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RESEARCH PAPER

Evaluation of Agronomical Traits and Aroma in Tepi Boro Rice Landraces of Bangladesh

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ABSTRACT

Twenty-five duplicate or similar named TepiBoro group of rice landraces were studied to evaluate variations through15 quantitative Agronomical traits and aroma using sensory test and SSR markers at Bangladesh Rice Research Institute during Boro 2020-21 season. The sensory test of leaf aroma by using 1.7% KOH solution and molecular characterization using high aroma trait linked SSR markers as RM282 (for QTL *aro3.1*), RM273 (for QTL *aro4*) and RM223 (for QTL *aro8.1* represented by *badh2* gene) were performed. The highest leaf area (39.6 cm^2) was observed in Tepu IRRI (Acc. no.6213). The growth duration ranged from 128 to 176 days. TepuDhan (acc. 7986) had the highest number of filled grains per panicle (253). The longest grain (10.26 mm) was found in TopaBoro (acc. 4981) and the highest dehulled LB ration (3.82) was recorded in TopaBoro (acc. 4981). The highest grain yield (930.2 g/plot) was observed in Tepa IRRI (acc. 7855). Besides, the combined result of the sensory test revealed that all the evaluators found leaf aroma in TepiBoro (Acc. 4526), TepuDhan (Acc. 7931) and TopaBoro (Acc. 4373). For PCA, the studied TepiBoro genotypes were grouped into five clusters, where the inter-cluster D^2 values ranged from 11.77 to 43.13. The genotypes under clusters V may be selected for crossing with the genotypes of cluster I and cluster IV for developing improved TepiBoro rice variety. All the three aroma linked SSR markers showed polymorphism and RM223 had the highest PIC value and was considered to be the best marker for studying TepiBoro rice germplasm. Topa Boro (Acc. 4373), Tepi Boro (Acc. 4526) and Tepu Dhan (Acc. 7931) showed aroma for SSR primers RM223, RM273 and RM282. The unrooted neighbour-joining tree revealed that the genotypes were grouped into three major clusters. Finally, this study would be useful for breeders to choose and identify the potential pre-breeding materials for developing aromatic TepiBoro rice variety. Moreover, the candidate genes for rice grain aroma could be identified by combining QTL mapping and transcriptome profiling approaches.

Keywords: Variability, sensory test, aroma, SSR marker, TepiBoro, rice, landraces, Bangladesh

Introduction

Rice (*Oryza sativa* L.) is the most important cereal crop and serves as the staple food in Asia. It is the people's livelihood in our country, because of its agriculture-based economy. Historically, abundant and diversified rice landraces were cultivated in all over Bangladesh from the time immemorial. But, rice yield and its total production need to be increased with less land and inputs to meet the food demand of its increasing population. Consequently, higher yielding climate smart varieties are now needed to develop. For this, broadening of the genetic base through utilization of rice landraces in breeding of newrice varieties may be able to break the yield barrier that rice breeders are now trying to address. Rice genetic resource is the primary material for rice breeding and makes a concrete contribution to global wealth creation and food security (Zhang *et al.*, 2011). It is a rich reservoir of valuable genes that plant breeders can harness for crop improvement (Yadav *et al.*, 2013). Mining elite genes within rice landraces is of importance for the improvement of cultivated rice (Zhang *et al.*, 2014). To assess phenotypic variation, morphological

and yield related features have been used as criteria as well as sources of enhancing the rice yield potential (Ali *et al.*, 2024). Moreover, identifying germplasm accessions for different agronomical characters in phenotypically divergent sources would help in pre-breeding and breeding programs (Pachauri *et al.*, 2017). However, 12,487 names of rice germplasm were listed season and Thana wise and identified that duplicate(s) named rice germplasm were in all over Bangladesh (Hamid *et al.*, 1982). Hence, similar or duplicate named TepiBoro rice landrace groups need to be agronomically studied for exploiting their morphological genetic variation. Because, TepiBoro, also known as TepaBoro rice, is a local variety of Boro rice that has traditionally been cultivated in Bangladesh, particularly along the Padda and Meghna river banks-where the regions are characterized by low-lying, flood-prone areas. It is also known for its distinct taste and is often favored for 'Khichuri'-a famous traditional dish. The rice grains are typically oval-shaped and thick, sometimes with slight breakage, and elongate when cooked.

