

Agro-morphological and molecular characterization of traditional scented Radhatilak rice of lower Gangetic plains of West Bengal

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ABSTRACT

Radhatilak, an indigenous tall-indica type scented rice, is presently cultivated in small pockets in 3-4 districts within lower gangetic plains of West Bengal, India. With a view to register as a farmers' variety, its agro-morphological characterization was done at 'C' Block Farm, B.C.K.V., Kalyani, West Bengal during kharif (wet) season of 2012, 2013 and 2014 following DUS test guidelines of Protection of Plant Varieties and Farmers' Rights Authority (PPV&FRA). The variety has late heading (115-120 days) and late maturity (140-150 days), which produces long-statured plants (130-140 cm height) with no anthocyanin colouration on leaf blade and sheath, nodes and internodes. The colour of lemma and palea is golden-yellow with red or reddish-purple spot at tip at maturity, while sterile lemma is red in colour. The awnless grains are short in length (6.3 mm) with very low test weight (10.95 g). The white- coloured kernels are short-bold in shape with low amylose (18.6%), medium gelatinization temperature (alkali value 3.3) and medium-strong aroma. Twenty three SSR markers were used for DNA amplification profiling of Radhatilak rice against non-aromatic international check variety IR 36. The study could identify two markers (RM 339 and RM 341) which can clearly distinguish Radhatilak and IR 36 (182.39 vs. 143.09 bp and 135.39 vs. 174.97 bp, respectively).

Key words: Aromatic rice, DUS characteristics, Grain quality, SSR polymorphism

Among 35-40 non-Basmati type scented rice landraces of West Bengal, Radhatilak is a short-grain aromatic rice, which is marginally cultivated by traditional farm families in small pockets in 3-4 districts like Nadia, Hooghly, Purba Medinipur, etc. of lower gangetic plains of South Bengal (Yadav et al., 2014). The name 'Radhatilak' has probably been originated about 400-500 years ago from the social life system of 'Vaishnavas', the disciples of Shree Chaitanya Mahaprabhu. The word has two parts: 'Radha' and 'Tilak', where 'Radha', is indicated as the lover or Gopini of 'Lord Krishna' in Hindu mythology and 'tilak' means sectarian mark painted by the Vaishnavas mostly on their foreheads, chests and arms (Ghosh, 2019). Farmers in native areas cultivate Radhatilak rice for their own use in small portions of their agricultural lands

following traditional practices intermixing with a few modern technologies in recent times during *kharif* season. The variety has some special end-uses like bhog (rice intermixed with pulses), payash (desert), pistak or pitha (home-made cake), etc. during social functions and religious festivals in the native areas.

Being a signatory to the general agreement on Trade and Tariffs during 2014, the Government of India has enacted its *sui generis* system Protection of Plant Varieties and Farmers' Rights Act (PPV&FRA), 2001 for providing protection to plant varieties based on distinctiveness, uniformity and stability (DUS) test apart from novelty. With a view for registration of Radhatilak rice as a farmers' variety under PPV&FR Act, 2001; the plant characteristics including grain quality needs

to be determined. It may also strengthen the right of the farming community to conserve, cultivate and protect the variety against counterfeit ones and / or multinational corporate seed sectors in present-day agricultural system.

Seeds of Radhatilak rice were collected from Agricultural Training Centre, Department of Agriculture, Government of West Bengal, Fulia, Nadia, West Bengal, India (Fig. 1). Twenty five days old seedlings of Radhatilak rice @ single hill⁻¹ were transplanted in an open puddled field with five replications at 'C' Block Farm (22°59'N, 88°27'E and 9.75 m above mean sea level) of Bidhan Chandra Krishi Viswavidyalaya, 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 organic 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 Radhatilak. Grain quality parameters like size and shape of grain and kernel, amylose content (Juliano, 1971), gelatinization temperature (Little et al., 1958) and aroma (Nagaraju et al., 1991) were determined at Aromatic Rice Laboratory, Department of Agronomy, Bidhan Chandra Krishi Viswavidyalaya Kalyani, Nadia, West Bengal, India.

The molecular characterization of Radhatilak rice was done at the Division of Plant Biology, Bose Institute, Kolkata, West Bengal during 2006-2007. Three-days old seedlings of Radhatilak along with

international non-aromatic check variety IR 36 were used for genomic DNA isolation following the method of Walbot (1988). DNA amplification was carried out by standard PCR method with 23 pairs of 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 were determined with the reference of 100 bp DNA ladders (Sib Enzyme Ltd., Russia) by the Molecular Analyst software (BioRad, USA) and were compared.

