

## Original Research Article

# Unravelling the genetic variability of rice (*Oryza sativa* L.) genotypes for enhanced drought stress tolerance

**Comment [D1]:** If you can be more specific

### ABSTRACT

**Comment [D2]:** Objectives, methods and results/conclusions are shown

Identification of rice genotypes based on drought tolerant attributes is one of pivotal exploration work in the changing environmental situation with occurrence of drought has become more often. BCKV-Regional Research Station (Red & Laterite zone) Jhargram, was the area where the work was carried out, where thirty-eight rice genotypes were evaluated with checks under drought stress and normal condition. ANOVA revealed significant differences between the various genotypes regarding the traits under consideration, under the two screening environments. High variability along with maximum broad sense heritability were present for the characters namely leaf proline content, relative water content, recovery percentage and no. of grains per panicle. Indices related to drought stress resilience, root length, root shoot ratio and grain yield per plant in normal & stress condition also exhibited high heritability and genetic advance. These traits may offer a basis for direct selection of genotypes for drought prone areas.

Keywords- Drought stress, DSI, genetic variability, proline content, yield stability index

### Introduction

Indian subcontinent is a major producer of rice. It is extensively cultivated under different environmental conditions as an irrigated, rainfed-highland and rainfed lowland crop. India had over 46 million ha of under rice cultivation at the end of fiscal year 2022 (Anonymous, 2023), of which major area falls under irrigated land. Among the various abiotic stress, drought severely limits the rice productivity (Vallivodan and Nguyen, 2006; Sinclairs, 2011). Further, lack of adaptable rice varieties to waster stress conditions remains one of the major challenges for the country's rice production. Uncertain water availability in rainfed upland areas, coupled with infertile soils that can be acidic or saline, and varying crop management practices, present a diverse range of breeding targets with different environmental conditions that impact the phenotypic response of genotypes. This complex and diverse situation often occurs within small geographical regions. Enhancing heritable traits under such complex conditions poses a significant challenge for any breeding program.

Physiological systems that rely on cell volume expansion are particularly vulnerable to water shortage, including increased leaf area as well as the rates at which the gases are exchanged. Water stress also affects transpiration, photosynthesis, respiration, and assimilate translocation, as well as phenological stages. Reduction in leaf area is likely the primary means by which plants regulate water as drought response (Sadras and Milroy, 1996). Quantifying the morpho-physiological feedback of stress observed in rice to water is crucial for assessing the influence of rainfall and soil conditions on the productivity of rice. The scientific pursuit of genetic improvement in rice for drought tolerance involves the careful selection, breeding, and incorporation of desirable traits. By capitalizing on the genetic variability in rice germplasm, scientists aim to develop resilient cultivars that can thrive under water-limited conditions. These efforts play a crucial role in addressing the challenges posed by climate change and securing the productivity and nutritional quality of rice in drought-prone areas. Therefore, quantifying drought tolerance in rice genotypes along with delineating inheritance of associated traits which come under the purview of not much stressed and non-stressed environments need to be studied in order to develop a better understanding regarding the tolerance mechanisms in rice and improving yield under drought conditions

## **MATERIALS & METHODS**

**Comment [D3]:** Clarified subsections

The research was carried out at BCKV-RRS-Jhargram experimental field during the 2016 kharif season. The prevailing agro-climate at the experimental site is of semi-arid type with uneven rainfall distribution. The soil is sandy loam, rich in Fe and Al with slightly acidic reaction (Garg et al., 2017).

A set of 38 rice genotypes along with checks IR-36, Vandana, IR-64 and Sahabhabidhan were screened under both irrigated and water-stress environment in randomized block design (RBD) which included three replications. The method of sowing adopted was direct seeding method under both normal as well as stress conditions, keeping in view the agroclimatic condition. Irrigated experiment represented favorable conditions, and the plots were watered as and when required to avoid water stress. Applications of NPK and S fertilizers were given @ 60:40:30 & 10 kg ha<sup>-1</sup> respectively. An initial dose of 1/2 N along with a full dose of P, K, and S were applied basally. A split dose of half of N was provided at the sites of tillering and panicle initiation. In the other set of experiment, a fifteen-day drought stress was imposed at panicle initiation stage. Data were recorded for a total of thirty-two traits. Phenological attributes included days to 50% flowering, flowering delay and days to maturity. These were determined on per plot basis. Plant height (cm), chlorophyll content, panicle length (cm), number of tillers/plant, flag leaf area (cm<sup>2</sup>), number of grains/panicle, thousand grain weight, harvest index

