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RESEARCH ARTICLE

Morpho-Genetic Characterization of Scented Radhunipagal Rice Landrace of South Bengal

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Radhunipagal is a traditional small-grained non-Basmati type scented rice of *rahr* (red and laterite) and *gangetic* plain region of West Bengal. With a view to develop the phenotypic and genetic database of the unique variety, the agro-morphological characterization of Radhunipagal rice was done following DUS test guidelines of PPV&FRA at 'C' Block Farm, B.C.K.V., Kalyani, West Bengal during *kharif* (wet) season of 2012, 2013 and 2014. The variety had long statured plants (scale 7, 135-145 cm height) with distinct anthocyanin colouration on lower nodes and internodes; and it had late heading (120-125 days) and late maturity (scale 7, 150-155 days). The flower was bi-sexual including six yellow coloured anthers, and an ovary with light purple coloured feathery stigma. The colour of lemma and palea of matured grain was straw with purple spot at tip and the grains were awnless, short in length (5.8 mm) with very low test weight (10.03 g). The white-coloured kernels were short-bold (length 3.57 mm and width 1.86 mm) in shape, and the cultivar had low amylose content (17.0%), medium gelatinization temperature (alkali value 3.2) and medium-strong aroma. The DNA-based study using 23 simple sequence repeat (SSR) markers for Radhunipagal rice against non-aromatic international check (IR 36) revealed that two markers (RM 42 and RM 310) recorded near-similar molecular weights for both the varieties, while other two markers (RM 339 and RM 341) made greater genetic distances (184 *vs*. 143 bp and 138 *vs.* 175 bp, respectively) between Radhunipagal and IR 36.

Key Words: Aromatic rice, Morpho-agronomic traits, Grain quality, SSR polymorphism

Introduction

The cultivation and use of small and medium-grained non-Basmati type scented rices in Bengal region of eastern India have been documented in *punthi* (manuscript), district gazetteers, books, folk literatures, doggrels, etc. for a long period. Among such 40 aromatic rice landraces, Radhunipagal is traditionally cultivated in *rahr* (red and laterite) and lower gangetic plains of West Bengal for about 400–500 years. The earliest record of Radhunipagal rice was found in two district gazetteers on 24 Parganas (Hunter, 1875) and Medinipur (Hunter, 1876), and thereafter in two books entitled 'The Dictionary of the Economic Products of India' (Watt, 1891) and 'The Handbook of Agriculture' (Mukherji, 1901). Although it was very popular in Bengali society and culture for preparation of *bhog* (rice intermixed with pulses), *payash* (dessert), *pistak* or *pitha* (home-made cake), etc. during social functions and religious festivals for a long period, but its cultivation has been marginalized to small pockets of a few districts (Birbhum, Burdwan, Hooghly, Bankura, etc.) during last four decades due to large-scale adoption of high-yielding varieties in the region. Farmers in native areas cultivate Radhunipagal rice following traditional practices intermixed with a few modern technologies in recent times during *kharif* (wet) season.

In the present-day agricultural system, it has become a necessity to register important landraces as farmers' varieties under PPV&FRA, 2001 to strengthen the right of the farming community to conserve, cultivate and protect the same against unauthorized utilization by multi-national seed companies at national and global level. Hence, agro-morphological, physico-chemical and molecular characterization of Radhunipagal rice needs to be done as legal evidence to find out phenotypic and genetic distances from other closely related genotypes as well as to avoid duplication in rice germplasm conservation system.

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Materials and Methods

DUS Testing and Determination of Grain Quality

The seeds of Radhunipagal rice were collected from the Regional Research Sub-Station (RRSS), Red and Laterite Zone, Bidhan Chandra Krishi Viswavidyalaya (BCKV), Sekhampur, Birbhum, West Bengal. 25 days old seedlings of Radhunipagal rice @ single / hill were transplanted in an open puddled field with five replications at 'C' Block Farm (22º59′N, 88º27′E and 9.75 m above mean sea level) of BCKV, Kalyani, Nadia, West Bengal, India during *kharif* (wet) season of 2012, 2013 and 2014. 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 Radhunipagal 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, BCKV, Mohanpur, Nadia.

