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Research Article

Unearthing the genetic potential of drought resilient rice (*Oryza sativa* L.) landraces through multipronged approaches

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Abstract
Drought has become principle and devastating abiotic stress due to the erratic shifts in climate patterns. Stress breeding programme in rice has extensively explored drought tolerant potential of cultivated *indica* types. Present investigation aims to ascertain the genetic potential of Indian rice landraces for their drought tolerance ability coupled with high yield. The PEG 6000 concentration of -7.0 bar was standardized as the optimal stress threshold for effectively screening the rice germplasm *invitro*. Twenty-six rice landraces sourced from NBPGR were subjected to *invitro* drought screening at -7.0 bar. Based on seedling vigor index at -7 bar, 10 drought tolerant genotypes *viz.,* IC115439, RL4015, RL10330, RL5648, RL6807, RL192, RL10823, RL10844, RL11113 and RL6812 were selected. These genotypes exhibited better performance in terms of vigor index and germination percentage than the drought tolerant checks Anna (R) 4, Apo and Wayrarem. To confirm the phenotypic performance of these selected genotypes in natural environmental condition, they were evaluated under stress in rain out shelter (ROS) and well irrigated field conditions. Evaluation based on biometrical, biochemical traits and drought scores resulted in identification of five landraces *viz.,* IC115439, RL4015, RL5648, RL10330 and RL10823. Among the 26 land races, IC115439, a land race collected from the farmers field of Raipur, was identified to be a valuable pre-breeding resource for drought tolerance breeding in rice.

Keywords: Rice, polyethylene glycol, landrace, drought, osmotic potential.

INTRODUCTION

Rice (*Oryza sativa* L.) is a major cereal crop that feeds half of the world's population. It is cultivated in a wide range of environmental conditions. However, during cultivation, it is subjected to a number of biotic and abiotic stresses, which reduces yield. Abiotic stress *viz*., drought, submergence and salinity contribute to more than 50 per cent of productivity losses. Drought stress is the most destructive of these, accounting for 70 per cent of produce loss (Manohar *et al*., 2022). India's food security heavily depends on the rice production. Rice is cultivated across 43.90 million hectares in India, with a production of 114.45 million metric tons and productivity of 2607 kg/ hectare of yield (Agricultural Statistics at a Glance 2022). The cultivation of rice requires about 3000- 5000L water/ kg (Shereen *et al*., 2019). The seedling stage emerges as a pivotal phase for crop establishment, recognized widely for its ability to mirror tolerance levels during subsequent maturity stages (Sagar *et al.,* 2020). This crucial early germination period not only accurately reflects tolerance but also reduces the time and space required for assessment (Iyem *et al.,* 2021). The genetic makeup of the traditional rice landraces remains underexplored yet resiliently cultivated by local communities due to their adaptability to arid conditions. Exploring the traits and mechanisms that make these landraces resilient and adaptive to drought stress across various growth stages is paramount. This exploration aids in precise selection and development of genotypes suited for drought-prone regions.

The present study involves standardizing the optimum stress level to be imposed using PEG 6000 to identify drought-tolerant genotypes at seedling stage and followed by selection under field conditions for further utilization. The combination of laboratory screening and subsequent field assessment under natural conditions, utilizing a rainout shelter to induce stress and well-irrigated conditions to reveal genetic potential, yields valuable insights for the recognition of drought-tolerant genotypes.

MATERIALS AND METHODS

Polyethylene glycol (PEG) – induced drought screening: Polyethylene glycol is an inert polymer which mimics the drought stress by reducing water availability and creating osmotic stress to plants thereby limits their growth and development (Nahar *et al*., 2018). It is a simple, reliable and rapid method for screening rice landraces at their early development stages. Laboratory screening was carried out using five drought-tolerant and susceptible checks along with 26 rice landraces in the Department of Plant Biotechnology, Centre for Plant Molecular Biology and Biotechnology, Tamil Nadu Agricultural University, Coimbatore. The experimental design followed was two factor completely randomized design with two replications.

Initially, three drought-tolerant rice genotypes *viz.,* Apo, Anna R4 and Wayrarem and two susceptible genotypes, Jaya and IR 64 obtained from TNAU, Coimbatore, were used to standardize the moisture stress level using PEG 6000. Uniform sized seeds were surface sterilized with 0.1% sodium hypochlorite solution for 2-3 min and washed with distilled water. For standardization, equal quantity of varying concentrations of PEG 6000 solution *viz*., -5.0 bar, -6.0 bar, -7.0 bar and -8.0 bar along with control (0 bar – distilled water) were using. The sterilized seeds of each genotype were placed on the blotting paper in petri dishes containing various concentrations of PEG solution. Seedling observations *viz.,* germination percentage (GP), shoot length (SL), root length (RL) and number of roots (NR) were recorded on tenth day and vigor index was calculated. The PEG 6000 concentration of -7.0 bar that induced a fifty per cent decrease in growth and germination in the tolerant check genotypes was chosen as the optimum dose for screening 26 rice landraces (Sourced from NBPGR, New Delhi) with drought tolerant (AnnaR4, Apo, Wayrarem) and drought susceptible checks (Jaya, IR64). The screening process followed the same methodology used for standardization. Seedling observations were recorded on the tenth day and other parameters *viz.,* vigor index (VI), shoot length stress tolerance index (SLSI) and root length stress tolerance index (RLSI) were computed as per Manonmani *et al.* (2020) and Shah *et al*. (2020).

