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RESEARCH ARTICLE

Yield Improvement of Tulaipanji Rice through Recombination Breeding and Selection

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Tulaipanji is a famous aromatic rice from West Bengal (GI tagged), cultivated only in the district of Uttar Dinajpur. Recombination breeding was attempted and crosses were made between Tulaipanji and three high yielding cultivars of rice namely, Ranjit, IR64 and Pusa Basmati 1460 to combine high yield with superior grain quality. One of the breeding lines of the cross (Tulaipanji × Ranjit), showed unique new trait which was red pericarp. Two most promising lines were selected from the cross (Tulaipanji × IR64) with enhanced yield and aroma. Grain length was 9.75-9.85 mm, width was 2.39-2.41 mm, 1000 seeds weight was 27.86-28.38 g, just with 27.09, 34.63 and 87.69 % improvement over Tulaipanji. Grain number per panicle was 186 in F5 lines but only 94 in Tulaipanji. Maturity time has been reduced to 135 days instead of 145-150 days in Tulaipanji. One F2 progeny line of a triparental cross (Tulaipanji × IR64 × PB1460) had enhanced yield potentiality and quality. Grain quality was judged using physicochemical parameters (ASV, sensory based aroma and GT).

Key Words: Pedigree selection, Pure lines, Rice landrace Tulaipanji, Yield increase

Introduction

Rice [*Oryza sativa* L.], the most important cereal crop, provide staple food for half of the World's population (> 3.3 billion). This staple food grain is the main energy resource providing 40-75% of the daily calorie intake to the world's poor people (Yu *et al*., 2002; Khush, 2005). Asia is considered as 'Rice Basket' because it produces 90% of the world's production (748 million tons, paddy rice, Mt). Total world production was 748 Mt from 163.1 million hectares with productivity of 4.6 tons/hectare (t/ha) in 2016 of which 676.5 million tons was produced by Asian countries. Global rice production must be doubled by 2050 (Ray *et al*., 2013; Arbelaez *et al*., 2015) to feed the more than 9 billion people (9BPQ). Narrow genetic base in released rice varieties has make the improvement in plateaus (Tanksley and McCouch, 1997; Tian *et al*., 2006). Genetic bottlenecks during domestication causes narrow genetic diversity (Tanksley and McCouch 1997) in the well adapted cultivars leading to yield stagnation. Breeder could manage the yield increase over released varieties through genetic gain by combining the yield related genes/QTLs from various genetic resources of rice germplasms either from cultivated local landraces (CLR) or from wild varieties. Germplasm diversity is the mainstay for crop improvement and genetic dissection of complex traits. Rice germplasm shows tremendous genetic diversity in both within the species and among the varietal groups (Godfray *et al*., 2010; Tester and Langridge 2010; Gill *et al*., 2011) and can be exploited to introgress these traits using knowledge of molecular breeding techniques such as marker assisted breeding (MAB) (Kilian and Graner, 2012; McCouch *et al*., 2012, 2013; Li and Zhang, 2013; Agarwal *et al*., 2016) or marker assisted selection (MAS). Efficient breeding designs are much needed to transfer the useful diversity to breeding lines. The MAGIC (Multi-parent Advanced Generation InterCross) breeding program has been designed to produce highly recombined populations with increased genotypic diversity leading to genetic enhancement (Bandillo et al 2013; Leung *et al*., 2015; Meng *et al*., 2017). Following Green Revolution, farmers stopped growing specific local fragrant rice varieties and replaced them with the new, fast growing, disease resistant, non-fragrant and high yielding varieties (Bhattacharjee *et al*., 2002; Itani *et al*., 2004) due to low yield of these fragrant varieties compared to HYVs. This led to tremendous reduction in the genetic diversity of fragrant rice. Many of the local fragrant landraces were out-competed and lost from the gene pool (Singh *et al*.,

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2000; Bhattacharjee *et al*., 2002; Garg *et al*., 2006). Nowadays there is a worldwide demand for fragrant rice 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 (Lorieux *et al*., 1996; Sriboonchitta and Wiboonpongse, 2005; Toojinda *et al*., 2005) which is compounded by management practices (Yoshihashi *et al*., 2002; Itani *et al*., 2004). Fragrant rice exhibits higher aroma levels when grown in sub-optimal conditions.