Molecular Characterization by SSR Markers

The molecular characterization of Radhunipagal rice was done at the Division of Plant Biology, Bose Institute, Kolkata, West Bengal during 2006-2008. 3-day old seedlings of Radhunipagal 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). Each reaction mixture contained 1 µl of genomic DNA (100 ng), 0.5 µl of each primers (at a concentration of 10 pmole/µl), 2.5 µl of 10× PCR buffer, 0.75 µl of 50 mM MgCl2, 0.25 µl of 2.5 mM dNTP mixture, 0.2 µl (1 unit) of 5 unit/µl Taq DNA polymerase and 19.3 µl of PCR-grade water. The temperature profile of the first PCR cycle was 97°C for 5 minutes, 55-60°C (as necessary in accordance to Table 2) for 2 min; followed by 35 cycles of 1 min at 95°C, 1 min at 55-60°C and 2 min at 72°C. The final extension was at 72°C for 10 min.

The PCR products were resolved by native polyacrylamide gel electrophoresis (PAGE) following the protocol given by Sambrook *et al.* (1989). The size (in nucleotides) of the most intensely amplified band for each microsatellite marker was determined using the Molecular Analyst software (BioRad, USA), based on the migration of the band relative to standard molecular weight size markers (100 bp DNA ladder SibEnzyme Ltd., Russia). IR36 was used as a molecular weight reference in each gel because a sequence-based estimate of allele size in this germplasm was available. The different alleles amplified from the genomic DNA of Radhunipagal rice along with the check were identified on the basis of their length or base pairs (bp) for making genetic characterization of Radhunipagal rice in the study.

Results and Discussion

Agro-morphological Characteristics and Grain Quality

Radhunipagal rice was usually adaptable to rainfed medium land in gangetic alluvium and *rahr* region of West Bengal. The characteristics of Radhunipagal rice following 'DUS Test Guidelines for Rice' of PPV&FRA are described in Table 1.

Plant: Radhunipagal rice belonged to long-duration type with late heading (scale 7, 122 days) and late maturity (scale 7, 151 days) (Fig. 1).

Fig. 1. Radhunipagal rice field at dough stage

Table 1. Plant characteristics of Radhunipagal rice following DUS Test Guidelines.

Sl. No.	Characteristics	Scale	Remarks measured values etc.
1	Coleoptile: colour	2	Green
2	Basal leaf sheath colour	3	Purple lines
3	Leaf : Intensityof green colour	5	Medium
4	Leaf : anthocyanin colouration	1	Absent
5	Leaf : distribution of anthocyanin colouration	–	
6	Leaf sheath : anthocyanin colouratin	9	Present (inner side of lower leaf sheath)
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	White
16	Leaf : length of blade	7	Long (70.0 cm)
17	Leaf : width of blade	3	Narrow (0.98 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 (122 days)
21	Flag leaf attitude of blade (early observation)	3	Semi-erect
22	Spikelet : density of pubescence of lemma	3	Weak
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	7	Strong
27	Spikelet : colour of stigma	4	Light purple
28	Stem: thickness	5	Medium (0.52 cm)
29	Stem: length (excluding panicle)	5	Medium (129.3 cm)
30	Stem: anthocyanin coloration of nodes	1	Absent (Present usually at lower nodes)
31	Stem : intensity of anthocyanin colouration of nodes	3	Weak
32	Stem : anthocyanin colouration of internodes	9	Present
33	Panicle: length of main axis	5	Medium (21.9 cm)
34	Flag leaf: attitude of blade (late observation)	5	Horizontal
35	Panicle: curvature of main axis	7	Drooping
36	Panicle: number per plant	3	Few (9.57)
37	Spikelet: colour of tip of lemma	5	Purple
38	Lemma & Palea : Colour	7	Purple spots / furrows on straw
39	Panicle: awns	1	Absent
40	Panicle: colour of awns (late observation)	–	
41	Panicle: length of largest awn	–	
42	Panicle: distribution of awns	–	
43	Panicle : presence of secondary branching	9	Present