and seed yield/plant were also recorded. Yield attributes as well as physiological traits related to drought stress resilience viz. leaf rolling at vegetative stage, leaf drying at vegetative stage, spikelet fertility, relative water content, proline content, recovery after stress, reduction (%), seed vigour index, root length (cm), shoot length (cm), root-shoot ratio, fresh and dry weight of root (g), fresh and dry weight of shoot (g) were estimated based on 5 competitive plant from individual plots.

Following drought-tolerance related seed yield based indices were also worked out-

(1) Drought Susceptibility Index (DSI) -  $(1 - Y_{\text{stress}}/Y_n) / (1 - \text{Mean } Y_{\text{stress}} / \text{Mean } Y_n)$

(2) Stress tolerance, TOL -  $Y_n - Y_{\text{stress}}$

(3) Stress tolerance index, STI -  $(Y_{\text{stress}} * Y_n) / (Y_n)^2$

(4) Yield index, YI =  $Y_{\text{stress}} / \text{Mean of } Y_{\text{stress}}$

(5) Yield stability index, YSI =  $Y_{\text{stress}} / Y_n$

Here,  $Y_{\text{stress}}$  denotes the mean yield of genotypes under stress, while  $Y_n$  denotes the mean yield of genotypes in non-stress conditions (irrigated).

After carrying out the various experiments, the collected data were analysed for variance followed by estimation of genetic variability parameters, using Ms-Excel. The broad-sense heritability ( $H^2$ ) was estimated ensuing the method proposed by Hanson et al. (1956). Further, phenotypic (PCV) and genotypic coefficients of variation (GCV) were computed based on the approach described by Burton and Devane (1952). Genetic advance, which indicates a percentage of the mean (GAM), was determined using the technique outlined by Johnson et al. (1955).

## RESULTS & DISCUSSION

The variance analysis for a number of quantitative and qualitative attributes in rice genotypes showed significant variation under both stress and irrigated conditions (Table-1). This suggests that further investigation into water stress tolerance is meaningful, considering the significant mean sum of squares observed under water stress as well as irrigated (non-stress) conditions. Interestingly, most traits exhibited greater mean sum of squares in contrast with irrigation systems under drought stress, indicating desired level of variation for these characters is more prominent under water stress. This is expected since different genotypes respond differently to water stress conditions. Previous studies, including those by Mina et al. (2011), Ganapathy et al.

(2007), Pantuwan et al. (2002), Ouk et al. (2006), Chen et al. (2001), Muthuswamy and Kumar (2006), Allah (2009), and Gomez and Kalamani (2003), have also concluded that greater variation exists for different traits in the genotypes grown under moisture stress regimes. Highest GCV under normal (non-stressed) condition was recorded for leaf proline content whereas lowest was recorded for days to maturity (Table 2). On the other hand, highest GCV under stressed condition was recorded for flowering delay whereas lowest value was evident for yield index. In case of PCV under normal condition, highest and lowest values were recorded for leaf proline content and relative water content, respectively. Under stress condition, highest and lowest PCV were recorded for leaf drying at vegetative stage and days to maturity, respectively. The phenotypic variations of the examined traits were typically greater compared to the genotypic variances, indicating that environmental influences play a role in the behaviour of the traits under investigation.

In crop development programs, selection can be carried out through direct or indirect methods. Direct selection involves considering parameters such as heritability and genetic advance, while indirect selection relies on correlation and path coefficient analysis. Highest  $H^2$  was recorded for flag leaf area under normal sown condition whereas for spikelet fertility and proline content under stressed condition. Also, lowest  $H^2$  was recorded for days to maturity and leaf rolling at vegetative stage under normal and late sown condition, respectively. In our study, high broad-sense heritability was observed for traits viz. days to 50% flowering, seed vigour index, root length, root-shoot ratio, dry weight of root, plant height, relative water content, reduction percentage, drought susceptibility index, seed yield per plant, stress tolerance index, tolerance index, yield stability index, and proline content. These findings align with studies conducted by Venkataramana and Shailaja (1999), Wu et al. (2004), Muthuswamy et al. (2006), Manickavelu et al. (2006), and Allah (2009).

