Plant Archives Vol. 25, Supplement 1, 2025 pp.107-117 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org
DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2025.v25.supplement-1.016

DEVELOPMENT AND EVALUATION OF F_5 BREEDING LINES FOR YIELD AND YIELD ATTRIBUTES IN RICE (*ORYZA SATIVA* L.)

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(Date of Receiving : 05-08-2024; Date of Acceptance : 09-10-2024)

ABSTRACT

The objective of experiment is to detect genetic purity in the F_1 rice plants comprising of total 128 samples from four crosses *viz*., Lalkada Gold × GR-18, NVSR-2435 × Sardar, Maudamani × NVSR-2435 and Swarna Sub-1 × NVSR-2435 and to analyse the genetic variability in F_5 breeding lines by applying field rapid generation advancement (FRGA) approach under field condition. To assess the hybridity of the resulting F_1 population, polymorphic markers within the parental combination with varied positions of female and male parent specific bands were identified. Total 16, 9, 12 and 11 primes were found to be polymorphic for parents out of different 37 SSR markers tested for polymorphism among the four crosses, respectively. The hybrid purity test findings revealed that 85 out of 128 samples of 4 cross combinations were found true hybrids and forwarded for selection with precision in succeeding generations from crossing programme to F_5 during *Kharif*-2021 to *Kharif*-2023. Forty-two genotypes of rice were examined for the genetic variability parameters for 10 yield contributing traits in F_5 breeding lines. An analysis showed that there is a significant degree of variability among these genotypes. Higher value of GCV for GPP and SYPP and higher value of PCV for PTPP, GPP, 100GW, SYPP and GYPP were observed. High heritability coupled with high GAM was observed for DFF, 100GW and SYPP. The progenies 23KDSBF$_5$-35, 23KDSBF$_5$-13 and 23KDSBF$_5$-19 were identified as elite genotypes which shown significantly higher grain yield over the check varieties.

Keywords **:** Hybridity, polymorphic, SSRs, variability, heritability, GAM

Introduction

Rice (*Oryza sativa* L.) is an enormously popular food crop which farmed in more than a hundred nations. It is primary source of food and calories for more than half of the world's population. In Asia, where 60 % of the earth's people live, 90 % of the world's rice is grown and more than 3 billion Asians obtain 35-75 % of their calories from rice and its products (Khush, 2005). It provides 20% and 15% of the calories and protein consumed by the world's population, respectively (Randhawa *et al.*, 2006).

Due to the rapid increase in population, it is mandatory to fulfil the production requirements. The increased need for rice production might be met by combining traditional breeding methods with cutting-edge plant biotechnological tools like molecular markers for crop improvement, rather than relying solely on traditional hybridisation and selection (Stuber

et al., 1999). It has been demonstrated that using molecular markers based on DNA sequence polymorphism for genetic purity evaluation is an objective way to evaluate cultivar purity in a number of crop species (Karakousis *et al.*, 2003). Genetic purity testing by molecular markers is one of the most accurate and conventional method. Simple sequence repeats (SSR), one of the many DNA-based markers available, are frequently employed to assess the genetic integrity of rice hybrids and varieties. Hybridity testing should be encouraged so that isolating heterozygous samples becomes much easier, selection becomes easier, and the breeding programme can be advanced with high precision. In modern breeding approaches, hybridity testing through molecular markers is mandatory to ascertain purity in F_1S, so that confirmed crossed materials should be advanced with high efficiency and accuracy. This ultimately saves the resources and selection will be effective in advanced generations.

Several researchers have studied the relationship between yield and its main economic components in segregating rice populations (Prajapati *et al.*, 2022). An essential component of every crop development effort is genetic diversity. The progeny's variability can be attributed to environmental factors or genetic makeup. Determining whether the observed variability is heritable or not is a challenging task. For a crop development program to be successful, the traits with variability to be present must be highly heritable as selection progresses according to the character's genetic advance, heritability and severity of selection. For various targeted qualities, estimations of genetic progress and heritability aid breeders in selecting the best breeding techniques for crop development programs (Cobb *et al.*, 2019).

Materials and Methods

Plant Materials

The present experiment was carried out by using modified field rapid generation advancement approach under field condition at Regional Rice Research Station, Navsari Agricultural University, Vyara, Gujarat during *kharif* 2021 to *kharif* 2023.

For the study of genetic variability, broad sense heritability (h^2_{bs}) and genetic advance as *per cent* of mean (GAM), breeding lines were developed from four crosses *viz*., Lalkada Gold × GR-18, NVSR-2435 × Sardar, Maudamani × NVSR-2435 and Swarna Sub-1 × NVSR-2435 were made during *kharif*-2021. Detail characteristics of parental material utilized for crossing presented in Table 1.

In the *Rabi*-2021, the F_1 progenies were grown and leaf samples were took from 30 days old seedlings and stored at -20 °C for the prevention of moisture loss. A total of 128 samples were taken for hybrid purity testing. The experiment was conducted at Molecular Breeding Laboratory of the Department of Genetics and Plant Breeding, N. M. College of Agriculture and Forest Biotechnology Lab, CoF, NAU, Navsari, Gujarat.