On the other hand, based on 169 nuclear simple sequence repeats (SSRs), rice has been classified into five distinct groups known as *indica*, aus, aromatic, *temperate japonica*, and *tropical japonica* (Glaszmann, 1987). Fragrance or aroma in rice is considered as a special trait with huge economic importance that determines the premium price in global trade. With the availability of molecular maps and genome sequences, a major gene for fragrance (badh2) was identified on chromosome 8. An 8-bp deletion in the exon 7 of this gene was reported to result in truncation of betaine aldehyde dehydrogenease enzyme whose loss-of-function lead to the accumulation of a major aromatic compound, 2-acetyl 1-pyrroline (2AP) in fragrant rice (Neeraja *et al.*, 2009). Aromatic rice is a special type of rice that highly preferred by people in Asia due to the presence of aroma. Aroma is one of the primary grain quality traits that play a considerable role in the consumer preferences; the others are grain physical appearance, cooking and eating quality, grain physical appearance and nutritional value (Nadaf *et al*., 2016).

2-acetyl-1-pyrroline (2-ACP), a volatile compound, was discovered to be a major contributor to the pleasant scent of aromatic rice, primarily in Basmati and Jasmine rice (Buttery *et al*., 1983). Ocan*et al.* (2020) reported that three compounds, namely: toluene, 1-hexanol, 2-ethyl and heptane, 2, 2, 4, 6, 6-pentamethyl- are potential volatile biomarkers for the rice aroma trait. Since rice aroma, a polygenic quantitative trait with complex inheritance pattern is highly influenced by environment; it is difficult to identify genes that undermine this trait (Pachauri *et al.* 2010). Initially, it was reported that rice aroma is controlled by a single dominant gene with a segregation ratio of 3:1 (Kadam and Patankar, 1938). Subsequently, Jodon (1944) proposed that aroma is controlled by a single recessive fragrance gene (*frg* gene). Fragrance in rice, a recessive trait, has been shown to be due to an eight bp deletion and three SNP's in a gene on chromosome8 which encodes a putative betaine aldehyde dehydrogenase 2 (BAD2) (Bradbury *et al.*, 2005a). It was hypothesized that loss of BADH2 activity causes 2-ACP accumulation (Bradbury *et al.*, 2005b). The fgr gene in rice is located on chromosome 8 (Jin *et al.*, 2010). However, rice aroma is controlled by three Quantitative Trait Loci (QTLs), *aro3.1, aro4.1* and *aro8.1* located on short arm of chromosome 3 and long arms of chromosome 4 and 8, respectively (Amarawathi *et al.*, 2008). Gene for *aroma QTL* frg4-1 due to its similarity to the *badh2* gene on chromosome 8 *aro8.1* is the most significant and is represented by a non-functional (recessive) allele of the *BADH2* gene.

Besides, Singh *et al.* (2007) mapped the Aroma QTLs in Basmati Rice (*Oryza sativa* L) on chromosomes 3, 4 and 8. Previous studies also reported that chromosomes (3, 4, 8 and 12) were implicated through mapping with aromatic trait (Amarawathi *et al*., 2008). Lorieux *et al.* (1996) confirmed close linkage between RG28 and fgr (5, 8 cM) on chromosome 8 and identified two quantitative trait loci for fragrance, on chromosome 4 and chromosome 12. Then, Bradbury *et al*. (2005) showed that a functional BADH2 enzyme inhibits 2ACP biosynthesis which is a major component of aroma. Non-fragrant varieties possess a fully functional copy of the gene encoding BAD2, while fragrant varieties possess a copy of the gene containing eight base pair deletion resulting in a frame shift mutation disabling the BAD2 enzyme. Kovach *et al*. (2009) also confirmed that BADH2 is the major genetic determinant of fragrance in rice. In majority of the aromatic rice lines, fragrance is caused due to accumulation of the fragrant compound2-Acetyl -1-pyrroline (2-ACP). The aromatic character was characterized by three methods, 1.7% KOH sensory test, gas chromatography-mass spectrometry-selected ion monitoring (GC-MS-SIM) and polymerase chain reaction (PCR) analysis (Sarhadi*et al.*, 2009). Smritia *et al.* (2016) reported that the mutation in the *badh2* gene was explored previously towards development of a perfect co-dominant marker to discriminate between aromatic and non-aromatic lines at molecular level.

