidance mechanism. Molecular screening was also done with SSR markers viz. RM71, RM520, RM256, RM217 and RM431, out of which RM256 showed polymorphism among the genotypes. The molecular characterisation confirmed that Madumulungi, Garudan Samba, Kattuyanam, Keerai Samba, Kannan Samba and Manag Kuruvai showed the presence of a resistant allele with 127 bp. These identified drought-tolerant landraces can be used as a donor parent in further breeding programs to develop drought-tolerant varieties.

Keywords: drought; landraces; PEG; proline; rice

Introduction

Rice (*Oryza sativa* L.) is an important cereal that contributes more than 70 % of the everyday diet of the people in India. In Asia, rice productivity is severely affected due to drought, as it can occur at any stage of crop growth and development, over varied periods of time and with different intensities (1). The vegetative and reproductive stages of rice are the most vulnerable to drought stress (2, 3). Drastic reduction in yield is seen during the reproductive stages at times of drought stress (3, 4). The economic yield of rice is reduced significantly, up to 45 %-94 % and 60 %, respectively, by severe moisture stress during the reproductive and grain filling stages (5). Drought tolerance in rice is a quantitative trait governed by polygenes, due to which developing drought-tolerant varieties is quite challenging (6). I*n vitro* selections of genotypes were effective for the identification of drought-tolerant genotypes by screening them with different levels of moisture stress. PEG, a chemical compound with a higher molecular weight which is commonly used to maintain a lower osmotic potential under hydroponic conditions (7). Under reduction of osmotic potential, germination rate, root length, shoot length and R/S ratio also showed wide variations (8).

In this study, a screening for drought tolerance in traditional land races, both *in vitro* and at field conditions, along with molecular studies, was carried out. The present study aimed to characterize the rice genotypes to understand the response of rice genotypes to the induced moisture stress and to identify the most promising drought-tolerant genotypes to be used as donors in future breeding programs.

Materials and Methods

The present investigation was undertaken to identify potential donor parents for drought tolerance in rice landraces. The research work was conducted at the Agriculture College and Research Institute, Tamil Nadu Agricultural University, Killikulam, Vallanadu, during the year of *Kharif* 2023. The experiment was carried out both *in vitro* and in the field. The field experiment was conducted in field numbers 13A and 13B, D-block of the Department of Plant Breeding and Genetics, V.O.C. Agricultural College and Research Institute, Killikulam, Vallanadu, Tuticorin District. The experimental area is located at 8°46' N latitude and 77° 42' E longitude in the southern part of Tamil Nadu, at an altitude of 40 m above MSL.

In vitro screening for drought tolerance

In vitro screening of landraces for drought tolerance was carried out using PEG 6000. In wet germination paper, 25 seeds of each genotype were placed at the marked point with a spacing of 1 cm over a horizontal line. Another moistened paper towel was carefully placed over the seeds. The paper towels were then loosely rolled into a tube and secured with a rubber band, along with a polythene layer underneath. The rolls were placed in containers of different PEG concentrations (9). Drought stress was simulated at three different concentrations, 5 %, 10 % and 20 % at four osmotic potential levels, including 0.001, 0.27, 0.54 and 1.09 MPa. Control was also maintained. Germination percentage, root length, shoot length, fresh shoot weight, root volume and fresh root weight were observed after 12 days of PEG treatment at different concentrations.

Under *in vivo* conditions

Stress was applied during the active tillering stage (four weeks of crop growth) for screening in the vegetative stage. Under moderate to severe stress, leaves begin to roll and some will dry out. The stress was maintained until the susceptible check showed leaf rolling, tip drying and total withering. The International Rice Research Institute Standard Evaluation System (IRRI SES) was followed for scoring (1 to 9 scales) (Table 1) leaf rolling, drying and recovery symptoms (10). The landraces that showed earlier leaf rolling symptoms were susceptible and the late rolling ones were tolerant and recovered faster. To determine the proline content in leaves, the standard method was used (11). 0.1 g of rice leaves was ground with 5 mL of 3 % sulfosalicylic acid and the mixture was then filtered. In a test tube, 2 mL of the filtrate was added. 2 mL of acid ninhydrin and 2 mL of glacial acetic acid were also added to the filtrate. The contents were mixed well and boiled at 100 ˚C for an hr. The mixture was kept in ice and 4 mL of toluene was added and mixed. Then the mixture is undisturbed for 5-10 min. Absorbance of the reddish pink upper phase was measured at 520 nm against a toluene blank.