The estimation of heritability and genetic advance, together with the components of variation including genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV), can assist in identifying traits suitable for selection and improvement. In our research, traits such as leaf proline content, flag leaf area, fresh shoot weight and spikelet fertility exhibited high heritability and high GAM. The outcomes inferred were in tune with the results observed by Ganapathy et al. (2007) and Mina et al. (2011), suggesting least amount of environmental influence indicating the prevalence of the additive gene effects. As a consequence, under water stress conditions, genotypes can be altered and chosen for these features to improve drought tolerance. The combination of high heritability and high genetic advance indicates the observed variation to be primarily attributed towards additive gene effects. Thus, implementing a selection scheme targeting these traits can lead to the development of widely adapted genotypes by harnessing the fixable genetic variance available.

## CONCLUSION

The traits that showed the highest effectiveness for selecting drought-resilient as well as high-yielding rice genotypes under drought conditions included drought tolerance indices, flag leaf area, chlorophyll content, relative water content, root traits, and proline content. These traits may be incorporated in the selection of genotypes for cultivation in water-scarce areas. Further, genotypes identified based on these traits can be used for hybridization to obtain segregating generations. The segregating generation can then either be used for identification of some transgressive segregants for future breeding or may be used as mapping population for delineating chromosomal regions governing these traits.

## REFERENCES

- Allah, A.A.A. (2009). Genetic studies on leaf rolling and some root traits under drought conditions in rice (*Oryza sativa* L.). *Afr. J. Biotech.*, **8**(22): 6241-6248.
- Anonymous (2023). <https://www.statista.com/statistics/765691/india-area-of-cultivation-for-rice/#:~:text=At%20the%20end%20of%20fiscal,across%20the%20south%20Asian%20nation>. Accessed on 11.06.2023.
- Burton, G.W. and Devane, E.H. (1952). Estimating heritability in tall fescue (*Festuca arundinaceae*) from replicated clonal material, *Agron. J.*, **45**: 478-481.
- Chen, FengMei., Long, YuanMei., Cheng, JianFeng., Pan, XiaoYun and Liu, YiBai. 2001. Genetic analysis of the drought-resistant indices in indica rice. *Acta Agric Universitatis Jiangxiensis*. **23**(1): 41-45
- Fengmei, C., Yuanmei, L., Jianfeng, C., Xiaoyun, P., & Yibai, L. (2001). Genetic analysis of the drought-resistant indices in indica rice. *Acta Agric. Univ. Jiangxiensis*, **23**(1): 41-45.
- Ganapathy, S., Ganesh, S.K., Vivekanandan, P., Shanmugasundaram, P., & Babu, R.C. (2007). Variability and interrelationship between yield and physio-morphological traits in rice (*Oryza sativa* L.) under moisture stress condition. *Crop Res.*, **34**(1/3): 260-262.
- Gomez, S.M., & Kalamani, A. (2003). Scope of landraces for future drought tolerance breeding program in rice (*Oryza sativa* L.). *Plant Arch.*, **3**(1): 77-79.
- Garg, H.S., Kumari, P., Bhattacharya, C., Panja, S., & Kumar, R. (2017). Genetic parameters estimation for yield and yield related traits in rice (*Oryza sativa* L.) with drought tolerance trait under stress condition. *J. Crop Weed*, **13**(1): 83-88.
- Hanson, C. H., Robinson, H. F., & Comstock, R. E. (1956). Biometrical studies of yield in segregating populations of Korean lespedeza 1. *Agron. J.*, **48**(6): 268-272.
- Manickavelu, A., Gnanamalar, R.P., Nadarajan, N., & Ganesh, S.K. (2006). Genetic variability studies on different genetic populations of rice under drought condition. *J. Pl. Sci.*, **1**(4): 332-339.
- Mina, A., Abarshah, Babak, R., Habibollah, & Samizadeh, L. (2011). Assessing Genetic Diversity of Rice Varieties under Drought Stress Conditions. *Notulae Sci. Biol.*, **3**(1): 114-123.
- Muthuswamy, A., & Kumar, C.R.A. (2006). Variability studies in drought-resistant cultivars of rice. *Res. Crops*, **7**(1): 130-132.

