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Environmental response and stability analysis in muti-parent advanced breeding lines of rice (*Oryza sativa* L.)

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Abstract
The improvement of yield stability is paramount to increase the grain yield. Hence, it is a prerequisite for the newly generated advanced breeding lines to undertake multi environmental yield trials for selection of superior and stable genotypes suitable for different environments. The present investigation was carried out in order to identify the superior and stable multi-parent advanced breeding lines in the test locations. Thirty multi-parent advanced breeding lines along with six check varieties were evaluated at three locations in randomized block design. The stability performance was analyzed by using Eberhat and Russel (1966) model and GGE bi-plot technique. The pooled Analysis of variance for stability showed that the mean sums of squares due to environments + (varieties × environment) was found significant for characters such as number of tillers per plant, number of spikelets per panicle and days to 50% flowering. Some of the multi-parent advanced breeding like ML1-6-3-1-23, ML2-5-21-3-154, ML2-7-2-3-160, ML1-8-1-1-31 and ML3-5-2-1-105 showed higher grain yield (kg/ha) and showed stable performance in all three locations. These lines could be further analyzed for mega environment tests in farmer's fields to identify the most desirable advanced breeding line. Study of molecular diversity using sixty polymorphic SSR markers revealed the existence of high molecular diversity between the breeding lines that grouped them into eight clusters.

Keywords: Stability analysis, GGE bi-plot, multi-parent advanced breeding lines, SSR markers

1. Introduction
Rice, *Oryza sativa* is one the most important crop in Asia and is the staple food for more than half of the world's population. It belongs to the family *Gramineae* and is grown in about 120 countries with China and India together accounting for more than 50 percent of the rice production globally. Rapid population growth is imparting increased pressure on already strained food-producing resources. According to the reports of the UN Department of economic and social affairs, India's population may reach 1.5 billion by 2030 and 1.64 billion by 2050. Currently the production of rice in India is 121.46 MT and the demand of rice is estimated to increase to 197.40 MT by 2050 (FAOSTAT, 2018). In addition to this, elevating drift in climate change like rise in temperature, altered precipitations, melting of glaciers and shifting seasons are threat to agriculture and food security to the growing population. It is necessary to develop new technologies in order meet this need and contribute to global efforts which are directed towards poverty alleviation.
In order to increase the rice production there is a need to identify suitable high yielding varieties which can be cultivated in different topographical conditions and also develop high yielding, nutritionally superior and biotic and abiotic stress tolerant varieties. Hence, there is a need to combine all the desirable traits of different varieties using Multi-parent advanced generation inter-cross population (MAGIC) and identify suitable rice genotypes suited to different topographic regions. MAGIC – combines high diversity (from multiple parents) with high recombination and also has the potential to increase the speed and efficiency of breeding. Magic populations can serve as source material for extraction and development of breeding lines and varieties with several agronomically beneficial traits. A variety which can be adopted to several diverse regions or which is suitable for diverse climatic conditions can be developed. These populations bring model shift toward QTL analysis, gene mapping, variety development etc. in plant species.
Yield is a complex quantitative character which is highly influenced by environmental changes or fluctuations and therefore the selection for superior genotypes based on yield at a single location in a year is not very useful.

In any breeding programme, the selection of superior genotypes is based on its phenotype. Generally yield and its contributing traits along with the phenotypic expression are often used by the breeders to select superior genotypes in a single mega environment test. The genotypes tend to perform differently in varied environments if Genotype × Environment interactions are significant. Therefore, assessment in one environment does not guarantee the selection of superior genotypes whose performance would be uniform over different locations or years.

The assessment of the stability of a genotype under different environments helps to recommend the genotypes or cultivars suitable for that particular location. The stability of the cultivars over a wide range of environments along with high yielding potential is desirable. Hence, it is emphasized by breeders to assess for stability before releasing an ideal variety for commercial cultivation. For commercial crop production, it is prerequisite that the newly developed crop varieties to perform consistently across years (stability) and across locations (adaptability).

Many biometrical models have been proposed to measure the stability of individual genotypes across environments. Eberhart and Russel's model (1966) [7], and GGE biplot methods are some of the techniques, which have been widely used to measure the stability of genotypes in many crop plants. These methods have been utilized in the present investigation to analyze the stability analysis in 30 multi-parent advanced breeding lines belonging to the MF_6 generation.

DNA markers have different benefits over morphological and biochemical markers for obtaining a genotype specific profile. DNA markers are more polymorphic since they span the entire genome. These are phenotype-neutral, have no epistatic impact, and are unaffected by environmental variables or developmental stages. Microsatellites or simple sequence repeats (SSRs) are well-known as molecular instruments among PCR-based DNA markers because of their potentially high information richness, adaptability, and preference for outcomes consistency. Thousands of microsatellite markers have been created for rice study, and their chromosome position and polymorphism levels have been determined. In rice, SSRs were routinely utilised to assess genetic purity. Whether there is significant diversity among the breeding lines is important to determine. Because it can give information, how these lines can be used in heterosis breeding and further crop improvement programme. Hence the molecular diversity of the advanced breeding lines was also studied using SSR markers.

