International Journal of Biotechnology and Biomedical Sciences
p-ISSN 2454-4582, e-ISSN 2454-7808, Volume 4, Issue 2; July-December, 2018 pp. 22-27
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http://www.krishisanskriti.org/Publication.html

Improvement of Tulaipanji Rice through Molecular Breeding

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Abstract—*Tulaipanji is non-Basmati aromatic rice (GI tagged) of Uttar Dinajpur district, West Bengal, famous for fragrance and quality with medicinal value. Yield rate is low 1.8 -2.1 t/h, because not responsive to any fertilizer and excess water. Reason is that genetic architecture is not responsive to high input of resources due to narrow genetic base or minimum genetic buffer prevailed in this gene pool. Hybridization was initiated by crossing with Ranjit, IR64, and Pusa Basmati 1460, to widen the genetic base. Two lines were selected from the cross between Ranjit x Tulaipanji. One line (Progeny-Awn) was with awn and aroma but other line (Progeny-Awnless) without awn and aroma. Progeny-Awnless line evolved new trait at F4 stage such as red pericarp colouration which was not present either of the parental lines. New gene recombination may be reshuffled during selfing within the Rc/Rd gene location. Genetic marker (SNP and InDel) variations were studied in these rice lines using Illumina-NGS based GBS technique. Total SNPs was 52810 identified including 4327 InDels at read depth 10. Parental genome introgression was not at per Mendelian genetics but segregation distortion (SD) was observed. Out of 10013 alleles, 92.52% was introgressed into progeny-awn from Tulaipanji and 7.48% from Ranjit, whereas progeny-awnless carried 89.19% alleles from Ranjit and only 10.81% alleles from Tulaipanji. Parental genome introgression into the F5 (RIL) were investigated to gain genetic architectural knowledge of SNPs variations associated with traits. Four progeny lines were selected at F5 stage from the cross Tulaipanji x IR64. Morpho-agronomical characters were transgressive in nature, which are linked to the yield increase. Third cross was made Tulaipanji and Pusa Basmati1460. Two progeny lines were showing good quality in terms of aroma and fluffiness. Present outcome of different hybridization lines are promising containing genes for high yield performance with grain quality.*

Keywords*: Rice landrace Tulaipanji, Hybridization, genome introgression, Illumina-NGS, GBS technique, SNP/InDels variations.*

1. INTRODUCTION

Rice (*Oryza sativa* L.) is an important and staple food grain of the world because more than half of the world's human population are dependent on it [1]. Global rice production must be doubled by 2050 [2-3] to feed the more than 9 billion people (9BPQ). Narrow genetic base in released rice varieties has make the improvement in plateaus [4-5]. Though rice has a wide genetic base, the germplasm with which breeders are working, in general, has limited variability, which probably is the major bottleneck in improving productivity.

Yield increase was the prime aim of the Green Revolution without considering the grain quality. Farmers stopped growing specific local fragrant rice varieties and replaced them with the new, fast growing, disease resistant, high yielding, non-fragrant varieties [6-7] due to low yield of these fragrant varieties in compared to HYV. It has a tremendous detrimental effect on the genetic diversity of fragrant rice. Many of the local fragrant landraces were out-competed and lost [6, 8, 9] from the gene pool. Nowadays there is a high demand of fragrant rice worldwide due to progression of living standard and increasing income. Low yield in fragrant rice varieties is due in part to its susceptibility to diseases and insect pests [10-12] and compounded by management practices [7, 13]. Fragrant rice exhibits higher aroma levels when grown in sub-optimal conditions. Tulaipanji is a non-Basmati aromatic rice of West Bengal and famous for its quality and fragrance but low yielder. Thus, the present project is designed to broaden the genetic base of the local rice landrace cultivar Tulaipanji of Uttar Dinajpur district by hybridization with different genetic stock to increase the yield through introgression of yield related gene/QTLs.