**Comment [D4]:** Many are too old, preferably 25% (last 5 years)

Ouk, M., Basnayake, J., Tsubo, M., Fukai, S., Fischer, K.S., Cooper, M., & Nesbitt, H. (2006). Use of drought response index for identification of drought-tolerant genotypes in rainfed lowland rice. *Field Crops Res.*, **99**(1): 48-58.

Sadras, V.O., & Milory, S.P. (1996). Soil-water thresholds for the responses of leaf expansion and gas exchange: A review. *Field Crops Res.*, **47**: 253-266.

Sinclair, T.R. (2011). Challenges in breeding for yield increase for drought. *Trends Plant Sci.*, **16**: 289-293.

Vallivodan, B., & Nguyen, H.T. (2006). Understanding regulatory networks and engineering for enhanced drought tolerance in plants. *Curr. Opin. Plant Biol.*, **9**:189-195.

Venkataramana, P., & Shailaja, H. (1999). Genetic variability on some important traits in two F<sub>2</sub> segregants of rice (*Oryza sativa* L.) under non-submergence condition. *Crop Res.*, **18**(1): 53-56.

Johnson, H. W., Robinson, H. F., and Comstock, R. E. (1955). Estimates of genetic and environmental variability in soybeans. *Agron.J.*, **47**(7): 314-318.

Wu, M.G., Lin, J.R., & Zhang, G.H. (2004). Genetic analysis of leaf relative water content in paddy upland hybrid rice. *Chin. J. Rice Sci.*, **18**(6): 570-572.

Reddy, Y.R., Medda, P.S., Roy, S., & Datta, S. (2022). Genetic variability studies of garlic (*Allium sativum* L.) germplasm in Terai region of West Bengal. *J. Crop Weed*, **18**(3): 172-180.

UNDER PEER REVIEW

**Table: 1 Analysis of variance for different quantitative and qualitative characters of rice under stress and normal condition**

Traits	Mean sum of squares					
	Replication		Treatments		Error	
	Normal	Stress	Normal	Stress	Normal	Stress
<b>DF</b>	0.07	5.71	69.13**	81.93**	0.49	8.15
<b>FD</b>	-	0.38	-	12.29**	-	0.74
<b>DM</b>	44.32	2.35	166.14**	65.34**	157.02	1.03
<b>PH(cm)</b>	20.06	12.15	251.00**	363.72**	10.93	1.43
<b>FLA (cm<sup>2</sup>)</b>	0.16	2.19	123.42**	165.51**	0.16	0.75
<b>CHL.C.</b>	1.38	0.84	22.55**	10.00**	0.26	1.19
<b>NTPP</b>	0.21	0.18	10.98**	10.20**	0.96	0.27
<b>LR</b>	0.94	0.71	0.72**	0.59**	0.43	0.49
<b>LD</b>	1.95	0.11	0.69**	0.76**	0.56	0.54
<b>RWC</b>	10.12	1.11	24.90**	44.67**	1.19	0.94
<b>PL(cm)</b>	0.64	0.54	19.34**	36.60**	0.73	0.40
<b>SF</b>	2.35	0.16	477.48**	480.02**	2.18	0.90
<b>RAS</b>	0.18	2.64	89.99**	98.95**	3.12	0.51
<b>NGPP</b>	8.76	0.42	390.53**	139.90**	14.40	2.33
<b>TGW(g)</b>	0.40	0.06	20.99**	22.27**	0.63	0.19
<b>R %</b>	-	48.18	-	739.66**	-	51.41
<b>HI</b>	0.35	3.60	71.15**	97.04**	0.33	0.96
<b>SYPP(g)</b>	0.01	0.31	13.22**	10.19**	0.29	0.39
<b>SVI</b>	18780.03	38450.57	1753831.13**	1316984.30**	3708.34	8069.95
<b>RL(cm)</b>	0.07	4.88	14.61**	20.56**	0.43	0.57
<b>SL (cm)</b>	1.36	3.02	226.68**	170.27**	0.41	1.31
<b>RSR</b>	0.01	0.14	0.43**	1.51**	0.01	0.09
<b>DWR(g)</b>	0.60	1.30	18.98**	14.53**	0.21	0.42
<b>FWR(g)</b>	0.09	1.20	61.07**	12.45**	0.42	0.59