Tulaipanji is a non-Basmati aromatic rice of West Bengal (GI tagged) and famous for its quality and fragrance but with low yield potential. Uttar Dinajpur district lies between latitude 25°11' N to 26°49' N and longitude 87°49' E to 90°00' E occupying an area of 3142 km². Total area under Tulaipanji rice cultivation in Uttar Dinajpur district was 6700 hectare during 2017-18 *kharif* crops and total production was 14,740 metric tons. Thus, the present project is designed to broaden the genetic base of the local rice landrace cultivar Tulaipanji of Uttar Dinajpur district by recombination breeding through hybridization with different genetic stock and to increase the yield potentiality through introgression of yield related gene/QTLs from different genetic resources of rice.

Materials and Methods

Plant Materials

Local rice landrace cultivar Tulaipanji of Uttar Dinajpur district was used as main parent for its improvement. It is a non-Basmati aromatic rice landrace of West Bengal (GI tagged, No 530, 2017, Govt. of India). It is characterized by medium grain quality with good aroma, drought avoidance, unresponsive to chemical fertilizer application and low yield (1.8 t/ha). Three varieties namely, Ranjit, IR64, Pusa Basmati-1460 were used as parents for genetic enhancement and yield improvement of Tulaipanji rice in this recombination breeding program.

Conventional Artificial Pollination for Hybridization

Hybridization was performed between cultivar Tulaipanji and Ranjit (2011), Tulaipanji and IR64 (2013) and Tulaipanji and PB-1460 (2016). Hybridization was done according to the standard protocols of artificial pollination in rice (Sleper and Poehlmer 2007; Sha 2013; Roy, 2017). Morpho-agronomic traits were recorded according to standard evaluation system of rice (SES, IRRI, 2002, www.irri.org) for evaluation of the rice lines. Alkali spreading value (ASV) (in a scale of 1 to 7) was measured according to the standard method (Little *et al*., 1958). A low ASV corresponds to a high gelatinization temperature (GT), conversely, a high ASV indicates a low GT. Sensory based aroma (in a scale of 0 to 3) was evaluated using standard procedure (Sood and Siddiq, 1978).

Statistics and Anova Analysis

Statistical significance of the field data were analyzed using Excel Stat data analysis software (MS office). Anova and t-test was performed at $p < 0.05$ (5% significance level).

Results and Discussion

Agromorphological and Physicochemical Data Analysis

Crosses were made between Tulaipanji × Ranjit (2011), Tulaipanji × IR64 (2012) (named as IRT64F1). One line IRT64F4 was again crossed with PB1460 in 2016 to increase the genetic base and F2 population seeds were harvested from the cross (IRT64F4 × PB1460) in 2017 *kharif* season. Agro-morphological studies were done based on DUS test protocol of rice for all the progeny lines (Table 1, Figures 2, 3 and 4). Kernel shape and size of different progeny lines were morphologically summarized (Figure 5). Nine morphological traits were considered for the present analysis–plant height, flag leaf length, flag leaf width, maturity days, awn length, panicle length, grain per panicle, 1000 seeds weight, grain length, grain width and grain length/width ratio (Table 1) and three physicochemical properties were considered for the evaluation of the progeny lines (Table 2, figures 6 and 7) to judge the grain quality.

Observed plant height was 135.1 cm, 135 cm, 135.2 cm in Tulaipanji, progeny F7 awn and progeny F7 awnless respectively. Short height was recorded in IR64, which was 100.9 cm and F5 progeny lines showed similar to mid-parent value, 125.3 to 125.4 cm.

F2 progeny lines of cross IRT64F4 × PB1460 also showed reduced plant height (125 cm) in compared to Tulaipanji parental line 135 cm. Plant height in subsequent F3 generation again decreased to 110 cm which is quite similar to PB1460 plant height of 105 cm. The plant height controlling gene *Sd1* had been introduced in IR8 through crossing from *Dee-Geo-*

Table 1. Summary of the statistical analysis of the Agro-morphological data in the parental and progeny lines.

