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Trait associated studies in diversified rice genotypes (*Oryza sativa* L.) under irrigated and drought stress conditions

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Abstract
Drought is a major abiotic constraint for growing rain-fed rice in India. In the present investigation, a total of 30 rice genotypes were evaluated under drought stress and irrigated (non-stress) condition. Fifteen morpho-physiological and biochemical characters *viz*, root length, plant height, number of tillers per plant, panicle length, panicle weight, number of spikelets per panicle, filled grains per panicle, grain weight per panicle, 1000 grain weight, chlorophyll content flavonoids, phenols, carbohydrates, proline and plant yield were recorded on five randomly selected plants from each replication under stress and control for statistical analysis. Under both environments, association analysis revealed that the plant yield exhibited positive and significant correlation with root length, plant height, number of tillers, panicle length, panicle weight, number of spikelet's, per panicle, number of filled grains per panicle, grain weight per panicle and 1000 grain weight. Path analysis showed direct and high positive effect on plant yield under irrigated and stress was exhibited by number of tillers per plant, panicle weight and chlorophyll content. Further, the selection of traits with positive association towards yield and other attributes will be rewarding for crop improvement programmes for development of drought tolerant lines.

Keywords: drought, trait association, direct selection, positive effect

Introduction

Rice is a self-pollinated cereal crop belonging to the family Gramineae under the order Cyperales and class monocotyledon having chromosome number 2n=24. The genus Oryza is known to consist of two cultivated species i.e., Asian rice (*O. sativa*, 2n=24=AA) and African rice (*O. glaberrima*, 2n=24=AA) and 22 wild species (2n=24, 48). The river valleys of Yangtze, Mekon River area in China could be the primary centre of origin of *Oriza sativa*. *Oryza glaberrima* is indigenous to the upper valley of the Niger River and it is cultivated only in western tropical Africa. Rice is the main food for more than half of the world's population.

Global climate change accompanied by unpredictable and uneven rainfall patterns created concern to the plant breeders for limited water supply and to take the gigantic task to evolve lines adapted to water stress condition with less fluctuation in grain yield compared to favourable water availability (Pushpalatha *et al*., 2017) [5]. Drought is a major abiotic stress that limits rice productivity in rain fed and upland ecosystems and worldwide. Drought stress is not only limited to arid or semiarid areas, but also sometimes, due to irregular distribution of rain, causes a significant decrease of plant yield. Drought stress is a multidimensional stress that affects plants at different growth stages like, stress in the tillering, grain filling and panicle initiation stage happened to have some inverse effect on plant growth and development. For development of drought tolerant varieties and traits contributing for drought tolerance and their association were studied by focusing on correlation and path -co-efficient analysis. The study of correlation and path co-effect analysis is essential to know the degree of mutual association between yield and its component traits, which forms the basis for selecting desirable genotypes in plant breeding. For any crop, to setup a suitable breeding program. interrelationship among and between yield contributing characters is necessary and this will provide an opportunity to compute the magnitude and direction of their association along with other direct and indirect components. A study of genetic diversity studies will thus help in identifying suitable selection criteria for improving yield, and related traits for drought tolerant traits. Hence, the present study was undertaken to access correlation and path coefficients in selected set of 30 rice cultivars under irrigated and drought stress conditions.

Material and Method

Material

The experiment was carried out by using 30 rice genotypes (Table 1) in randomized block design (RBD) with three replications under optimum irrigation and drought stress conditions. Plot size for each genotype was 3m x

2m with spacing of 15 x 15 cm. The recommended agronomic practices were followed up to tillering stage. After tillering stage irrigation was withheld for 10 days in one set of experiment to impose artificial drought. Recorded the data on fifteen morphological characters under both conditions. Such morphological traits include Root length at 35 DAS (cm), Plant height at maturity stage (cm), Number of tillers per plant, Panicle length (cm), Panicle weight (g), Number of spikelets per panicle, filled grains per panicle, Grain weight per panicle (g), 1000 grain weight (g) and Yield per plant (g). Various biochemical analysis was also conducted for leaf sample of 90 days old were used for chlorophyll, phenols, flavonoids and proline estimation. While harvested rice grains were used for estimation of carbohydrate content from both well irrigated and drought field.