Molecular screening for drought tolerance

The Cetyltrimethylammonium Bromide (CTAB) technique was used to isolate genomic DNA (12). Young fresh leaves (200 mg) were ground with an autoclaved pre-chilled pestle and mortar to a fine paste in approximately 2 mL of pre-warmed CTAB buffer. Following that, the mixture was transferred to a microcentrifuge tube and continuously shaken every 10 min for an hr at 60 °C in a water bath. After incubation, the mixture was centrifuged for 10 min at 12000 rpm to remove cell debris and the supernatant was then transferred into fresh microcentrifuge tubes. In each tube, an equal amount of 24:1 chloroform and isoamyl alcohol was added and mixed thoroughly. Centrifuge at 12000 rpm for 10 min after the supernatant has been collected into sterile microcentrifuge tubes. To precipitate the DNA, an equal volume of ice-cold isopropanol was added and the tube was gently inverted several times. The tube was stored at -20 °C overnight to allow the DNA to be precipitated by alcohol. The sample was then centrifuged at 12000 rpm for 10 min, the supernatant was decanted without disturbing the pellet and 200 µL of ice-cold, 70 % ethanol was added. Decanted the ethanol and dried the pellet just long enough to get rid of the extra alcohol while leaving the DNA wet. The DNA pellet was dissolved in 50 µL of TE buffer (10 mM Tris, pH 8, 1 mM EDTA) and 5 µL of RNase A solution was added. The mixture was then incubated at 37 °C for an hr, dissolved in TE buffer and then kept at -20 °C. Five SSR markers (RM71, RM520, RM256, RM217 and RM431) were used to test the polymorphism of the landraces. The PCR amplification was carried out using a thermocycler with a total reaction volume of 10 µL. The PCR products obtained were resolved by agarose gel electrophoresis on a 1.2 % agarose gel.

Results and Discussion

The present drought screening study was performed using 20 traditional rice genotypes and two check varieties that includes Anna 4 as resistant check and IR64 as susceptible check to study the responses of rice genotypes to varying degrees of PEG-induced drought stress, genetic analysis of rice genotypes using SSR markers linked to drought tolerance includes the screening of above rice genotypes for drought stress induced by PEG using molecular markers (SSR markers) linked to drought tolerant QTLs, proline estimation and field level screening of the landraces after inducing the stress and rating different parameters like tip drying and leaf rolling.

In vitro screening for drought tolerance

The effect of PEG on seed germination was measured to determine the drought tolerance of rice genotypes to drought stress. Seed germination was significantly affected by different levels of induced drought stress conditions, while, at the same time, it differed substantially among genotypes under study. The germination percentage of rice decreased to different extents with increasing drought stress. In the study, it was observed that relative to the control, increasing PEG concentration steadily reduced germination percentage of seeds, the experiment was carried out at four levels of drought stress created by adding PEG 6000 at four concentrations: 0 %, 5 %, 10 % and 20 % at four osmotic potential levels, including 0.001, 0.27, 0.54 and 1.09 MPa. With the increase in moisture stress level using different concentrations of PEG (5 %, 10 % and 20 %), the mean germination percentage found to be significantly decreased with respect to the control. Out of 22 genotypes, 17 genotypes, along