Field screening: Field screening was carried out at the Paddy Breeding Station, Department of Rice, CPBG, Tamil Nadu Agricultural University, Coimbatore, during summer, 2023. Ten drought-tolerant rice landraces identified in the initial screening using PEG 6000 @ -7.0 bar were chosen. These selected landraces, along with five check genotypes (Jaya, IR64, Anna R4, Apo and Wayarem) were raised for field screening in both the rainout shelter to induce stress and a well-irrigated control (non-stress) condition. The screening was conducted in both ecosystem in a randomized block design with two replications. Seeds were directly sown in rain out shelter with two replications, while for control bed twenty-eight days old seedlings were transplanted with the spacing of 20 x 20 cm in 12m^2 area. Drought stress was imposed during the flowering stage, particularly from panicle initiation stage (70 DAS) to harvesting stage by withholding irrigation in rain out shelter (ROS), while the control plots were consistently maintained with ample irrigation. Observations on biometrical traits *viz.,* days to fifty per cent flowering*,* plant height, flag leaf length, flag leaf breadth, number of productive tillers and single plant yield were recorded at the time of harvest in both control and ROS. The drought score, leaf tip drying and leaf rolling were observed in ROS based on Standard Evaluation System, IRRI, 1996. Biochemical parameters *viz.,* proline and peroxidase activity were recorded immediately after the drought stress symptoms appeared (twenty-five days) after stress enforcement as per the method proposed by Bates *et al.,* 1973, Saddique *et al*., 2020 and Kar and Mishra, 1976, respectively. The data were statistically analyzed for analysis of variance (ANOVA) as per the method suggested by Gomez and Gomez (1984) by using statistical package GRAPES version 1.1.0. DMRT (Post-Hoc test) for field data was carried out using SPSS software version 16.0.

RESULTS AND DISCUSSION

PEG induced drought screening: PEG is an inactive polymer extensively employed in plant studies to induce osmotic stress. It's non-penetrating nature enables it to decrease the osmotic potential of the solution, consequently generating a stressful environment for plants. PEG does not infiltrate plant cells but modifies the surrounding solution, emulating a water scarcity or drought-like scenario (Rajput *et al*., 2022).

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A standardization experiment was set up with five checks comprising both susceptible and resistant genotypes to optimize the stress level to be imposed for screening the landrace germplasm collection obtained from NBPGR, New Delhi. The results pointed out that there was a significant decrease in germination percentage, seedling length and vigor index with an increase in water potential from -5.0 bar to -8.0 bar (**Table 1**). At -7.0 bar, the tolerant checks Apo, Anna (R) 4, and Wayrarem recorded mortality rates of 40 to 50 per cent. Conversely, in susceptible checks the mortality per cent was 80 per cent at -7.0 bar. The substantial variation in germination rates between the tolerant and susceptible checks underscored that -7.0 bar is the optimal stress threshold for effectively screening the germplasm collection for drought tolerance for rice.

Based on standardization, stress was imposed at -7.0 bar along with distilled water as control (0 bar) for screening of twenty-six genotypes. ANOVA revealed significant differences among the genotypes for all the five seedling traits (**Table 2**). The decrease in germination percentage, shoot length, root length, number of roots and vigor index were significantly altered by individual and interactive effects of the genotypes at control and -7.0 bar. This revealed the presence of variability among genotypes and hence amenable for selection which is in accordance with the findings of Manonmani *et al.,* (2020) in rice.

The parameters studied during laboratory condition exhibited considerable variability among the genotypes. Highest germination percentage of 70 was observed in IC115439 under -7.0 bar compared to other genotypes (**Fig. 1**) (**Table 3**). Conversely, in 4 genotypes *viz.,* RL2308, RL 9986, RL10092 and RL10203, the germination was completely arrested. Essential enzymes such as alpha-amylase, protease, lipases, phosphatase, cellulose, and other nutritional reserves play a crucial role in facilitating embryo growth. To activate these enzymes, a suitable level of moisture is essential. The decrease in water potential upon stress led to decreased seed germination across all genotypes, possibly stemming from the inhibition of enzyme activity due to a decline in the rate of water absorption. Similarly, Sabesan and Saravanan, (2016) also observed that reduced water potential resulted in a decreased germination rate of rice genotypes.