	Tulaipanji	IR64	Progeny Aw F5	Progeny AL F5	PB1460	Progeny F2	Ranjit	Progeny Aw F7	Progeny AL F7
Plant height (cm)	*135.1±0.433	100.9±0.622	*125.3±1.042	*125.4±0.541	105±1.011	*125.4±0.702	103±0.843	135±0.930	135.2±0.827
Flag leaf length (cm)	*26±0.293	30.05±1.065	*29.03±0.523	*29.08±0.639	29.10±0.759	*35.09±0.917	27.02±0.435	30.08±0.507	30.04±0.559
Flag leaf width (mm)	9±0.137	15.07±0.280	15.02±0.192	15.03±0.186	14.04±0.165	13.51±0.229	17.02±0.143	9.02±0.075	10.02±0.144
Maturity days	*150.2±0.466	120±0.596	*135±0.516	*135±0.333	135±0.516	140.4±0.561	145±0.537	145±0.475	145±0.537
Awn length (mm)	30±0.542	00	23.07±0.271	00	31.06±0.516	12.08±0.381	00	18.01±0.380	00
Panicle length (cm)	*26±0.414	24±0.423	*30.01±0.314	*28.02±0.201	27±0.235	23±0.203	24.61±0.248	27.01±0.193	27±0.229
Grain/panicle	*94.1±0.525	130.1±0.433	*185±0.977	*186.1±1.159	120.4±0.819	*140.4±0.686	180.3±0.869	145.1±1.242	140.2±0.827
1000 seeds wt (g)	*15.12±0.053	25.02±0.203	*27.86±0.115	*28.38±0.155	24.60±0.240	*26.46±0.128	19.27±0.084	19.38±0.161	16.50±0.120
Grain length (mm)	*7.75±0.014	9.60±0.022	*9.75±0.017	*9.85±0.025	10.60±0.020	*10.55±0.045	7.84±0.028	8.47±0.021	8.87±0.018
Grain width (mm)	*1.79±0.007	2.40±0.009	*2.41±0.005	*2.39±0.004	1.94±0.011	*2.14±0.023	2.21±0.027	2.37±0.013	2.14±0.019
GL/GW ratio	4.32±0.013	4.00±0.021	4.04±0.026	4.12±0.036	5.45±0.021	4.92±0.015	3.54±0.021	3.57±0.013	4.14±0.036

Mean value of 10 plants± standard error. * Significant at p value < 0.05 level in t-Test in row wise.
[Progeny F5 (Tulaipanji × IR64), Progeny F2 (Tulaipanji × IR64 × PB1460), Progeny F7 (Tulaipanji × Ranjit), Aw- Awn, AL-Awnless].

Woo-Gen, a Taiwan variety. Due to short height and other genetic components, IR8 was high responsive to chemical fertilizer and water along with lodging resistant (Khush 1987; Mackill 2018; http://www.irri.org/ir8). The progeny lines of F5 and F3 were lodging resistant (Fig. 1) compared to the parental line Tulaipanji in the field. Genetic components of high tillering and increased grain number in these F3 and F5 progeny lines might have been inherited from parental line IR64. IR64 itself is a multiparental high yielding mega variety developed and released by IRRI in 1985 (Mackill and Khush, 2018). The F3 progeny lines had the characteristics of all the three parents namely Tulaipanji, IR64 and PB1460. Analysis of variance revealed that at least one of the three samples had significantly different means (Table 3).

Table 2. Summary of the physicochemical properties of the parental and progeny lines of different crosses.

Cultivars/progenies	Aroma	ASV	GT
Tulaipanji	3	4	3
IR64	0	1	7
F5AL (Tulaipanji × IR64)	3	4	3
F5AW (Tulaipanji × IR64)	3	3	5
F5Md (Tulaipanji × IR64)	1	3	5
F5Blk (Tulaipanji × IR64)	2	4	3
PB1460	1	7	1
Ranjit	0	1	7
F7AW (Tulaipanji × Ranjit)	2	3	5
F7AL (Tulaipanji × Ranjit)	0	2	7
F2 (IRT64F4 × PB1460)	2	4	3

[AVS: Alkali spreading value, GT: Gelatinization temperature]

Table 3. Summary of the ANOVA analysis for the morphological traits in parental and progeny lines.

a.	Anova of plant height (cm)		
	Source of Variation	*MS*	*P-value*
	Between Groups	2046.853	5.24E-58
	Within Groups	6.374	
b.	Anova of Flag leaf length (mm)		
	Source of Variation	*MS*	*P-value*
	Between Groups	63.90614	1.2E-12
	Within Groups	4.53148049	
c.	Anova for maturity days		
	Source of Variation	*MS*	*P-value*
	Between Groups	805.4778	2.054882
	Within Groups	2.592593	
d.	Anova for panicle length (cm)		
	Source of Variation	*MS*	*P-value*
	Between Groups	46.4987	1.25E-29
	Within Groups	0.842093	
e.	Anova for grain per panicle		
	Source of Variation	*MS*	*P-value*
	Between Groups	9958.128	3.58E-82
	Within Groups	7.655556	
f.	Anova for 1000 Grain weight (g)		
	Source of Variation	*MS*	*P-value*
	Between Groups	250.5303778	2.22E-79
	Within Groups	0.226058765	
g.	Anova for grain length (mm)		
	Source of Variation	*MS*	*P-value*
	Between Groups	11.55992	2.72E-88
	Within Groups	0.00626	

