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Morpho-agronomic and Molecular Characterization of Gobindabhog, a Traditional Aromatic Rice of West Bengal, India

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The agro-morphological characterization of Gobindabhog, a traditional non-Basmati type aromatic rice of lower gangetic plains and *rahr* (red and laterite) region of West Bengal was done at B.C.K.V., Kalyani, West Bengal, India during *kharif* season of 2011, 2012 and 2013 following DUS test guidelines of Protection of Plant Varieties and Farmers' Rights Authority (PPV&FRA). The variety had long statured (scale 7, 130-140 cm height) plants with no anthocyanin colouration on leaf blade, sheath, nodes and internodes; which had late heading (110-115 days) and late maturity (scale 7, 140-150 days). The flower was bi-sexual including six yellow coloured anthers, and an ovary with white/yellowish-white feathery stigma. The lemma and palea of grain were straw or golden-yellow in colour and the grains were awnless, short in length (6.1 mm) with very low test weight (10.17 g). The kernels were short-bold (length 3.97 mm and width 1.95 mm) in shape with white colour, which had low amylose content (17.9%), medium gelatinization temperature (alkali value 3.3) and medium-strong aroma. 23 simple sequence repeat (SSR) markers were used for DNA amplification profile to develop molecular base-pair length database of Gobindabhog rice against non-aromatic international check variety IR 36 in the study. Among them, two markers (RM 341 and RM 339) made greater genetic distance (95.33 *vs*. 174.97 bp and 180.75 *vs.* 143.09 bp, respectively) between Gobindabhog and IR 36.

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

Introduction

The state of West Bengal has precious wealth of genetic diversity in aromatic rice (Shovarani and Krishnaiah, 2001). Among 35-40 such non-Basmati premium scented rices, Gobindabhog, is a native cultivar of lower gangetic plains and *rahr* (red and laterite) region of Bengal, which is traditionally cultivated for about 400–500 years. The name of Gobindabhog was originated probably due to its common use by the Hindus for preparation of *bhog* offered to their worshipped God 'Lord Gobinda' as a religious tradition in gangetic Bengal region for a long time. The earliest record of Gobindabhog rice cultivation was found in a district gazetteer (Hunter, 1877), and then in a book (Mukherji, 1901). At present, it is cultivated in about 30,000–35,000 ha. land in the districts of Burdwan, Bankura, Hooghly, Nadia, Murshidabad, Birbhum and North 24 Parganas in South Bengal, with an average production of 90,000–1,00,000 tonnes paddy every year. Farmers in native areas cultivate Gobindabhog rice in small portions of their agricultural lands following traditional practices intermixed with a few modern technologies in recent times during *kharif* (wet) season. Although it is very popular in domestic market for preparation of *bhog* (rice intermixed with pulses), *payash* (desert), *pistak* or *pitha* (home-made cake), *chira* (flattened rice), etc. during social functions and religious festivals for a long period, but the milled rice obtained from one year aged paddy is marketed in southern states of the country (*viz*. Karnataka, Tamilnadu, Kerala, etc.) during last three decades mainly for preparation of *polao* and *biryani*. In addition, the Standing Committee on Commerce, Parliament of India recommended the export of Gobindabhog rice during 2011 (Rajya Sabha, 2011) based on a Proposal submitted by the RKVY Project on 'Bengal Aromatic Rice' of Bidhan Chandra Krishi Viswavidyalaya, West Bengal. The short-grained variety having pleasant aroma is much potential for international trade especially in the countries like Bangladesh, U.K., Brazil, etc.

Being a signatory to the general agreement on Trade and Tariffs, Government of India has enacted its *sui generis* system Protection of Plant Varieties and Farmers'

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Rights Act (PPV&FRA), 2001 for providing protection to plant varieties based on distinctiveness, uniformity and stability (DUS) test apart from novelty. Thus, agro-morphological, physico-chemical and molecular characterization of Gobindabhog rice needs to be done as legal evidence of DUS, which may also strengthen the right of the farming community to conserve, cultivate and protect the variety against counterfeit ones and/or aggressiveness of multi-national corporate seed sectors in present-day agricultural system.

Materials and Methods

DUS Testing and Determination of Grain Quality

The seeds of Gobindabhog rice was collected from Rice Research Station, Department of Agriculture, Government of West Bengal, Chinsurah, Hooghly, West Bengal, India. Twenty five days old seedlings of Gobindabhog 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 Bidhan Chandra Krishi Viswavidyalaya, Kalyani, Nadia, West Bengal, India during *kharif* 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 Gobindabhog 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.

Molecular Characterization by SSR Markers

The molecular characterization of Gobindabhog rice was done at the Division of Plant Biology, Bose Institute, Kolkata, West Bengal during 2006-2008. Three day old rice seedlings of Gobindabhog along with international non-aromatic check variety (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 Gobindabhog rice along with the check were identified on the basis of their length or base pairs (bp) for making genetic characterization of Gobindabhog rice in the study.

Results and Discussion

Agro-morphological Characteristics and Grain Quality

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

Plant: Gobindabhog rice belonged to long-duration type with late heading (scale 7, 114 days) and late maturity (scale 7, 143 days) (Fig. 1).