The F_2 population of four crosses were grown under narrow spacing (10 cm row to row) and bulk harvesting of each cross was carried out during *summer* - *kharif* 2022. The F_3 population of each cross space planted at 20 cm × 15 cm and 50 individual plant selection (IPS) made from each cross during *kharif* 2022 - *Rabi* 2022-23. While in F_4 generation, 50 progenies of each cross space planted at 20 cm between rows and 15 cm within row and total 38 promising IPS made from two crosses *viz*., NVSR-2435 × Sardar and Lalkada Gold × GR-18 during summer-2023. While, no promising material was identified from crosses *viz*., Maudamani × NVSR-2435 and Swarna Sub-1 × NVSR-2435 due to late flowering of F_4 progenies in July-2023 resulted in bird damage (Table-2).

The development and evaluation of F_5 breeding lines were carried out during *kharif* 2021 to *Kharif* 2023. The experimental material involved forty-two rice genotypes and out of which, thirty-eight were F_5 breeding lines and four cultivars *viz*., Lalkada Gold, GR-18, NVSR-2435 and Sardar were the check varieties. All 42 progenies were analyzed in a RBD with three replications. There were fifteen plants in each row, spaced 20 centimetres apart from one another and 15 centimetres between each plant. By using all fundamental agronomic and plant protection techniques, the crop was produced successfully. Total ten quantitative traits *i.e*., days to 50 % flowering (DFF), days to maturity (DM), plant height (cm) (PH), panicle length (cm) (PL), number of grains per panicle (GPP), productive tillers per plant (PTPP), 100 grain weight (g) (100GW), straw yield per plant (g) (SYPP), grain yield per plant (g) (GYPP) and harvest index (%) (HI) were studied. For the biometrical study, five randomly chosen plants from each genotype and replications were observed, and their means were utilised. However, DFF and DM were evaluated on a population basis.

METHODS

DNA extraction

The genomic DNA of all the selected individuals were isolated from tender fresh leaves of each

genotype by using CTAB (Cetyl Trimethyl Ammonium Bromide) extraction method described by Doyle and Doyle (1990) with some modifications. The purity of genomic DNA isolated from selected plants was checked by agarose gel electrophoresis. The quantification (in terms of protein and RNA contamination) of isolated genomic DNA in the samples were measured using Nanodrop (Thermo, U.S.A.) at an absorbance ratio of 260/280 nm. The DNA from each sample was diluted with an appropriate amount of TE buffer to yield a working concentration of 50 µg/µl and these diluted samples were stored at 4 °C. The remaining stock DNA samples were kept at -20 °C.

PCR Amplification

A total of 37 SSR markers were used for the purpose of parental polymorphism and they are listed in Table-3. PCR was done with 10.0 µL reaction volume containing template DNA of 1.0 µL, forward primer 1.0 µL, reverse primer of 1.0 µL, nuclease free water of 2.0 µL and an EmeraldAmpGT PCR master mix of 5µL that contains Taq polymerase, PCR buffer, dNTPs, gel loading dye and fluorescence dye. The PCR protocol is comprised of initial denaturation at 95 °C for 5 min, followed by 35 cycles with denaturation for 30 seconds at 95 °C, annealing for 30 seconds at 56 °C and primer extension at 72 °C for 45 seconds and a final extension step of 72 °C for 10 min. All PCR reactions were performed in Thermal cycler (BIO-RAD, U.S.A.). The amplified PCR products were separated and visualised using 3.5 % poly acrylamide gels stained with ethidium bromide (0.5µL/ml) and visualised under UV light using gel documentation system (Gel Doc™ XR+ Imaging system). The standard size of 100 bp DNA ladders is used to estimate the size of the amplified products.

Results and Discussion

Parental polymorphism

The markers, which were polymorphic within the parental combination and had different positions of female and male parent specific bands were used to assess the hybridity of their derived F_1 population. Parental polymorphism is required to evaluate the genetic purity of F_1 samples because, by knowing the relative locations of alleles of both parents at a particular size (bp), we can analyse the same alleles in the F_1 samples and determine whether the sample is genetically pure or a parental type. More polymorphism is anticipated between the parents, implying that the amplicon size between the alleles should be bigger, preventing overlapping between the alleles. Thirty-seven rice SSR markers were used to identify parental polymorphism in all four crosses. Among 37 primers, only 16 SSR markers found polymorphic in nature for Lalkada Gold × GR-18; 9 SSR markers found polymorphic in nature for NVSR-2435 × Sardar; 12 SSR markers found polymorphic in nature for Maudamani × NVSR-2435 and 11 SSR markers found polymorphic in nature for Swarna Sub-1 × NVSR-2435 (Table-4).

Testing genetic purity of F_1 using SSR markers:

Cross 1: Lalkada Gold × GR-18

Among 16 primers, RM-225 were used for testing hybridity for this cross. The primer RM-225 had amplified an allele of size 123 bp in the female parent and 145 bp in male parent. However, this F_1 cross exhibited the alleles of both parents (123 bp and 145 bp) confirming the heterozygosity of the hybrid by having two bands of sizes 123 bp and 145 bp (Table-5). For testing of F_1 hybridity of 54 individual plants for this cross was analysed, and it was observed that 37 were true hybrid and 17 were self as seen in Plate-1.