Chlorophyll Content (mg/g)

Chlorophyll was extracted by using 80% acetone and absorption were recorded at 663nm and 645nm in spectrophotometer. Using the absorption coefficients. The amount of chlorophyll (a) and (b) were measured as per the formula suggested by Aronon (1949).

$$\text{Chl-a} = 12.7(\text{value of 663nm} - 2.69 \times \text{value of 645nm}) \times \frac{V}{1000 \times W}$$

$$\text{Chl-b} = 22.9(\text{value of 645nm} - 4.68 \times \text{value of 663nm}) \times \frac{V}{1000 \times W}$$

$$\text{Total chlorophyll} = 20.2(\text{value of 645nm} + 8.02 \times \text{value of 663nm}) \times \frac{V}{1000 \times W}$$

V = volume of the sample
W = weight of the sample

The total chlorophyll content was expressed in mg chlorophyll per g leaf sample.

Total Phenol Content (mg/g)

The total phenol content in leaf samples were estimated by using Folin-Ciocateau reagent method (Mlick and Singh, 1980). Reading was recorded in spectrophotometer at 650nm wave length using 80% ethanol as blank solution.

$$\text{Total phenol content} = \frac{C \times V}{M}$$

C= the concentration of Folin- Ciocateau reagent from calculation
V= the volume of leaf sample,
M = weight of the leaf sample used.
The total phenol content was expressed mg phenol per g leaf sample.

Proline Content (µ moles/ g)

The amount of proline was estimated using Acid Ninhydrin method given by (Bates *et al.*, 1973). Noted the readings in UV spectrophotometer at 520 nm wavelengths using Proline as blank solution.
Express the proline content on fresh weight basis as fallows

$$\mu\text{ Moles per (g) tissue} = \frac{\mu gproline/ml \times mltoluene}{115.5\mu Moles} \times \frac{5}{gSample}$$

115.5 is the molecular weight of proline.
The proline content was expressed µM per g leaf sample.

Flavonoids content (mg/g)

The total flavonoids content was estimated using aluminium Chloride Colorimetric method._Absorbance was measured at 415nm by using ethanol as the blank solution. The total expressed flavonoids content was calculated using formula recommended Zhishen *et al.* (1999).

$$\text{Total flavonoids content} = \frac{C \times Df \times V}{M}$$

C = concentration obtained from the standard curve
Df = dilation factor
V = volume of stock solution
W = weight of sample used

The total flavonoids content was expressed mg per g leaf sample.

Carbohydrates Content (mg/g)

The total carbohydrates content was estimated by Anthron method (Hedge *et al.*, 1962). Absorbance was recorded at 630nm to the dark green colour solution using glucose as the blank.

The amount of carbohydrates presents in 100 mg/g of sample

$$\text{Total carbohydrates} = \frac{mg\ Of\ Glucose}{volume\ of\ test\ sample} \times 100$$

The total carbohydrate content was expressed in mg per g grain sample.

The recorded data of individual plant basis were subjected to the statistical analysis by using software windo-stat version 9.2 for estimation of Simple correlation co-efferent both at phenotypic and genotypic levels along with path co-efficient analysis, fallowing the methods suggested by Dewey and Lu, 1959, Lenka and Mishra 1973, Singh and Chaudhary 1977.

Results and Discussion

Yield is a complex and polygenic trait shows low effectiveness to direct selection as it is highly influenced by many genetic factors and environmental fluctuations. The degree of association among the characters is an important factor can be used as the best guide for successful yield improvement by indirect selection. The value of phenotypic and genotypic correlation provides the information about the relationship between the two or more than two independent variables. Hence association analysis was carried out to determine the direction of selection and number of characters to be consider in improvement of grain yield. In plant breeding and genetics knowledge with respect to association traits with seed yield would be of immense help in formulation of an effective and efficient selection and screening programme. In general, genotypic correlation was higher than phenotypic correlation under both control and stress condition. Indicating a low influence of environmental factors and relative stability of the characters. In the current study, genotypic and phenotypic correlation among the fifteen characters of rice genotypes was computed under control and drought stress condition. The influence of each character on yield was known through correlation studies to determine the relationship among the yield and yield attributing traits.