Assessing shoot and root length under stress conditions serves as a key criterion for evaluating genotype performance under stressed conditions (Manonmani *et al.,* 2020). The relationship between rice shoot and root length and the concentration of PEG 6000 exhibited an inverse correlation, as documented by Sagar *et al.* (2020). The maximum shoot length of 1.10 cm was observed in RL 4015 followed by IC115439 (1.03) Similarly, for the root length at -7.0 bar, the highest root length was observed in IC115439 (1.88 cm) followed by RL4105 (1.41 cm). Being a semi-aquatic shallow rooted crop, rice necessitates a higher count of lengthier roots to effectively adapt to challenging environmental circumstances. Notably, both these genotypes that recorded the highest primary root lengths were characterized with the maximum of four roots (**Table 3**). The decrease in both shoot and root length under stress can be attributed to reduced water uptake by the cell wall, which arises from disruption in

Table 1. Details on germination percentage, seedling length and vigor index of 5 checks under varying concentrations of PEG 6000

S.No.	Genotypes	Germination percentage (GP)%					Seedling length (cm)					Vigor index (VI)				
		Control	-5.0 bar	-6.0 bar	-7.0 bar	-8.0 bar	Control	-5.0 bar	-6.0 bar	-7.0 bar	-8.0 bar	Control	-5.0 bar	-6.0 bar	-7.0 bar	-8.0 bar
1	JAYA	99	99	40	20	0	11.63	6.00	0.40	0.29	0.00	1153.20	594.60	16.00	5.81	0.00
2	IR64	100	99	30	20	0	11.33	2.48	0.26	0.20	0.00	1131.46	245.77	7.83	4.00	0.00
3	APO	100	98	60	60	40	12.43	4.88	0.79	1.72	0.70	1242.50	479.54	47.33	103.00	27.65
4	ANNA R4	100	98	65	55	40	12.65	4.22	0.45	0.78	0.30	1265.00	414.78	29.25	43.08	12.00
5	WAYRAREM	100	99	55	50	0	11.05	4.85	0.03	0.75	0.00	1103.16	482.25	1.83	37.50	0.00

Table 2. Analysis of variance for seedling traits for 26 genotypes under *invitro* condition

Source of variation	df	Germination Percentage	Shoot length	Root length	Vigor index	Number of roots
Control & -7.0 bar (A)	1	152781.50*	2364.00*	2841.90*	106531918.20*	1179.75*
Genotypes (B)	25	699.05*	6.52*	13.69*	278508.58*	7.10*
A x B	25	653.40*	4.00*	8.09*	176278.94*	3.34*
Error	104	96.84	0.09	0.68	7888.21	0.37

*Significant at 5%probability

Table 3. Mean performance of 26 rice genotypes for seedling traits under control and -7.0 bar PEG 6000

