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# Assessment of Genetic Diversity in F2 Rice Seed Population of a Cross between Tulaipanji and Ranjit Using Morphological, Physicochemical and SSR Markers

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**Abstract**

Tulaipanji rice (*Oryza sativa* L.) variety is a region specific traditional cultivar of North Dinajpur district (West Bengal). It is low yielding (1.8 t/h) cultivar due to their poor harvest index and other genetic factors such as tendency to lodging and susceptibility to foliar diseases such as blast and bacterial blight, tungro virus etc. Tulaipanji is non-Basmati aromatic rice and people prefer it due to its soft fluffy grain quality with mild fragrance and easy to digest. Hence it has high demand in the market and costs Rs. 80/kg. Demand gap can be fulfilled by developing a new improved variety of Tulaipanji combining its grain quality attributes with high yield potential genes/QTLs and resistance to diseases. Hybridization was made (during kharif season 2011) between Tulaipanji and Ranjit (HYV) for the introgression of short stature, high yielding responsible genes/QTLs from Ranjit to Tulaipanji for its improvement. $F_2$ seeds population were collected and analysed based on morphological and physicochemical parameters for their genetic variability and screening. Genetic diversity among the F1 plants and parental lines were analysed using microsatellite based SSR primer in PCR amplification. SSR markers in relation to disease resistance gene/QTL such as Xa21, Pita440 and Pib sub 3-5 were used to screen the breeding lines. Xa21specific amplified band was observed in cultivar Ranjit (HYV) which was located on chromosome 6 (resistance gene/QTL for bacterial blight) and accordingly introgressed into one of the line of F1 plants (Tulaipanji x Ranjit). There was no gene/QTL specific band was amplified for SSR marker of Pi440 and Pib sub 3-5 in the parental or F1 lines for blast resistance. Grain quality QTL specific markers such as Waxy, Sbe 2 and RM225 were employed to screen the F1 and parental lines for apparent amylose content (ACC). Apparent amylose content (ACC) was 22% in Tulaipanji and 25% in Ranjit and F2 seed showed in between 22 and 25% amylose. Breeding lines showing desired traits in relation to disease resistance and quality traits can be used for the development of improved Tulaipanji rice of North Dinajpur through marker assisted selection (MAS) using SSR markers.

**Keywords:** Tulaipanji rice, F2 seeds, SSR markers, Xa21, Waxy gene, Amylose content.

## Introduction:

Cultivated rice belongs to two species- *Oryza sativa* (L.) and *Oryza glaberrima* (Steud.). *Oryza glaberrima,* the African cultivated rice, is grown on a limited scale in West Africa. *Oryza sativa* has two subspecies- *O. sativa* sp indica and *O. sativa* sp japonica. The subspecies japonica is mainly cultivated in Japan, Korea, Taiwan and Italy, and their genetic diversity is narrow. On the other hand, the subspecies indica is mainly cultivated in China, India, Thailand, Indonesia, and their genetic diversity is wide. About 90% of the cultivated rice belongs to the indica type and not japonica.

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Cultivated rice *Oryza sativa* (L.) subspecies indica is grown worldwide especially in Asian subcontinent. Rice is grown in a wide range of agro-ecological conditions in India. Based on the harvested area of 45 million ha (Mha), about 55% of the crop is planted in Eastern India, 21% in North, 18% in the South and about 6% in West. Rice is central to the food security of over half the world's population – it is vital for the nutrition of much of the population in Asia, as well as in Latin America, the Caribbean and in Africa, totalling over 3.5 billion people worldwide that eat it every day, and depend on it for more than 30-40% of their daily calories. Asia can be considered as *'Rice Basket'* of the world because where 90 per cent of world's rice is

grown. Maximum rice (90%) are grown in Asia as well as consumed with 60% of population and where about two-thirds of world's poor live (Khush and Virk, 2000). Hence, *'Rice is life'* in Asian subcontinent. Only 4-5 per cent of world rice production enters the Global market. That means, any shortfall in rice production in the major rice growing countries could be disaster for food security. Rice occupies pivotal role in Indian agriculture. It is the staple food for more than 70% Indians and source of livelihood for 120-150 million rural households. It contributes 43% to the total food grain and 53 per cent to the cereal production and thus holds the key to sustain food sufficiency in the country (Siddiq *et al.*, 2004). To feed 5 billion rice consumers in 2025, we have to develop rice varieties with high yield potential and greater yield stability (1.1% yield increase every year) with quality grains for food sufficiency. To keep rice prices stable and affordable, IRRI estimates that an additional 8-10 million tons of rice needs to be produced every year. The population of rice consumers is increasing at the rate of 1.8% annually. The present annual rice production of 700 million tons must be increased to 850 million tons by 2025. There are no additional lands available for rice cultivation. In fact, the area planted to rice is going down in several countries due to pressures of urbanization. Thus, we need the rice varieties with higher yield potential and yield stability for meeting the challenges of increased rice production. During this intensive breeding effort, varieties have been developed which have genes from various ecotypes of rice. Even the genes from wild species have been introduced into modern varieties. Thus, ecotypic differentiation present in the landraces of rice no longer exists in the improved varieties. Environmentalists have expressed concern about the reduction in biodiversity due to replacement of numerous old varieties by a selected number of improved varieties. However, genes of numerous landraces have been incorporated into the new varieties. For example, IR64 has 20 landraces in its ancestry. Moreover, most of the traditional varieties grown before the green revolution have been preserved in the gene banks and are available for future rice improvement (Khush, 1997).