Table 4. Depicted the t-Test results showing significant variances between Tulaipanji and Progeny F5 Awn line (Tulaipanji × IR64).

a. t-test: Two-Sample assuming equal variances in plant height		
	137	125
Mean	134.889	125.333
Variance	1.611	12.25
P(F<=f) one-tail	0.0048	

b. t-Test: Two-Sample Assuming Equal Variances in Maturity days		
	150	*135*
Mean	150.222	135
Variance	2.4444	2.666
P(T<=t) two-tail	1.70731E-13	

c. t-Test: Two-Sample Assuming Equal Variances in panicle length		
	26	30.5
Mean	26.002	29.956
Variance	1.931	1.282
P(T<=t) two-tail	5.93035E-06	

d. t-Test: Two-Sample Assuming Equal Variances in grain per panicle		
	94	188
Mean	94.111	184.666
Variance	3.1111	9.5
P(T<=t) two-tail	6.00544E-22	

e. t-Test: Two sample assuming equal variances in grain length		
	7.73	9.75
Mean	7.752222222	9.75
Variance	0.002419444	0.0033
P(T<=t) two-tail	3.41858E-22	

Fig. 1. Experimental field trial in *Kharif* crop 2017, at Kaliyaganj, Dist. Uttar Dinajpur. Above: Transplanted in 30 cm × 20 cm based on DUS test protocol of parental and progeny lines F5 (Tulaipanji × IR64), and F7 (Tulaipanji × Ranjit). Clearly showing that F5 lines (Tulaipanji × IR64) was lodging resistance but Tulaipanji has been totally lodged in the field (bottom).

One of the progeny lines of the cross between Tulaipanji × Ranjit, developed a new unique trait, which is red pericarp formation from F4 generation. The trait red pericarp was totally absent in the parental lines (Tulaipanji and Ranjit) (Figure 5). Red pericarp is common in wild rice genotypes while cultivated genotypes mostly have white colour pericarp (Sweeney *et al*., 2007). This phenomenon of red pericarp coloration is associated with the domestication of rice crop (Izawa *et al*., 2009). Red pericarp has high nutritional value (Shirley, 1998; Furukawa *et al*., 2007).

Red pericarp colouration leads to accumulation of proanthocyanidin in grains of wild rice which helps in plant defense against pathogens or predators. Present observation might be consistent with the earlier findings (Brooks *et al*., 2008) that one wild type allele (*Rc*) changed to recessive null allele (*rc*) by deleting 14 bp in the exon seven of *Rc* allele of the parental line (Tulaipanji and Ranjit). There might be second mutation in the exon resulting in recovery of the reading frame of the wild type Rc allele leading to red pericarp development (Roy and Reddy, 2017).

Mainly four progeny lines were selected using pedigree selection method from the cross Tulaipanji × IR64 which was medium grained with awn, black husk coloured, long grained with awns and long grained without awns (Figures 2, 3 and 4). Field performance of two F5 progeny lines showed lodging resistance with strong and stout stem (Figure 1 lower panel). As the parental line Tulaipanji completely lodged in the field (Figure 1), lodging resistant gene must have been inherited from parental line IR64 in the progeny lines. Plant height in the progeny F5 line was similar to mid-parent value (125 cm) (Table 1), flag leaf character was as like as IR64. Maturity time reduced to 135 days instead of 145-150 days needed for Tulaipanji. The IR64 is early maturity high yielding variety takes 120 days. Panicle length has increased in progeny line 28-30 cm with high grain numbers (185-186) compared to Tulaipanji (94 grain/panicle) (Table 1). Grain length increased in F5 progeny lines (9.75 mm – 9.85 mm), whereas grain length was 7.75 mm in Tulaipanji. 1000