Cross 2: NVSR-2435 × Sardar

Among 9 primers, RM-164 were used for 172 testing hybridity for this cross. The primer RM-164 had amplified an allele of size 274 bp in the female parent and 254 bp in male parent. However, this F_1 cross exhibited the alleles of both parents (274 bp and 254 bp) confirming the heterozygosity of the hybrid by having two bands of sizes 274 bp and 254 bp (Table-5). For testing of F_1 hybridity of 22 individual plants for this cross was analysed, and it was observed that 15 were true hybrid and 7 were self as seen in Plate-2.

Cross 3: Maudamani × NVSR-2435

Among 12 primers, RM-225 were used for testing hybridity for this cross. The primer RM-225 had amplified an allele of size 147 bp in the female parent and 125 bp in male parent. However, this F_1 cross exhibited the alleles of both parents (147 bp and 125 bp) confirming the heterozygosity of the hybrid by having two bands of sizes 147 bp and 125 bp (Table-5). For testing of F_1 hybridity of 30 individual plants for this cross was analysed, and it was observed that 19 were true hybrid and 11 were self as seen in Plate-3.

Cross 4: Swarna Sub-1 × NVSR-2435

Among 11 primers, RM-279 were used for testing hybridity for this cross. The primer RM-279 had amplified an allele of size 129 bp in the female parent and 167 bp in male parent. However, this F_1 cross exhibited the alleles of both parents (129 bp and 167 bp) confirming the heterozygosity of the hybrid by having two bands of sizes 129 bp and 167 bp (Table-5). For testing of F_1 hybridity of 22 individual plants

for this cross was analysed, and it was observed that 14 were true hybrid and 8 were self as seen in Plate-4.

Genetic variability in F_5 breeding lines for ten quantitative characters

The ANOVA (Table 6) demonstrates significant MS (mean square) values for every character in the F_5 breeding lines, suggesting that there is sufficient variation (Table-7 and Graph-1) in the breeding lines for every attribute.

The mean values indicated a wide range for DFF (57.33 - 106.67 days), DM (85.33 - 134.67 days), PH (93.20 - 140.27 cm), PL (19.20 - 28.53 cm), ETPP (4.40 - 8.93), GPP (111.40 - 249.47), 100GW (1.56 - 3.46 g), SYPP (13.28 - 34.64 g), HI (27.30 - 48.69 %) and GYPP (6.53 - 23.09 g). Based on mean values, the breeding line 23KDSBF$_5$-13 (22.20 g) of NVSR-2435 × Sardar cross and breeding line 23KDSBF$_5$-35 (23.09 g) and 23KDSBF$_5$-19 (19.76 g) of Lalkada Gold × GR-18 cross had shown significantly higher grain yield over the check varieties *viz*., NVSR-2435 (13.38 g), Sardar (19.57 g), Lalkada Gold (14.01 g) and GR-18 (16.04 g).

The progenies exhibited higher GCV values for GPP (22.00%) and SYPP (23.44%) and higher values of PCV for PTPP (20.12%), GPP (29.25%), 100GW (20.38%), SYPP (29.41%) and GYPP (30.33%) indicating greater scope of improving these characters by applying selection in appropriate direction. Moderate GCV values were observed for the traits *viz*., DFF (11.53%), PTPP (11.90%), 100GW (16.65%) and GYPP (19.25%) and moderate PCV values were observed for the traits *viz*., DFF (12.14%), PH (11.47%) and HI (18.05%) indicating environmental influence on these traits and limited scope for improvement by selection. Low GCV values were recorded for the characters *viz*., DM (8.29%), PH (5.66%), PL (4.47%) and HI (9.61%) and low PCV values were observed in DM (8.81%) and PL (9.46%) indicating narrow range of variability for these traits and restricting the scope of selection for these traits.

High GCV and high PCV were observed in GPP by Kumar *et al.* (2020), Manohara *et al.* (2020), Shrivastav *et al.* (2020), Singh *et al.* (2020) and Manju *et al.* (2021) and SYPP by Karthikeyan *et al.* (2010), Patel *et al.* (2018), Singh *et al.* (2021), Chendake *et al.* (2023) and Demeke *et al.* (2023). Moderate GCV and high PCV were observed in PTPP by Kumari *et al.* (2019), Nithya *et al.* (2020) and Kumar *et al.* (2020), 100GW by Singh *et al.* (2021), Reddy *et al.* (2022), Chendake *et al.* (2023) and Demeke *et al.* (2023) and GYPP by Priyatham *et al.* (2018), Amegan *et al.* (2020) and Bisen *et al.* (2020). Moderate GCV and moderate PCV were observed in DFF by Nithya *et al.* (2020), Bhargavi *et al.* (2021), Manju *et al.* (2021) and Reddy *et al.* (2022). Low GCV and moderate PCV were observed in PH by Yuvaraja *et al.* (2019), Shrivastav *et al.* (2020) and Kumar *et al.* (2023) and HI by Zaid *et al.* (2022) and Chendake *et al.* (2023). Low GCV and low PCV were observed in DM by Chendake *et al.* (2023), Demeke *et al.* (2023), Kumar *et al.* (2023) and Moukoumbi *et al.* (2023) and PL by Edukondalu *et al.* (2017), Yadav *et al.* (2017), Priyatham *et al.* (2018), Singh and Verma (2018), Singh *et al.* (2021) and Demeke *et al.* (2023).