In the present investigation, the morphological traits like root length, plant height, number of tillers, panicle length, panicle weight, number of spikelets, per panicle, number of filled grains per panicle, grain weight per panicle, 1000 grain weight exhibited positive and correlation with plant yield under both control as well as stress conditions. The findings were consonance with Sabar and Arif (2014) [9], Aswathi and Lal (2014) [10], Terra *et al.*, (2017) [11], Yang *et al.*, (2019) [12], chander *et al.*, (2014), Perween *et al.*, (2020) [14], Bhutta *et al.*, (2019) Navya *et al.*, (2019) [15]. Hence the selection of these traits will be rewarding for development of the drought tolerant lines under stress conditions. Flavanoids, phenols, carbohydrates, chlorophyll, Proline exhibited positive significant associations with plant yield under control as well as stress conditions. Similar results were found with the findings of Navya *et al.*, (2019) [15]. Selection process of these traits will be rewarding based on the experiment under taken, like when the study only associated stress trait Proline can be the ideal trait for studying drought stress levels in plants.

Information obtained from correlation study does not give ample idea about the contribution of each component character towards yield. When the relation due to manifold effect of genes it would be tough to separate these effects by selecting particular character therefore, the technique of path coefficient analysis enables the researcher to get an idea of direct and indirect causes of correlation and helps to compare the causal factors on the genetic basis of their relative contribution. Path coefficient analysis revealed that traits like number of tillers per hill, panicle weight and chlorophyll content exhibited high and positive direct effect on plant yield under both conditions. The selection method of these traits will be more beneficial for the plant breeder for developing of drought tolerant lines by using various breeding procedures. These results were found similar to the findings of Kumar *et al.*, (2008) [17], Jeevula *et al.*, (2019) [18], Haider *et al.*, (2012) [19] Manjappa *et al.*, (2014) [20].

Table 1: Genotypic (G) and phenotypic (P) correlation co-efficient among yield and yield attributes in thirty rice genotypes under normal condition.

Characters		Root length (cm)	Plant height (cm)	Number of tillers/ plants	Panicle length (cm)	Panicle weight (g)	Number of spikelets per panicle	Filled grain/ panicle	Grain wt./ panicle (g)	1000grain wt. (g)
Root length (cm)	G	1	0.08	0.3 ***	0.38 ***	0.26 **	0.42 ***	0.37 ***	0.29 **	0.55 ***
	P	1	0.07	0.29 ***	0.35 ***	0.23 **	0.39 ***	0.34 ***	0.24 **	0.52 ***
Plant height (cm)	G		1	0.45 ***	0.36 ***	0.43 ***	0.60 ***	0.26 **	0.22 **	0.51 ***
	P		1	0.38 ***	0.34 ***	0.40 ***	0.57 ***	0.23 **	0.26 **	0.49 ***
Number of tillers/ plants	G			1	0.46 ***	0.45 ***	0.37 ***	0.47 ***	0.42 ***	0.58 ***
	P			1	0.45 ***	0.44 ***	0.35 ***	0.44 ***	0.41 ***	0.55 ***
Panicle length (cm)	G				1	0.46 ***	0.49 ***	0.33 ***	0.4 ***	0.49 ***
	P				1	0.45 ***	0.46 ***	0.32 ***	0.38 ***	0.48 ***
Panicle	G					1	0.28 ***	0.35 ***	0.55 ***	0.44 ***

weight (g)	P					1	0.26 **	0.34 ***	0.52 ***	0.43 ***
No of spikelet/ tpanicle	G						1	0.53 ***	0.28 ***	0.36 ***
	P						1	0.52 ***	0.26 **	0.35 ***
Filled grain/ panicle	G							1	0.45 ***	0.23 **
	P							1	0.43 ***	0.21 **
Grain wt./ panicle (g)	G								1	0.52 ***
	P								1	0.51 ***
1000 grain wt. (g)	G									1
	P									1
Yield/ Plant	G	0.194	0.453	0.832	0.686	0.869	0.701	0.817	0.869	0.823
	P	0.201	0.450	0.831	0.683	0.865	0.696	0.810	0.864	0.821

Table 2: Genotypic (G) and phenotypic (P) correlation co-efficient among yield and yield attributes in thirty rice genotypes under stress condition.