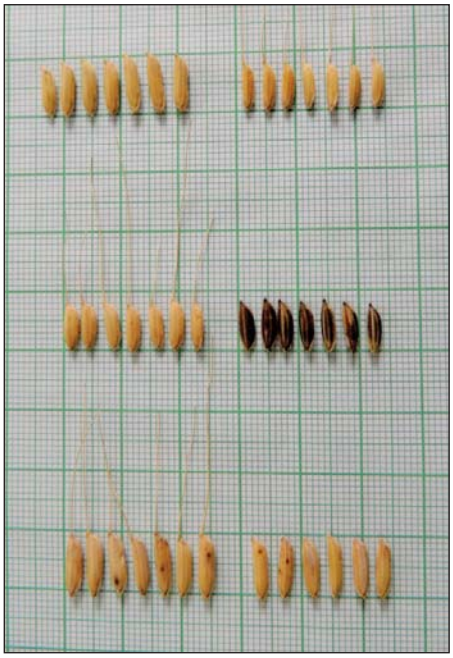

Fig. 2. Grain morphology of progeny lines in the cross Tulaipanji and IR64. First panel, IR64, Tulaipanji, second panel, F5 medium grain with awn, F5 black husk grain, third panel, F5 progeny Awn and F5 progeny Awnless

Fig. 3. Grain morphology of progeny lines. First panel, PB-1460, F5 Medium grain (Tulai × IR64), second panel, progeny F5 Awn (Tulaipanji × IR64), F5 awnless (Tulaipanji × IR64), third panel, Tulaipanji, progeny F7 AL, F7 AW, and Ranjit

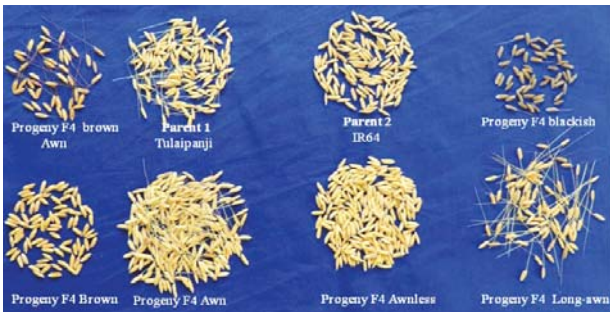

Fig. 4. Grain morphology of progeny lines of cross between Tulaipanji and IR-64, at F4 generations, *kharif* 2016

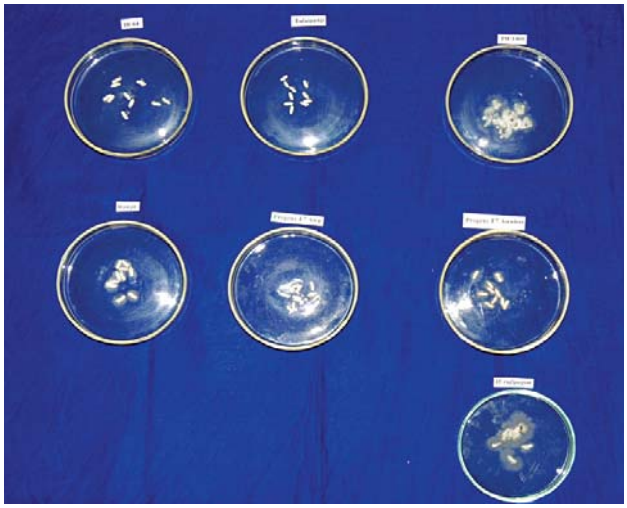

Fig. 6. Physicochemical properties of the progeny rice lines (Tulaipanji × Ranjit) at F7 stage was depicted for quality check. Showing the alkali spreading value, gelatinization temperature and sensory based aroma test

seeds weight was high in F5 lines (27.86 g–28.38 g) but only 15.12 g in Tulaipanji.

Aroma index was 3 in the progeny lines of the cross (Tulaipanji × IR64) in F5 stage with ASV values 3-4 and GT value 3 or 5. ASV showed distinct genetic variability among the parental and progeny lines (Figures 6 and 7). Cultivar IR64 showed low ASV (1), and high value of GT (7). ASV depends on the nature of the amylopectin molecules and is reported to be dependent on soluble starch synthase gene on chromosome 6. Both the traits (GT and ASV) are known to be governed by the enzymes of granule bound starch synthase I (GBSSI) encoded by the *Waxy* gene locus on short arm of chromosome 6 (Umemoto *et al*., 2002 and 2004).