S.No.	Genotypes	Germination percentage (GP)		Shoot length (SL)		Root length (RL)		Vigor index (VI)		Number of roots (NR)		SLSI	RLSI
		Control	-7.0 bar	Control	-7.0 bar	Control	-7.0 bar	Control	-7.0 bar	Control	-7.0 bar	-7.0 bar	-7.0 bar
1	RL4015	100.00	65.00	11.38	1.10	11.14	1.41	2251.50	163.41	9	4	9.67	12.69
2	RL10844	100.00	53.00	8.15	0.67	9.83	0.60	1797.50	67.31	8	3	8.22	6.11
3	RL10823	99.00	56.00	9.50	0.73	7.62	0.89	1694.52	90.88	6	3	7.71	11.68
4	RL10203	99.00	0.00	7.53	0.00	7.20	0.00	1457.28	0.00	7	0	0.00	0.00
5	RL10894	100.00	40.00	10.00	0.10	7.00	0.20	1700.00	12.00	7	1	1.00	2.86
6	RL10311	99.00	48.00	9.58	0.10	8.13	0.25	1749.94	16.80	7	1	1.04	3.08
7	RL9986	98.00	0.00	7.05	0.00	8.10	0.00	1491.77	0.00	8	0	0.00	0.00
8	RL11185	99.00	39.89	7.68	0.10	9.13	0.54	1668.18	25.52	7	1	1.30	5.91
9	RL11113	100.00	46.78	9.25	0.40	13.75	0.63	2293.68	47.95	9	2	4.32	4.55
10	RL10330	100.00	60.00	10.38	0.90	12.67	0.93	2307.69	109.80	7	3	8.67	7.34
11	RL6807	100.00	51.27	7.70	0.69	8.73	0.63	1638.67	67.85	6	2	8.96	7.26
12	RL11372	98.00	20.00	7.30	0.25	8.07	0.45	1505.93	14.00	8	1	3.42	5.58
13	RL10418	99.00	20.00	7.45	0.00	9.00	0.00	1629.10	0.00	8	1	0.00	0.00
14	RL10007	100.00	30.00	6.80	0.10	4.88	0.13	1162.44	7.00	8	1	1.47	2.74
15	RL2308	98.00	0.00	2.73	0.00	7.90	0.00	1042.07	0.00	5	0	0.00	0.00
16	RL10345	99.00	46.00	9.50	0.00	6.25	0.00	1564.37	0.00	7	0	0.00	0.00
17	RL369	100.00	20.00	6.63	0.10	7.88	0.43	1450.00	10.50	6	1	1.51	5.40
18	RL9977	99.00	33.33	7.13	0.00	7.99	0.00	1499.41	0.00	5	0	0.00	0.00
19	RL192	100.00	52.50	8.09	0.18	8.75	0.33	1684.00	26.78	6	2	2.18	3.81
20	RL10410	98.00	40.00	5.78	0.10	10.50	0.03	1595.44	5.33	6	3	1.73	0.32
21	RL5648	99.00	60.00	9.06	0.50	9.38	0.73	1821.99	74.00	7	3	5.52	7.82
22	RL6812	100.00	50.00	7.40	0.17	11.75	0.92	1915.00	54.33	8	3	2.30	7.80
23	RL10389	98.00	30.00	7.08	0.10	8.43	0.00	1519.82	3.00	7	0	1.41	0.00
24	IC 115439	100.00	70.00	11.63	1.03	16.25	1.88	2787.50	204.17	9	4	8.89	11.59
25	RL8144	99.00	20.00	7.33	0.10	3.43	0.00	1065.90	2.00	8	0	1.36	0.00
26	RL10092	99.00	0.00	7.88	0.00	9.18	0.00	1682.84	0.00	5	0	0.00	0.00
		SE	**CD**	**SE**	**CD**	**SE**	**CD**	**SE**	**CD**	**SE**	**CD**		
	A	1.58	3.13	0.05	0.10	0.13	0.26	14.22	28.20	0.10	0.19		
	B	5.68	11.27	0.18	0.35	0.48	0.95	51.28	101.69	0.35	0.70		
	A x B	8.04	15.93	0.25	0.50	0.68	1.34	72.52	143.81	0.50	0.99		