Characters		**Root length (cm)**	**Plant height (cm)**	**Number of tillers/ plants**	**Panicle length (cm)**	**Panicle weight (g)**	**No of spikelet per panicle**	**Filled grain /Panicle**	**Grain wt./ panicle (g)**	**1000 grain wt. (g)**
Root length (cm)	G	1	0.14	0.46 ***	0.56 ***	0.53 ***	0.51 ***	0.56 ***	0.23 **	0.41 ***
	P	1	0.13	0.45 ***	0.54 ***	0.5 ***	0.49 ***	0.54 ***	0.22 **	0.39 ***
Plant height (cm)	G		1	0.39 ***	0.57 ***	0.23 **	0.23 **	0.44 ***	0.28 ***	0.56 ***
	P		1	0.37 ***	0.54 ***	0.21 **	0.22 **	0.43 ***	0.26 **	0.54 ***
Number of tillers/ plants	G			1	0.28 ***	0.47 ***	0.51 ***	0.29 ***	0.37 ***	0.22 **
	P			1	0.24 **	0.46 ***	0.49 ***	0.38 ***	0.34 ***	0.2 **
Panicle length (cm)	G				1	0.32 ***	0.51 ***	0.58 ***	0.35 ***	0.49 ***
	P				1	0.3 ***	0.5 ***	0.57 ***	0.34 ***	0.48 ***
Panicle weight (g)	G					1	0.33 ***	0.52 ***	0.55 ***	0.52 ***
	P					1	0.32 ***	0.49 ***	0.52 ***	0.5 ***
No of spikelets per panicle	G						1	0.26 **	0.58 ***	0.43 ***
	P						1	0.25 **	0.56 ***	0.41 ***
Filled grain/panicle	G							1	0.47 ***	0.2 **
	P							1	0.28 ***	0.18
Grain wt. /panicle (g)	G								1	0.45 ***
	P								1	0.44 ***
1000 grain wt. (g)	G									1
	P									1
Yield/ Plant	G	0.3366	0.4141	0.857	0.8364	0.806	0.5442	0.6703	0.803	0.719
	P	0.3414	0.4137	0.856	0.8338	0.8025	0.5404	0.6668	0.801	0.717

Table 3: Genotypic (G) and phenotypic (P) correlation co-efficient among yield and biochemical attributes in thirty rice genotypes under control condition.

Characters		**Flavonoids (mg/g)**	**Phenols (mg/g)**	**Proline (µmoles/g)**	**Carbohydrates (mg/g)**	**Chlorophyll (mg/g)**
Flavonoids (mg/g)	G	1	-0.201 **	0.268**	- 0.429***	-0.37***
	P	1	-0.189	0.237**	- 0.387***	-0.35***
Phenols (mg /g)	G		1	0.223**	- 0.238 **	-0.05
	P		1	0.215**	- 0.229 **	-0.03
Proline (µmoles/g)	G			1	-0.228**	-0.36***
	P			1	-0.209**	-0.34***
Carbohydrates (mg/g)	G				1	-0.35***
	P				1	-0.32***
Chlorophyll	G					1

(mg/g tissue)	P					1
Yield/ plant (g)	G	0.4842	0.2922	0.2095	0.5088	0.5376
	P	0.4864	0.296	0.2133	0.5109	0.5396

Table 4: Genotypic (G) and phenotypic (P) correlation co-efficient among yield and biochemical attributes in thirty rice genotypes under stress condition.

Characters		**Flavonoids (mg/g)**	**Phenols (mg/g)**	**Proline (µmoles/g)**	**Carbohydrates (mg/g)**	**Chlorophyll (mg/g tissue)**
Flavonoids (mg /g)	G	1	-0.28 **	0.36***	- 0.38***	- 0.32***
	P	1	-0.24***	0.33***	- 0.35***	- 0.31***
Phenols (mg / g)	G		1	0.25**	- 0.26 **	-0.15
	P		1	0.22**	- 0.24 **	-0.12
Proline (µmoles/g)	G			1	-0.25**	- 0.31***
	P			1	-0.21**	- 0.28***
Carbohydrates (mg/g)	G				1	-0.41***
	P				1	-0.37***
Chlorophyll (mg/g tissue)	G					1
	P					1
Yield/plant (g)	G	0.5455	0.594	0.3591	0.538	0.5392
	P	0.5481	0.5956	0.364	0.5405	0.5412

Table 5: Path co-efficient analysis showing direct (bold) and indirect effect of component traits on yield per plant in rice genotypes under control condition.