High heritability values were observed for DFF (90.13%), DM (88.59%), 100GW (66.73%) and SYPP (63.53%) indicating that phenotype could provide good measure of genotypic effect and improvement for these traits could be made by simple phenotypic selection. Moderate heritability was observed for PTPP (34.97%), GPP (56.56%) and GYPP (40.30%). This result indicates moderate influence of environmental factors in the expression of this trait showing limited scope for phenotypic selection. PH (24.39%), PL (22.27%) and HI (28.33%) showed low heritability values, which indicate direct selection for these traits would not be effective. Similar results were observed for DFF (96.11%), DM (88.04%), 100GW (88.42%) and SYPP (96.86%) by Demeke *et al.* (2023); ETPP (51.50%) and GPP (59.60%) by Asati and Yadav (2020); GYPP (58.12%) by Noatia *et al.* (2021); PH (28.19%) by Manohara *et al.* (2020) and PL (18.00%) by Chozin and Sumardi (2019).

High magnitude of GAM was recorded for DFF (22.54%), GPP (34.08%), 100GW (28.02%), SYPP (38.49%) and GYPP (25.18%). Moderate magnitude of GAM was observed for DM (16.07%), PTPP (14.49%) and HI (10.53%). Low estimate of GAM was observed for

PH (5.76%) and PL (4.34%). Similar results were observed for DFF (21.78%), GPP (58.71%), 100GW (47.26%) and GYPP (61.77%) by Manju *et al.* (2021); SYPP (38.57%) by Patel *et al.* (2018); DM (16.25%) and HI (15.45%) by Naik *et al.* (2022); ETPP (16.26%) by Singh *et al.* (2020); PH (9.68%) and PL (8.60%) by Sadia *et al.* (2020).

When forecasting the response of selection, heritability (h^2_{bs}) combined with GAM is more beneficial than heritability alone. High h^2_{bs} coupled with high GAM was observed for DFF (90.13% and 22.54%), 100GW (66.73% and 28.02%) and SYPP (63.53% and 38.49%) indicating the role of additive gene effects and less effects of environmental factors on expression of the traits. Thus, the improvement of

these traits could be achieved through direct phenotypic selection. High h^2_{bs} coupled with moderate GAM was observed for DM (88.59% and 16.07%) which indicates this trait is governed by additive gene effects and selection is effective for this trait. Moderate h^2_{bs} with high GAM was observed for GPP (56.56% and 34.08%) and GYPP (40.30% and 25.18%) which indicated that the additive gene effects. The moderate heritability is being exhibited due to high environment effect. So, selection would be effective for this trait. Selection would be fairly effective for improvement of PTPP (34.97% and 14.49%) due to moderate h^2_{bs} with moderate GAM. Low h^2_{bs} with moderate GAM was observed for HI (28.33% and 10.53%) indicated that this trait was govern by non-additive gene action, thus direct selection is not beneficial for this trait. Low h^2_{bs} and GAM were observed for PH (24.39% and 5.76%) and PL (22.27% and 4.34%) indicating higher environmental influence. Hence, little gain is expected through selection for these traits. Similar results of heritability and GAM were observed for DFF (93.16% and 20.14%), 100GW (94.11% and 32.65%) and SYPP (81.40% and 40.48%) by Reddy *et al.* (2022) and DM (94.27% and 16.11%), GPP (36.70% and 27.56%), ETPP (30.14% and 10.34%) and PH (19.45% and 8.62%) by Amegan *et al.* (2020).

Conclusion

By employing RM-225 primer, 37 plants were found true F_1 out of 54 plants in cross Lalkada Gold × GR-18, whereas, 19 plants were found true F_1 out of 30 plants in cross Maudamani × NVSR-2435 by. While 15 plants were observed true F_1 out of 22 plants in cross NVSR-2435 × Sardar by employing RM-164 primer and 14 plants were observed true F_1 out of 22 plants in cross Swarna Sub-1 × NVSR-2435 by employing RM-279 primer. The progenies $23KDSBF_5$-35, $23KDSBF_5$-13 and $23KDSBF_5$-19 were identified as elite genotypes which shown significantly higher grain yield over the check varieties. The magnitude of GCV and PCV was high for straw yield per plant, which indicated the potential for selection based genetic enhancement. High heritability combined with high GAM was recorded for days to 50% flowering, 100 grain weight and straw yield per plant. Days to 50% flowering, productive tillers per plant, grains per panicle, 100 grain weight, straw yield per plant and grain yield per plant were recorded moderate to high GCV and PCV value, heritability and genetic advance as *per cent* of mean. So, improvement of these traits could be achieved through direct phenotypic selection.