Table 1. Scale for leaf rolling and leaf drying at the vegetative stage

Leaf rolling		Leaf drying	
Scale	**Observation**	**Scale**	**Observation**
0	Leaf Healthy	0	No symptom
1	Leaf starts to fold or shallow V shape	1	Slight tip drying
3	Leaves folding and a deep V shape	3	Tip drying extended up to half-length in most leaves
5	Leaves are fully cupped and U-shaped	5	One fourth to half of all leaves are fully dried
7	Leaf margin touching O shape	7	More than 2/3 of all leaves are fully dried
9	Leaves tightly rolled	9	All plants are apparently dead

with the drought-tolerant check and parent, showed germination percentage at 20 % PEG concentration. Due to reduced water potential, drought stress has been shown to negatively impact seed germination and hinder the seedling growth (13). Similarly, the germination percentage of rice genotypes dropped from 95.8 % in the control to 6.6 % in the highest stress (20 % PEG) level as water stress increased (14).

From the above observation, it can be noted that Keerai Samba (83 %) has the highest germination at concentration of 20 % PEG along with the tolerant check Anna 4 (93.3 %) (Table 2). Shoot length of 22 genotypes was measured from the root base to the tip of the shoot after 12 days of PEG treatment at different concentration. In the study, it was observed that relative to the control, increasing PEG concentration steadily reduced shoot length. With the increase in moisture stress level using different concentrations of PEG (5 %, 10 % and 20 %), mean shoot length was found to be decreased with respect to the control. Out of 22 genotypes, 17 lines showed shoot growth up to 20 % indicating drought tolerance. Shoot length decreases as external water potential increases due to a decline in turgor pressure as well as a decrease in cell division and elongation (15, 16). Out of the genotypes, Anna 4 (10.67 cm), Garudan samba (10.08 cm), Keerai Samba (11.6 cm) and Orissa Kuttai (10.16 cm) showed greater shoot length at a concentration of 20 % PEG (Table 2).

Fresh shoot weight of 22 genotypes was measured after 12 days of PEG treatment at different concentrations. In the study, it was observed that relative to the control, increasing PEG concentration steadily reduced shoot weight, as there was a decrease in the shoot length. With the increase in moisture stress level using different concentrations of PEG (5 %, 10 % and 20 %), shoot weight was found to be decreased with respect to the control. On interpreting the results, it was found that the genotypes Anna 4 (0.175), Kollan Samba (0.145), Garudan Samba (0.17), Vadan Samba (0.135), Chinkar (0.135) had higher shoot weight compared to the other genotypes at concentration of 20 % PEG (Table 2). An important trait of seedlings is their roots, which sense and transmit water deficit signals to the shoot, causing a range of physiological, morphological and molecular responses across the whole plant (17). Root length of 22 rice genotypes was measured from the root base to the tip of the root after 12 days of PEG treatment at different concentrations of 5 %, 10 % and 20 %. It was observed that the root length also declined with increased external water potential and consequently, all PEG treatments caused a decrease in root elongation in 24 rice genotypes compared to their controls. With the increase in external water potential using different concentrations of PEG (5 %, 10 % and 20 %), the mean root length was found to be decreased with respect to the control (18). Out of 22 genotypes, 17 genotypes showed root growth up to 20 % along with drought tolerance, indicating drought tolerance. PEG affects the water uptake and reduces turgor pressure, resulting in shortening of root

Table 2. Results for *in vitro* screening for drought tolerance (T_1 at 5 % PEG, T_2 at 10 % PEG, T_3 at 20 % PEG)