SLSI – Shoot length stress tolerance index, RLSI – Root length stress tolerance index

cell division, cytoplasm volume, turgor pressure, and nutritional equilibrium. Mehmandar *et al.* (2023) and Shah *et al.* (2020) also documented parallel conclusions, highlighting that water stress impacts meristematic cell division and elongation in *Cucumis melo* and chickpea, respectively. This, in turn, reduces cytokinin production, leading to a decline in shoot and root growth. Shoot length stress tolerance index and root length stress tolerance index was calculated using the shoot length and root length under stress and control condition as per the method suggested by Shah *et al.*, 2020. Among all the genotypes, IC115439 exhibited the highest Shoot length stress index (SLSI) and Root length stress index (RLSI), indicating that a decrease in water potential had a relatively minor impact on both shoot and root lengths.

As defined by the International Seed Testing Association (ISTA), vigor index encompasses all the characteristics of a seed that collectively influence its performance and growth in diverse environmental conditions. The vigor index (Manonmani *et al.*, 2020) calculated based on seedling length and germination percentage at -7.0 bar, indicated that maximum vigor index was recorded by IC115439 followed by RL4015 and RL10330 (**Table 3**). Genotypes exhibiting greater seedling vigor index under stressful conditions could be indicative of strong field

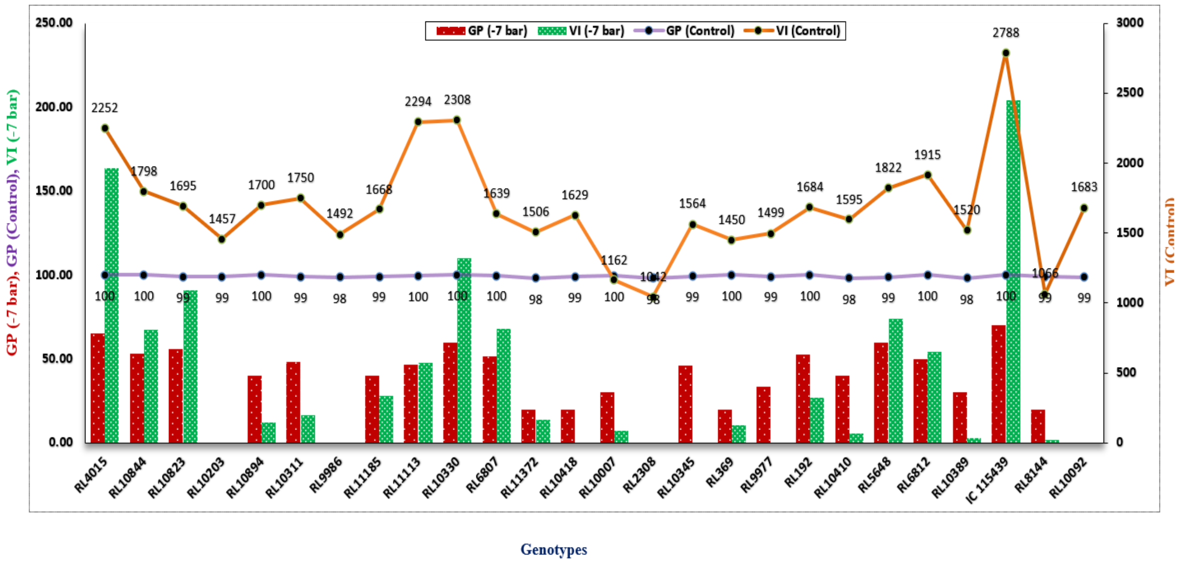

Fig. 1. Relative comparison of germination percentage, vigor index of 26 rice genot under control and -7 bar PEG 6000

performance (Nivethitha *et al.,* 2020). Therefore, the top ten genotypes *viz.,* IC115439, RL4015, RL10330, RL10823, RL5648, RL6807, RL10844, RL6812, RL11113 and RL192 that exhibited highest vigor index at -7.0 bar were selected and evaluated along with checks under field condition to gain a holistic understanding of their ability to cope with water deficit conditions.

Filed screening: The selected 10 genotypes along with checks were evaluated under both drought stressed (rain out shelter) and control condition in field. ANOVA revealed significant difference among the genotypes and interaction between stress *Vs* control (**Table 4**). All the genotypes showed considerable reduction in yield and other traits under drought compared to the control (**Table 5**). Drought stress adversely reduced the days to fifty percent flowering, plant height, leaf length, productive tillers which ultimately led to the yield reduction (Maurya *et al*., 2021). On the basis of IRRI SES scores (IRRI 1996), the 10 landraces were classified into three groups *viz.,* highly tolerant, moderately tolerant and susceptible based on their ability to tolerate drought stress. It was found that five genotypes were grouped as highly tolerant, four as moderately tolerant and one genotype was found susceptible (**Table 5**). Notably, the superior drought-tolerant genotype was associated with the lowest SES score of one. Favorably, these genotypes recorded higher single plant yield under stress on compared with rest of the genotypes. Therefore, the five highly tolerant genotypes *viz.,* IC115439, RL4015, RL5648, RL10330 and RL10823 were outperforming in terms of yield and other traits.

Specifically, under stress condition, IC115439 outperformed with highest number of productive tillers (12.50), panicle length (26.85cm), flag leaf breadth (1.80 cm) and single plant yield (15.15g) (**Table 5**). The flag leaf length of IC115439 was also observed to be higher than all the three drought tolerant checks. The size of the flag leaf (both length and breadth) directly influences the surface area available for photosynthesis. Larger flag leaves can potentially maintain higher photosynthetic rates even under drought conditions, allowing the plant to produce the energy it needs for survival and reproductive

Table 4. Analysis of variance for six biometrical traits under field evaluation

Source of variation	df	Plant height	Flag leaf length	Flag leaf breadth	Number of productive tillers	Panicle length	Single plant yield
Replication	1	64.07	0.08	0.00	0.04	1.94	36.19
Control and stress(A)	1	3226.67*	251.740*	0.140*	58.01*	31.97*	471.52*
Genotypes(B)	14	712.06*	132.24*	0.068*	8.28*	24.60*	98.79*
AxB	14	75.06*	71.23*	0.018*	3.19*	2.89*	65.85*
Error	29	88.43	53.41	0.012	3.24	4.27	12.63

*Significant 5%probability

Table 5. Mean performance of six biometrical traits with drought scores under field evaluation