Characters	**Root length (cm)**	**Plant height (cm)**	**Number of tillers/ plants**	**Panicle length (cm)**	**Panicle weight (g)**	**No of spikelet/ panicle**	**Filled grain/ panicle**	**Grain wt. /panicle (g)**	**1000 grain wt. (g)**
Root length (cm)	0.0352	0.0042	-0.0012	0.0019	0.0121	0.0065	0.0087	0.0122	0.0146
Plant height (cm)	-0.0031	-0.0264	-0.0052	-0.0152	-0.0163	-0.0148	-0.0152	-0.0168	-0.0162
Number of tillers/ plants	-0.0185	0.1067	0.5378	0.2657	0.2645	0.2005	0.2551	0.2717	0.2508
Panicle length (cm)	0.0002	0.0024	0.002	0.0041	0.0029	0.0023	0.0026	0.0028	0.0027
Panicle weight (g)	0.3212	0.5788	0.4607	0.6539	0.9368	0.7635	0.843	0.9337	0.9069
No of spikelet/ panicle	-0.005	-0.0153	-0.0101	-0.0154	-0.0221	-0.0271	-0.0248	-0.0224	-0.0192
Filled grain/panicle	-0.0001	-0.0002	-0.0002	-0.0002	-0.0003	-0.0003	-0.0004	-0.0003	-0.0003
Grain wt. /panicle (g)	-0.0218	-0.04	-0.0318	-0.0434	-0.0627	-0.052	-0.0573	-0.0629	-0.0606
1000 grain wt. (g)	-0.1069	-0.158	-0.1203	-0.1682	-0.2498	-0.1828	-0.202	-0.2487	-0.258
Yield/ plant (g)	0.2012	0.4523	0.8317	0.6832	0.865	0.6957	0.8098	0.8693	0.8206
Partial R²	0.0071	-0.0119	0.4473	0.0028	0.8103	-0.0189	-0.0003	-0.0547	-0.2117

R SQUARE = 0.9700; RESIDUAL EFFECT = 0.1733

Table 6: Path co-efficient analysis showing direct (bold) and indirect effect of component traits on yield per plant in rice genotypes under stress condition.

Characters	**Root length (cm)**	**Plant height (cm)**	**Number of tillers/ plant**	**Panic le length (cm)**	**Panicle weight (g)**	**No of spikelet/ panicle**	**Filled grain/ panicle**	**Grain wt./ panicle(g)**	**1000 grain wt. (g)**
Root length (cm)	0.0316	0.0056	0.0115	0.0083	0.007	0.0076	0.0025	0.0072	0.0094
Plant height (cm) at maturity	-0.0213	-0.1208	-0.0212	-0.0696	-0.0826	-0.0529	-0.0634	-0.0822	0.0787

Number of tillers/ plants	0.2002	0.0967	0.5512	0.3211	0.2473	0.116	0.1664	0.2422	0.2351
Panicle length (cm)	0.037	0.0807	0.0816	0.1402	0.1121	0.0953	0.1049	0.1138	0.096
Panicle weight (g)	0.1186	0.3643	0.2391	0.4262	0.5329	0.3543	0.4259	0.5299	0.4 93
No of spikelets per panicle	-0.0012	-0.0022	-0.001	-0.0034	-0.0033	-0.005	-0.0043	-0.0034	0.0023
Filled grain/panicle	-0.0088	-0.0582	-0.0334	-0.083	-0.0886	-0.0949	-0.1108	-0.0885	0.0595
Grain wt. /panicle	0.1012	0.3018	0.195	0.3601	0.4412	0.3049	0.3542	0.4437	0.4 13
1000 Grain wt. (g)	-0.1159	-0.2533	-0.1658	-0.2661	-0.3596	-0.181	-0.2086	-0.3618	0.3887
Yield per plant	0.3414	0.4147	0.857	0.8338	0.8065	0.5444	0.6668	0.801	0.7173
Partial R^2	0.0108	-0.0501	0.4724	0.1169	0.4298	-0.0027	-0.0739	0.3554	0.2788

R SQUARE = 0.9798; RESIDUAL EFFECT = 0.1423

Table 7: Path co-efficient analysis showing direct (bold) and indirect effect of biochemical attributes on yield per plant in rice genotypes under control condition.