A rapid rise in world population and decreasing agricultural land exert pressure on the production and productivity of rice cultivars (Khush, 2005). Currently India produces 104 million tonnes of rice equal to 43% of the total grains from 45 million hectares of land out of 297 million hectares total agriculture land. We need approximately 150 million tonnes of rice by 2030 to feed 1.378 billion Indian people (Goyal and Singh 2002). Increasing world population, shrinking cultivable rice land area, water scarcity and excess, evolution of new biotypes of pests and diseases, deteriorating soil health and climate change pose major cause of concern to rice breeders to increase production and productivity with multiple resistances to biotic and abiotic stresses which causes low yield and quality (average yield 2.1 t/ha in compare to world yield 4.1 t/ha). Thus there is a plateau in yield levels of existing rice varieties. Different breeding strategies such as introduction, selection, recombination breeding, heterosis breeding etc were practiced during and after green revolution to increase the yield levels of rice. Although, yield was improved using these breeding strategies, the yield levels have stagnated subsequently.

Not only the biotic/abiotic stress problems but also narrow genetic diversity in modern cultivars poses major constraint to further increases in productivity. Molecular markers provide opportunities to map resistance genes and accelerate the application of marker assisted backcross (MAB) breeding through the precise transfer of target genomic regions into the recurrent parent (Jena and Mackill, 2008; Lewis and Kernodle, 2009). Backcross breeding is often used in a conventional breeding program to transfer specific genes into elite cultivars since long back (Allard, 1961). The basis of MAB breeding is to transfer a specific gene/allele of the donor parent into the recurrent parent genome while selecting against donor introgressions across the rest of the genome. The effectiveness of MAB breeding depends on the availability of closely linked DNA markers for the target locus, the size of the population, the number of backcrosses and the position and number of markers for background selection (Frisch and Melchinger, 2005). In addition, molecular markers precisely estimate the

introgression of chromosome segments from donor parents and can speed up the recipient genome recovery via background selection (Suh *et al.*, 2009). Recent advances in rice genomics research (IRGSP, 2005) has made it possible to identify and map precisely a number of genes through linkage to DNA markers. Some important markers are tightly linked to tolerance/resistance to blast, bacterial blight, virus diseases, brown plant hopper, drought, submergence, salinity, low temperature and improved agronomic and grain quality traits (http://www.gramene.org). Historically, many breeding programs took yield potential as the primary target. As a result many popular high-yielding cultivars and hybrids have relatively poor quality. But there is also a tremendous need for improvement of rice grain quality because it has market demand due to increased living standard. So, the improvement in cooking, eating, and appearance quality of the rice grain has become a priority area in rice breeding programme (Zhang, 2007). So, rice varieties with increased yield potential in adverse conditions as well as in normal conditions have to be developed in order to sustain the rice production across wide range of environments and over the years. The challenge is to produce these additional quantities with less land, less water, and less labor, in more efficient, environmentally-friendly production systems that are more resilient to climate change. Earlier, wild and wild relatives were frequently used in resistance breeding programs to improve simple traits such as resistance to various pests and diseases. They were rarely used to improve complex traits such as yield, drought tolerance, salinity tolerance etc. Currently, advanced backcross Quantitative Trait Locus method followed by molecular mapping studies showed that phenotypically poor wild species can contribute genes for improving yield and such loci can be mapped and introgressed in to elite cultivars (Gur and Zamir, 2004). This enables the efficient use of wild and wild relatives to broaden the genetic base of the existing cultivars and also to improve complex traits by marker aided introgression of superior wild alleles (Laxuman *et al.*, 2011).

Tulaipanji is a non-Basmati aromatic scented rice of West Bengal and specifically cultivated in the district of North Dinajpur, West Bengal and sold at the rate of Rs.70-80/kg and it has high demand in the rice market of West Bengal and other states.

Total cultivated land of North Dinajpur is 2,41,292 hectare, out of this kharif aman rice area is 1,82,975 hectare in 2012. District has two Subdivisions- Islampur and Raiganj. Islampur subdivision having 1,04,950 hectare agricultural land in 5 block and Raiganj Subdivision having 78025 hectare in 4 block which are under Aman rice cultivation. Raiganj Block has total cultivated land area 35,200 hectare, out of which 29,950 hectare is used for Aman rice in 2012 and out of Aman area 2120 hectare is used for Tulaipanji cultivation (which is 14.97% land of this block). Total area under Tulaipanji rice cultivation in North Dinajpur is: 4485 hectare in 2012 (2.45% in respect to other aman rice in the District) and yield is 1.8 t/h on an average. Main problem facing by the farmers: Main constrains of the cultivation of this high demand aromatic rice (Tulaipanji) is susceptible to various diseases and pest and moreover low yielder (1.8 t/h). In spite of low yield and disease susceptibility farmers are still cultivating this Tulaipanji for high price in the market and to conserve their inherited agricultural practices and cultural heritage.