2. Materials and Methods

Plant material and location of the Experimental site

Thirty multi-parent advanced breeding lines of MF_6 developed from crossing eight parents (JGL1798 × KPR2, Hemavathi × Gandhasali, BPT5204 × H3, Jaya × Mysore Mallige) in structured mating design along with six checks *viz.*, JGL1798, KPR2, Hemavathi, Gandhasali, BPT5204 and Mysore mallige were collected from the Department Of Genetics and Plant Breeding, KSNUAHS, College of Agriculture, Shivamogga, Karnataka, India. The list of the multi-parent advanced breeding lines along with the six checks used in the present investigation is presented in Table 1. The study was conducted during *Kharif* 2021 at three different locations in Karnataka (Table 2). Twenty-one days old seedlings were transplanted manually into the main field with single seedling per hill in Randomized block design with two replications with spacing of 20 cm row to row and 15 cm plant to plant. The recommended package of practices was followed to maintain a healthy and good crop stand. Five plants were randomly selected from each multi-parent advanced line and labelled for recording the observations in each treatment. Mean of the observations recorded on these five plants was considered for statistical analysis. The characters for which observations were recorded are as follows: days to 50% flowering, number of tillers, number of spikelets per panicle, and grain yield (kg/ha).

The stability was analyzed by Eberhat and Russel (1966) [7] model and GGE bi-plot technique using the R-4.2.0 software. Three parameters were determined by using the Eberhat and Russel (1966) [7] model, *viz.* mean of the genotype across environments, regression of genotype on environmental index and the function of the squared deviation from the regression. A genotype having regression coefficient as unit i.e., b=1 and non-significant deviation from Zero i.e., S^2d = average, was considered to be the stable genotype.

Table 1: List of MF_6 rice advanced lines taken for the screening for submergence and stability analysis

Line No.	Genotype	Line No.	Genotype
1	ML1-2-3-1-6	16	ML3-5-2-1-105
2	ML1-2-5-1-8	17	ML3-6-7-1-107
3	ML1-6-1-1-22	18	ML3-7-1-1-108
4	ML1-6-3-1-23	19	ML3-7-3-1-110
5	ML1-7-2-1-26	20	ML3-7-9-1-113
6	ML1-8-1-1-31	21	ML1-2-7-4-121
7	ML1-11-4a-2-45	22	ML1-7-1-1-126
8	ML1-11-5-1-49	23	ML2-5-21-3-154
9	ML1-11-9-1-50	24	ML2-7-2-2-156
10	ML1-12-6-1-52	25	ML2-7-2-3-160
11	ML1-14-1-1-54	26	ML2-7-3-2-164
12	ML1-15-3A-1-17	27	ML2-5-21-2-152(P2*)
13	ML2-5-3-1-19	28	ML2-8-10-1-177(P2*)
14	ML2-6-1-1-76	29	ML2-8-13-2-178(P2*)
15	ML3-2-4-4-96	30	ML1-11-4a-3-47(P1)

GGE bi-plot methodology was used for visual interpretation of patterns of GE interaction. Polygon view of GGE bi-plot based on symmetrical scaling for determining 'which-won-where' pattern of genotypes with test locations and average-environment coordination (AEC) view of bi-plot based on environment-focused scaling for interpreting mean performance of the genotypes vs. their adaptability patterns were used to understand the pattern of genotype-environment interaction. IPCA1 scores were plotted against their IPCA2 scores to visually identify accessions with specific/wide adaptation and similarity between accessions and locations. The genotypes/accessions that are more similar to each other in terms of their trait expression are closer to each other in the GGE bi-plot than those that are less similar. The genotypes located near the origin of IPCA1 vs. IPCA2 bi-plot are those with wide adaptation across locations than those located far from the origin.

Table 2: Location of experiments conducted to evaluate rice advanced breeding lines for stability analysis

Sl. No.	Particulars	Environments		
		E1	E2	E3
1	Locations	AHRS, Kathalagere	ZAHRS, Shivamogga	AHRS, Honnavile
2	Latitude	16°12' N	13.054° N,	13.930° N
3	Longitude	74°54' E	75.03930° E	75.568 °E
4	Elevation	598 meters	569 meters	570.00 meters
5	Average temperature	25.5 °C	24.8 °C	25.60 °C
6	Method of Cultivation	Transplanting	Transplanting	Transplanting
7	Spacing	20 × 15 cm	20 × 15 cm	20 × 15 cm
8	Date of Sowing	25/07/2017	05/07/2017	11/07/2017
9	Date of Planting	16/08/2017	31/07/2017	10/08/2017
10	Average Rainfall	567 mm	909 mm	1166 mm

The mean value of five randomly selected plants on each genotype from each of the two replications was statistically analysed using Windostat 9.2 [12] and Genstat [13] software on the data on the individual character. The mean data was analyzed, and the following statistical procedures were used in the analysis:

Table 3: Structure of pooled analysis of variance

Source of variation	DF	MSS	Expected MSS
Environnent (E)	(E-1)	MSS(e)	-
Génotypes (G)	(G-1)	MSS (i)	$\sigma^2_E + \sigma^2_{GE} + e\sigma^2_E + \sigma^2_G$
Génotypes x Environnent (G×E)	(E-1) (G-1)	GLMSS	$\sigma^2_E + \sigma^2_{GE}$
Pooled error	(EG – 1)	EMSS	σ^2_E

DF: degree of freedom, MSS: Mean Sum of Square, σ^2_E:Error variance, σ^2_{GE}:G x E variance, σ^2_G:Genotypic variance

Eberhart and Russel Model

Stability performance by Eberhart and Russel model requires the estimation of three parameters, each of which is defined by a mathematical formula.