	Germination percentage (%)				Shoot length (in cm)				Fresh shoot weight (mg)			
Genotype	**Control**	**T_1**	**T_2**	**T_3**	**Control**	**T_1**	**T_2**	**T_3**	**Control**	**T_1**	**T_2**	**T_3**
Anna 4	95	100	100	93.3	14.75	14.21	13.58	10.67	0.37	0.29	0.17	0.175
IR 64	93.3	75	56	35	10.9	10	8.06	7.24	0.14	0.2	0.2	0.15
Garudan Samba	72	50	40	30	12.62	11.63	10.39	10.08	0.13	0.11	0.195	0.17
Athur Kichili Samba	75	56.6	33.3	26.6	13.22	12.94	8.58	7.48	0.085	0.16	0.065	0.07
Katyanam	83	69	52	43.3	16.84	17.69	13.12	5.01	0.21	0.27	0.22	0.065
Kannan Samba	83.3	86.6	70	67	9.52	10.92	10.23	9.52	0.195	0.215	0.2	0.15
Chinnar	53.3	33.3	23.3	20	7.9	9.66	7.4	6	0.09	0.14	0.075	0.08
Kollan Samba	86.6	83.3	76.6	73.3	17.08	18.03	17.88	9.63	0.285	0.305	0.29	0.145
Kasturi samba	76	60	43.3	23.3	10.15	14.22	5.02	2.16	0.07	0.11	0.045	0.025
Cengalpattu Sirumani Samba	70	60	46.6	13.3	8.8	4	2.6	2.5	0.11	0.1	0.05	0.01
Karuthakar	76.6	66.6	53.3	33.3	18.99	15.27	12.37	7.25	0.305	0.195	0.17	0.115
Vasanai Seraka Samba	66.6	53.3	33.3	23.3	8.21	2.88	1.9	1.5	0.105	0.003	0.035	0.02
Orissa Kuttai	63.3	53	48	26.6	14.03	15.96	11.19	10.16	0.28	0.205	0.155	0.12
Kalanamak	66.6	60	30	20	11.48	9.38	6.32	3.68	0.135	0.09	0.035	0.05
Keerai samba	90	86.6	85.3	83	12.04	12.84	12.5	11.6	0.245	0.24	0.255	0.13
Thanga samba	63.3	56.6	33.3	23.3	5.07	7.7	7.25	0.95	0.38	0.1	0.006	0.01
Chinkar	60	46.6	33	26.6	8.15	14.9	10	9.39	0.2	0.18	0.15	0.135
Sivan Samba	50	0	0	0	9.8	0	0	0	0.01	0	0	0
Vadan Samba	63.3	56.6	46.6	33.3	12.96	14.78	11.21	9.39	0.125	0.185	0.0395	0.135

Table 3. Results for *in vitro* screening for drought tolerance (T_1 at 5 % PEG, T_2 at 10 % PEG, T_3 at 20 % PEG)

	Root length (cm)				Fresh root weight (mg)				Shoot root ratio			
Genotype	**Control**	**T_1**	**T_2**	**T_3**	**Control**	**T_1**	**T_2**	**T_3**	**Control**	**T_1**	**T_2**	**T_3**
Anna 4	19	23.7	17.4	13.2	0.095	0.135	0.1	0.06	0.776	0.599	0.78	0.808
IR 64	7.1	16.3	16.4	10.9	0.02	0.037	0.06	0.1	1.535	0.613	0.491	0.664
Garudan Samba	13.3	19.5	15.6	10.1	0.045	0.065	0.065	0.095	0.948	0.596	0.666	0.998
Athur Kichili Samba	11.2	19.7	9	9.1	0.01	0.06	0.155	0.065	1.18	0.65	0.953	0.821
Katyanam	16.2	18.9	15.5	8.3	0.045	0.09	0.09	0.055	1.039	0.935	0.846	0.603
Kannan Samba	15.4	19.6	17.1	10.8	0.075	0.07	0.065	0.09	0.618	0.557	0.598	0.88
Chinnar	18.4	19	15.5	8	0.06	0.04	0.015	0.06	0.429	0.508	0.477	0.75
Kollan Samba	16.7	22.6	19.5	8.5	0.085	0.1	0.205	0.09	1.022	0.797	0.916	1.13
Kasturi Samba	12.4	13.8	12.3	12	0.035	0.05	0.01	0.01	0.818	1.03	0.408	0.18
Cengalpattu Sirumani Samba	8.7	2	1.7	5.5	0.02	0.015	0.01	0.01	1.011	2	1.529	0.45
Karuthakar	23.3	20.7	18.3	10.9	0.045	0.1	0.145	0.07	0.815	0.737	0.675	0.665
Vasanai Seraka Samba	9	4.7	4.2	3.8	0.03	0.015	0.01	0.005	0.912	0.612	0.452	0.394
Orissa Kuttai	13.7	18.7	13.9	7.9	0.06	0.06	0.08	0.06	1.024	0.853	0.805	1.286
Kalanamak	8	5.1	7.6	7.7	0.03	0.05	0.065	0.01	1.435	1.839	0.831	0.477
Keerai Samba	22.6	19.9	19.2	10	0.105	0.135	0.155	0.1	0.532	0.645	0.651	1.16
Thanga Samba	8.1	9.7	17.2	4.7	0.03	0.035	0.035	0.01	0.625	0.793	0.421	0.202
Chinkar	13.2	14.3	7.2	6.7	0.015	0.27	0.15	0.1	0.617	1.041	1.388	1.401
Sivan Samba	12.8	0	0	0	0.01	0	0	0	0.765	0	0	0
Vadan Samba	11.9	16.8	13.2	6.7	0.03	0.055	0.0285	0.012	1.089	0.879	0.849	1.401