S.No.	Genotypes	Days to 50 percent flowering		Plant Height		Flag leaf length		Flag leaf Breadth		Number of productive tillers		Panicle length		Single Plant Yield		DS	LTD	LTR
		Control	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress			
1	RL4015	87.00^{ab}	79.00^{bc}	138.0^{cde}	135.00^{abc}	61.00^{bcdef}	60.65^{a}	1.70^{abc}	1.55^{cdef}	12.00^{ab}	11.00^{bcd}	26.50^{ab}	23.50^{abcd}	16.95^{bcde}	14.00^{a}	1	1	1
2	RL10844	79.50^{cde}	76.50^{c}	148.00^{abcd}	118.00^{c}	65.65^{abcde}	63.50^{a}	1.70^{abc}	1.65^{abcd}	12.50^{ab}	9.00^{cd}	25.35ab	24.35^{abc}	14.30^{cdef}	13.20^{a}	3	3	3
3	RL10823	79.50^{cde}	78.00^{b}	130.50^{de}	114.50^{c}	56.70^{def}	49.50^{a}	1.65^{abc}	1.45^{def}	12.00^{ab}	11.00^{bcd}	25.90^{abc}	25.40^{ab}	16.40^{bcde}	13.80^{a}	1	1	1
4	RL11113	83.50^{bc}	81.00^{ab}	143.0^{bcde}	128.00^{bc}	61.20^{bcdef}	54.65^{a}	1.85^{ab}	1.80^{ab}	10.00^{b}	9.00^{cd}	20.65^{ef}	18.30^{cd}	18.90^{bcde}	11.20^{ab}	3	1	3
5	RL10330	83.00^{bcd}	79.50^{bc}	140.50^{cde}	123.50^{bc}	56.00^{ef}	47.35^{a}	1.60^{bcd}	1.50^{cdef}	13.00^{ab}	9.50^{cd}	25.35^{ab}	21.20^{abcd}	18.10^{bcde}	13.85^{a}	1	1	1
6	RL6807	79.00^{de}	78.00^{bc}	130.50^{e}	127.00^{bc}	60.85b^{cdef}	55.30^{a}	1.70^{bcd}	1.60^{bcde}	13.50^{ab}	11.00^{abc}	24.95^{bcd}	23.85^{ab}	23.20^{bc}	10.95^{ab}	3	3	1
7	RL192	79.00^{de}	76.50^{c}	123.50^{e}	118.00^{c}	53.50^{a}	52.60^{a}	1.65^{abc}	1.40^{ef}	10.50^{ab}	9.00^{d}	19.00^{f}	17.50^{d}	11.60^{def}	4.70^{c}	5	5	5
8	RL5648	84.50^{ab}	81.00^{ab}	148.00^{abcd}	119.00^{c}	59.50^{cdef}	57.60^{a}	1.65^{abc}	1.50^{cdef}	14.00^{ab}	12.20^{ab}	25.00^{abc}	22.80^{abcd}	15.10^{fg}	13.90^{a}	1	1	1
9	RL6812	85.00^{ab}	77.00^{c}	152.50^{abc}	137.50^{abc}	68.95^{abcd}	68.80^{a}	1.60^{bcd}	1.45^{a}	11.50^{ab}	8.50^{d}	21.80^{def}	21.10^{abcd}	25.10^{b}	11.10^{ab}	3	3	1
10	IC115439	88.50^{a}	79.50^{bc}	162.50^{ab}	154.50^{a}	71.00^{a}	67.50^{a}	1.90^{a}	1.80^{ab}	14.00^{ab}	13.00^{ab}	28.50^{bcd}	26.85^{a}	17.80^{efg}	15.15^{a}	1	1	1
11	JAYA	79.50^{cde}	81.00^{ab}	125.50^{e}	113.00^{c}	59.30^{cdef}	58.15^{a}	1.35^{d}	1.20^{def}	12.00^{ab}	9.00^{cd}	24.50^{bcd}	21.05^{abcd}	8.40^{g}	6.90^{bc}	7	7	9
12	IR64	78.00^{e}	83.50^{a}	131.75^{de}	117.00^{c}	66.00^{abcd}	55.35^{a}	1.55^{cd}	1.35^{f}	11.50^{ab}	10.00^{cd}	21.85^{cefd}	20.80^{bacd}	21.00^{bcd}	6.55^{bc}	5	5	7
13	APO	84.50^{ab}	78.50^{bc}	133.75^{cde}	111.50^{c}	69.50^{ab}	51.50^{a}	1.85^{ab}	1.60^{bcde}	14.50^{ab}	12.50^{ab}	23.35^{bcde}	20.30^{bcd}	27.80^{bcde}	15.95^{a}	1	1	0
14	ANNA R4	87.00^{ab}	78.00^{bc}	162.50^{ab}	147.50^{ab}	68.30^{abc}	63.65^{a}	1.80^{abc}	1.60^{bcde}	14.50^{a}	13.00^{a}	26.00^{ab}	25.00^{ab}	36.35^{a}	15.00^{a}	1	1	0
15	WAYRAREM	88.50^{a}	80.50^{ab}	166.00^{a}	147.50^{ab}	72.50^{a}	56.30	1.80^{abc}	1.70^{abc}	16.00^{a}	11.00^{bcd}	28.00^{a}	26.30^{ab}	25.35^{b}	9.70^{a}	1	1	3
		SE	**CD**	**SE**	**CD**	**SE**	**CD**	**SE**	**CD**	**SE**	**CD**	**SE**	**CD**	**SE**	**CD**			
	A	0.42	0.85	2.43	4.97	1.89	3.86	0.03	0.06	0.35	0.71	0.53	1.09	0.92	1.88			
	B	1.14	2.33	6.65	13.6	5.17	10.57	0.07	0.16	0.95	1.94	1.46	2.99	2.51	5.14			
	A x B	1.61	3.30	-	-	-	-	-	-	-	-	-	-	3.55	7.27			