$Y_{ij} = \mu_i + \beta_i I_j + S_{ij}$,

Where, Y_{ij} is the mean of the i^{th} variety in the j^{th} environment, μ_i is the mean of the i^{th} variety across all environments, β_i is the phenotypic index to environmental index regression coefficient that measures the response of i^{th} variety to varying environment, S_{ij} is the deviation from regression of the i^{th} variety in the j^{th} environment, and I_j is the environmental index obtained by subtracting the grand mean from the mean of all varieties in the j^{th} environment. The following equations were used to calculate these variables.

$$Ij = \sum_i \frac{Y_{ij}}{t} = \sum_{ij} \frac{Y_{ij}}{t.s}$$

Where, t is the number of lines and s denotes the number of environments such that ΣjIj =0.

The regression coefficient for each variety (bi) is given by

$$bi = \frac{\sum_j Y_{ij} I_j}{\sum_i i_j^2}$$

The deviation from regression is determined as follows:

$$S^2 d_i = \frac{\sum_j \delta_{ij}^2}{S-2} - S_e^2/r$$

where δ_{ij}^2 is the deviation of i^{th} variety in j^{th} environment from regression.

Yij = Xi + biIj was used to calculate the genotypes predicted performance.

X_i is the estimate of μ_i. Environment (linear), genotype x environment (linear), and deviation from the regression coefficient are used to split the variance due to environments and Genotype x Environment in this model.

For each character and genotype, the amplitude of the G x E interaction was determined. This was done according to Eberhart and Russell's instructions [5]. According to this paradigm, the analysis of variance (Table 4) for stability is algebraically represented as n = number of environments, r = number of replications, and g = number of genotypes. CF stands for correction factor. Mean square against the pooled error mean square is used to determine the importance of pooled deviation.

$$F = \frac{MS3}{MS4}$$

If pooled deviation mean square is determined to be significant, it should be used as the numerator to assess the significance of all components, including genotypes and genotype x environment (linear). Otherwise, the denominator should be pooled error square. To determine the relevance of variances in genotype mean and to demonstrate that genotypes do not differ in their regression on environmental indicator this is calculated by

$$F = \frac{MS2}{MS3}$$

Furthermore, the significance of b_i deviation from unity is tested using the t test.

Table 4: Eberhart and Russell Model analysis of variance for stability

Source of variation	d. f	SS	MSS	F Value
Genotype (G)	(g-1)	$\Sigma i\Sigma jYij$-CF	MS1	MS1/ MS3
Environment (E)	(n-1)	$1/n\Sigma jYi^2$-$1/n\Sigma jYi$		
G x E	(g-1) (n-1)	$\Sigma i\Sigma jYij^2$-$1/n\Sigma jYi^2$		
Environment (linear)	1	$1/g[(\Sigma jYj\text{-}Ij)^2\Sigma jIj^2$		
G x E (Linear)	(g-1)	$\Sigma j[(\Sigma Yij\ Ij^2/\Sigma j\ Ij^2]$- $1/g[(\Sigma jYj\text{-}Ij)^2\ \Sigma jYj^2]^2$	MS2	MS2/ MS3
Pooled deviation	g(n-2)	$\Sigma i\Sigma jd^2ij^2$	MS3	MS3/ MS4
Genotype 1	(n-2)	$[\Sigma jYij^2\text{-}Yi^2/n]$-$[\Sigma jYijIj]^2/\Sigma jIj^2$		
Genotype 2	(n-2)	$[\Sigma j\ Ygj^2\text{-}Yg^2.\ /n]$-$[\Sigma jYgjIj]^2/\Sigma jIj^2$		
Pooled error	n(g-1) (r-1)		MS4	
Total	(n g -1)	$\Sigma i\Sigma jYij^2$-CF	TSS	

df: degree of freedom, CF: Correction Factor, SS: Sum of Square, MSS: Mean Sum of Square, g:number of genotypes, n: number of environments, r:number of replication.,

A combination of three characteristics, the genotypes average performance across environments (locations) Xi, Bi coefficient of regression and deviation from linear regression S^2d_i is a tool for determining genotype stability (variety). The divergence from regression estimate indicates how much trust on linear regression should be placed in data interpretation. The expected phenotype cannot be predicted satisfactorily if these values are significantly different from zero. When the deviations are not substantial, the conclusions can be reached by combining the mean yield and regression values [14, 5]. Because the average slope across all kinds on the environment index will be unity, a regression value of unity is interpreted as average stability (Table: 5). Stability performance by Eberhart and Russel model was done using Windostat 9.2 [12].

Table 5: Mean performance of the genotype

Regression	Stability	Mean yield	Remarks
bi = 1	Average	High	Adaptable to a variety of environments
bi = 1	Average	Low	Adapts poorly to all environments
bi > 1	Below average	High	Adapted to a variety of favourable environments
bi < 1	Above average	High	Adapted specifically to unfavorable conditions

The GGE bi-plot criteria to interpret GEI

GGE-biplot, which is a combination of AMMI bi-plot and GGE concepts [16] was used for visual interpretation of patterns of GEI. The GGE bi-plot is based on the following model.

$$Y_{ij} - Y_j = \lambda_1\alpha_{i1}\gamma_{j2} + \lambda_2\alpha_{i2}\gamma_{j2} + \varepsilon_{ij}$$

Where, Y_{ij}= trait mean of i^{th} inbred line in the j^{th} environment; Yj= trait mean of all the inbred lines in the jth environment; λ1 and λ2 are the square root of eigen values of first and second IPC axes, respectively; αi1and αi2 are the scores of the first and second IPC, respectively, for the i^{th} inbred line; γj2 and γj2 are the first and second IPCs respectively for j^{th} environment.