length. Out of the 22 genotypes, Anna 4 (13.2), Garudan samba (10.17), Kasturi samba (12.075), Kannan Samba (10.8) and Karuthakar (10.9) showed greater root length at a concentration of 20 % PEG (Table 3).

Fresh root of 22 genotypes was measured after 12 days of PEG treatment at different concentrations. In the study, it was observed that relative to the control, increasing PEG concentration steadily reduced root weight, as there was a decrease in the root length and volume. With the increase in moisture stress level using different concentrations of PEG (5 %, 10 % and 20 %), root weight was found to be decreased with respect to the control. On interpreting the results, it was found that the genotypes Keerai Samba (0.49), Vadan Samba (0.475) and Sinkar (0.475) had higher root weight compared to the other genotypes at a concentration of 20 % PEG (Table 3). The interdependence of shoot and root is required as, shoot relies on the root for water, nutrients and mechanical support while the roots depend on the shoot for organic nutrients. With the increase in external water potential using PEG treatment (5 %, 10 % and 20 % concentrations), we observed a decrease in shoot root ratio compared to the control. A decrease in shoot root ratio under PEG-induced external water potential indicates that PEG-induced osmotic stress positively influences drought growth compared to shoot growth. High shoot-to-root ratio has been reported as a component trait for drought tolerance (18). On comparing the interpreted results, it was found that the genotypes Keerai Samba, Orissa Kuttai, Vadan Samba, Sinkar and Kollan Samba are found to perform better than other genotypes at higher concentrations of PEG (Table 3).

Genetic analysis of rice genotypes using SSR markers linked to drought tolerance

Selection for drought-tolerant lines based on phenotypic traits may be accelerated by using molecular markers associated with the trait. The phenotypically obtained seedling traits of 22 rice genotypes (Table 4) were screened for drought tolerance by using 5 SSR markers (RM71, RM520, RM256, RM217 and RM431) linked to drought tolerance (Fig. 1). Out of 20 markers, one SSR marker, namely RM256, was found to exhibit polymorphism among two check varieties. Hence, RM256 was utilised in screening of 22 rice genotypes (Fig. 2). The SSR marker RM256 was utilized in the present study for validation of the QTL DTY 8.1 in the rice genotypes (19). The amplification profile of the SSR marker RM256'specific to the QTL DTY 8.1 located at chromosome 8 at 24.2 Mb with the 127 bp in the resistant check Anna 4. The distinguishable polymorphism obtained between the resistant and the susceptible check aided the further screening of genotypes. The SSR marker RM256 was polymorphic among the checks and effectively used in the genotyping of the landraces. The results of molecular analysis of the SSR marker RM256 show that the genotypes Madumulungi, Garudan Samba, Kattyanam, Kannan Samba, Mananguruvai and Keerai Samba were found to have the resistant allele with 127 bp and the genotypes 14, 15 have greater than 127 bp and the rest of the genotypes coincide with the susceptible check.