2. MATERIALS AND METHODS

Plant materials

Tulaipanji rice: Well adapted local rice landrace *Oryza sativa* L. subspecies *indica* cultivar Tulaipanji of Uttar Dinajpur district was used as recurrent parent. Tulaipanji is a non-Basmati aromatic rice landrace of West Bengal (GI tagged, No 530, 2017, Govt. of India). This cultivar is characterized by medium grain quality with good aroma having drought avoidance characteristics, not responsive to chemical fertilizer and low yielder (1.8-2.1 t/ha). Germplasm is being maintained in the Department of Botany, University of North Bengal. Other three varieties such as Ranjit, IR64, Pusa Basmati-1460 were used as donor parent for yield enhancement through crossing. Hybridization was performed between cultivar Tulaipanji and Ranjit (2011), Tulaipanji and IR64 (2013) and

Tulaipanji and PB-1460 (2016) using standard artificial pollination system [14-16].

Agromorphological and Physicochemical data analysis

Morphological data recording and analysis was done based on DUST protocol (Govt. of India).

Genomic DNA isolation and GBS library preparation with SNP data analysis

Total genomic DNA was isolated from fresh leaf samples of four lines (2 parental and 2 progeny) following standard protocol (DNeasy Plant Mini Kit, QIAGEN, Germany) to study the SNPs variation into the progenies of the cross between Tulaipanji and Ranjit. GBS library from four lines was constructed using the standard protocol [17-19]. The sequencing was done based on V4 Chemistry on HiSeq 2500 platform of Illumina. GBS data was analyzed for SNPs variation analysis.

3. RESULTS AND DISCUSSION

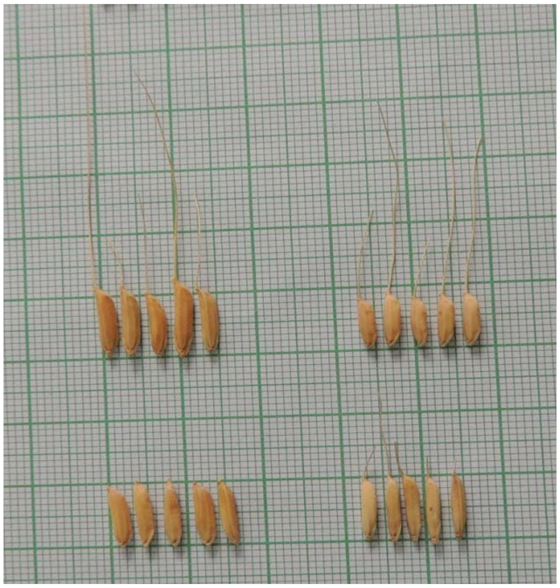
Tulaipanji is an aromatic rice (non-Basmati) variety of West Bengal, India, famous for its fragrance, fluffiness and mouth feeling (GI tagged, No 530, 2017, Govt. of India). The local rice landrace Tulaipanji was cross with Ranjit (HYV) in the year 2011 (First cross). Two distinct lines were identified from the progenies- one line with awn and aroma traits but second line without awn and aroma. It was noticed that awn containing line is giving aroma in grains but awn less line without aroma. Germination rate and seedling vigour was studied (Fig. 2). Vigorous root growth was observed in Progeny awnless line. Both the progeny lines are showing mid-parent value in respect to the seed morphology and grain quality. New trait was appeared in the fifth generation seed of progeny-awnless with more brown coloration in the rice bran (Progeny awn less), which showing improvement of the grain quality in the fifth generation line (Awn less). This improvement may be more nutritious in compared to parental lines. Both the progeny lines were vigorous in morphological growth. They had good root system and fast growing habitat in compared to parental lines (Fig. 1). Second cross was made between Tulaipanji and IR64 in the year 2013 (Fig. 2) and third cross between Tulaipanji and PB-1460 in 2016. The F5 progeny was harvested from the field level experiment with four segregation lines in case of second cross. Two progeny lines were identified from the segregation lines at F2 generation in third cross (Tulaipanji x PB-1460). Among these developed progeny lines, two progeny lines of the cross between Tulaipanji and IR-64 was most promising and may be considered for varietal trial (Fig. 2). Agromorphological characteristics such as grain length and width was showing transgressive segregation by increasing the length and width of the F5 seeds (Tulaipanji x IR-64). Plant height was mid-parent value 125 cm instead of 135 in Tulaipanji and 95 cm in IR-64 (Table 1). Grain and kernel morphology of the different progeny lines were summarized (Fig. 3-5). Physicochemical properties of the different progeny rice lines were evaluated (Fig. 6). The ASV (alkali spreading value) was measured in the different rice grain based on the standard protocol [20]. Sensory based aroma was evaluated using standard procedure [21]. It was identified that aroma was high in Tulaipanji and progeny F5 lines (Tulaipanji x IR64) and AVS value was moderate (Fig. 6). Grain characteristics were of transgressive pattern, because length and weight was increased in the progeny F5 lines (Table 1, Fig. 3-5). Tillering was also high in F5 progeny lines (Tulaipanji x IR64) (Fig. 2).