<b>DWS(g)</b>	0.65	0.12	69.48**	29.86**	0.36	1.04
<b>FWS(g)</b>	0.03	7.46	323.07**	219.06**	0.44	1.65
<b>DSI</b>	-	0.00	-	0.29**	-	0.00
<b>TOL</b>	-	0.48	-	11.35**	-	0.85
<b>STI</b>	-	0.17	-	35.48**	-	0.51
<b>YI</b>	-	0.01	-	0.00**	-	0.01
<b>YSI</b>	-	0.00	-	0.01**	-	0.00
<b>PROL.C.</b>	0.02	0.00	0.23**	0.66**	0.01	0.00

\*\* Significance at 1 % level, \* Significance at 5% level

**Abbreviations-** **DF**-Days to 50% Flowering, **FD**-Flowering Delay, **DM**-Day to Maturity, **PH**-Plant Height (cm), **FLA**-Flag Leaf Area (cm<sup>2</sup>) **CHL.C.**-Chlorophyll Content, **NTPP**-Number of Tillers/plant, **LR**-Leaf rolling at Veg. Stage, **LD**-Leaf Drying at Veg. Stage, **RWC**-Relative Water Content, **PL**-Panicle Length (cm), **SF**-Spikelet Fertility, **RAS**-Recovery After Stress **NGPP**-No. of Grains/Panicle, **TGW**-Thousand Grain Weight, **R%**-Reduction (%), **HI**-Harvest Index, **SYPP**-Seed Yield/Plant **SVI**-Seed Vigour Index, **RL**-Root Length (cm), **SL**-Shoot Length (cm), **RSR**-Root-Shoot Ratio, **DWR**-Dry Weight of Root (g), **FWR**-Fresh Weight of Root (g), **DWS**-Dry Weight of Shoot(g), **FWS**-Fresh Weight of Shoot(g), **DSI**-Drought susceptibility index, **TOL**-Tolerance index, **STI**-Stress tolerance index, **YI**-Yield index, **YSI**-Yield stability index, **PROL.C.**-Proline Content

**Table: 2 Genetic parameters for various quantitative and qualitative traits of rice genotypes under stress and non-stress conditions.**