Germplasm diversity is the mainstay for crop improvement and genetic dissection of complex traits. Due to aggressive introduction of modern high yielding varieties (HYV) in the region has resulted in the loss of a large number of landraces especially from irrigated lands, which leads to narrowing down the gene pool of the rice diversity. The present investigation looks at the genetic variability and specific morpho-quality traits to conserve rice germplasms on farm and to maximise its use in rice breeding. Wide genetic resources may be required to either increase the genepool for germplasm improvement or for the development of new cultivated varieties. Accurate assessment of the levels and patterns of genetic diversity can be invaluable in crop breeding for diverse applications including introgression desirable genes from diverse germplasm into the available genetic base. Conservation of the rice gene pool is necessary so that breeders can get access of these genetic resources for future use and for widening

the narrow genetic base of the developed varieties.

Yield attributing characters are complex and controlled by many physio-morphological traits which are independently governed by polygenes and are much influenced by environmental factors. Although conventional approach was used to address the genetic improvement of yield and yield attributing traits and its stability. But they show low heritability in the field and expensive in attaining progress (Nguyen *et al.*, 1997). Alternatively molecular breeding may achieve the target specificity at faster rate in compared to conventional approach. Use of marker assisted breeding (MAB) would offer opportunity in identifying specific genes through QTLs for complex traits combining yield and yield related traits in crop plants (O'Toole, 1989). Marker assisted selection (MAS) of genes for pyramiding bacterial blight resistance genes, Xa13 and Xa21 (Mahapatra *et al.*, 2006) and QTLs controlling root traits (Steele *et al.* 2006) in elite backgrounds are example for success stories of molecular breeding in rice improvement. The introgression of useful traits from diverse donors into elite background by back crossing to develop introgressed lines (BILs) is the best approach for QTL mapping as well as genetic improvement of elite cultivars (Li, 1999). It would allow the simultaneous identification of desirable alleles of many QTLs and generation of backcross inbred lines (BILs) with incorporated QTLs for evaluation in target environments.

After reviewing the literatures and considering the origin of the problem, the present investigation will be made first time to evaluate the pattern and extent of genetic diversity exists in Tulaipanji rice employing important morpho-quality, physico-chemical and agronomic traits and introgression of yield and disease resistance traits using Ranjit (HYV) as donor parent. In the present study F2 seeds population is used as mapping population for screening the breeding lines with desired traits and targeting to broaden the gene pool for genetic improvement of Tulaipanji rice variety using marker assisted selection (MAS) in molecular breeding.

## Materials and Methods

### *Experimental site*

The present investigation was carried out during kharif 2011 and 2012 at Experimental Rice Field, Department of Botany, University of North Bengal, WB, India, representing the low land with sandy-loam soil, acidic pH which is located at latitude of 26° 84A North and longitude of 88° 44A East.

### *Experimental material*

The experimental material for the present investigation comprised of Tulaipanji rice (*Oryza sativa* L.) variety of North Dinajpur district as recurrent parent and Ranjit as donor parent. Ranjit is a high yielding variety (HYV) has genealogy of Pankaj and Mashuri (including Taichung 65 and Tangkai rotan).

### *Experimental Layout*

Hybridization was performed according to IRRI protocol between Tulaipanji and Ranjit during kharif 2011 for introgression of Yield/quality/disease resistance gene/QTLs in recurrent parent through marker assisted selection. The introgressed population is genotyped and mapped with six well distributed SSR markers selected from the linkage map developed at Cornell University (Temnykh *et al.*, 2001) and http://www.gramene.org for the following trait specific gene/QTL markers- Waxy, Sbe2, RM225, Xa21, Pita 440 and Pib sub 3-5. The mapping population of F2 seeds are evaluated along with parental lines for morphological and physicochemical analyses. The mapping population is evaluated with two replications in randomized complete block design during kharif season (2012). Each line was planted in two rows of 2m length with row spacing of 20cm and 10cm between plants. The recommended package of practice is followed for raising a good and healthy crop.

### *Procedure of artificial cross-pollination*

The rice inflorescence is a panicle bearing singled-flowered spikelets. The flower is surrounded by a lemma and palea, structures that form the hull or husk that enclose the caryopsis. The outer glumes are sterile and usually obscure. The blooming of rice normally occurs between 10 am and 2 pm. Pollen is generally shed at the time the flower opens

with blooming of the spikelet, starting at the apex of the panicle and proceeding downward. Rice flowers are emasculated in preparation for crossing by cutting the tip of the floret and removing the anthers. (Fig. 7) About one-third to one-half of each

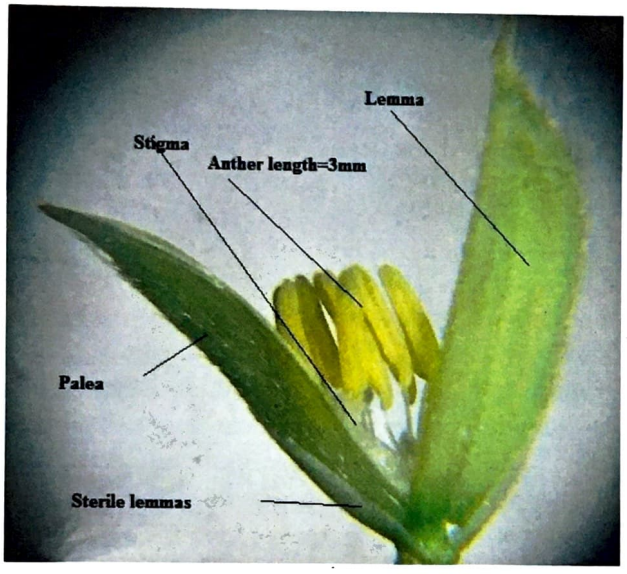


Figure 7: Rice flower showing anther and stigma and other parts of the spikelet of cultivar Tulaipanji.