Morpho-agronomic characteristics and grain quality

Radhatilak rice is usually adapted to rainfed medium land in lower gangetic alluvial region of West Bengal. The characteristics of Radhatilak rice following 'DUS Test Guidelines for Rice' of PPV & FRA are described in Table 1.

Plant

Radhatilak rice belongs to long-duration type with late heading (scale 7, 119 days) and late maturity (scale 7, 149 days) (Fig. 6).

Stem

It is a long statured landrace with average stem length of 138.0 cm excluding the panicle. The thickness of stem is medium (scale 5) with mean diameter of 0.55 cm. Anthocyanin colouration is usually absent or rarely present on nodes, but the pigment is totally absent on internodes (Fig. 2). The attitude of the culm is categorised as semi-erect (score 3) at booting stage.



Fig. 1. Seed



Fig. 2. Node and internode.

Table 1. Plant characteristics of Radhatilak rice following DUS guidelines.

Sl. No.	Characteristics	Scale	Remarks / Measured values
1	Coleoptile : colour	2	Green
2	Basal leaf sheath colour	1	Green
3	Leaf : Intensityof green colour	5	Medium
4	Leaf : anthocyanin colouration	1	Absent
5	Leaf: distribution of anthocyanin colouration	-	-
6	Leaf sheath : anthocyanin colouratin	1	Absent (inner side of 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	Green
16	Leaf : length of blade	7	Long (69.5 cm)
17	Leaf : width of blade	3	Narrow (9.7 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 (119 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	5	Medium
27	Spikelet : colour of stigma	1	White
28	Stem : thickness	5	Medium (0.55 cm)
29	Stem : length (excluding panicle)	7	Long (138.0 cm)
30	Stem : anthocyanin coloration of nodes	1	Absent (but present usually 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 (24.2 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 (8.6)
37	Spikelet : colour of tip of lemma	4	Red
38	Lemma & Palea : Colour	8	Purple spot on tip
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
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 (149 days)
48	Leaf : senescence	5	Medium
49	Sterile lemma : colour	3	Red
50	Grains : weight of 1000 fully developed grains	1	Very low (10.95 g)
51	Grain : length	1	Very short (6.3 mm)
52	Grain : width	2	Narrow (2.3 mm)
53	Grain : phenol reaction of lemma	-	-

Continued.....

54	Decorticated grain : length	1	Very short (4.03 mm)
55	Decorticated grain : width	1	Very narrow (1.96 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 (18.6 %)
60	Varieties with endosperm of amylose absent only-polishedgrain : exertion of white core	-	-
61	Gelatinization temperature through alkali spreading value	3	Medium (Alkali score 3.3)
62	Decorticated grain : aroma	9	Present (Medium-strong)

Leaf

The landrace produces long, narrow and green leaves. The colour of basal leaf sheath is green (score 1), while the intensity of green colour of the leaf is medium (scale 5) without any anthocyanin colouration. The average length and width of leaf blade are noted as 69.5 mm and 9.7 mm respectively. The split-type (scale 3) ligule and sickle-shaped auricle at leaf base are found in the plant. The attitude of the flag leaf is semi-erect (scale 3) at early observation and horizontal (scale 5) at late observation.

Inflorescence

The length of panicle of Radhatilak is categorized as medium (scale 5, 24.2 cm) with the curvature of the main axis as drooping (scale 7) (Fig. 8). The plant produces very few (scale 3, mean 8.6) well-exerted panicles in the field. The colour of the lemma and palea is green at anthesis, which turns to golden-yellow at ripening stage (Fig. 5). But red or reddish-purple spot appears at the tip of the spikelet (apicular pigmentation) starting from post-anthesis period, which becomes darker at maturity (Fig. 7).