A GGE bi-plot can be used in a variety of ways, but the polygon view is the most useful. Interaction between genotype and environment the accessions with specific/wide adaptability and similarities across inbred lines and settings were visually identified by plotting their PC 1 (IPC) scores against their IPC 2 scores. In the GGE bi-plot, the inbred lines that are more similar in terms of their illness responses are closer together than those that are less similar. Inbred lines at the origin of the IPC 1 vs. IPC 2 bi-plot are thought to be more adaptive to different settings than those farther away. Straight lines are used to connect inbred lines that are further away from the bi-plot origin. Inbred lines at the origin of the IPC 1 vs. IPC 2 bi-plot are thought to be more adaptive to different settings than those farther away. The inbred lines that are further away from the bi-plot origin are joined with straight lines to build a polygon that contains all other inbred lines. A set of lines perpendicular to each side of the polygon were drawn from the bi-plot origin. The polygon is divided into sectors by perpendicular lines to the polygon sides, each with its own winning genotype, which is the vertex genotype for that sector [17]. GGE biplot analysis was performed using metan - R package.

3. Results and Discussions

The pooled analysis of variance revealed that significant genotypes × locations (G × L) differences for all the traits studied across the three tested locations. The important source of variations such as varieties or genotypes, environment + (varieties × environment) and environment (linear) and pooled deviation are statistically significant for majority of the traits. The results are presented in the Table 3. The stability parameters *viz*., mean, regression coefficient (Bi) and deviation from regression (S2Di) of the advanced breeding lines for various characters in the three environments based on Eeberhart and Russel model are mentioned in Table 4 and 5.

Table 6: Pooled analysis of variance for stability based on Eberhart and Russel model

Source of variation	DF	Days to 50% flowering	number of tillers per plant	Number of spikelets per panicle	Yield (kg/ha)
REP. with ENV	3	0.5231	1.898	70.049	432616.984
Varieties	35	14.8737**	3.065**	321.773	880469.801
ENV. + (VAR. × ENV)	72	173.192**	3.028**	901.750**	1075818.102
Environments	2	6045.781**	74.343**	25355.510**	17213509.503**
VAR. × ENV	70	5.404**	0.990	203.071	614741.205
Environments (Lin.)	1	12091.562**	148.685**	50711.010**	34427019.006**
VAR. × ENV. (Lin)	35	326.538**	0.691	153.858	386472.909
Pooled deviation	36	51.733	1.254**	245.277**	819592.571**
Pooled error	105	208.493	0.408	71.525	107193.460
Total	107	12990.414	3.040	712.038	1011919.125

, * significance at 5% and 1% levels respectively

With respect to days to 50% flowering none of the thirty multi-parent advanced breeding lines showed significant (Bi) value and only twelve multi-parent advanced breeding lines showed significant deviation from regression. Multi-parent advanced breeding lines like ML1-6-1-1-22, ML1-11-4a-2-45, ML1-11-5-1-49, ML1-14-1-1-54, ML1-15-3A-1-17, ML3-5-2-1-105, ML1-2-7-4-121, ML1-7-1-1-126, ML2-7-2-2-156, ML2-5-21-2-152(P2*), ML2-8-10-1-177(P2*), BPT 5204 and Hemavathi showed significant Deviation from regression and Mysore mallige showed significance regression coefficient (bi) for number of tillers per plant while ML1-12-6-1-52, ML1-7-1-1-126 and ML2-8-13-2-178(P2*) multi-parent advanced breeding lines showed significant deviation from regression indicating that these lines were highly sensitive to environmental changes.

None of the multi-parent advanced breeding lines recorded significant regression coefficient (bi) values for number of spikelets per panicle and grain yield per hectare. However, the advanced breeding lines ML1-2-3-1-6, ML1-7-2-1-26, ML1-8-1-1-31, ML3-2-4-4-96, ML2-8-10-1-177(P2*), ML2-8-13-2-178(P2*), JGL-1798 and BPT-5204 were found significant for deviation from regression (S^2Di) with respect to number of spikelets per panicle. The advanced breeding lines ML1-2-5-1-8, ML1-6-1-1-22, ML1-7-2-1-26, ML1-6-1-1-22, ML1-14-1-1-54, ML2-5-3-1-19, ML3-2-4-4-96, ML3-7-1-1-108, ML2-7-3-2-164, ML2-5-21-2-152(P2*), ML2-8-10-1-177(P2*), JGL-1798 and KPR-2 were found significant for deviation from regression (S^2Di) with respect to grain yield per hectare (kg/ha). These lines were more sensitive to environmental changes as they were found significant for deviation from regression.

