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Morpho-genetic Characterization of Traditional Aromatic Tulaipanji Rice of North Bengal, India

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Abstract

Tulaipanji is a non-Basmati type aromatic rice landrace of old alluvial region of North Bengal in eastern part of.India, which is presently cultivated in about 7,000 ha. land with production of 14,000–15,000 tonnes paddy every year. With a view to develop the phenotypic and genetic database of the unique variety, its agro-morphological characterization was done at 'C' Block Farm, B.C.K.V., Kalyani, West Bengal during *kharif* (wet) season of 2011, 2012 and 2013 following the DUS test guidelines of Protection of Plant Varieties and Farmers' Rights Authority (PPV&FRA). The variety had late heading (110-115 days) and late maturity (140–150 days), which produced long statured plants (125-135 cm height) with no anthocyanin colouration on leaf blade, sheath, nodes and internodes. The flower was bi-sexual including six yellow coloured anthers, and an ovary with white or yellowish-white feathery stigma. The lemma and palea were straw in colour with long awns (mean length 17.3 mm), and the grains were short in length (7.5 mm) with low test weight (15.6 g). The kernels were medium-slender (length 5.27 mm and width 1.91 mm) in shape and white in colour, which had low amylose content (17.2%), medium gelatinization temperature (alkali value 4.3) and medium aroma. 23 simple sequence repeat (SSR) markers were used for DNA amplification profile to develop molecular base-pair length database of Tulaipanji rice against non-aromatic international check variety IR 36 in the study. Among them, one marker (RM 182) recorded similar molecular weights for both the varieties, while two markers (RM 207 and RM 339) made greater genetic distances between Tulaipanji and IR 36 (75.82 vs. 128.48 bp and 182.76 vs. 143.09 bp, respectively).

Keywords: Aromatic rice, grain quality, morpho-agronomic traits, SSR polymorphism

1. Introduction

The state of West Bengal is known as a land of thousands of folk varieties including about 40 non-Basmati type scented rice varieties for a long period. Among them, Tulaipanji rice has long been documented in the stories, doggerels, folk literatures, etc. due to its unique quality features and importance in agro-social system in old alluvial districts of North Bengal. The name of Tulaipanji was probably originated from the 'Tulai' river of the region or soft-textured, white-coloured rice with resemblance of white cotton (*tula* in Bengali)-like clouds (Ghosh, 2010). The earliest record of Tulaipanji rice cultivation was found in two district gazetteers (Hunter, 1876a and 1876b) of North Bengal. At present, it is cultivated in about 7,000 ha. land comprising 6,000 ha. in Uttar Dinajpur and 1,000 ha. in Dakshin Dinajpur district, with a production of 14,000–15,000 tonnes paddy every year (Ghosh, 2014). Farmers in native areas cultivate Tulaipanji rice following traditional practices intermixed with a few modern technologies in recent times during *kharif* (wet) season. It is very popular in domestic market for preparation of scented table rice, *polao* and *biryani*, *payash* (desert), *pistak* or *pitha* (home-made cake), *chira* (flattened rice), etc. during social functions and religious festivals in the region for a long period. Based on a proposal submitted by the RKVY Project on 'Bengal Aromatic Rice' of Bidhan Chandra Krishi Viswavidyalaya, West Bengal; the Standing Committee on Commerce, Parliament of India recommended the export of Tulaipanji rice during 2011 (Rajya Sabha, 2011). The medium-grained variety having pleasant aroma is much potential for large-scale marketing and international trade especially in the countries like Nepal, Bangladesh, U.K., Brazil, etc.

Although the switch-over to high-yielding rice varieties with the spread of modern agriculture has posed a great threat to the security of the age-old practice of growing indigenous varieties during last four decades in the state, but Tulaipanji rice is still cultivated by the traditional farmers mainly due to its unique quality features and special uses in social life system. In the present-day agricultural system, it is necessary to register the landraces as farmers' varieties under Protection

of Plant Varieties and Farmers' Rights Act (PPV&FRA), 2001, which may also strengthen the right of the farming community to conserve and cultivate for production-based marketing as well as to protect the variety against counterfeit ones and/or aggressiveness of multi-national corporate seed sectors at national and global levels. Thus, agro-morphological, physico-chemical and molecular characterization of Tulaipanji rice needs to be done as legal evidence of DUS (distinctiveness, uniformity and stability), and molecular characterization to find out genetic distances and/or avoid duplication in rice germplasm conservation system.