Total genomic DNA was isolated from the four rice lines (Tulaipanji x Ranjit) at F5 generation DNA quality was checked in agarose gel electrophoresis and concentration was measured in Qubit analyzer (Fig. 7).

Figure 1: Seed germination in the four progeny lines (Tulaipanji x Ranjit) at F5 generation.

Figure 2: Progeny lines F4 (Tulaipanji x IR64) and parental line IR64 (early maturity right side).

Figure 3: Grain morphology of progeny lines in the cross Tulaipanji and IR64. First panel, IR64, Tulaipanji, second panel, F5 medium grain with awn, F5 black grain, third panel, F5 Awn and F5 Awnless,



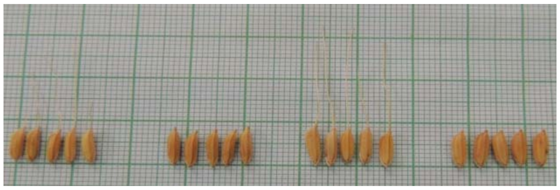


Figure 4: Grain morphology of progeny lines. First panel, PB1460, F4 Medium grain (Tulai x IR64), second panel, progeny F5 Awn, F5 awnless, third panel, Tulaipanji, progeny F7 AL, F7 AW, and Ranjit.

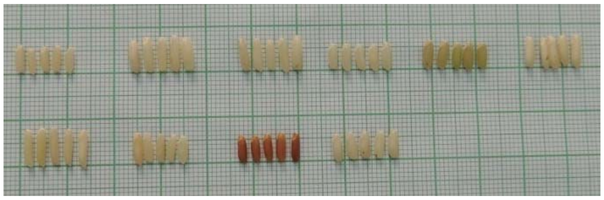


Figure 5: Kernel shape and size of different progeny lines. Above: Tulaipanji, progeny F5 awnless, F5 awn, F5 medium grain with awn, F5 black, and IR64, and below: PB1460, progeny F7 Awn, F7 awnless, Ranjit.

Table 1: Morphological characteristics of the Parental and progeny lines based on DUS test protocol.

Rice cultivars	PH	FL L	FL W	Maturity	Aw L	Pn L
Tulaipanji	135	26	9	150	30	26
IR64	100	30	15	120	0	24
Progeny Aw (Tulai x IR64) F5	125	29	15	135	23	30
Progeny AL (Tulai x IR64) F5	125	29	15	135	0	28
PB1460	105	29	14	135	31	27
Progeny F2 (TulaixIR64x PB1460)	125	35	13.5	140	12	23
Ranjit	103	27	17	145	0	24.6
Progeny AwF7 (Tulai x Ranjit)	135	30	9	145	18	27
Progeny AL F7 (Tulai x Ranjit)	135	30	10	145	0	27

Rice cultivars	Gr/Pani	1000 GrWT	GL	GW	GL/GW
Tulaipanji	94	15.12	7.75	1.79	4.32
IR64	130	25.00	9.60	2.40	4.00
Progeny Aw (Tulai x IR64) F5	185	27.86	9.75	2.41	4.04
Progeny AL (Tulai x IR64) F5	186	28.38	9.85	2.39	4.12
PB1460	120	24.60	10.60	1.94	5.46