The advanced breeding line ML1-7-2-1-26, ML1-11-9-1-50, ML2-5-3-1-19, ML3-6-7-1-107, ML1-7-1-1-126, ML2-5-21-3-154, BPT 5204 and Hemavathi showed mean value less than population mean for days to fifty per cent flowering and also had regression coefficient near unity and least deviation from regression and hence were identified as stable lines for early flowering across the three environments. These findings were in concurrence with those of Koli *et al.* (2015) [9], Rashmi *et al.* (2017) [13] and Dushyanthaumar *et al.* (2020) [6].

The advanced breeding lines such as ML1-6-3-1-23, ML1-15-3A-1-17, ML2-7-3-2-164, ML2-8-13-2-178(P2*) and Hemavathi exhibited more mean values than the population mean (16.43) and also showed regression coefficient value around unity and less deviation from regression and hence these lines were identified as stable across the environments for number of tillers per plant. The multi-parent advanced breeding lines *viz.*, ML1-11-9-1-50(232.667), ML3-5-2-1-105(229.667), ML3-6-7-1-107(228.667), ML2-7-2-2-156(239.333), BPT 5204(230.667) exhibited more mean value than the population mean (224.96) and also had regression coefficient value is around unity and less deviation from regression for number of spikelets per panicle across the environments. These results were in concurrence with Koli *et al.* (2015) [9], Rashmi *et al.* (2017) [13] and Dushyanthakumar *et al.* (2020) [6].

Table 7: Stability parameters over three locations

	Days to 50% flowering			Number of tillers per plant		
Advanced breeding Lines	**Mean**	**Bi**	**S2Di**	**Mean**	**Bi**	**S2Di**
ML1-2-3-1-6	90.333	0.721	0.736	15.667	0.633	0.102
ML1-2-5-1-8	92.500	0.606	0.668	16.000	0.827	0.266
ML1-6-1-1-22	94.667	1.253	1.286	16.000	1.254	-0.406
ML1-6-3-1-23	89.667	0.781*	-1.980	17.500	0.918	-0.389
ML1-7-2-1-26	93.667	1.019	0.724	15.833	0.776	-0.226
ML1-8-1-1-31	96.833	0.662	1.765	16.167	0.969	-0.124
ML1-6-1-1-22	98.167	1.053	1.904	15.500	0.827	0.266
ML1-11-5-1-49	97.333	1.024	-1.288	18.500	1.527	3.966**
ML1-11-9-1-50	92.167	1.015	-1.931	15.833	1.021	0.452
ML1-12-6-1-52	95.500	1.133	0.679	17.667	1.669	5.253**
ML1-14-1-1-54	96.000	1.173	-1.325	16.500	0.245	-0.157
ML1-15-3A-1-17	93.500	1.109	3.2129	17.833	0.930	2.184
ML2-5-3-1-19	93.333	1.024	-1.288	15.500	0.827	0.266
ML2-6-1-1-76	94.167	1.089	7.0418*	17.000	0.246	-0.157
ML3-2-4-4-96	94.333	1.090	-1.194	16.000	1.254	-0.406
ML3-5-2-1-105	93.167	0.979	-1.347	17.167	1.124	2.538
ML3-6-7-1-107	93.000	1.001	-1.318	15.500	1.009	-0.111
ML3-7-1-1-108	91.333	0.957	-1.376	16.333	1.203	-0.216

ML3-7-3-1-110	93.667	1.084	-1.202	15.833	1.021	0.452
ML3-7-9-1-113	93.833	1.094	-1.712	15.667	0.724	-0.407
ML1-2-7-4-121	93.667	1.082	-1.954	16.000	0.673	-0.276
ML1-7-1-1-126	91.833	0.957	-1.376	17.833	1.475	5.772**
ML2-5-21-3-154	92.833	1.024	-1.288	16.167	1.242	1.888*
ML2-7-2-2-156	97.167	1.179	-1.059	17.167	0.815	0.016
ML2-7-2-3-160	92.500	0.935	-1.404	15.667	0.815	0.016
ML2-7-3-2-164	95.500	0.738	-1.323	19.000	1.099	1.098
ML2-5-21-2-152(P2*)	95.833	0.688	-0.374	18.333	1.203	-0.216
ML2-8-10-1-177(P2*)	94.833	1.058	-1.726	15.667	0.815	0.016
ML2-8-13-2-178(P2*)	96.167	1.215*	-1.981	16.833	1.085	7.396**
ML1-11-4a-3-47(P1)	95.833	1.094	-1.712	16.000	0.491	0.597
JGL1798	98.500	0.868	-1.484	15.333	0.621	0.166
BPT 5204	90.833	0.957	-1.376	17.167	1.888	0.042
KPR2	92.000	0.935	-1.404	15.500	0.827	0.266
Hemavathi	91.000	0.935	-1.404	15.667	1.151	0.286
Gandhasali	96.333	1.291	-0.876	18.000	0.582	-0.305
Mysore mallige	95.167	1.179	-1.059	17.333	2.212*	0.558
Mean	94.088			16.55		

, * significance at 5% and 1% levels respectively

The advanced breeding lines ML1-6-3-1-23(8255.667kg/ha), ML3-5-2-1-105 (3-5-2-1-105kg/ha), ML3-6-7-1-107 (7600.087kg/ha), ML2-5-21-3-154(7891.528kg/ha) and ML2-7-2-3-160(8083.955kg/ha) showed more mean value than the population mean (7122.23kg/ha), regression coefficient value is around unity and less deviation from regression for grain yield across the three environments. Similar results were obtained by Dushyanthakumar *et al.* (2020) [6].