Sensory test may be done by tasting of grains and/or cooked rice and smelling of leaf tissue. The sensory test of aromatic in rice can performed by using solution of KOH 1.7% (Soodand Siddiq, 1978) or the molecular markers-based study using a specific marker (Bradbury *et al.*, 2005) closely linked to the fgr gene, controlling the expression of aromatic trait. Molecular markers, such as single nucleotide polymorphism (SNPs) and simple sequence repeats (SSRs) that are genetically linked to fragrance, have been developed for the quick selection of aromatic/ fragrant rice (Cordeiro *et al*., 2002).

Carsono*et al.* (2020) reported that seventy-two (81.82%) genotypes were categorized as aromatic rice based on sensory test and molecular markers. But due to inconsistency results from each method alone, it is advised both methods to be applied to ensure the reliability and the accuracy since aroma in rice is affected by genetic composition and environment conditions. Thandar *et al.* (2023) characterized 15 rice cultivars for their aroma by three methods as sensory analysis, proline determination and SSR marker study and found that the three methods were strong enough to distinguish aromatic rice varieties. Hien *et al.* (2006a) reported that aroma in rice is a quantitativecharacter that several genes are involved. On the other hand, Pachauri *et al.* (2017) characterized 124 rice germplasm accessions on the basis of 11 agronomical traits. Islam *et al.* (2018) studied 36 similar named aromatic rice landraces of Bangladesh based on 14 quantitative traits. Similarly, Noorzuraini *et al.* (2024) evaluated 33 rice

genotypes comprising thirty Malaysian rice landraces and three modern rice cultivars for aroma using sensory tests and molecular markers and found aroma in 11 genotypes. Hien *et al.* (2006a) evaluated the aroma in 70 rice genotypes from nine Asian countries using KOH method, molecular markers and quantitative analysis of aromatic compound.

Since, TepiBoro rice is produced completely naturally and additional fertilizers and chemical pesticides are not required. Moreover, it is one of the famous rice in the country as it is very good for diabetics due to low amylase and has nice aroma along with high elongation ratio when cooked. Therefore, more detailed studies on similar named group of TepiBoro rice landraces of Bangladesh need to be done for its more effective utilization. Therefore, the present study was carried outto characterize and evaluate the 25 similar or duplicate named TepiBoro group of rice landraces through quantitative traits as well as by sensory test and SSR markers for QTL *aro3.1*, *aro4* and *aro8.1* (represented by *badh2* gene) of rice aroma.

Materials and Method

Morphological Characterization: Twenty-five similar or duplicate named TepiBoro accessions from BRRI Genebank (Table 1) were characterized through 15 quantitative Agronomical traits at Genetic Resources and Seed Division, Bangladesh Rice Research Institute, Gazipur. The unit plot comprised with two rows of each 5.4 m long. The thirty-days-old single seedling was transplanted in Boro 2020-21season with a spacing of 20×20 cm between rows and plants, respectively. Fertilizers were applied @ 80:20:40 kg N, P and K per hectare respectively. Crop management such as weeding, irrigation etc. were done in time. Appropriate control measures were taken for insect pests, diseases and weeds when needed. The recorded 15 quantitative Agronomical characters were leaf length (cm), leaf width (cm), leaf area (cm^2), culm diameter (mm), effective tiller number, panicle length (cm), plant height (cm), days to maturity, filled grains per panicle, un-filled grains per panicle, grain length (mm), grain width (mm),dehulled grain length breadth ratio, 1000-grain weight (g) and grain yield per plot (g). The ANOVA and mean separation tests were analyzed by R programme and multivariate analyses were performed by GENSTAT 5.5 programme.

Table 1. List of TepiBoro group of rice germplasm used in Boro 2020-21 season.