Table 1: Salient features of parents *viz.*, Lalkada Gold, GR-18, NVSR-2435, Sardar, Maudamani and Swarna Sub-1 used in the present experiment

Particulars	Lalkada Gold	GR-18	NVSR-2435	Sardar	Maudamani	Swarna Sub-1
Parentage / pedigree	IR-28 / LALKADA	GAR-13 / JGJ-3828	GAR-13 / JAYA	GURJARI / JAYA	(DANDI / NAVIN) / DANDI	SWARNA / IR49830 (Sub-1 donor)
Days to flowering	88-90	78-80	110-112	84-86	108-117	120-125
Plant height (cm)	105-115	120-130	120-126	110-115	109-119	85-90
Panicle length (cm)	21-24	25-28	22.5-26.5	20-24	22.1-26.1	26.0
Productive tillers per plant	6-8	5-8	5-8	8-10	5-8	10-12
Grains per panicle	140-170	210-240	226-253	220-250	119-146	150-180
Grain type	Long slender	Medium slender	Medium slender	Long bold	Short bold	Medium slender
Grain yield (kg/ha)	4000-4500	5400-5800	5500-6000	5500-6000	6000-8000	5000-5500

Table 2: F_5 breeding lines of all crosses evaluated in the present experiment

Cross	Number of breeding lines	Breeding lines
NVSR-2435 × Sardar	18	$23KDSBF_5$-1 TO $23KDSBF_5$-18
Lalkada Gold × GR-18	20	$23KDSBF_5$-19 TO $23KDSBF_5$-38
Maudamani × NVSR-2435	0	-
Swarna Sub-1 × NVSR-2435	0	-

Table 3: List of primers used for detecting polymorphism

No.	Marker	Primer Sequence (5' to 3')
1	HvSSR01-01	F- TCAATCCTATGTTCAATCCC
		R- GCAGTCGACATAATGCATAC
2	HvSSR11-13	F- TGAAACCACAATGAGTCAAA
		R- GCCCTAAACCCAAATAGAAG
3	RM-13	F- TCCAACATGGCAAGAGAGAG
		R- GGTGGCATTCGATTCCAG
4	RM-35	F- GGTGGCATTCGATTCCAG
		R- CGACGGCAGATATACACGG
5	RM-126	F- CGCGTCCGCGATAAACACAGGG
		R- TCGCACAGGTGAGGCCATGTCG
6	RM-144	F- TGCCCTGGCGCAAATTTGATCC
		R- GCTAGAGGAGATCAGATGGTAGTGCATG
7	RM-164	F- TCTTGCCCGTCACTGCAGATATCC
		R- GCAGCCCTAATGCTACAATTCTTC
8	RM-171	F- AACGCGAGGACACGTACTTAC
		R- ACGAGATACGTACGCCTTTG
9	RM-174	F- AGCGACGCCAAGACAAGTCGGG
		R- TCCACGTCGATCGACACGACGG
10	RM-206	F- CCCATGCGTTTAACTATTCT
		R- CGTTCCATCGATCCGTATGG
11	RM-212	F- CCACTTTCAGCTACTACCAG
		R- CACCCATTTGTCTCTCATTATG
12	RM-224	F- ATCGATCGATCTTCACGAGG
		R- TGCTATAAAAGGCATTCGGG
13	RM-225	F- TGCCCATATGGTCTGGATG
		R- GAAAGTGGATCAGGAAGGC
14	RM-246	F- GAGCTCCATCAGCCATTCAG
		R- CTGAGTGCTGCTGCGACT
15	RM-277	F- CGGTCAAATCATCACCTGAC
		R- CAAGGCTTGCAAGGGAAG
16	RM-279	F- GCGGGAGAGGGATCTCCT
		R- GGCTAGGAGTTAACCTCGCG
17	RM-287	F- TTCCCTGTTAAGAGAGAAATC
		R- GTGTATTTGGTGAAAGCAAC
18	RM-310	F- CCAAAACATTTAAAATATCATG
		R- GCTTGTTGGTCATTACCATTC
19	RM-332	F- GCGAAGGCGAAGGTGAAG
		R- CATGAGTGATCTCACTCACCC
20	RM-335	F- GTACACACCCACATCGAGAAG
		R- GCTCTATGCGAGTATCCATGG
21	RM-337	F- GTAGGAAAGGAAGGGCAGAG
		R- CGATAGATAGCTAGATGTGGCC
22	RM-413	F- GGCGATTCTTGGATGAAGAG
		R- TCCCCACCAATCTTGTCTTC
23	RM-450	F- AAACCACAGTAGTACGCCGG
		R- TCCATCCACATCTCCCTCTC
24	RM-488	F- CAGCTAGGGTTTTGAGGCTG
		R- TAGCAACAACCAGCGTATGC
25	RM-490	F- ATCTGCACACTGCAAACACC
		R- AGCAAGCAGTGCTTTCAGAG

26	RM-495	F- AATCCAAGGTGCAGAGATGG
		R- CAACGATGACGAACACAACC
27	RM-513	F- TCTAGTGGCCTCAAAAAGGG
		R- GCAACGAAATCATCCCTAGC
28	RM-517	F- GGCTTACTGGCTTCGATTTG
		R- CGTCTCCTTTGGTTAGTGCC
29	RM-520	F- AGGAGCAAGAAAAGTTCCCC
		R- GCCAATGTGTGACGCAATAG
30	RM-572	F- CGGTTAATGTCATCTGATTGG
		R- TTCGAGATCCAAGACTGACC
31	RM-5699	F- ATCGTTTCGCATATGTTT
		R- ATCGGTAAAAGATGAGCC
32	RM-6987	F- AGCTTGACACGATCCTTACC
		R- CATCGCAGCAATATCCACTG
33	RM-7193	F- ATGTGGGAATTTCTAGCCCC
		R- CCCTAGTTTTCCAAATGGCC
34	RM-18639	F- CATCATGTGGTAAGTGTGCAACG
		R- GGTTGCGATGAGATTACGAGACC
35	RM-19255	F- TTAAGCTAGGGAATCAGCGGTTAGC
		R- GGAGTTGCAGTGTGGTGTGTGG
36	RM-19771	F- AACCAATGCACACTTCTTCTGTGC
		R- CAACTGTAGAGGTTGGAATGATCTGC
37	RM-20025	F- GCTGTTCCTGTAGCTAGCCATGC
		R- GGCATATCCAGATTGTCACATCC