No.	Traits	$\sigma^2_g$		$\sigma^2_p$		GCV		PCV		$h^2$ (Broad sense) %		Genetic Advance as % of Mean	
		Normal	Stress	Normal	Stress	Normal	Stress	Normal	Stress	Normal	Stress	Normal	Stress
1	DFF	22.87	24.59	23.37	32.74	6.26	6.64	6.33	7.66	97	75	12.76	11.85
2	DM	3.03	21.43	160.06	22.47	1.72	4.43	12.53	4.54	01	95	0.49	8.92
3	PH (cm)	80.02	120.76	90.95	122.20	9.81	12.29	10.46	12.36	88	98	18.96	25.18
4	FLA (cm <sup>2</sup> )	41.08	54.91	41.25	55.67	19.13	22.05	19.17	22.20	99	98	39.34	45.12
5	CHL.C.	7.43	2.93	7.69	4.13	7.21	4.76	7.34	5.64	96	71	14.60	8.27
6	NTPP	3.34	3.31	4.30	3.58	16.70	21.53	18.97	22.41	77	92	30.31	42.63
7	LR	0.09	0.03	0.53	0.52	25.88	18.28	60.25	72.64	18	06	22.90	9.47
8	LD	0.04	0.07	0.61	0.62	18.06	36.11	68.55	104.43	06	12	9.80	25.72
9	RWC	7.90	14.57	9.09	15.52	3.58	5.01	3.84	5.17	86	93	6.88	10.01
10	PL (cm)	6.20	12.06	6.93	12.46	9.65	14.52	10.20	14.76	89	96	18.80	29.42
11	SF	158.43	159.70	160.62	160.61	18.82	19.47	18.95	19.53	98	99	38.51	40.01
12	RAS	28.95	32.81	32.07	33.32	7.25	7.91	7.63	7.97	90	98	14.2	16.17
13	NGPP	125.37	45.85	139.78	48.19	9.92	6.70	10.47	6.87	89	95	19.35	13.47
14	TGW	6.78	7.36	7.42	7.55	11.05	13.49	11.56	13.67	91	97	21.77	27.44
15	HI	23.60	32.02	23.94	32.99	10.25	13.37	10.32	13.57	98	97	20.97	27.15
16	SYPP	4.31	3.26	4.60	3.66	17.97	19.73	18.57	20.89	93	89	35.83	38.41
17	SVI	583374.30	436304.80	587082.60	444374.80	16.28	15.33	16.33	15.47	99	98	33.44	31.29
18	RL	4.72	6.66	5.16	7.23	8.70	11.91	9.10	12.41	91	92	17.15	23.54
19	SL	75.42	56.32	75.83	57.63	13.68	12.46	13.72	12.61	99	97	28.11	25.39
20	RSR	0.14	0.47	0.15	0.56	20.69	34.48	21.48	37.80	92	83	41.07	64.79
21	DWR	6.25	4.70	6.47	5.13	22.26	34.38	22.65	35.92	96	91	45.09	67.80
22	FWR	20.21	3.95	20.64	4.55	27.57	22.27	27.86	23.90	98	86	56.21	42.75
23	DWS	23.04	9.60	23.40	10.65	23.91	26.85	24.09	28.27	98	90	48.88	52.54

24	FWS	107.54	72.47	107.98	74.12	17.55	20.09	17.58	20.32	99	97	36.08	40.93
25	FD	-	3.85	-	4.59	-	81.52	-	89.07	-	83	-	153.72
26	R %	-	229.41	-	28.83	-	18.83	-	20.83	-	81	-	35.72
27	DSI	-	0.096	-	0.10	-	51.06	-	52.39	-	95	-	102.50
28	TOL	-	3.49	-	4.35	-	77.99	-	87.03	-	80	-	143.98
29	STI	-	11.65	-	12.17	-	16.48	-	16.84	-	95	-	33.23
30	YI	-	-0.00	-	0.01	-	3.87	-	5.49	-	49	-	5.64
31	YSI	-	0.02	-	0.02	-	18.79	-	20.79	-	81	-	34.98
32	ProL.C.	0.07	0.22	0.086	0.22	131.46	60.67	141.31	60.90	86	99	251.95	124.51

**Abbreviations-** **DF**-Days to 50% Flowering, **FD**-Flowering Delay, **DM**-Day to Maturity, **PH**-Plant Height (cm),**FLA**-Flag Leaf Area (cm<sup>2</sup>) **CHL.C.**-Chlorophyll Content, **NTPP**-Number of Tillers/plant, **LR**-Leaf rolling at Veg. Stage, **LD**-Leaf Drying at Veg. Stage, **RWC**-Relative Water Content, **PL**-Panicle Length (cm),**SF**-Spikelet Fertility, **RAS**-Recovery After Stress**NGPP**-No. of Grains/Panicle, **TGW**-Thousand Grain Weight, **R%**-Reduction (%),**HI**-Harvest Index, **SYPP**-Seed Yield/Plant **SVI**-Seed Vigour Index, **RL**-Root Length (cm),**SL**-Shoot Length (cm),**RSR**-Root-Shoot Ratio, **DWR**-Dry Weight of Root (g),**FWR**-Fresh Weight of Root (g), **DWS**-Dry Weight of Shoot(g),**FWS**-Fresh Weight of Shoot(g), **DSI**-Drought susceptibility index,**TOL**-Tolerance index, **STI**-Stress tolerance index, **YI**-Yield index, **YSI**-Yield stability index,**PROL.C.**-Proline Content