Sl. No.	Characteristics	Scale	Remarks measured values etc.
44	Panicle : secondary branches	2	Strong
45	Panicle : attitude of branches	3	Erect to Semi-erect
46	Panicle: exertion	7	Well exerted
47	Time of Maturity	7	Late (151 days)
48	Leaf : senescence	5	Medium
49	Sterile lemma: colour	4	Purple
50	Grains: weight of 1000 fully developed grains	1	Very low (10.03 g)
51	Grain : length	1	Very short (5.78 mm)
52	Grain : width	2	Narrow (2.35 mm)
53	Grain : phenol reaction of lemma	–	
54	Decorticated grain: length	1	Very short (3.57 mm)
55	Decorticated grain: width	1	Very narrow (1.86 mm)
56	Decorticated grain shape	2	Short bold
57	Decorticated grain: colour	1	White
58	Endosperm: presence of amylose	9	Present
59	Endosperm: content of amylose	3	Low (17.0 %)
60	Varieties with endosperm of amylose absent only-polishedgrain : exertion of white core	–	
61	Gelatinization temperature through alkali spreading value	3	Medium (Alkali score 3.2)
62	Decorticated grain : aroma	9	Present (Medium-strong)

Table 2. Details of SSR markers and base pair length of Radhunipagal rice

SSR Marker	Motif	Rice Chromosome No.	Annealing temperature (^{0}C)	Base pair (bp)	
				Radhunipagal	IR 36 (International check)
RM 42	(GA)6	8	65	155	156
RM 44	(GA)16	8	55	109	113
RM 72	(TAT)5C(ATT)15)	8	55	149	166
RM 80	(CTT)20	8	65	118	122
RM 112	(GAA)5	2	55	130	142
RM 149	(AT)10	8	59	262	247
RM 152	(GGC)10	8	60	142	150
RM 182	(AT)16	7	59	294	296
RM 207	(GA)25	2	65	149	128
RM 210	(GA)23	8	55	136	150
RM 218	(GA)24	3	55	150	155
RM 223	(GA)25	8	55	148	164
RM 250	(CT)17	2	60	156	151
RM 251	(CT)29	3	55	114	120
RM 282	(GA)15	3	59	132	140
RM 284	(GA)8	8	55	147	140
RM 310	(GT)19	8	55	106	108
RM 337	(CTT)4-19(CTT)8	8	59	156	161
RM 339	(CCT)8(CCT9CCT)5	8	59	184	143
RM 341	(CTT)20	2	55	138	175
RM 505	(CT)12	7	55	123	126
RM 530	(GA)23	2	59	157	168
RM 569	(CT)16	3	59	161	168

Stem: Plants were long-statured with average stem length of 129.3 cm excluding panicle. The thickness of stem was medium (scale 5) with mean diameter of 0.52 cm. Anthocyanin colouration was usually present on lower nodes and internodes of Radhunipagal rice (Fig. 2), while the colour of inner side of leaf sheath mainly at plant base of closely related landrace Radhatilak was green. The attitude of the culm could be categorised as erect (scale 1) at booting stage.

Fig. 2. Node and internode

Leaf: Leaves were long, narrow and green. The colour of basal leaf sheath was green (scale 1) with medium intensity (scale 5), but there was anthocyanin colouration on inner side of lower leaf sheath. The average length and width of leaf blade were noted as 70.0 mm and 9.8 mm, respectively. The split-type ligule (scale 3) 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 Radhunipagal rice was categorized as medium (scale 5, 21.9 cm) with the curvature of the main axis as drooping (scale 7). The plant produced very few (scale 3, mean 9.57) well-exerted panicles in the field. The colour of the lemma and palea was green with purple spot at tip at anthesis (Fig. 3), which turned to straw with purple spot at tip at maturity.

Flower: The variety produced bi-sexual flowers including six yellow coloured anthers, and an ovary with light purple coloured feathery stigma (Fig. 4). But the colour of feathery stigma of Radhatilak rice was white.

Grain: The grains of Radhunipagal rice were short in size (mean length 5.8 mm and width 2.4 mm) and awnless. The weight of 1000 fully-developed grains was very low (10.03 g). The colour of lemma and palea was straw with purple spot at tip (scale 7) (Figs. 5 and 6), while that of sterile lemma was purple (scale 4). But the colour of tip of lemma in the spikelet of Radhatilak rice was reddish purple, while that of sterile lemma was red (Rey *et al.,* 2019).