In many breeding programs, the improvement of the quality of rice, especially its eating and cooking quality (ECQ), is an important objective as rice is mainly consumed in cooked form (Pang *et al*., 2016).

Fig. 5. Kernel shape and size of different progeny lines. Above: Tulaipanji, progeny F5 awnless (Tulaipanji × IR64), F5 awn (Tulaipanji × IR64), F5 medium grain with awn (Tulaipanji × IR64), F5 black husk (Tulaipanji × IR64), and IR64, and below: PB-1460, progeny F7 Awn (Tulaipanji × Ranjit), F7 awnless (Tulaipanji × Ranjit) with red pericarp, and Ranjit

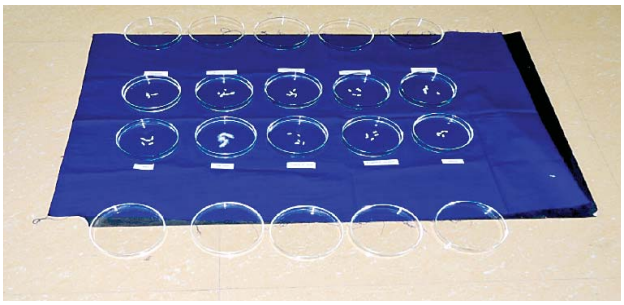

Fig. 7. Physicochemical properties of the progeny rice lines (Tulaipanji × IR64) at F5 stage was depicted for quality check. Showing the alkali spreading value, gelatinization temperature and sensory based aroma test

Rice grain consists primarily of starch (~90%) and is comprised of two components, amylose and amylopectin. It has many properties such as apparent amylose content (AAC), amylopectin structure, pasting viscosity, GT, gel consistency and texture. All the starch-related properties have various degrees of effect on the ECQ of cooked rice (Bao, 2012). A low or intermediate GT is desired for a high-quality rice variety (Juliano and Villareal, 1993). Some of the progeny lines had recorded low GT scores. The F5 awnless progeny line showed GT value 3 with ASV 4, and high aroma index 3. Progeny F2 lines (IRT64F4 × PB1460) had high grain quality with GT value 3, ASV 4 and aroma index 2.

Cross combining ability of Tulaipanji was maximum when crossed with IR64. Grain yield was doubled to 3.6 t/h in the progeny compared to the parental line Tulaipanji (1.8 t/h). In the same way other traits such as grain length, grain/panicle, short and stout stem related genes/QTLs introgressed appropriately into the genome of Tulaipanji and expressed the traits with maximum penetrance and expressivity. Thus yield increase doubled in the F5 progeny lines (Tulaipanji × IR64).

Recombination breeding was targeted to widen the gene pool of Tulaipanji rice by crossing ITR64F4 line with 3rd parental line PB-1460, to improve its grain quality. Progeny F2 lines showed grain length similar

to PB-1460 (Table 1, Figures 3, 4 and 5). F3 generation showed morphologically similar phenotype to parental line IR64. Grain quality was soft with aroma indicator value 3 (Figure 7). Plant height reduced to 110 cm, compared to F2 progeny lines, which was 125.4 cm (Table 1).

Conclusion

Intraspecific crosses were made for the improvement of local rice germplasm Tulaipanji. The RIL line (F7) of the cross between (Tulaipanji × Ranjit) has been registered under Breeders' Right act of Govt. of India and deposited to the NBPGR Gene Bank with IC no 626287 (Breeding line/NBUTRNF7-18). This F7 RIL line has unique trait of red pericarp development at the stage F4 and is stably inherited. Two promising breeding lines were identified from the cross, Tulaipanji × IR64. Line-1 is with awn and aroma but other line-2 is without awn but with aroma. Grain shape, size and weight were enhanced and showed transgressive segregation. The present study clearly showed better performing F5 recombinants (Tulaipanji × IR64) at the field level maintaining high grain quality and fragrance with improved yield (3.6 t/h), just double that of the Tulaipanji parental line (1.8 t/h). The F2 progeny line (IRT64F4 × PB1460) showed enhance grain quality based on physicochemical parameters. From this cross (IRT64F4 × PB-1460), two lines were selected. Both the lines performed better with respect to morphological and grain quality at F3 generation. Present investigation suggests that traditional rice varieties remain as a large reservoir of genetic diversity with potential to accelerate rice improvement if used properly in breeding program. Recombination breeding of local rice landraces, if improve their yield can retain them in farmers' fields in the long run.

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