floret is cut off at a slant to expose the anthers. Anthers are removed with forceps. Emasculations performed in the morning may risk anthers opening during their removal, and so it is safer to remove them in the afternoon (when anthesis has ceased for the day at 3 pm). After emasculation, flowers are covered with paper bag to protect them from natural cross-pollination until they open and are ready for pollination. The rice panicles are pollinated in the morning following emasculation. The male panicles are cut and flag leaf removed. These panicles are watched closely for anther extrusion, and used thereafter for pollination. The paper bag is taken out from the female and the pollen shaken over the emasculated panicle. After pollination, the panicles are covered by the same paper bag for protection from stray pollen and tagged properly. Pollen of rice is short-lived, only remaining viable for about 5 minutes. After 25 days of pollination F1 seeds are collected from the parent plant (October 2011) and labelled accordingly (cross I: Tulaipanji x Ranjit and cross II: Ranjit x Tulaipanji). F1 seeds along with lines were germinated on earthen pot with soil mixture in next year kharif season (2012) for further analysis. Seedlings were transplanted after 21 days of germination and maintained with proper agronomical practices for good crop. F2 seeds were analysed for grain morphology, quality and physicochemical analysis. F3 seed population will be collected from this kharif crop during October, 2013.

*Recording of observation*

The data on morphological, physicochemical and other traits are recorded from three randomly selected representative plants in all the genotypes in each replication. The standard method (DUS-Distinctness over Uniformity and Stability, Govt. of India) is used for recording observation for each of the character which includes the following- plant height, stem thickness, penultimate leaf length, penultimate leaf breadth, flag leaf length, flag leaf breadth, panicle length, panicle branching, seed/ panicle, grain weight.

*Phenotyping for grain quality traits*

The grain quality was measured according to IRRI protocol (Juliano and Villareal 1993)- grain length (GL), grain breadth (GB), awn length, kernel length (KL), kernel breadth (KB), ratio of GL/GB, ratio of KL/KB, cooked kernel length (CKL), cooked kernel breadth (CKB), ratio of CKL/CKB, linear elongation ratio, cooking time etc. In this present investigation other quality parameters such as aroma, gelatinous temperature (GT) and alkali spreading value (ASV) were also considered. These quality traits was represented through distinct number only (0, 1, 2, 3 etc) to denote their magnitude. The alkali spreading value (ASV) was determined by the method of Little *et al.* (1958) with minor modifications. A set of five polished rice grains from each line was immersed in a freshly prepared 1.7% KOH solution and incubated at 30 f C for 23 h and spreading of the rice grains was recorded by visual observation (Fig. 4) in seven categories from 1(unaffected) to 7 (completely dissolved). The aroma of polished rice grains was determined by a sensory evaluation protocol according to the method of Sood and Siddiq (1978) with minor modification. Ten milled rice grains were placed in a 50 mm Petri plate containing 10 ml of 1.7% KOH and incubated at room temperature for 10 min with

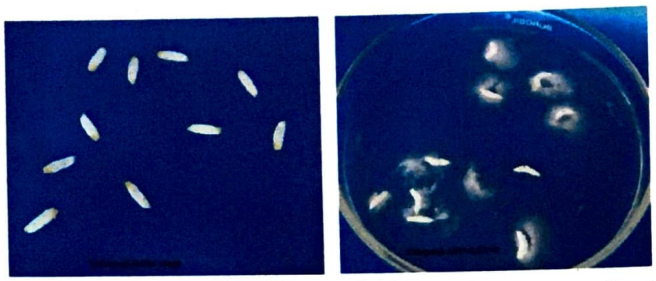

Figure 4. Alkali spreading value (ASV) was determined in Tulaipanji for quality assessment.

lids on. The lids were then opened one by one and samples were smelled and rated for aroma by sensory evaluation in a scale of 0-3, where 0 was non-aromatic and 3 was highly aromatic. Two blind checks, Pusa Basmati 1(moderately aromatic) and Ranjit (non aromatic) were included in this observation.

*Amylose measurements by Spectrophotometer*

Starch is a major component (90-93%) of rice seeds. Starch is composed of a mixture of two forms of glucose polymer, amylose and amylopectin. Amylose is principally a linear polymer of α (1-4) linked glucose with small amounts of α (1-6) branch linkages. Amylopectin is a more complex mixture due to the extensive branching introduced by many more (5%) α (1-6) linkages of the α (1-4) linked chains of glucose. Starch is synthesised by the activity of several enzymes, each of which occur as a number of different isoforms that display tissue specific expression (Fitzgerald, 2004). Amylose content was measured on the F2 rice flour by iodine affinity based colorimetric standard method (Juliano 1971). One hundred (100) mg flour was transferred to volumetric flask and homogenized with 1ml of 95% ethanol and 9 ml of 1N NaOH. The samples were heated for 10 min in the water bath to gelatinize the starch. After cooling, it was made up with 100 ml water. Half ml aliquots of each test solution were separately placed in two test tubes. Five (5.00) ml of water, 0.10 ml of acetic acid and 0.20 ml of iodine solution were added. An additional 4.20 ml of water was added into each tube to make the total volume of reaction mixture to 10 ml. Absorbance was measured using a spectrophotometer (Systronic, India) at a wavelength of 620 nm. The AC was determined using a standard curve developed from known quantities of purified potato amylose from Sigma, USA.