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

SSR Marker	Motif	Rice Chromosome No.	Annealing temperature (°C)	Length of base pair (bp)	
				Radhatilak	IR 36 (International check)
RM 42	(GA)6	8	65	154.71	156.36
RM44	(GA)16	8	55	110.33	112.78
RM72	(TAT)5C(ATT)15)	8	55	146.62	165.65
RM80	(CTT)20	8	65	112.68	121.82
RM112	(GAA)5	2	55	131.05	141.98
RM149	(AT)10	8	59	261.84	246.99
RM152	(GGC)10	8	60	144.29	149.96
RM182	(AT)16	7	59	293.39	296.27
RM207	(GA)25	2	65	139.13	128.48
RM210	(GA)23	8	55	138.24	149.75
RM218	(GA)24	3	55	152.98	155.29
RM223	(GA)25	8	55	148.15	164.34
RM250	(CT)17	2	60	156.99	150.96
RM251	(CT)29	3	55	118.08	119.88
RM282	(GA)15	3	59	128.64	140.39
RM284	(GA)8	8	55	144.69	139.68
RM310	(GT)19	8	55	105.75	107.57
RM337	(CTT)4-19(CTT)8	8	59	157.38	161.29
RM339	(CCT)8(CCT9CCT)5	8	59	182.39	143.09
RM341	(CTT)20	2	55	135.39	174.97
RM505	(CT)12	7	55	124.89	126.25
RM530	(GA)23	2	59	161.05	168.02
RM569	(CT)16	3	59	178.45	167.61

Flower

The landrace produces bi-sexual flowers including six yellow coloured anthers, and an ovary with yellow feathery stigma (Fig. 3 and Fig. 4).

Grain

The grains of Radhatilak rice are short in size (mean length 6.3 mm and width 2.3 mm) and awnless (Fig. 9). The weight of 1000 fully-developed grains is low (10.95 g). The colour of lemma and palea is golden-yellow with purple spots at tip (scale 8) (Fig. 7), while that of sterile lemma is red (scale 4).

The kernels are short-bold in shape and white in colour (Fig. 10), which have low amylose content (18.6%), medium gelatinization temperature (alkali value 3.3) (Fig. 11) and medium-strong aroma.

DNA amplification profile and molecular weight

Twenty three simple sequence repeat (SSR) markers used in the study were selected from chromosome 2,



Fig. 3. Flower



Fig. 4. Flowering stage



Fig. 5. Change of grain colour



Fig. 6. Maturity stage

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 on these chromosomes. The SSR markers revealed clear and consistent amplification profile in the investigation, which developed the molecular base-pair length database of Radhatilak against the non-

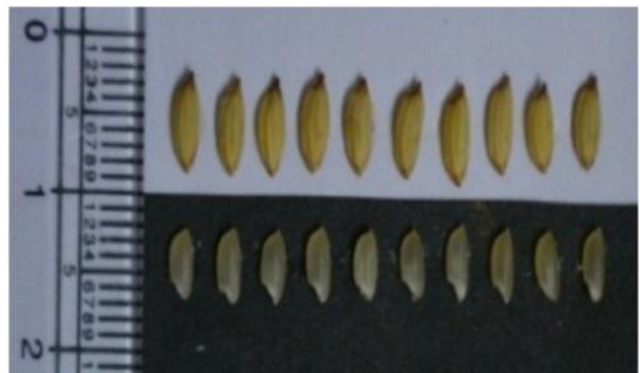


Fig. 7. Grains and kernels



Fig. 8. Compactness of the panicle



Fig. 11. Alkali digestion test of kernels



Fig. 9. Grains

aromatic international check variety IR 36 because of availability of sequence-based estimate of allele size of the later as reference variety (Table 2). Out of 23 SSR markers, 17 recorded lower molecular weights for Radhatilak compared to IR 36, while 6 markers (RM 149, RM 207, RM 250, RM 284, RM 339 and



Fig. 10. Kernels

RM 531) showed greater base-pair length values for the tested variety than the check. Perusal of molecular weight database in Table 2 revealed two markers (RM 339 and RM 341) which showed greater differences in allele size due to large variation in amplified repeat length motifs between Radhatilak and IR 36 (182.39 vs. 143.09 bp and 135.39 vs. 174.97 bp, respectively).

CONCLUSION

Radhatilak, a traditional aromatic rice variety of West Bengal, India has late maturity (140-150 days), which produces long statured plants (130-140 cm height) with no anthocyanin colouration on leaf blade and sheath, nodes and internodes. The lemma and palea of grain are golden-yellow in colour with red or reddish-purple spot at tip and the grains are short in length (6.3 mm) with very low test weight (10.95 g). The white-coloured kernels are short-bold in shape, having low amylose (18.6%), medium gelatinization temperature (alkali value 3.3) and medium-strong aroma as per the DUS test guidelines of Protection of Plant Varieties and Farmers' Rights Authority (PPV&FRA). Molecular base-pair length database of Radhatilak rice was developed using 23 SSR markers taking IR36 as the reference genotype.

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