Sl. no.	Code name	Local name	Acc. No.*	Upazila	District	Season
1	TPB1	Tape Boro	7297	Kessorgonj	Kessorganj	Boro
2	TPB2	Tepa IRRI	7855	Dashmina	Patuakhali	Aus
3	TPB3	Tepakain	1532	---	Dinajpur	Aus
4	TPB4	TepiBoro	258	Trisal	Mymensingh	Boro
5	TPB5	TepiBoro	930	Nabigonj	Sylhet	Boro
6	TPB6	TepiBoro	3998	Derai	Habiganj	Boro
7	TPB7	TepiBoro	4526	SunamganjSadar	Sunamganj	Boro
8	TPB8	TepiBoro	4959	--	Khulna	Boro
9	TPB9	TepiKhorch	931	Nabigonj	Sylhet	Boro
10	TPB10	Tepi Sail	1712	--	Faridpur	Boro
11	TPB11	Tepi Sail	1724	--	Khulna	Aus
12	TPB12	TepiBoroDhan (Kalo)	8058	Kumarkhali	Kushtia	Boro
13	TPB13	Tepu	7382	Patuakhali Sadar	Patuakhali	Boro
14	TPB14	Tepu	7473	Rahamgonj	Bakerganj	Aus
15	TPB15	Tepu IRRI	6213	Patuakhali Sadar	Patuakhali	Aus
16	TPB16	TepuDhan	7931	Golachipa	Patuakhali	T. Aman
17	TPB17	TepuDhan	7986	Patuakhali Sadar	Patuakhali	Aus
18	TPB18	Topa	2256	--	Kishorganj	Boro
19	TPB19	TopaBoro	4373	MoulvibazarSadar	Moulvi Bazar	Boro
20	TPB20	TopaBoro	4981	Pabna Sadar	Pabna	Boro
21	TPB21	TopaBoro	4983	Pabna Sadar	Pabna	Boro
22	TPB22	TopaBoro	5041	Pabna Sadar	Pabna	Boro
23	TPB23	TopaBoro	62	--	Dhaka	Boro
24	TPB24	Tupa	1811	Hossainpur	Kishorganj	Boro
25	TPB25	Tupa	2257	--	Kishorganj	Boro

*= BRRI Genebank accession number

Sensory Test of Leaf Aroma: The sensory test of leaf aroma was performed by smelling the leaf samples by using a 1.7% KOH solution (Sood and Siddiq 1978). For this, one gram of green leaf blade from the heading growth stage of rice plant was cut into small pieces and put into Petri dishes with 5 ml of a 1.7% KOH solution at room temperature in Molecular lab of Genetic Resources and Seed Division. After 30 minutes, the dishes were opened one by one and the smell was smelled immediately by each persons. The presence (+) or more moderately presence (++) or absence (-) of leaf aroma was then scored for each entry. All individual genotypes (25 plants) were scored from five sets of sensory test by five expert persons from the same division who have experience in rice aroma evaluation.

Molecular Characterization for Leaf Aroma: Molecular characterization of 25 TepiBoro germplasm along with three check varieties *viz.* Kalizira (Acc. no. 1589), Bangladesh Kalijira (Acc. 247) and BRRI dhan34 were conducted using three most popular and specific aroma trait linked SSR markers *viz.* RM282 linked with rice aroma QTL *aro3.1* and RM273 linked with rice aroma

QTL *aro4*. The other SSR marker RM223 was linked with rice aroma QTL *aro8.1* representing by the *badh2* gene. DNA was extracted from three weeks old leaves following the quick DNA extraction protocolof Ferdous *et al.* (2012). PCR analysis was performed in 10 µl reaction sample containing 3 µl of DNA template, 4.5 µl of Master Mix (Promega), 1.5 µl of Nuclease-Free Water, 0.5µl each of 10 µM forward and reverse primers using a GeneAtlas G (Astec, Japan) 96-well thermal cycler. The mixture was overlaid with 10 µl of mineral oil to prevent evaporation. After initial denaturation for five minutes at 94°C, each cycle comprised 30 sec denaturation at 95°C, 30 sec annealing at 55°C, and 25 sec extension with a final extension for 5 min at 72°C at the end of 32 cycles.

The PCR products were mixed with bromophenol blue gel loading dye for analyzing by electrophoresis on 8% polyacrylamide gel using mini vertical polyacrylamide gels for high throughput manual genotyping (CBS Scientific Co. Inc., CA, USA). Total 2.5 µl of amplification products were resolved by running gel in 0.5X TBE buffer for 1.5-2.5 hrs depending upon the allele size at around 100 voltages and 500 mA current. The gels were stained in 5 µl SYBR Safe DNA gel stain (10,000X concentration in DMSO, USA) with 200 ml 0.5X TBE buffer for 15 min and exposed to UV light using a molecular imager gel documentation unit (XR System, Uvitec Cambridge, France) for visualization.

The size of the band for each marker was measured by Alpha Ease FC 4.0 software. The summary statistics of the marker data *viz* average number of alleles per locus, major allele frequency, gene diversity and polymorphism information content (PIC) were done by using Power Marker V3.25 (Liu and Muse 2005). Genetic distance based unrooted neighbor-joining tree was constructed using MEGA 5.1 software (Tamura *et al.*, 2011).