Characters	**Flavonoids (mg/g)**	**Phenols (mg/g)**	**Proline (µmoles/g)**	**Carbohydrates (mg/g)**	**Chlorophyll (mg/g tissue)**
Flavonoids (mg /g)	0.0315	0.0066	0.0053	0.0278	0.0119
Phenols (mg / g)	0.0500	0.2367	0.0551	0.0577	0.0015
Proline (µmoles/g)	-0.0129	-0.018	-0.0772	-0.0177	-0.0283
Carbohydrates (mg/g)	0.2457	0.0678	0.0638	0.2782	0.1012
Chlorophyll (mg/g tissue)	0.1721	0.0028	0.1664	0.1649	0.4534
Yield per plants	0.4864	0.296	0.2133	0.5109	0.5396
Partial R^2	0.0153	0.0701	-0.0165	0.1421	0.2447

R SQUARE = 0.4557; RESIDUAL EFFECT = 0.7378

Table 8: Path co-efficient analysis showing direct (bold) and indirect effect of biochemical attributes on yield per plant in rice genotypes under stress condition.

Characters	**Flavonoids (mg/g)**	**Phenols (mg/g)**	**Proline (µmoles/g)**	**Carbohydrates (mg/g)**	**Chlorophyll (mg/g tissue)**
Flavonoids (mg /g)	0.0865	0.044	0.0368	0.0661	0.0504
Phenols (mg / g)	0.167	0.328	0.1257	0.2003	0.1345
Proline (µmoles/g)	0.041	0.0369	0.0962	0.038	0.0203
Carbohydrates (mg/g)	0.0861	0.0688	0.0445	0.1127	0.0484
Chlorophyll (mg/g tissue)	0.1675	0.1179	0.0608	0.1235	0.2875
Yield per plants	0.5481	0.5956	0.364	0.5405	0.5412
Partial R^2	0.0474	0.1954	0.035	0.0609	0.1556

R SQUARE = 0.4943; RESIDUAL EFFECT = 0.7112

Plant height at maturity, 1000 grain weight, grain weight per panicle, filled grains per panicle and number of spikelets per panicle had negative and direct effects towards plant yield under two different conditions of control and stress. The selection of these traits will be ineffective for various drought tolerant development lines. The current results were consonance with the findings of Singh *et al.,* (2014) [23]. While other attributes like phenols, flavonoids, carbohydrates, Proline, root length and grain weight resulted with negligible to moderate direct effects to plant yield under control as well as stress. The selection criteria of these traits will be not effective in crop improvement program. These results are consonance with the finding of Charles and Joseph (2015) [22] Shukla *et al.,* (2012) [25]. The most of traits had high to negligible indirect effects on plant yield under stress and control conditions the selection of these traits will not be useful and prefer for development of drought tolerant lines. The results were similar to the findings of Hossain *et al.,* (2018) [21], Kumar *et al.,* (2012) [25]. The traits which have direct effect on yield are having true relationship with yield. Hence, selection for these traits would be much rewarding for the improvement of grain yield and trait which are having indirect effect through another component traits, the indirect selection through such traits may improve the yield.

Percentage contribution of thirty rice genotypes with twenty three characters were presented in table (7 and 8) among these traits highest manifestation of genetic divergence was exhibited by yield per plant 26.9 % fallowed by number of unfilled grain 22.53 %, flavanoids 10.57 % and chlorophyll content 9.89 % exhibited maximum contribution percentage to total divergence in control, whereas in drought stress yield per plant 45.06 % fallowed by number of unfilled grain 10.8 %, test weight 7.36 % and chlorophyll 11.49 % were found with the maximum percentage contribution towards to the genetic divergence, the results observed that under both conditions the most of the traits found same in contributing genetic divergence. This indicates that selection of genotypes for based on these traits may be rewarding for future utilization in breeding programme.

Conclusion

High heritability with genetic advance has been presented as an attribute to additive gene action. Under stress conditions, high heritability with high genetic advance was recorded for traits plant yield, flavonoid content leaf area index, test weight, total grains per panicle and root length. Highly significant correlation co-efficient among yield attributes indicated a high level of divergence in the genotypes used. Overall, the study provides many genotypes to explore further at molecular-assisted breeding and direct towards crop improvement for abiotic stress tolerance.

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