***Genotyping of F1lines and F2 seed populatio***

*DNA isolation of parents and mappin populations*

DNA isolation was carried out using standard protocol (Murray and Thomson, 1980). Fresh leaf tissue (21 days old plant) of 1g was taken from FI and parental lines (Tulaipanji and Ranjit) and made pulverised powder with liquid nitrogen in mortar and pestle. The pulverized powdery material was transferred to 15 ml polypropylene tube containing 4 ml of preheated CTAB extraction buffer (2% Cetyltrimethylammonium bromide, 1.5% polyvinyl-pyrrolidone PVP K-30, 1.4M NaCl, 20mM EDTA, 1.7% SDS, 100mM Tris-HCl (pH 8.0), 0.1% β-mercaptoethanol, add before use) with 50µg/ml Proteinase K. Incubated at 65°C in a water bath for 30 minutes with occasional gentle shaking. After cooling at room temperature equal volume of phenol: chloroform: isoamylalcohol (25:24:1) mixture was added, and mixed well. Centrifuged at 10,000 rpm for 10 minutes and upper aqueous supernatant was taken out with wide bore Pasture pipette to a new centrifuge tube. DNA was precipitated by adding double volume of chilled (-20ÚC) absolute ethanol followed by addition of 1/ 10th volume of 3M Sodium acetate (pH 5.5) and kept at -20ÚC for overnight for total DNA precipitation.

The precipitated DNA was spooled out and washed 2-3 times in 70% ethanol and dissolved in 500µl RNase A treatment buffer containing 20µg/ml RNase A solution for purification in a 1.5 ml eppendorf tube. It was incubated at 37°C for 1h to remove the RNA contamination. Then extracted with equal volume of chloroform: isoamylalcohol (24:1) and centrifuged at 10,000 rpm for 8 minutes and the supernatant was transferred to a new tube. DNA was precipitated by adding double volume of chilled (-20ÚC) absolute ethanol followed by addition of 1/10th volume of 3M Sodium acetate (pH 5.5) and kept at -20ÚC for overnight for total DNA precipitation. The purified DNA pellet was collected by centrifuging at 5000 rpm for 5 minutes in a microfuge. The excess ethanol was air dried and purified DNA was finally dissolved in 250µl TE buffer (pH 8.0) for further use in PCR amplification. Concentration of the purified genomic

DNA in each case was adjusted to10ng/µl in a different aliquots using UV-*vis* Spectrophotometer (Shimadzu-160) and stored at -20°C for further use.

*Protocol for PCR amplification*

QTL/gene linked six SSR markers was constructed from Operon technology, USA (given below) for genetic diversity analysis of the breeding lines according to McCouch *et al* (2002) and www.gramene.org. PCR amplification was carried out for genotyping the parental/F1 lines and F2 seed population for specific gene(s) or QTLs according to manufacturer protocol (Promega, USA). The 25µl PCR contained 50 mM KCl, 10 mM Tris-HCl (pH 8.8), 1.5 mM $MgCl_2$, 200µM dNTPs, 0.2µM primer, GoTaq 1µl (1.25U) and 10µg of genomic DNA as template. PCR amplification was performed in thermal cycler BioRad MJ Gradient (USA) for 35 cycles in the following temperature. 95 f C for 2 min for denaturation and then 35 cycles as follows- 95 f C for 30s, 55 f C for 30s, 72 f C for 30s and one cycle final extension at 72 f C for 7 min and kept at 4 f C forever. The SSR products were resolved in 1.0% agarose gel by electrophoresis at 100 volt for 1h in 0.5X TBE buffer. The gel was stained with ethidium bromide and photographed under UV- transilluminator.

Six primer pairs of SSR markers

| **Gene and linked SSR marker** | **Forward Primers 5′ - 3′** | **Reverse primer 5′ – 3′** |
|---|---|---|
| RM 225(AC) | TGCCCATATGGTCTGGATG | GAAAGTGGATCAGGAAGGC |
| Waxy(AC) | CTCTCTCACCATTCCTTCAG | CACAAGCAGAGAAGTGAAGC |
| sbe2(sbe2) | CCGAGGGAATGCCAGGAGTACCAG | GAACCACAACCAAGTCCAAGGCAA |
| PTA248 (Xa21) | AGACGCGGAAGGGTGGTTCCCGGA | AGACGCGGTAATCGAAAGGATGAAA |
| Pita440 | CAACAATTTAATCATACACG | ATGACACCCTGCGATGCAA |
| Pib sub3-5 | AGGGAAAAATGCAATGTGC | AGTAACCTTCTGCTGCCCAA |