The kernels were short-bold in shape (length 3.57 mm and width 1.86 mm) and white in colour (Fig. 6). The cultivar had low amylose content (17.0%), medium gelatinization temperature (alkali value 3.2) (Fig. 7) and medium-strong aroma.

Fig. 3. Spikelets at anthesis

Fig. 4. Flower

Fig. 5. Grain

Fig. 6. Grains and kernels

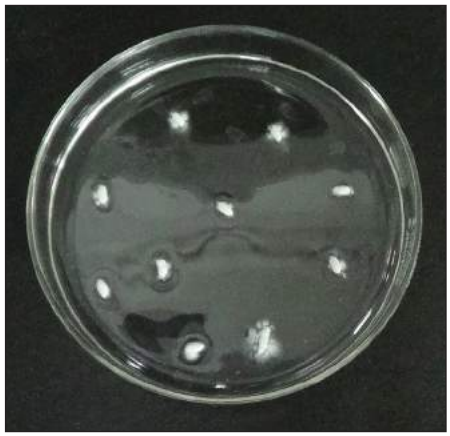

Fig. 7. Alkali digestion test

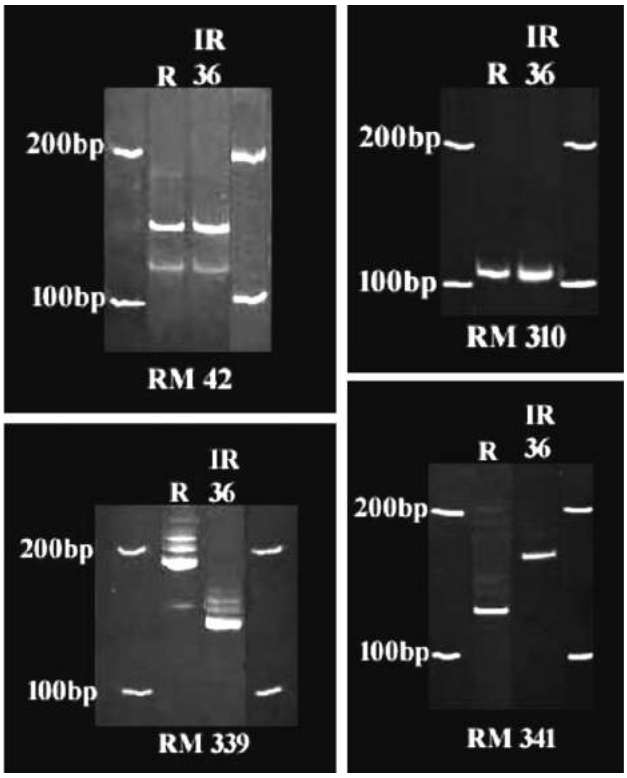

Fig. 8. SSR profile of scented Radhunipagal rice

DNA Amplification Profile

23 SSR markers used in the study were selected from chromosome 2, 3, 7 and 8 because two important traits like aroma (Ahn *et al*., 1992) and cooked kernel elongation ratio (Ahn *et al*., 1993) of scented rice 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 Radhunipagal 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 the markers used, two markers (RM 42 and RM 310) recorded near-similar molecular weights for both the varieties, while two markers (RM 339 and RM 341) made greater genetic distances (183.95 *vs*. 143.09 bp and 138.38 *vs*. 174.97 bp, respectively) between Radhunipagal and IR 36 varieties in the investigation.

Conclusion

Radhunipagal, an aromatic rice landrace of South Bengal, had late maturity (150-155 days) and the plants were long statured (135-145 cm height) with anthocyanin colouration on lower nodes and internodes. The colour

of lemma and palea of matured grain was straw with purple spot at tip and the grains were short in length (5.8 mm) with very low test weight (10.03 g). The kernels were short-bold in shape (length 3.57 mm and width 1.86 mm) and white in colour, which had low amylose content (17.0%), medium gelatinization temperature (alkali value 3.2) and medium-strong aroma. With these, Radhunipagal could be distinguished from another scented rice (Radhatilak) by the characteristics of anthocyanin colouration on internodes and inner side of leaf sheath, colour of tip of lemma and entire sterile lemma, grain size and weight. Based on molecular base-pair length database developed by 23 SSR markers, two (RM 339 and RM 341) made greater genetic distances between Radhunipagal and IR 36 in the study.

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