Progeny F2 (TulaixIR64x PB1460)	140	26.46	10.55	2.14	4.92
Ranjit	180	19.27	7.84	2.21	3.54
Progeny AwF7 (Tulai x Ranjit)	145	19.38	8.47	2.37	3.57
Progeny AL F7 (Tulai x Ranjit)	140	16.5	8.87	2.14	4.14

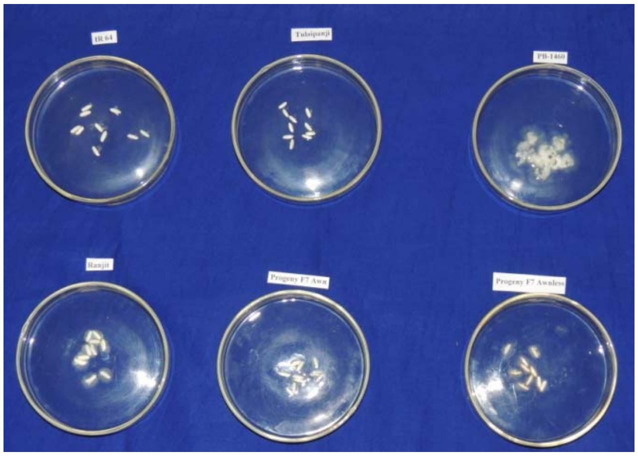
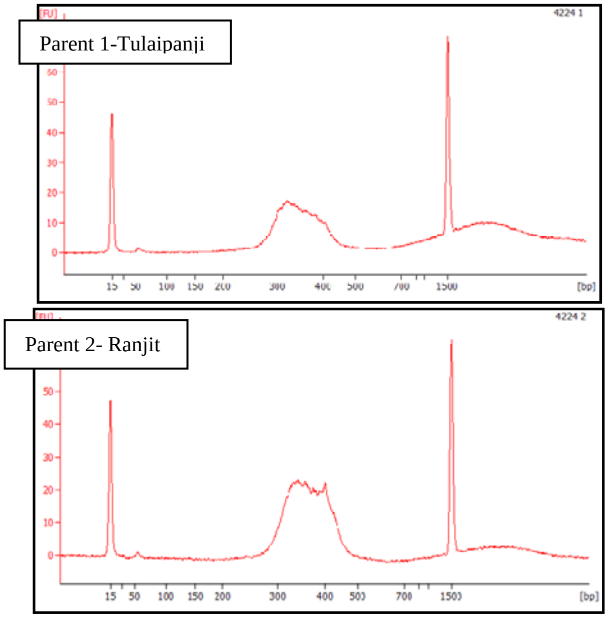


Figure 6: Physicochemical properties of the progeny rice lines (Tulaipanji x Ranjit) at F7 was depicted for quality check.



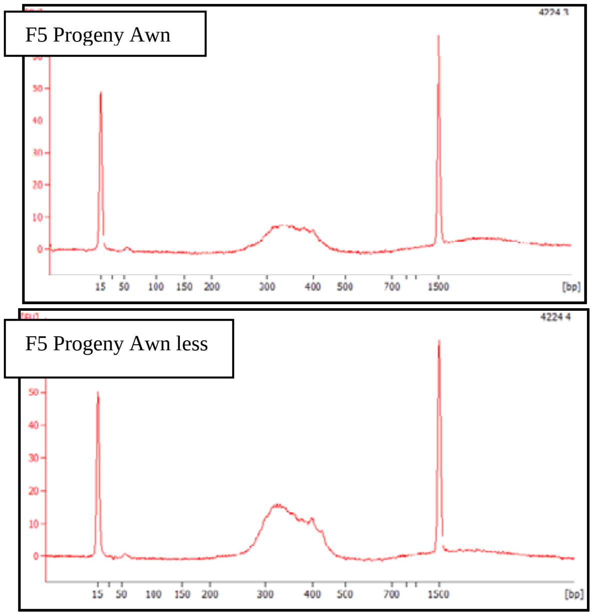
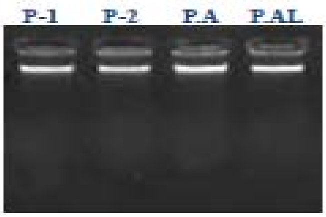
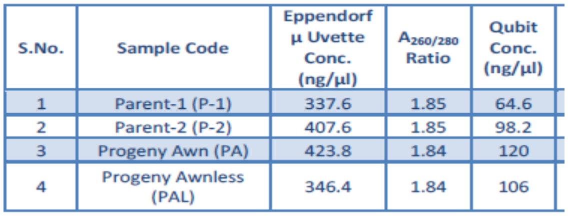