Table 4: SSR primers exhibited desired polymorphic banding pattern

Sr. No.	Cross	Polymorphic primers		
1	Lalkada Gold × GR-18	HvSSR01-01	RM-224	RM-413
		HvSSR11-13	**RM-225**	RM-517
		RM-13	RM-279	RM-6987
		RM-164	RM-287	RM-7193
		RM-206	RM-310	-
		RM-212	RM-337	-
2	NVSR-2435 × Sardar	**RM-164**	RM-310	RM-517
		RM-206	RM-332	RM-6987
		RM-212	RM-488	RM-7193
3	Maudamani × NVSR-2435	RM-206	RM-335	RM-572
		RM-225	RM-337	RM-6987
		RM-287	RM-413	RM-7193
		RM-310	RM-450	RM-19771
4	Swarna Sub-1 × NVSR-2435	HvSSR11-13	RM-413	RM-572
		RM-164	RM-488	RM-5699
		RM-279	RM-490	RM-6987
		RM-287	RM-513	-

Table 5: Useful SSR markers for identifying true F_1 hybrid plants from four crosses of rice

Sr. No.	Cross	Primer	Female amplicon bp	Male amplicon bp	Total F_1 plants	True F_1 plants	Self-plants
1	Lalkada Gold × GR-18	RM225	123	145	54	37	17
2	NVSR-2435 × Sardar	RM164	274	254	22	15	7
3	Maudamani × NVSR-2435	RM225	147	125	30	19	11
4	Swarna Sub-1 × NVSR-2435	RM279	129	167	22	14	8

Table 6: ANOVA of traits studied

Source of variation	d.f.	Mean sum of square									
		DFF	DM	PH	PL	PTPP	GPP	100 GW	SYPP	HI	GYPP
Replication	2	9.52	24.66	294.06	7.70	0.18	462.64	0.003	56.95	72.17	14.38
Progenies	51	209.09**	215.06**	170.24**	3.88**	2.95**	4726.55**	0.512**	119.76**	69.869**	34.42**
Error	102	7.36	8.85	149.45	4.14	1.13	963.27	0.073	19.23	31.97	11.38

** - Significant at 1% level of probability, * - Significant at 5.0 % level of probability

DFF= Days to 50% flowering DM= Days to maturity PH= Plant height (cm) PL= Panicle length(cm)

PTPP= Productive tillers per plant GPP= Grains per panicle 100 GW= 100 grain weight (g) HI= Harvest index (%)

SYPP= Straw yield per plant (g) GYPP= Grain yield per plant (g)

Table 7: Genetic variability parameters of the characters studied

Character	Range		GCV (%)	PCV (%)	ECV (%)	h^2_{bs}	GA	GAM (%)
	Min.	Max.						
Days to 50% flowering	57.33	106.67	11.53	12.14	3.81	90.13	1603.74	22.54
Days to maturity	85.33	134.67	8.29	8.81	2.97	88.59	1607.50	16.07
Plant height (cm)	93.20	140.27	5.66	11.47	9.97	24.39	706.29	5.76
Panicle length (cm)	19.20	28.53	4.47	9.46	8.34	22.27	105.88	4.34
Productive tillers per plant	4.40	8.93	11.90	20.12	16.22	34.97	94.86	14.49
Grains per panicle	111.40	249.47	22.00	29.25	19.28	56.56	5487.34	34.08
100 grain weight (g)	1.56	3.46	16.65	20.38	11.76	66.73	64.40	28.02
Straw yield per plant (g)	13.28	34.64	23.44	29.41	17.76	63.53	950.49	38.49
Harvest index (%)	27.30	48.69	9.61	18.05	15.28	28.33	389.69	10.53
Grain yield per plant (g)	6.53	23.09	19.25	30.33	23.43	40.30	362.45	25.18

GCV = Genotypic coefficient of variation (%)

PCV = Phenotypic coefficient of variation (%)