Proline content estimation in the genotypes

Proline acts as an osmoregulator and its concentration in many plant tissues is exposed to a variety of abiotic stresses. Genotypes with high proline contents in leaves were more dehydration-tolerant, relatively high-water content was maintained and leaf

Table 4. List of genotypes under study

S. No.	Genotype
25 R	Anna 4
24S	IR 64
1	Madumulungi
2	Garudan Samba
3	Athur Kichili Samba
4	Katyanam
5	Kannan Samba
6	Chinnar
7	Kollan Samba
8	Kasturi Samba
9	Cengalpattu Sirumani Samba
10	Karuthakar
11	Vasanai Seraka Samba
12	Orissa Kuttai
14	Kalanamak
15	Rathasali
16	Manang Kuruvai
17	Keerai Samba
18	Mani Samba
19	Thanga Samba
23	Vadan Samba

Fig. 1. Polymorphism check of SSR markers. R- Resistant check (Anna 4) and S- Susceptible check (IR 64).

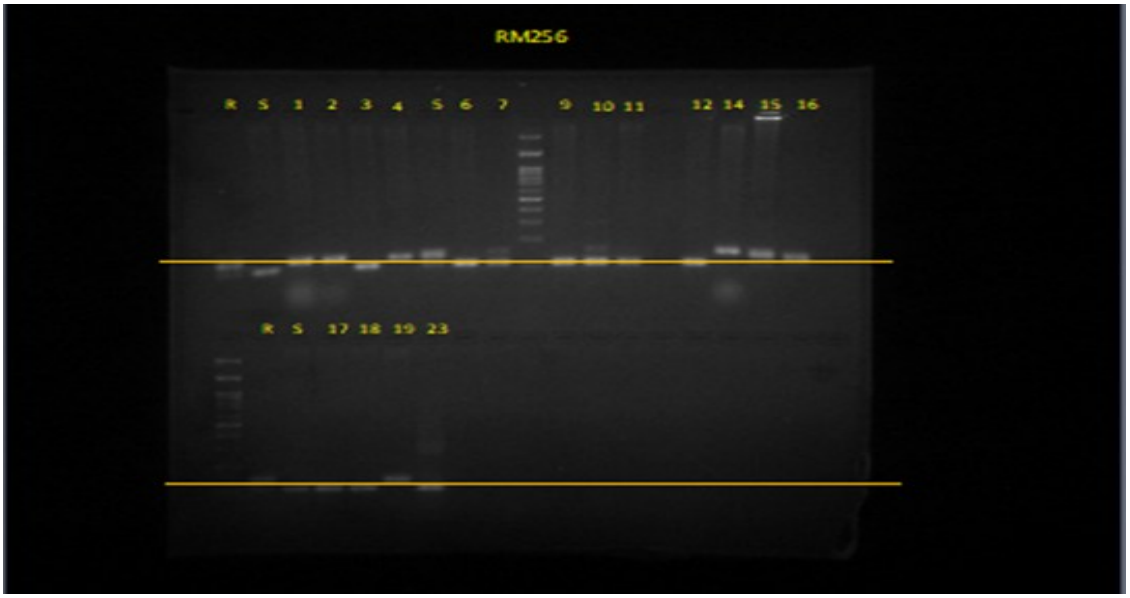

Fig. 2. Gel picture of RM 256.