Results and Discussion

Assessment of the Variability of the Agronomical Traits: Highly significant differences ($p \leq 0.01$) among the TepiBoro rice genotypes were observed for all the 15 studied morphological characters (Table 2). These results are compatible with the findings of Mohammad (2022), Ismaeel *et al*. (2018a), Ismaeel *et al*. (2018b) and Ranjith *et al.* (2020). The highest leaf length (51.2 cm) in Tupa (acc. no. 1811) and the highest leaf width (1.24 cm) and leaf area (39.6 cm^2) in Tepu IRRI (Acc. no..6213) were observed (Table 3 and Fig. 1). The highest culm diameter (4.8 mm) was recorded from TepiBoro (acc. 4526) and that of number of effective tiller per hill as 9 in few genotypes. The longest panicle length (30 cm) was observed in Tepu (acc. 7382) and that of plant height (138.6 cm) in Tepi Sail (acc. 1712), while the shortest plant (61 cm) was observed in TopaBoro (acc. 5041).The growth duration ranged from 176 days in Tepu (acc. 7382) to 128 days in Topa (acc. 2256).TepuDhan (acc. 7986) had the highest number of filled grains per panicle (253) as well as the highest number of un-filled grains per panicle (20) among the studied TepiBoro germplasms. Besides, the longest grain (10.26 mm) was found in TopaBoro (acc. 4981) and the highest dehulled LB ration (3.82) in TopaBoro (acc. 4981). The highest 1000-grain weight (26.8 g) was observed in TopaBoro (acc. 4981) and the lowest (10 g) in TepuDhan (acc. 7931). Consequently, the highest grain yield (930.2 g/plot) was observed in Tepa IRRI (acc. 7855) and the lowest (395.6 g/plot) in TepuDhan (acc. 7931). Finally, the identified superior genotypes were Tepa IRRI (Acc. no. 7855), TepiBoroDhan (Kalo) (Acc. 8058), Tepu (Acc. 7473) and TopaBoro (Acc. 4981). Ahmed *et al.* (2008) for rice (*Oryza sativa* L.) accessions having duplicate name, Pachauri *et al.* (2017) for Agro-morphological characterization and Gupta *et al.* (2023) using morphological descriptors also found highly significant variability among their studied rice germplasms.

Table 2. Variance analysis (ANOVA) and mean square from 25 TepiBoro genotypes for the 15 studied morphological characters.

Traits	Mean Squares		
	Replication (df= 2)	Genotypes (df= 24)	Error (df= 49)
Leaf length (LL) (cm)	0.045	205.613**	1.509
Leaf width (LW) (cm)	0.000	0.062**	0.002
Leaf area (LA) (cm^2)	0.015	204.577**	2.646
Culm diameter (CD) (mm)	0.115	0.925**	0.017
Effective tiller number (ETN)	5.120	5.500**	0.358
Panicle length (PL) (cm)	0.000	33.221**	0.190
Plant height (PH) (cm)	3.640	1322.940**	3.920
Days to maturity (DM)	0.000	407.980**	0.001
Filled grain per panicle (FGPP)	200.000	3325.600**	33.000
filled grain per panicle (UFGPP)	8.000	38.731**	5.714
Grain length (GL) (mm)	0.000	2.394**	0.006
Grain width (GW) (mm)	0.000	0.371**	0.002
Dehulled grain length breadth ratio (DGLBR)	0.002	0.402**	0.002
Thousand grain weight (TGW)(g)	8.611	51.910**	0.527
Grain yield (GY) (g/plot).	2812.000	64777.000**	172.000

** Significant at 1% levels of probability, df = degrees of freedom.

Table 3. Variability of 25 similar and duplicate named TepiBoro group of rice germplasm for 15 important morphological characters during Boro 2020-21 season