Table 8: Stability parameters over three locations

	Number of spikelets/panicle			Grain yield (kg/ha)		
Advanced breeding Lines	**Mean**	**Bi**	**S2Di**	**Mean**	**Bi**	**S2Di**
ML1-2-3-1-6	220.833	0.846	750.693**	7530.52	0.631	286736.00
ML1-2-5-1-8	243.167	1.499	-59.671	7144.638	0.868	1783440.00**
ML1-6-1-1-22	225.000	0.826	-54.998	7265.333	1.434	805814.60**
ML1-6-3-1-23	237.000	1.262	104.578	8255.667	1.094	-96648.90
ML1-7-2-1-26	240.333	1.345	943.140**	7560.792	0.757	626287.10*
ML1-8-1-1-31	215.667	0.952	317.028*	8098.167	0.835	-36544.90
ML1-6-1-1-22	213.333	0.823	-57.982	6809.5	0.667	2891202.00**
ML1-11-5-1-49	207.500	0.648	-71.519	6749.942	0.148	226220.60
ML1-11-9-1-50	232.667	1.046	-40.961	6371.613	1.268	-28780.60
ML1-12-6-1-52	232.167	0.071	-31.391	6554.08	1.003	-26013.50
ML1-14-1-1-54	221.833	0.638	41.816	6610.695	0.947	5504209.00**
ML1-15-3A-1-17	214.000	1.132	-71.163	6539.972	0.676	271341.10
ML2-5-3-1-19	210.000	0.728	186.382	7286.458	1.283	1133393.00**
ML2-6-1-1-76	212.500	0.797	24.878	7820.485	0.534	26799.81
ML3-2-4-4-96	218.000	1.175	1433.663**	7028.242	-0.77	587087.80*
ML3-5-2-1-105	229.667	1.086	-32.201	7690.312	1.086	-107056.00
ML3-6-7-1-107	228.667	0.954	-71.344	7600.087	1.001	-104006.00
ML3-7-1-1-108	218.667	1.068	-60.870	7077.653	1.269	1439135.00**
ML3-7-3-1-110	215.333	1.109	38.1792	6020.985	0.155	-95973.60
ML3-7-9-1-113	220.000	1.235	-10.858	7218.183	1.710	85807.92
ML1-2-7-4-121	222.000	1.104	-46.95	7346.350	2.278	202404.10
ML1-7-1-1-126	219.833	0.416	-69.669	7288.730	2.232	98250.38
ML2-5-21-3-154	236.167	1.556	-67.333	7891.528	0.967	-103487.00
ML2-7-2-2-156	239.333	1.057	-60.685	7697.095	1.418	104391.70
ML2-7-2-3-160	239.000	1.370	-46.244	8083.955	1.061	-85318.80
ML2-7-3-2-164	209.167	0.943	-48.278	6797.257	0.392	3292029.00**
ML2-5-21-2-152(P2*)	243.667	0.539	218.816	6391.447	0.286	540768.60*
ML2-8-10-1-177(P2*)	232.667	0.564	560.662**	6670.417	0.878	1608277.00**
ML2-8-13-2-178(P2*)	234.333	1.457	642.346**	6864.018	1.951	-59598.00
ML1-11-4a-3-47(P1)	230.333	1.455	24.943	7192.392	1.696	-86193.70
JGL1798	230.000	1.435	1391.269***	6865.833	0.304	1925634.00**
BPT 5204	230.667	1.078	701.171**	7133.445	2.020	-100312.00
KPR2	223.667	1.189	-16.802	6434.595	0.653	2715467.00**
Hemavathi	223.000	0.755	-69.332	6912.928	1.455	53152.54
Gandhasali	214.167	0.976	-67.965	6815.153	0.419	468135.40
Mysore mallige	214.167	0.862	-68.276	6781.680	1.389	-99626.90
Mean	224.96			7122.23		

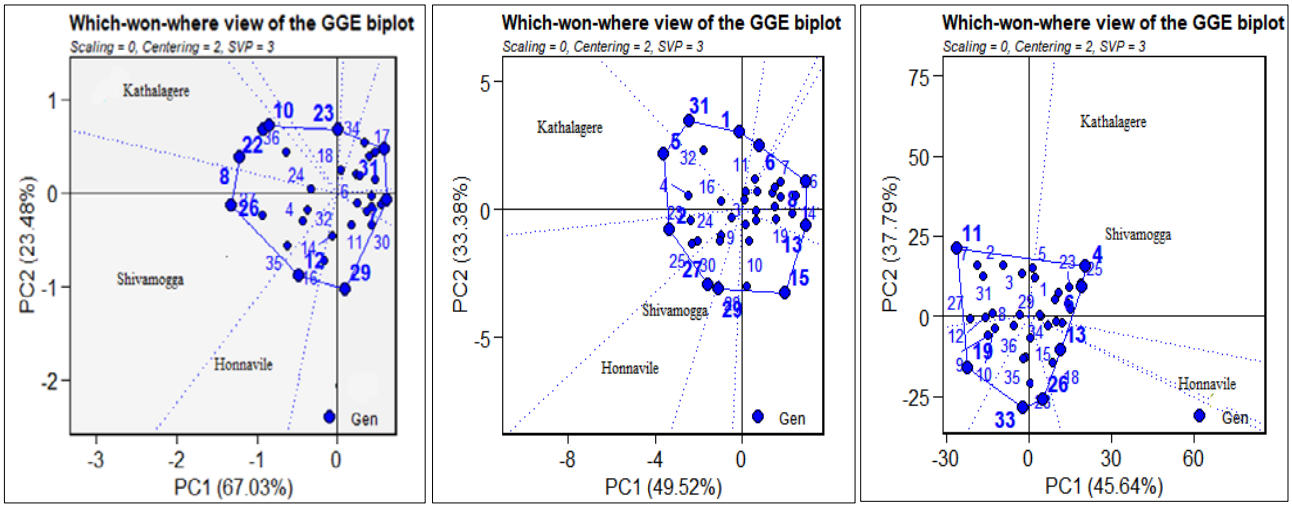
, * significance at 5% and 1% levels respectively