2. Materials and Methods

2.1. DUS testing and determination of grain quality

The seeds of Tulaipanji rice was collected from Regional Research Station, Old Alluvial Zone, Uttar Banga Krishi Viswavidyalaya, Majhian, Dakshin Dinajpur, West Bengal, India (Plate 1). Twenty five days old seedlings of Tulaipanji

Plate 1: Seeds of Tulaipanji rice

rice @ single hill^{-1} were transplanted in an open puddled field with five replications at 'C' Block Farm (22°59´N, 88º27´E and 9.75 m above mean sea level) of Bidhan Chandra Krishi Viswavidyalaya, Kalyani, Nadia, West Bengal, India during *kharif* (wet) season of 2011, 2012 and 2013. Each experimental unit consisted of 6-metre row length comprising 30 rows including row to row distance of 30 cm and plant to plant distance of 20 cm. Standard agronomic practices were adopted in trial plots during the course of investigation. The DUS descriptors following 'DUS Test Guidelines for Rice' of PPV&FRA, Government of India (www.plantauthority.gov.in) were used to define the morphological and related characteristics of Tulaipanji rice. Grain quality parameters like size and shape of grain and kernel, amylose content (Juliano, 1971), gelatinization temperature (Little et al., 1958) and aroma (Nagraju et al., 1991) were determined at Aromatic Rice Laboratory, Department of Agronomy, Bidhan Chandra Krishi Viswavidyalaya Kalyani, Nadia, West Bengal, India.

2.2. Molecular characterization by SSR markers

The molecular characterization of Tulaipanji rice was done at the Division of Plant Biology, Bose Institute, Kolkata, West Bengal during 2006–2007. 3-day old rice seedlings of Tulaipanji rice along with international non-aromatic check (IR 36) were used for isolation of genomic DNA following the method of Walbot (1988). DNA amplification was carried out by standard PCR method with 23 pairs of simple sequence repeat (SSR) markers in a Peltier Thermal Cycler (MJ Research, USA). The PCR products were resolved by native polyacrylamide gel electrophoresis (PAGE) following the protocol given by Sambrook et al. (1989). The length of the amplified DNA bands (SSR alleles) from two rice genotypes was determined with the reference of 100 bp DNA ladders (SibEnzyme Ltd., Russia) by the Molecular Analyst software (BioRad, USA).

The different alleles amplified from the genomic DNA of Tulaipanji rice along with the check were identified on the basis of their length or base pairs (bp) for making genetic characterization of Tulaipanji rice in the study.

3. Results and Discussion

3.1. Agro-morphological characteristics and grain quality

Tulaipanji rice was usually adaptable to rainfed medium land in old gangetic alluvium region of North Bengal. The characteristics of Tulaipanji rice following 'DUS Test Guidelines for Rice' of PPV&FRA are described in Table 1.

Plant**:** Tulaipanji rice belonged to long-duration type with late heading (scale 7, 113 days) and late maturity (scale 7, 146 days) (Plate 2).

Stem**:** It had long statured plant with average stem length of 118.0 cm excluding panicle. The thickness of stem was medium (scale 5) with mean diameter of 0.45 cm. Anthocyanin colouration was absent on nodes and internodes. The attitude of the culm could be categorised as semi-erect (scale 3) at booting stage.

Leaf: The variety produced long, narrow and green leaves. The colour of basal leaf sheath was green (scale 1), while the intensity of green colour of the leaf was light (scale 3) without any anthocyanin colouration. The average length and width of leaf blade were noted as 40.8 mm and 7.0 mm, respectively. The split-type (scale 3) ligule and sickle-shaped auricle at leaf base were found in the plant. The attitude of the flag leaf was semi-erect (scale 3) at early observation and horizontal (scale 5) at late observation.

Inflorescence: The length of panicle of Tulaipanji rice was categorized as medium (scale 5, 27.2 cm) with the curvature of the main axis as deflexed (scale 5). The plant produced very few (scale 3, mean 8.3) well-exerted panicles in the field. The colour of the lemma and palea was green at anthesis, which turned to straw (scale 1) or golden-yellow at ripening stage.