## Results and Discussion

F1 seeds were collected from the parent plants after 25 days of pollination and preserved in paper container in a dry place in room temperature (October, 2011). F1 seeds were germinated on pot conMorphological data recording and analysis was carried out according to DUS protocol (Table 1, Fig.1). Plant height during harvesting time was 92 cm on an average of cultivar Tulaipanji, (plant height ranges from 81-95 cm) and mean height was 61.6 cm in case of Ranjit. F1 plant (cross between Tulaipanji and Ranjit) showed 91.2 cm height but 82.8 cm height was measured in case of F1 plant where cross was made between Ranjit and

Table 1. Morphological traits of F1 plants and parental lines

| Morphological traits | Tulaipanji | Tulai x Ranjit | Ranjit x Tulai | Ranjit |
|---|---|---|---|---|
| Plant height (cm) | 92 ± 2.24 | 91.2 ± 1.94 | 82.8 ± 2.48 | 61.6 ± 2.94 |
| Stem thickness (cm) | 1.38 ± 0.232 | 1.72 ±0.172 | 1.66 ±0.307 | 1.88 ± 0.279 |
| Penaltimate leaf length (cm) | 43.34 ± 4.87 | 43.24 ± 3.39 | 33.08 ± 1.62 | 29.46 ± 4.72 |
| Penaltimate leaf breadth | 0.86 ± 0.08 | 0.56 ± 0.08 | 0.90 ± 0.06 | 1.02 ± 0.01 |
| Flag leaf length (cm) | 21.6 ± 2.31 | 29.34 ± 3.52 | 23.34 ± 3.46 | 21.06 ± 2.49 |
| Flag leaf breadth | 0.82 ± 0.04 | 0.76 ± 0.05 | 1.04 ± 0.05 | 1.46 ± 0.10 |
| Panicle length (cm) | 24 ± 2.12 | 18 ± 2.45 | 18 ± 1.97 | 21 ± 0.98 |
| Branching | 8 ± 1.98 | 7 ± 3.08 | 8 ± 2.98 | 9.5 ± 1.23 |
| Seed/panicle | 105± 1.26 | 80 ± 2.04 | 96 ± 1.45 | 123 ± 2.56 |

Mean of ten reading ± Sd

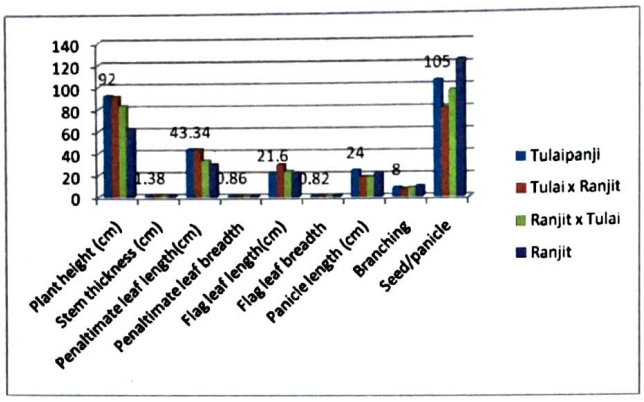


Figure 1. Graphical representation of morphological traits of F1 plants and parental lines.

Tulaipanji. Reciprocal cross showed different results which is not usual. Ranjit can be considered as semi-dwarf plant and Tulaipanji as tall plant as per DUS test. Maturity time is 140-150 days for Tulaipanji and 130-135 days for Ranjit.

F2 grains (seed population) were measured in respect to study their morpho-quality and physicochemical parameters (Table 2, 3 & Fig. 2, 3) - grain length is on an average 7.7 mm with awn (13.5 mm), width 2.1 mm, grain weight 15g/1000 seeds. Kernel length (KL) is measured as 6 mm, considered as medium slender grain according to Govt. of India notification (No. 67, 23 Jan, 2003, Ministry of Commerce), the minimum grain length for A grade basmati rice is 7.0 mm, while its minimum LBR is 3.5, kernel breadth (KB) 1.95 mm and KL/KB ratio is 3.07. Other quality parameters according to DUS protocol are: Aroma-3, ASV- 5, GT-3. Cooked kernel length (CKL) is 8.5 mm, cooked kernel to breadth ratio (CKB) is 2.3 mm, and CKL/CKB ratio is 3.69. The cooked kernel elongation ratio (ELR) is 2.06 in Basmati type grain that elongates length wise with minimal breadth wise swelling on cooking. In contrast, Tulaipanji showed a significantly lower ELR of 1.37 and Ranjit 1.57. Some F2 seed population showed transgressive segregation in respect to GL, GL/GB,

Table 2. Grain quality of F2 seed population based on physicochemical parameters.

| Name of the Cultivars | Weight of per seed (mg) | Grain length (mm) | Grain breadth | Ratio of GL/ GB | Awn (mm) | Aroma sensory score | GT | ASV |
|---|---|---|---|---|---|---|---|---|
| Tulaipanji | 15 | 7.7 ± 0.02 | 2.1 ± 0.60 | 3.6 | 13.5 ± 0.23 | 3 | 3 | 5 |
| Tulai x Ranjit | 17 | 8.63 ± 0.17 | 2.5 ± 0.12 | 3.8 | 17 ± 0.31 | 1 | 3 | 5 |
| Ranjit x Tulai | 17 | 7.64 ± 0.12 | 2.5 ± 0.25 | 3.2 | 11 ± 0.23 | 0 | 1 | 6 |
| Ranjit | 16.29 | 7.8 ± 0.21 | 2.5 ± 0.11 | 3.12 | Without awn | 0 | 7 | 2 |