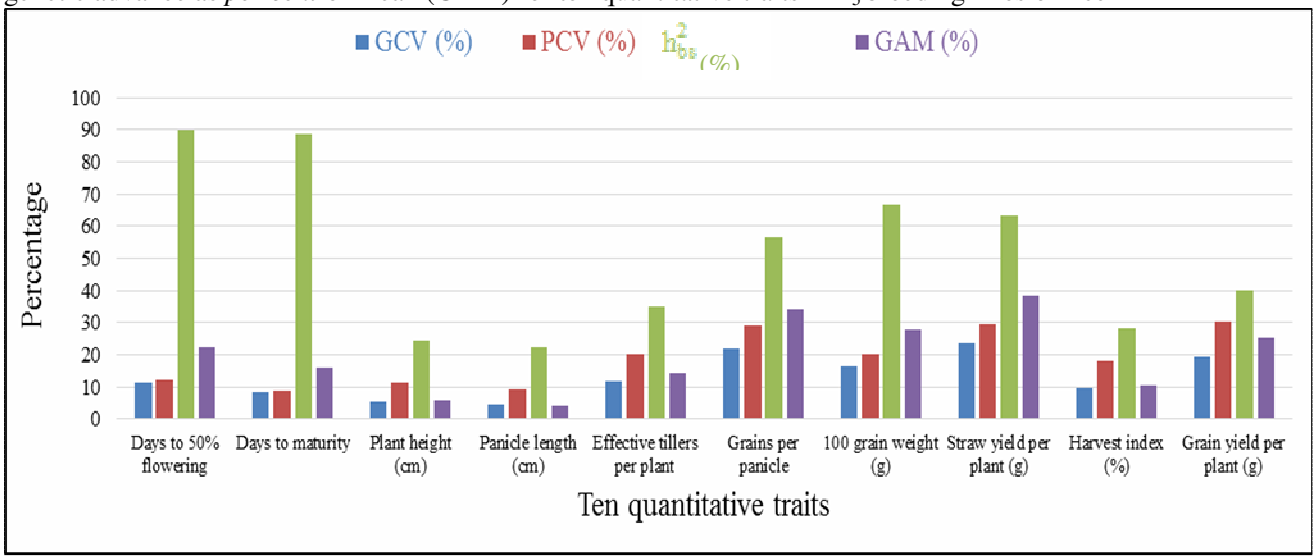
ECV = Environmental coefficient of variation (%)

GA = Genetic advance

GAM = Genetic advance as *per cent* of mean (%)

h^2_{bs} = Heritability (Broad sense) (%)

Fig. 1: Genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability (h^2) and genetic advance as *per cent* of mean (GAM) for ten quantitative traits in F_5 breeding lines of rice



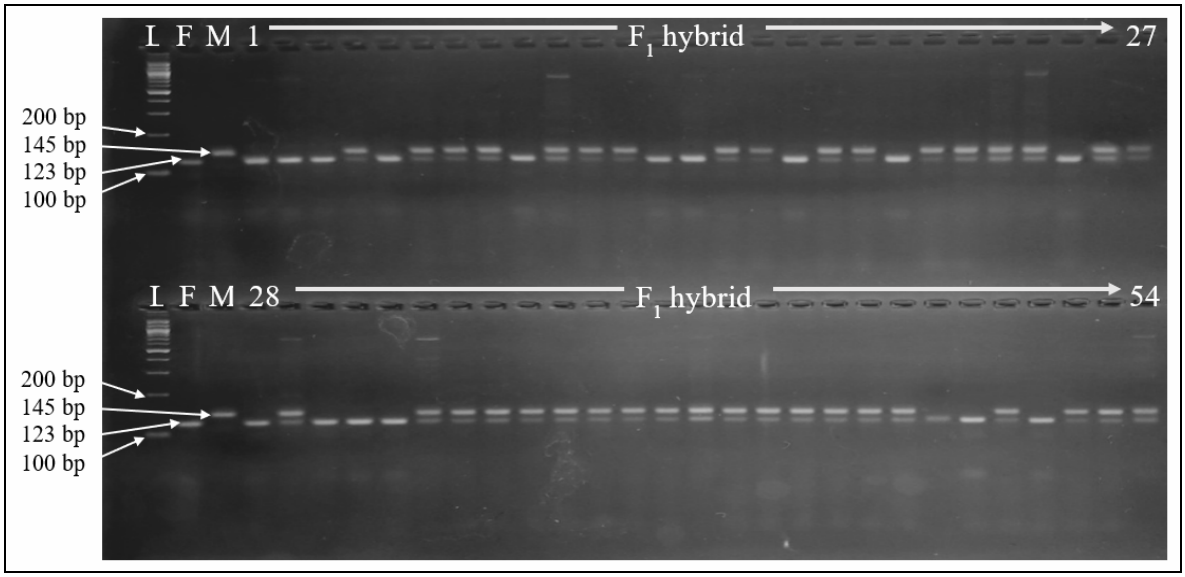


Plate 1: Hybridity test of 54 F_1 hybrid plants along with parents for Lalkada Gold × GR-18 cross by using RM-225 marker

Where, L: 100 bp ladder, F: Female, M: Male, F_1 hybrid: 54 F_1 hybrid plants of cross Lalkada Gold × GR-18