Flower: The variety produced bi-sexual flowers including six

Table 1: Plant characteristics of Tulaipanji rice following DUS guidelines

Sl. No.	Characteristics	Scale	Remarks measured values etc.
1.	Coleoptile: colour	2	Green
2.	Basal leaf sheath colour	1	Green
3.	Leaf: Intensityof green colour	3	Light
4.	Leaf: anthocyanin colouration	1	Absent
5.	Leaf: distribution of anthocyanin colouration	—	—
6.	Leaf sheath: anthocyanin colouratin	1	Absent
7.	Leaf sheath: intensity of anthocyanin colouration	—	—
8.	Leaf: pubescence of blade surface	5	Medium
9.	Leaf: Auricles	9	Present
10.	Leaf: anthocynin colorations of auricles	1	Colourless
11.	Leaf: collar	9	Present
12.	Leaf: anthocyanin colouration of collar	1	Absent
13.	Leaf: ligule	9	Present
14.	Leaf: shape of ligules	3	Split
15.	Leaf: colour of ligule	1	Green
16.	Leaf : length of blade	7	Long (40.8 cm)
17.	Leaf : width of blade	3	Narrow (0.7 mm)
18.	Culm : attitude (for floating rice only)	—	—
19.	Culm : attitude	3	Semi-erect
20.	Time of heading (50% of plants with panicles)	7	Late (114 days)
21.	Flag leaf attitude of blade (early observation)	3	Semi-erect
22.	Spikelet : density of pubescence of lemma	5	Medium
23.	Male sterility	1	Absent
24.	Lemma: anthocyanin colouration of keel	1	Absent
25.	Lemma: anthocynin of area below apex	1	Absent
26.	Lemma: anthocynin colouration of apex	1	Absent
27.	Spikelet : colour of stigma	1	White
28.	Stem: thickness	5	Medium (0.45 cm)
29.	Stem: length (excluding panicle)	7	Long (118.8 cm)
30.	Stem: anthocyanin coloration of nodes	1	Absent (occasionally present at lower nodes)
31.	Stem : intensity of anthocyanin colouration of nodes	—	—
32.	Stem : anthocyanin colouration of internodes	1	Absent
33.	Panicle: length of main axis	5	Medium (27.2 cm)
34.	Flag leaf: attitude of blade (late observation)	5	Horizontal
35.	Panicle: curvature of main axis	5	Deflexed
36.	Panicle: number per plant	3	Few (8.3)
37.	Spikelet: colour of tip of lemma	2	Yellowish
38.	Lemma & Palea : Colour	1	Straw
39.	Panicle: awns	9	Present
40.	Panicle: colour of awns (late observation)	2	Yellowish brown
41.	Panicle: length of largest awn	7	Long (17.3 cm)
42.	Panicle: distribution of awns	5	Whole length
43.	Panicle : presence of secondary branching	9	Present
44.	Panicle : secondary branches	1	Weak
45.	Panicle : attitude of branches	3	Erect to Semi-erect
46.	Panicle: exertion	7	Well exerted
47.	Time of maturity	7	Late (146 days)
48	Leaf : senescence	5	Medium
49.	Sterile lemma: colour	1	Straw
50.	Grains: weight of 1000 fully developed grains	3	Low (15.6 g)

Continue...

Sl. No.	Characteristics	Scale	Remarks measured values etc.
51.	Grain : length	3	Short (7.5 mm)
52.	Grain : width	2	Narrow (2.4 mm)
53.	Grain : phenol reaction of lemma	—	—
54.	Decorticated grain: length	3	Medium (5.27 mm)
55.	Decorticated grain: width	3	Narrow (1.91 mm)
56.	Decorticated grain shape	2	Medium slender
57.	Decorticated grain: colour	1	White
58.	Endosperm: presence of amylose	9	Present
59.	Endosperm: content of amylose	3	Low (17.2 %)
60.	Varieties with endosperm of amylose absent only-polishedgrain : exertion of white core	—	—
61.	Gelatinization temperature through alkali spreading value	3	Medium (Alkali score 4.3)
62.	Decorticated grain : aroma	9	Present (Medium)

Plate 2: Tulaipanji rice plants at post-flowering stage

yellow coloured anthers, and an ovary with white or yellowish-white feathery stigma.