Mean of ten reading ± Sd

Table 3. Assessment of genetic variation of F2 seed population based on quality parameters

| Name of the Cultivars | Kernel length | Kernel breadth | Ratio of KL/KB | Cooked kernel length | Cooked kernel breadth | Ratio of CKL/ CKB | Linear elongation ratio | Breadth elongation ratio | Cooking time |
|---|---|---|---|---|---|---|---|---|---|
| Tulaipanji | 6 | 1.95 | 3.07 | 8.5 | 2.3 | 3.69 | 1.37 | 1.17 | 5 min |
| Tulai x Ranjit | 5.8 | 2.1 | 2.76 | 8.0 | 2.95 | 2.73 | 1.36 | 1.50 | 5 min |
| Ranjit x Tulai | 5.8 | 2.1 | 2.76 | 8.2 | 2.96 | 2.83 | 1.41 | 1.50 | 5 min |
| Ranjit | 5.45 | 2.15 | 2.53 | 7.8 | 2.3 | 3.78 | 1.59 | 1.06 | 6 min |

Mean of ten reading

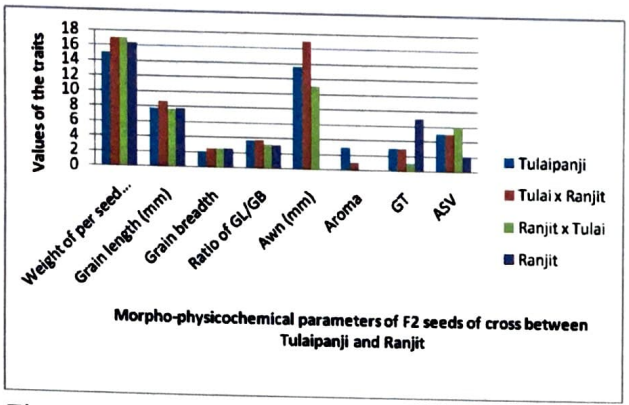


Figure 2. Morpho-physicochemical parameters of F2 seed population.

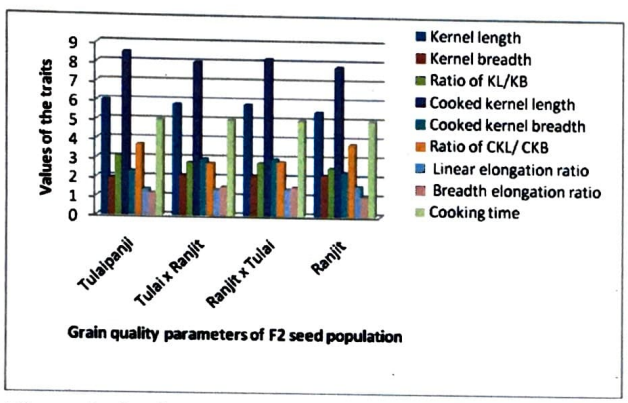


Figure 3. Grain quality analysis of F2 seed population.

awn length because they showed better result in compared to parental lines. Highest aroma was recorded in Tulaipanji at value 3 and F2 grain of cross Tulai x Ranjit showed value at 1. Ranjit as a non scented rice showed aroma value 0 (zero). Ranjit showed highest GT value at 7 but Tulaipanji has low GT value which is 3. Crosses showed GT value ranges from 1-3. Tulaipanji has ASV 5 in compared to 2 in Ranjit. Some F2 grains showed ASV 6. Both the traits are known to be governed by the enzymes of starch biosynthesis pathway, including granule bound starch synthase I (GBSSI- a 60 kDa molecular weight enzyme encoded by the *Waxy* gene locus), soluble starch synthase, and starch branching and de-branching enzymes (Umemoto *et al.*, 2002 & 2004). These enzymes are responsible for the synthesis of amylose during the development of starch granules in cereal endosperm and were not observed in waxy starch. GBSS, which is localized within the starch granule, is responsible for amylose biosynthesis and also has a role in the elongation of long chains in amylopectin. Mutations in the *Waxy* locus leading to loss of GBSSI activity result in amylose free (waxy) starch. While AC is almost entirely attributed to the GBSS1 gene located on the short arm of chromosome 6. The ASV depends on the nature of the amylopectin molecules and is reported to be dependent on soluble starch synthase gene on the same chromosome arm. It could be modulated by the other poorly characterized genes of the pathway. Amylose content of the rice grain determines whether it will be firm and fluffy on cooking, or it will turn sticky and glutinous. The japonica rice varieties have very low AC and hence turn sticky upon cooking, which the consumer prefers in China and Japan for eating with chopstick. Apparent amylose content (AC) is measured 22% in Tulaipanji and 25% in Ranjit. In contrast, basmati type varieties have intermediate AC of 20-25% and their grains remain firm and separated after cooking, at the same time they give a soft mouth feel while eating. Tulaipanji grain showed the same amylase content (22%) and giving the same mouth feel as basmati.