Understanding the pattern of genotype × environment interaction using the GGE bi-plot tool

Which-won-where graph was constructed by connecting the farthest multi-parent advanced breeding lines to form a polygon. Equality lines or perpendicular lines were then drawn from the biplot origin to each side of the polygon in order to divide the biplot into several sections. The multi-parent advanced breeding lines present on the vertices of the polygon showed either the best or poorest performance in one or more locations. The bi-plot for various characters *viz.*, number of tillers, number of spikelets and grain yield per hectare are presented in Fig. 1a-c, respectively. The equality lines of the number of spikelets per panicle and grain yield per hectare divided the test locations into two sections whereas the polygon view for number of tillers per plant indicates that the test locations were divided into three sections. But all the three test locations fell into single section with respect to test weight.

In case of number of tillers per plant, ML1-12-6-1-52, ML1-7-1-1-126 and ML1-11-5-1-49 were the winning advanced breeding lines in AHRS, Kathalagere which formed the first section. However, ZAHRS, Shivamogga which formed the second section had ML-2-7-3-2-164 as the most suitable advanced breeding line and AHRS, Honnavile formed the third section, in which, ML1-15-3A-1-17 was found to be the winner of that location and ML1-6-3-1-23, ML2-7-2-2-156 and BPT5204 were stable for all the three locations since they were located near the origin.

With respect to number of spikelets per panicle, ML1-7-2-1-26 was the successful advanced breeding line in AHRS, Kathalagere. However, ZAHRS, Shivamogga and AHRS, Honnavile which formed the second section had ML2-5-21-2-152(P2*) as the common leading genotype for both the locations. The advanced breeding lines, *viz.*, ML1-6-1-1-22, ML1-11-9-1-50, ML2-7-2-2-156 and ML3-5-2-1-105 showed stable performance for number of spikelets for all the three locations. All the three locations fell into single section with respect to test weight in which ML1-6-1-1-22 and ML2-7-2-3-160 were the outstanding advanced breeding lines and multi-parent advanced breeding lines, *viz.*, ML1-8-1-1-31, ML3-2-4-4-96, ML3-5-2-1-105 and ML1-7-1-1-126 were stable for all the three locations since they were located near the origin.



(a) Number of tillers per plant (b) Number of spikelets per panicle (c) Yield per hectare (kg/ha).

Fig 1: Polygon view of GGE biplot for identification of stable multi-parent advanced breeding lines across the tested environments.

With respect to grain yield per hectare, the first section consisted of two locations *viz.*, Kathalagere and ZAHRS, Shivamogga, in which ML1-6-3-1-23 and ML1-8-1-1-31 were the best performing advanced breeding lines. However, AHRS, Honnavile (E2) which formed the second section had ML1-2-7-4-121 as the most suitable genotype. The multi-parent advanced breeding lines, *viz.*, ML1-2-3-1-6, ML1-7-2-1-26, ML2-5-21-3-154, ML2-8-13-2-178(P2*) and Hemavathi showed stable performance for grain yield (kg/ha) according to GGE biplots since they were located near the origin.

Ranking of genotypes based on mean yield and stability performance

The GGE bi-plot technique, estimates the yield and stability of genotypes (Fig. 2) by using the average environment/ location (tester) coordinate (AEC) methods. The line passing through the biplot origin is called the average environment (tester) coordinate (AEC), which is defined by the average PC1 and PC2 scores for all environments. More close to concentric circle indicates higher mean yield. ML1-6-3-1-23 ranked first with respect to grain yield and stability performance in all three locations followed by ML2-5-21-3-154, ML2-7-2-3-160, ML1-8-1-1-31 and ML3-5-2-1-105.

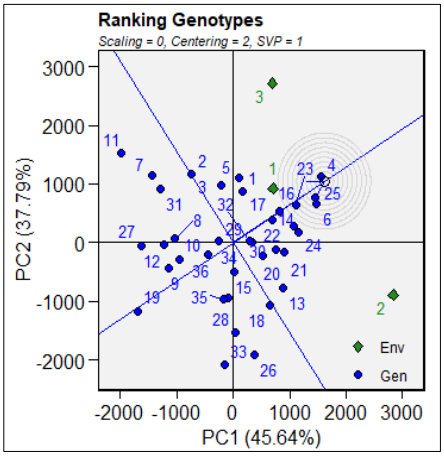
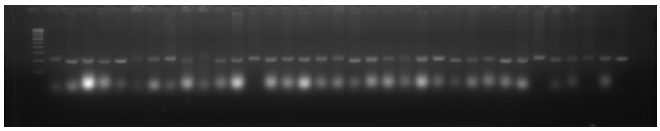