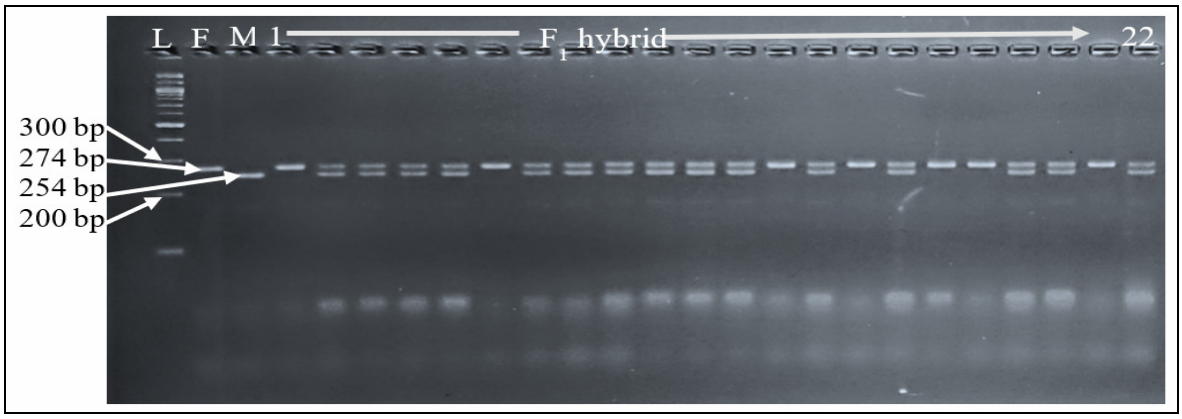


Plate 2: Hybridity test of 22 F_1 hybrid plants along with parents for NVSR-2435 × Sardar cross by using RM-164 marker

Where, L: 100 bp ladder, F: Female, M: Male, F_1 hybrid: 22 F_1 hybrid plants of cross NVSR-2435 × Sardar

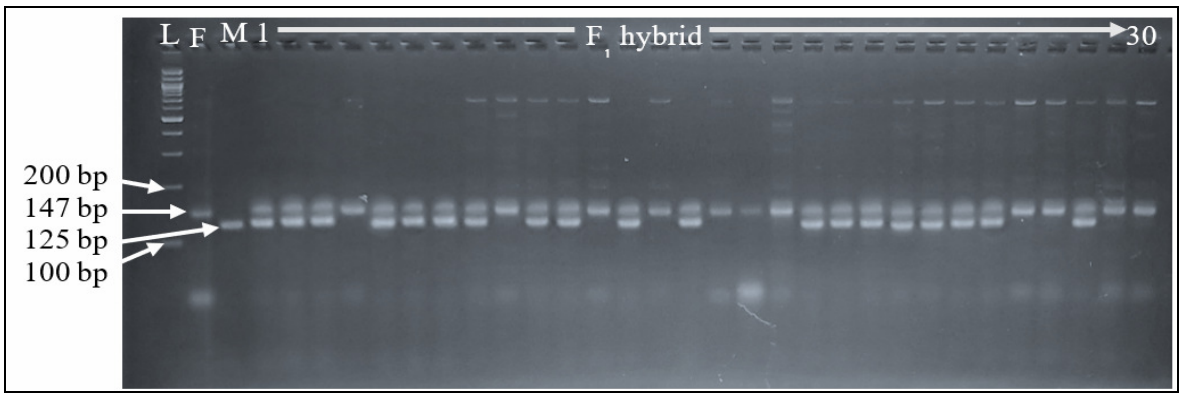


Plate 3: Hybridity test of 30 F_1 hybrid plants along with parents for Maudamani × NVSR-2435 cross by using RM-225 marker

Where, L: 100 bp ladder, F: Female, M: Male, F_1 hybrid: 30 F_1 hybrid plants of cross Maudamani × NVSR-2435

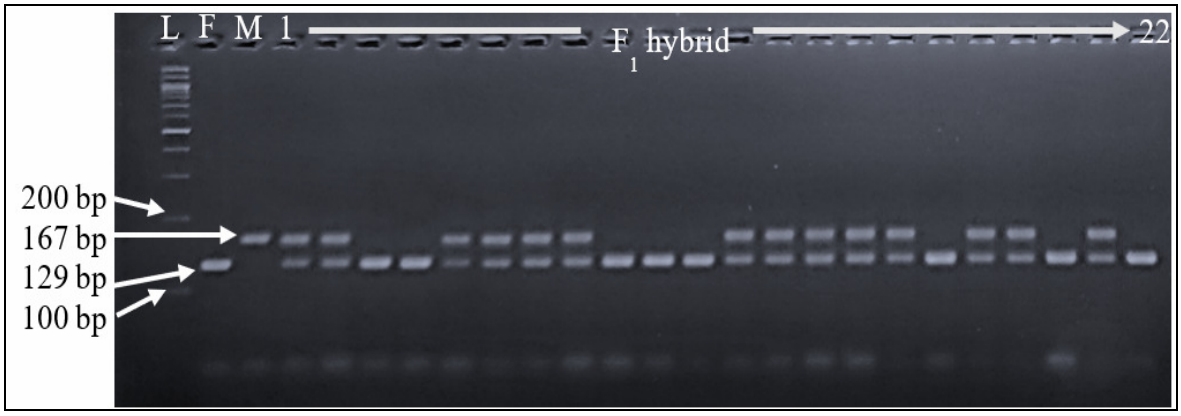


Plate 4: Hybridity test of 22 F_1 hybrid plants along with parents for Swarna Sub-1 × NVSR-2435 cross by using RM-279 marker

Where, L: 100 bp ladder, F: Female, M: Male, F_1 hybrid: 22 F_1 hybrid plants of cross Swarna Sub-1 × NVSR-2435

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