Apparent amylose content (ACC) falls into the following categories: glutinous = 0 to 5%, Low = 5 to 19%, Intermediate = 19 to 23%, and High > 23%. Tulaipanji is intermediate type in respect to amylose contend (22%) and Ranjit is under high category with 25% amylose. Mapping studies to identify quantitative trait loci (QTL) of cooking quality traits showed that amylose, gel consistency and some other viscosity parameters are mainly affected by the *Waxy* gene on chromosome 6 that encodes Granule Bound Starch Synthase I (Tian *et al.*, 2005). None of the studies developed markers for gel consistency, nor determined how the *Waxy* gene actually determines gel consistency. Waxy gene specific QTL was detected in both the parental lines Tulaipanji and Ranjit and also in the F1 breeding lines. Two other SSR markers RM225 and Sbe-2 have also been detected in all the breeding lines and associated with amylose biosynthesis QTLs (Fig. 5). Most of these grain quality attributes are controlled by quantitative trait loci (QTLs) as inferred from continuous phenotypic variation in the segregating progeny of intervarietal crosses. Genetics of rice quality has also been studied in various genetic backgrounds using molecular


Figure 5. PCR amplified SSR marker (Waxy and Sbe2 marker) specific bands fractionated on 1% agarose gel. Lane 1-for Waxy gene and lane 2-4 for Sbe2 gene in rice cultivar Tulaipanji.

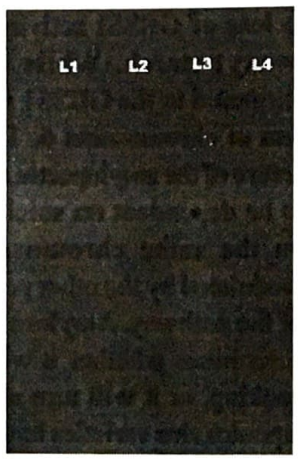


Figure 6. SSR marker (for Xa21 and Pita440) specific PCR amplified products were separated on 1% agarose gel. Marker Xa21 in rice cultivar, lane 1- Tulaipanji, no amplification, lane 2- F1 plant Tulai x Ranjit, showing band, lane 3-4, no marker specific band for Pita440 in Ranjit and Tulaipanji.

markers (Wang *et al.*, 1995). The F2 seed showed transgressive segregation for the traits GL, GL/GB ratio, and awn length (Table 2).

*QTL for amylose content*

QTL for amylase content was detected on short arm of chromosome 6 linked with SSR marker such as Waxy, Sbe-2 and RM225. The Waxy QTL was located in the waxy gene (GBSS1) region of chromosome 6.

*QTLs for leaf blight and blast*

Amplified PCR band was observed while Xa21 specific primer was mixed in the reaction of parental and breeding lines. Xa21 specific band was present in parental plant Ranjit (Fig. 6) and in cross between Tulaipanji and Ranjit but not in Ranjit x Tulai. Ranjit can be used as donor plant for bacterial blight resistance gene Xa21. Disease resistance QTL/ genes for bacterial blight has been identified (Xa genes) and introgressed into HYV (Xa21, Song *et al.*, 1995) from wild rice (*Oryza longistaminata*) and introgressed successfully into the cultivated rice to protect against the biotic stress and located on chromosome 11. Bacterial leaf blight (BLB) is caused by *Xanthomonas oryzae* pv. *oryzae* (that is why marker name is Xa). Blast resistance gene (RM529, Pib-sub 3-5, and Pita440) specific band have not been amplified in any one of the parental or breeding lines, while gene specific primer was used (Fig.6). Which suggested that blast resistance gene (QTLs) was not exist in any one of the parental lines. Blast disease of rice caused by the filamentous ascomycete *Magnaporthe oryzae* (*Magnaporthe grisea*) or *Pyricularia oryzae* (teleomorph, *Magnaporthe oryzae*) is a continuous thread to rice production and global food supply. Blast is recorded one of the main diseases of rice because of its worldwide distribution and destructiveness under favourable conditions. Rice blast has been reported in nearly every rice production region in the world and great concern in rice yield (Khush and Jena, 2009). R-gene Pita, a single copy gene, is located at 10.6 Mb near the centromere of chromosome 12, a region that often associates with recombination suppression (Chen *et al.* 2002). Resistance to *M. oryzae* in rice follows a gene-for-gene specificity where major resistance R genes (***Pi*** for ***Pyricularia***) are effective in controlling infection by races of *M. oryzae* possessing corresponding avirulance (AVR) genes (Flor 1971; Jia *et al.* 2000). Until now, at least 40 major ***Pi*** genes have been cloned (McCouch *et al* 1998, Chen *et al.* 2006; Liu *et al.* 2007). Out of these only Pita440 and Pib-sub 3-5 were used in the PCR reaction to study their existence in the parental lines. But no parental lines showed its presence in the PCR amplified band.

Grain and cooking quality traits are economically important for the traders and consumers of Tulaipanji rice which is comparable to Basmati rice to some extent. Therefore, new high-yielding disease tolerant variety of Tulaipanji rice need to be developed to cater for the growing domestic demand for this premium grade non-basmati aromatic rice of North Dinajpur district. Molecular markers tightly linked to the major QTLs governed the quality traits can be used in marker assisted breeding to develop new Tulaipanji variety to maintain its unique quality attributes while

introgressing the yield potential QTLs and resistance QTLs associated to various biotic (Xa21, Pita440, Pib-sub 3-5) and abiotic stresses.

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