Figure 8: BioAnalyzer (BA) based profiling for quality judge of the PCR amplified product during NGS library construction (Illumina) in ddRADSeq method (Four rice lines).





S.No.	Sample Code	Eppendorf µ Uvette Conc. (ng/µl)	$A_{260/280}$ Ratio	Qubit Conc. (ng/µl)
1	Parent-1 (P-1)	337.6	1.85	64.6
2	Parent-2 (P-2)	407.6	1.85	98.2
3	Progeny Awn (PA)	423.8	1.84	120
4	Progeny Awnless (PAL)	346.4	1.84	106

Figure 7. 1% Agarose gel electrophoresis for quantification of isolated DNA from four rice samples [Above panel]. Lane P-1 (Tulaipanji), P-2 (Ranjit), P-A (F5 Progeny with awn) and P-AL(F5 progeny without awn) and concentration and purity of the four DNA samples were measured in Qubit analyzer (below panel).

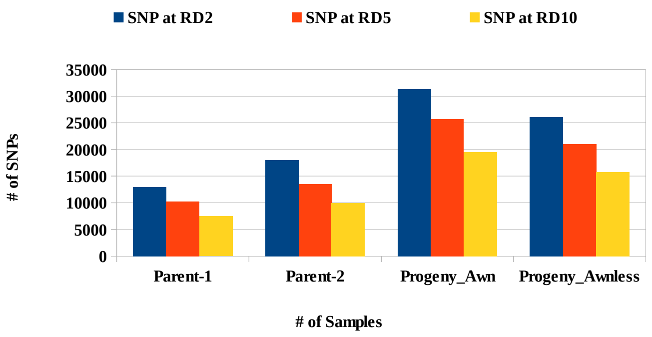


Figure 9: Sample-wise SNPs summary in the four rice lines (Tulaipanji x Ranjit) at F5 generation.

The NGS library was constructed from the total genomic DNA of four rice lines (F5, Tulaipanji x Ranjit) and analyzed through BioAnalizer (Fig. 8). Sample-wise SNPs were analyzed in these four lines (Fig. 9). Highest SNP number was 6195 in chromosome 2 and lowest 3169 in chromosome 10 in this present investigation. In the same way, highest InDels was 569 in chromosome 2 and least was 225 in chromosome 10. Total SNPs variant was 52810 and Indels variant was 4327 among these four rice lines and these were evenly distributed into the rice genome (12 chromosomes). Total SNPs were identified 52810 from the four rice lines, highest number of SNPs was 19559 in progeny awn, and least number of SNPs 7476 in parent 1 (Tulaipanji), 9966 SNPs in parent 2 (Ranjit) and 15809 in progeny awnless at RD10. Total InDels were identified 4327 from four rice lines at RD10, out of this highest was found in progeny awn 1514, least 489 in Tulaipanji, 950 in Ranjit and 1374 in progeny awnless. Progeny awn showed highest amount of variants (21073) considering both SNPs and InDels followed by progeny awnless (17183), Ranjit (10916) and Tulaipanji (7965).

In conclusion, it can be demonstrated that progeny F5 lines (Tulaipanji x IR64) may be considered as promising rice lines because these lines were showing transgressive segregation continuously from generation F3 to F5 in respect to grain length (GL), width (GW), and weight (1000 seed weight). Grain quality is *at par* with the Tulaipanji grain quality justified by ASV and aroma test. Plant height was also mid parent value 125 cm in F5 progenies (Tulaipanji x IR64) and tillering number was high 15-20 on average.

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