DS - Drought score   LTD -Leaf tip Drying   LTR- Leaf tip rolling

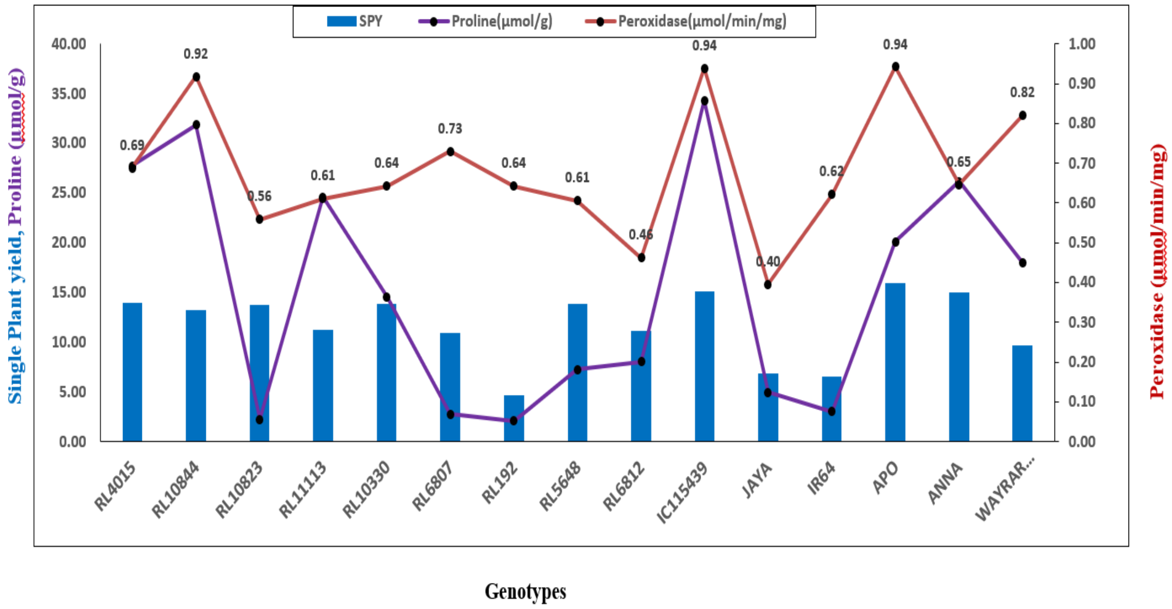

Fig. 2. Comparative performance of 10 rice genotypes along with checks under stressed environment for single plant yield, proline and peroxidase

success (Biswal and Kohli, 2013). Therefore, all the above traits in combination could have contributed to enhancing the yield of IC115439. The highest yield under stress recorded by IC115439 was on par with Apo. In addition, the levels of proline and peroxidase under stress were higher for IC115439 (**Fig.2**). The accumulation of proline contributes to the osmotic adjustment and tolerance of plants exposed to adverse environmental conditions. Substantially, correlation analysis involving biochemical parameters *viz.,* proline and peroxidase activity revealed a significant and positive correlation between proline levels and single plant yield under stress condition with correlation coefficient of 0.6. The significant increase of proline under drought was also observed by Moonmoon and Islam, (2017) in rice.

The research comprehensively evaluated the impact of drought stress during seedling and reproductive stages, both in laboratory and in natural environment conditions. Genotypes *viz.,* IC115439, RL4015, RL5648, RL10330, RL10823 and RL10844 emerged as promising lines due to their higher resilience to osmotic stress and better performance across multiple traits, including germination percentage, shoot and root growth, vigor index and yield-related traits. Particularly, IC115439 a land race collected from the farmers field of Raipur, Chhattisgarh locally named as *Sukara Phool* exhibited superiority over its counterparts, holds the potential to serve as a valuable pre-breeding resource in crop improvement initiatives. These findings hold significant implications for the identification of genotypes with improved drought tolerance, broadening genetic base and consequently contributing to the advancement of drought-resistant rice varieties.

The optimized PEG 6000 concentration of -7.0 bar can be utilized for rapid selection of drought tolerant rice genotypes at seedling stages. Vigor index serves as the reliable indicator to test the potential performance of genotypes that are better equipped to withstand and thrive in water limited area. The five selected genotypes (IC115439, RL4015, RL10330, RL5648, RL10823) based on the preliminary screening and field screening, can be further evaluated under target production environment for drought. In particular, the genotype, IC115439 outperformed and can forwarded for location testing and can also be utilized as pre breeding material for drought stress breeding. Furthermore, it can be recommended for multi-location trails aiming at varietal release. The development of such drought tolerant varieties can only ascertain "more crop per drop" possible to cope up with the changing climate.

REFERENCES

Bates, L.S., Waldren, R. P. and Teare, I. D. 1973. Rapid determination of free proline for water stress studies. *Plant soil*, **39**:205-207. [Cross Ref]

Biswal, A. K. and Kohli, A. 2013. Cereal flag leaf adaptations for grain yield under drought: knowledge status and gaps. *Molecular Breeding*, **31**:749-766. [Cross Ref]

International Rice Research Institute (RRI). 1996. Standard evaluation System for Rice, 4th edition, International Rice Research Institute, Los Bafios, *The Philippines*, 52pp.