Stem: It had long statured plant with average stem length of 124.0 cm excluding panicle. The thickness of stem was medium (scale 5) with mean diameter of 0.48 cm. Anthocyanin colouration was absent on nodes and internodes. The attitude of the culm could be categorised as erect (scale 1) 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 medium (scale 5) without any anthocyanin colouration. The average length and width of leaf blade were noted as 65.7 mm and 9.1 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 Gobindabhog rice was categorized as medium (scale 5, 25.2 cm) with the curvature of the main axis as deflexed (scale 5) (Fig. 2). The plant produced very few (scale 3, mean 9.5) well-exerted panicles in the field. The colour of the lemma and palea was green at anthesis, which turned to golden-yellow at ripening stage.

Table 1. Plant characteristics of Gobindabhog rice following DUS guidelines

S. No.	Characteristics	Scale	Remarks measured values etc.
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
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 (65.7 cm)
17	Leaf : width of blade	3	Narrow (9.1 mm)
18	Culm : attitude (for floating rice only)	—	—
19	Culm : attitude	1	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	5	Medium
27	Spikelet : colour of stigma	1	White
28	Stem: thickness	5	Medium (0.48 cm)
29	Stem: length (excluding panicle)	7	Long (124.0 cm)
30	Stem: anthocyanin coloration of nodes	1	Absent
31	Stem : intensity of anthocyanin colouration of nodes	—	—
32	Stem : anthocyanin colouration of internodes	1	Absent
33	Panicle: length of main axis	5	Medium (25.2 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 (9.5)
37	Spikelet: colour of tip of lemma	2	Yellowish
38	Lemma & Palea : Colour	1	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
44	Panicle : secondary branches	2	Strong
45	Panicle : attitude of branches	7	Semi-erect to spreading
46	Panicle: exertion	7	Well exerted
47	Time of Maturity	7	Late (143 days)
48	Leaf : senescence	7	Late
49	Sterile lemma: colour	1	Straw
50	Grains: weight of 1000 fully developed grains	1	Very low (10.17 g)
51	Grain : length	1	Very short (6.1 mm)
52	Grain : width	2	Narrow (2.2 mm)
53	Grain : phenol reaction of lemma	—	—
54	Decorticated grain: length	1	Very short (3.97 mm)
55	Decorticated grain: width	1	Very narrow (1.95 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 (17.9 %)
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)

Fig. 1. Gobindabhog rice field at dough stage

Fig. 2. Panicle of Gobindabhog rice

Fig. 3. Grains and kernels of Gobindabhog rice

Fig. 4. Alkali digestion test of Gobindabhog rice

Flower: The variety produced bi-sexual flowers including six yellow coloured anthers, and an ovary with white / yellowish-white feathery stigma.

Grain: The grains of Gobindabhog rice were short in size (mean length 6.1 mm and width 2.2 mm) and awnless (Fig. 3). The weight of 1000 fully-developed grains was very low (10.17 g). The colour of lemma and palea was straw (scale 1) or golden yellow, while that of sterile lemma was straw (scale 1).

The kernels were short-bold in shape (length 3.97 mm and width 1.95 mm) and white in colour (Fig. 3), which had low amylose content (17.9%), medium gelatinization temperature (alkali value 3.3) (Fig. 4) and medium-strong aroma.

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 Gobindabhog 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, five markers (RM 44, RM 112, RM 152, RM 207 and RM 251) recorded similar molecular weights for both

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

SSR Marker	Motif	Rice Chromosome No.	Annealing temperature (ºC)	Length of base pair (bp)	
				Gobindabhog	IR 36 (International check)
RM 42	(GA)6	8	65	160.88	156.36
RM44	(GA)16	8	55	112.20	112.78
RM72	(TAT)5C(ATT)15)	8	55	161.08	165.65
RM80	(CTT)20	8	65	125.03	121.82
RM112	(GAA)5	2	55	141.02	141.98
RM149	(AT)10	8	59	256.71	246.99
RM152	(GGC)10	8	60	149.87	149.96
RM182	(AT)16	7	59	312.65	296.27
RM207	(GA)25	2	65	129.02	128.48
RM210	(GA)23	8	55	145.32	149.75
RM218	(GA)24	3	55	144.14	155.29
RM223	(GA)25	8	55	154.72	164.34
RM250	(CT)17	2	60	152.71	150.96
RM251	(CT)29	3	55	120.11	119.88
RM282	(GA)15	3	59	128.49	140.39
RM284	(GA)8	8	55	144.96	139.68
RM310	(GT)19	8	55	103.44	107.57
RM337	(CTT)4-19(CTT)8	8	59	158.58	161.29
RM339	(CCT)8(CCT9CCT)5	8	59	180.75	143.09
RM341	(CTT)20	2	55	95.33	174.97
RM505	(CT)12	7	55	117.42	126.25
RM530	(GA)23	2	59	154.64	168.02
RM569	(CT)16	3	59	174.25	167.61

the varieties, while two markers (RM 341 and RM 339) made greater genetic distance (95.33 *vs*. 174.97 bp and 180.75 *vs.* 143.09 bp, respectively) between Gobindabhog and IR 36 varieties in the investigation.

Conclusion

Gobindabhog, a traditional aromatic rice variety of West Bengal, India had late maturity (140-150 days) and the plants were long statured (130-140 cm height) with no anthocyanin colouration on leaf blade, sheath, nodes and internodes. The colour of lemma and palea of grain was straw or golden-yellow and the grains were short in length (6.1 mm) with very low test weight (10.17 g). The kernels were short-bold (length 3.97 mm and width 1.95 mm) in shape and white in colour, which had low amylose content (17.9%), medium gelatinization temperature (alkali value 3.3) and medium-strong aroma. Based on molecular base-pair length database developed by 23 SSR markers, two (RM 341 and RM 339) made greater genetic distance between Gobindabhog and IR 36 in the investigation.

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