Table 5. Proline content in the rice landraces under study

Genotype	OD Value	Concentration	Proline content (µL)
Madumulungi	0.18	150	51.9
Garudan Samba	0.1	85	29.4
Athur Kichili Samba	0.12	106	36.7
Katyanam	0.63	53	18.3
Kannan Samba	0.07	62	21.5
Chinnar	0.03	29	10
Kollan Samba	0.09	81	28
Kasturi Samba	0.13	117	40.4
Cengalpattu Sirumani Samba	0.04	39	13.5
Karuthakar	0.03	31	10.72
Vasanai Seraka Samba	0.09	80	27.7
Orissa Kuttai	0.04	42	14.5
Bootha Kali Karupan	0.09	83	28.7
Kalanamak	0.05	46	15.9
Rathasali	0.06	55	19
Manag Kuruvai	0.04	35	12.1
Keerai Samba	0.03	27	9.3
Mani Samba	0.05	44	15.2
Thanga Samba	0.04	39	13.5

rolling and senescence were delayed under severe water deficit. From the estimation of the proline contents in leaves, it was found that the genotypes Madumulungi (51.95), Kasturi Samba (40.48) and Keerai Samba (39.34) have higher proline content in the leaves (Table 5).

Field screening of genotypes

From the scoring of the genotypes for leaf rolling at the vegetative stage, it was found that Kollan Samba, Mani Samba, Keerai Samba and Karuthakar had healthy leaves and started to fold or show shallow V-shape symptoms (Table 6). From the scoring of the genotypes for leaf drying at the vegetative stage, it was found that Kollan Samba, Mani Samba, Kalanamak, Kasturi Samba and Karuthakar had no symptoms to slight tip drying (Table 6).

Conclusion

Twenty-two rice genotypes, including one resistant (Anna 4) and one susceptible check (IR 64), were screened for drought tolerance using PEG-induced moisture stress at 5 %, 10 % and 20 % concentrations. Observations on shoot and root length ratios, weights and germination percentage were taken. Only 17 genotypes germinated at 20 % PEG, with Anna 4, Garudan samba and Orissa Kuttai showing higher shoot growth. Root length was greater in Anna 4, Garudan samba, Kasturi samba, Kannan Samba and Karuthakar at 20 % concentration. Molecular screening was done with 20 SSR markers, where RM256 exhibited polymorphism, indicating the presence of tolerant alleles in several genotypes. High proline content correlated with dehydration tolerance, with Madumulungi, Kasturi Samba and Keerai Samba showing the highest levels. During the vegetative stage screening, genotypes were scored for leaf rolling and drying. Kollan Samba, Mani Samba, Keerai Samba and Karuthakar exhibited healthy leaves and Kollan Samba, Mani Samba, Kalanamak, Keerai Samba, Kasturi Samba and Karuthakar showed no to slight symptoms of tip drying. Thus, these identified drought-tolerant landraces can be used as donors in further breeding programs to develop drought-tolerant varieties.

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Table 6. Scoring for leaf rolling and drying

	Leaf rolling			Leaf drying		
Genotypes	**S_1**	**S_2**	**S_3**	**S_1**	**S_2**	**S_3**
Kollan Samba	1	0	0	1	1	0
Mani Samba	0	1	0	1	1	0
Kalanamak	3	1	0	1	1	0
Keerai Samba	1	1	0	3	1	0
Madumulungi	3	1	1	3	1	1
Rathasali	1	1	1	1	3	1
Karuthakar	1	1	0	5	3	3
Orissa Kuttai	5	7	5	5	3	3
Kangan Samba	3	1	1	3	5	3
Kasturi Samba	5	3	3	1	1	1
Katyanam	1	1	1	3	1	1
Vadan Samba	3	1	0	3	1	1
Manag Kuruvai	1	1	1	3	1	1
Thanga Samba	3	3	3	5	5	3
Athur Kichili Samba	7	3	5	5	3	3
Vasanai Seraka Samba	3	1	3	3	1	1
Cengalpattu Sirumani Samba	1	3	3	1	1	1
Chinnar	3	5	3	3	5	3

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Authors' contributions

RJ, SSP, SGA, SR and MA conducted the morphological, molecular and biochemical studies. RJ drafted the manuscript. JJR and JHS helped to finalize the manuscript. JHS contributed to the development of the work and provided the field and laboratory facilities for the research. Every author contributed to the overall conduct of this experimental study. All authors read and approved the final manuscript.

Compliance with ethical standards

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

Ethical issues: None

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