Iyem, E., Yildirim, M. and Kizilgeci, F. 2021. Germination, Seedling Growth and Physio-Biochemical Indices of Bread Wheat (*Triticum Aestivum* L.) Genotypes under Peg Induced Drought Stress. *The Journal Agriculture and Forestry,* **67**(1):163-180. [Cross Ref]

Kar, M. and Mishra, D. 1976. Catalase, peroxidase, and polyphenoloxidase activities during rice leaf senescence. *Plant physiology*, **57**(2): 315-319. [Cross Ref]

Manohar, R. V., Nivethitha, T., Jadhav, B. N., Raveendran, M., Sritharan, N., Pushpam, R. and Joel, A. J. 2022. Utilising genetic variability and diversity analysis as a tool to identify drought tolerant pre-breeding genetic materials in rice (*Oryza sativa* L.).*The Pharma Innovation Journal*, **11**(8)**:** 1374-1381. [Cross Ref]

Manonmani, K., Jeyaprakash, P., Manonmani, S., Raveendran,,M. and Jeyakumar, P. 2020. Assessment of drought tolerance in Nagina 22 mutants of rice using Poly Ethylene Glycol (PEG). *Electronic Journal of Plant Breeding,* **11**(2):479-486. [Cross Ref]

Maurya, K., Joshi, H. C., Shankhdhar, S. C., Guru, S. K., Guar, A. K., Nautiyal, M. K. and Kumar, A. 2021. Evaluation of some rice (*Oryza sativa* L.) genotypes for drought tolerance. *Int. J. Curr. Microbiol. App. Sci*, **10**(2):3294-3301. [Cross Ref]

Mehmandar, M. N., Rasouli, F., Giglou, M. T., Zahedi, S. M., Hassanpouraghdam, M. B., Aazami, M. A., Tajaragh, R. P., Ryant, P. and Mlcek, J. 2023. Polyethylene glycol and sorbitol-mediated *in vitro* screening for drought stress as an efficient and rapid tool to reach the tolerant *Cucumis melo* L. Genotypes. *Plants*, **12**(4): 870. [Cross Ref]

Moonmoon, S. and Islam, M. 2017. Effect of drought stress at different growth stages on yield and yield components of six rice (*Oryza sativa* L.) genotypes. *Fundamental and Applied Agriculture*, **2**(3):1. [Cross Ref]

Nahar, S., Sahoo, L. and Tanti, B. 2018. Screening of drought tolerant rice through morpho-physiological and biochemical approaches. *Biocatalysis and Agricultural Biotechnology*, **15**:150–159. [Cross Ref]

Nivethitha, T., Ravikesavan, R., Kumari Vinodhana, N. and Senthil, N. 2020. Deciphering drought tolerance potential of sweet corn genotypes through polyethylene glycol induced drought stress. *Electronic Journal of Plant Breeding*, **11**(01): 217-223. [Cross Ref]

Rajput, G. S., Kuruwanshi, V. B. and Guhey, A. 2022. Polyethylene glycol induced screening for drought tolerance of different rice genotype, *The Pharma Innovation Journal*, **11**(9): 996 – 1000.

Sabesan, T. and Saravanan, K. 2016. In vitro screening of Indica rice genotypes for drought tolerance using polyethylene glycol. *International Journal of advances in agricultural and environmental engineering*,**3**(2): 2349-1531. [Cross Ref]

Saddique, M. A. B., Ali, Z., Sher, M. A., Farid, B., Ikram, R. M. and Ahmad, M. S. 2020. Proline, total antioxidant capacity, and OsP5CS gene activity in radical and plumule of rice are efficient drought tolerance indicator traits. *International Journal of Agronomy*, 1-9. [Cross Ref]

Sagar, A., Rauf, F., Mia, M., Shabi, T., Rahman, T. and Hossain, A. 2020. Polyethylene Glycol (PEG) induced drought stress on five rice genotypes at early seedling stage. *Journal of Bangladesh Agricultural University*, **18**(3):606-614. [Cross Ref]

Shah, T. M., Imran, M., Atta, B. M., Ashraf, M. Y., Hameed, A., Waqar, I., Shafiq, M., Hussain, K., Naveed, M., Aslam, M. and Maqbool, M. A. 2020. Selection and screening of drought tolerant high yielding chickpea genotypes based on physio-biochemical indices and multi-environmental yield trials. *BMC Plant Biology*, **20**(1):1-16. [Cross Ref]

Shereen, A., Khanzada, M., Wahid Baloch, M., Asma, A., Shirazi, M., Khan, M. and Arif, M. 2019. Effects of PEG induced water stress on growth and physiological responses of rice genotypes at seedling stage. *Pakistan Journal of Botany*, **51**(6):2013-2021. [Cross Ref]