Grain**:** The grains of Tulaipanji rice were short in size (mean length 7.5 mm and width 2.4 mm), with yellowish-brown coloured long awns (mean length 17.3 mm) (Plate 3). The weight of 1000 fully-developed grains was low (15.6 g). The

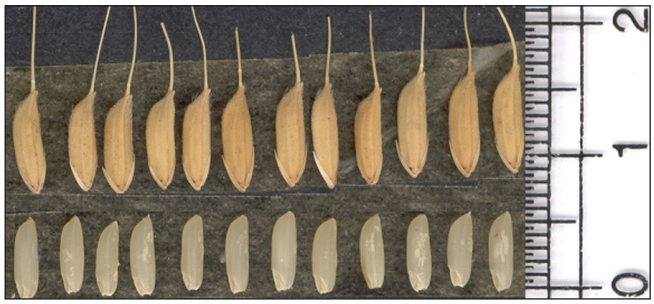

Plate 3: Grains and Kernels of Tulaipanji rice

colour of lemma and palea was straw (scale 1), and that of sterile lemma was straw (scale 1).

Plate 4: Kernels of Tulaipanji rice

The kernels were medium-slender in shape (length 5.27 mm and width 1.91 mm) and white in colour (Plate 4), which had low amylose content (17.2%), medium gelatinization temperature (alkali value 4.3) and medium aroma.

3.2. DNA amplification profile and molecular weight

23 SSR markers used in the study were selected from chromosome 2, 3, 7 and 8 because two important traits of scented rice, aroma (Ahn et al., 1992) and cooked kernel elongation ratio (Ahn et al., 1993) were mapped earlier using RFLP markers. The SSR markers revealed clear and consistent amplification profile in the investigation, which developed the molecular base-pair length database of Tulaipanji rice against the non-aromatic international check variety IR 36 because of availability of sequence-based estimate of allele size of the later reference variety (Table 2). Among 23 markers used, one marker (RM 182) recorded similar molecular weights for both the varieties, while 17 markers showed lower bp values for Tulaipanji rice compared to the check variety and rest five primers (RM 149, RM 250, RM 284, RM 339 and RM569) indicated greater molecular weights of the tested variety than the check. Perusal of molecular weight database revealed that two markers (RM 207 and RM 339) made greater genetic distances between Tulaipanji and IR 36 varieties (75.82 vs. 128.48 bp and 182.76 vs. 143.09 bp, respectively) compared to other markers in the investigation.

Table 2: Details of SSR markers and base pair length of Tulaipanji rice

SSR Marker	Motif	Rice chromo-some no.	AT (°C)	Length of base pair (bp)	
				Tulai-panji	IR 36
RM 42	(GA)6	8	65	152.93	156.36
RM44	(GA)16	8	55	108.28	112.78
RM72	(TAT)5C (ATT)15)	8	55	148.93	165.65
RM80	(CTT)20	8	65	117.59	121.82
RM112	(GAA)5	2	55	130.15	141.98
RM149	(AT)10	8	59	252.60	246.99
RM152	(GGC)10	8	60	140.55	149.96
RM182	(AT)16	7	59	296.58	296.27
RM207	(GA)25	2	65	75.82	128.48
RM210	(GA)23	8	55	136.37	149.75
RM218	(GA)24	3	55	150.25	155.29
RM223	(GA)25	8	55	146.57	164.34
RM250	(CT)17	2	60	156.49	150.96
RM251	(CT)29	3	55	116.58	119.88
RM282	(GA)15	3	59	127.70	140.39
RM284	(GA)8	8	55	149.50	139.68
RM310	(GT)19	8	55	103.01	107.57
RM337	(CTT)4-19(CTT)8	8	59	157.60	161.29
RM339	(CCT)8 (CCT9CCT) 5	8	59	182.76	143.09
RM341	(CTT)20	2	55	172.63	174.97
RM505	(CT)12	7	55	120.58	126.25
RM530	(GA)23	2	59	158.48	168.02
RM569	(CT)16	3	59	175.39	167.61

AT: Annealing temperature (°C); IR 36: IR 36 (International check)

4. Conclusion

Tulaipanji, a traditional aromatic rice variety of North Bengal, India had late maturity (140–150 days) and the plants were long statured (125–135 cm height) with no anthocyanin colouration on leaf blade, sheath, nodes and internodes. The colour of lemma and palea of grain was straw and the grains were medium in length (7.5 mm) with low test weight (15.6 g). The kernels were medium slender (length 5.27 mm and width 1.91 mm) in shape and white in colour, which had low amylose content (17.2%), medium gelatinization temperature (alkali value 4.3) and medium aroma. Based on molecular base-pair length database developed by 23 SSR markers, two (RM 207 and RM 339) made greater genetic distances between Tulaipanji and IR 36 in the investigation.

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