Fig 2: Ranking of the genotypes for yield and stability performance over all the three locations studied

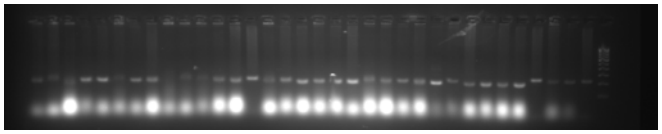
Eighty SSR markers were screened across thirty rice advanced lines and six parents to assess the molecular diversity. Out of the eighty markers screened, polymorphism was found for sixty SSR markers, while the remaining twenty were monomorphic. The number of alleles, highest frequency allele, and Polymorphism Information Content (PIC) values found among thirty rice advanced lines and six parents for sixty SSR markers were recorded using Power marker software Version 3.25 (Table 23). One hundred sixty alleles were detected at the loci of sixty microsatellite markers across thirty advanced rice lines. The number of alleles per locus ranged from five to two, with an average of 2.667 alleles across 160 loci. Polymorphism Information Content (PIC) value reflects allele diversity and frequency among varieties. PIC values ranged from 0.1029 to 0.8148, with an average of 0.4458.

The thirty rice advanced lines and six parents with sixty SSR markers were subjected to estimate Jaccard's similarity coefficient and the unweighted pair group method (UPGMA) clustering system-generated eight genetic clusters with a similarity coefficient of 62.40%. Cluster IV is the largest cluster, with nine multi-parent rice advanced lines each, followed by cluster VI having six rice advanced lines, cluster I, cluster II, cluster III, cluster V, and cluster VII with four rice advanced lines each and Cluster I is the smallest cluster with only one multi-parent rice advanced line (Fig).

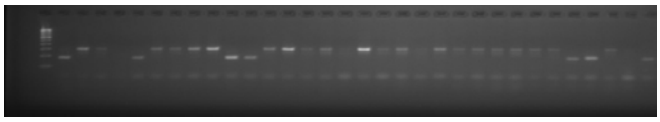
Amplification of multi-parent advanced breeding lines of rice for SSR marker RM130

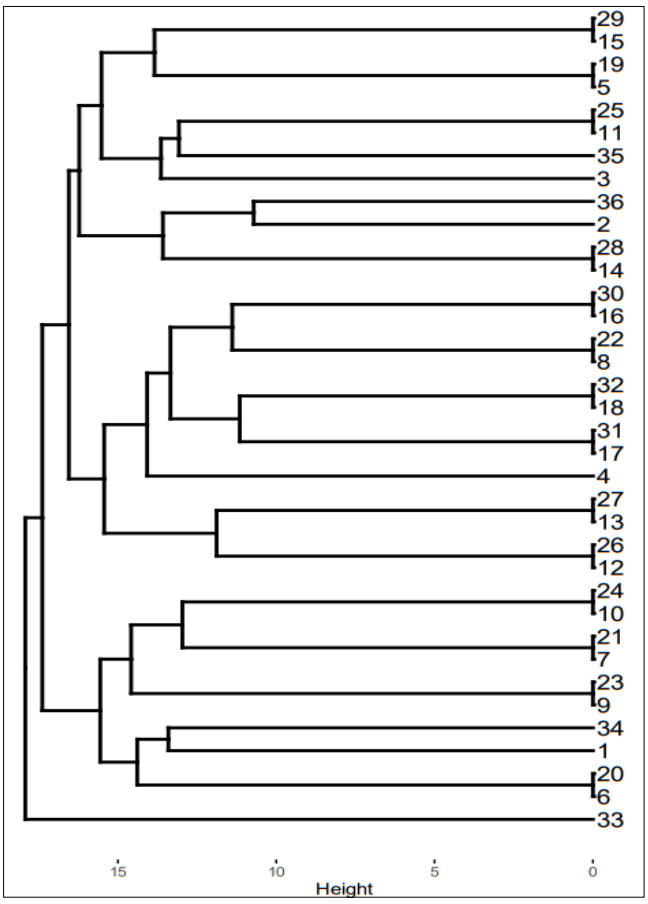


Amplification of multi-parent advanced breeding lines of rice for SSR marker RM229



Amplification of multi-parent advanced breeding lines of rice for SSR marker RM229





4. Conclusion

The stability analysis of the multi-parent advanced breeding lines using the Eberhart and Russel model and GGE biplot technique classified the advanced breeding lines based on overall performance of the multi-parent advanced across three different locations tested in Karnataka. The multi-parent advanced breeding lines that recorded highest mean yield and also performed well in all the three environments tested include ML1-6-3-1-23 and ML1-8-1-1-31 which were also the winning genotypes. These lines could be tested in largescale demonstration at farmer's field and also can be further analysed for the identification of multi-parent advanced breeding lines with desirable traits related to biotic and abiotic stress tolerance.

5. Author Contributions

D.B.M.: Standardization of methodology, Analysis. P.G.: Writing reviews, editing. H.K.V.: Data analysis, manuscript writing. M.M.G.: Experimentation, Manuscript writing. M.M.: Data analysis, Writing reviews, editing

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7. Conflicts of Interest

The authors declare no conflict of interest

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