Sl. no.	Local name	Acc. No.	Code name	LL (cm)	LW (cm)	LA (cm^2)	CD (mm)	ETN	PL (cm)	PH (cm)	DM (Days)	FGPP	UFGPP	GL (mm)	GW (mm)	DG LBR	TGW (g)	GY (g/plot)
1	Tape Boro	7297	TPB1	42.4efg	0.78e	24.8i	3.3efg	9a	23.4e	114.8f	138o	126bc	14cdefg	7.89f	3.15ef	2.04gh	24b	501.6kl
2	Tepa IRRI	7855	TPB2	40.4ghi	1.22a	37.0abc	4.0b	7bc	25.0cd	113.4fg	154g	126bc	10ghi	7.29i	2.92h	2.33d	18.8ef	930.2a
3	Tepakain	1532	TPB3	32j	1.04bcd	25.0i	3.0hi	5d	19.0i	94.4i	140m	70gh	14cdefg	7.34hi	3.40ab	1.71n	24b	571.7i
4	TepiBoro	258	TPB4	44.8cd	1.06bcd	35.6bcd	3.0hi	8ab	20.4h	101.0h	134q	68ghi	17abcd	7.26ij	2.73k	2.19ef	17.9fg	658.7efg
5	TepiBoro	930	TPB5	43.2cdef	0.98d	31.7gh	3.4def	7bc	25.2c	126.4c	145j	126bc	16abcde	7.21ijk	3.02g	1.85lm	20.5d	460.9m
6	TepiBoro	3998	TPB6	47b	1.08bc	38.1ab	3.4def	8ab	21.6g	113.4fg	143k	132bc	13efgh	7.57g	2.63lm	2.40cd	15.9h	515.8k
7	TepiBoro	4526	TPB7	50.4a	1.04bcd	39.3a	4.8a	5d	29.4a	133.0b	142l	81ef	9hi	6.85l	2.89hi	1.78m	17.8fg	540.3j
8	TepiBoro	4959	TPB8	45.2bc	1.04bcd	35.3bcde	3.5cde	7bc	21.6g	123.4cd	153h	60ij	14cdefg	7.14ijk	2.80j	2.02hi	16.7gh	637.2gh
9	TepiKhorch	931	TPB9	25.4lm	0.82e	15.6m	2.5lk	5d	20.4h	91.2ij	137p	74efgh	11fgh	8.98b	3.34bc	2.25e	24.7b	631.6h
10	Tepi Sail	1712	TPB10	44.6cde	1.12b	37.4abc	3.5cde	9a	24.6cd	138.6a	150i	97d	15bcdefg	7.13jk	2.70kl	2.34cd	16h	576.7i
11	Tepi Sail	1724	TPB11	41.6fgh	1.04bcd	32.5efgh	3.5cde	8ab	21.6g	125.2cd	150i	84e	12fgh	8.11e	2.62m	2.38cd	16h	661.5ef
12	TepiBoroDhan (Kalo)	8058	TPB12	29.4k	0.80e	17.6lm	2.3l	9a	23.4e	115.8ef	139n	40k	6i	7.56g	2.48n	2.51b	24.9b	915.9a
13	Tepu	7382	TPB13	41fghi	1.04bcd	31.9fgh	4.2b	9a	30.0a	103.6h	176a	134bc	18abc	8.40c	3.33bc	2.20ef	13.9i	582.2i
14	Tepu	7473	TPB14	33j	1.22a	30.2h	3.2fgh	8ab	21.4g	104.0h	156e	128bc	17abcde	8.28cd	3.17ef	2.12fg	23.8b	924.5a
15	Tepu IRRI	6213	TPB15	42.6defg	1.24a	39.6a	3.4def	6cd	23.6e	113.2fg	156e	136b	14cdefg	8.17de	3.21de	2.13f	19.9de	647.7fgh
16	TepuDhan	7931	TPB16	43.2cdef	1.04bcd	33.6defg	2.5kl	8ab	27.8b	90.2j	142l	146a	19ab	7.83f	3.26cd	1.92jkl	10j	395.6n
17	TepuDhan	7986	TPB17	23.8m	1.06bcd	18.9kl	2.7jk	6cd	21.4g	79.6k	158c	153a	20a	5.65n	1.98o	2.13f	23.9b	724.8d
18	Topa	2256	TPB18	39i	0.76e	22.2ij	3.7c	6cd	22.4f	122.6d	128r	75efg	13defgh	7.28i	3.17ef	2.23e	24.6b	741.8d
19	TopaBoro	4373	TPB19	24.2m	0.66f	11.9n	3.7c	6cd	25.0cd	119.0e	173b	64hi	10ghi	7.47gh	3.42a	1.69n	24.2b	582.4i
20	TopaBoro	4981	TPB20	26.8l	1.04bcd	20.9jk	2.8ij	7bc	21.2g	73.2l	155f	100d	11fgh	10.26a	2.66klm	3.53a	26.8a	797.3c
21	TopaBoro	4983	TPB21	40hi	1.06bcd	31.8fgh	3.0hi	8ab	19.4i	63.2m	156e	71fgh	12fgh	7.47gh	3.36ab	1.99hij	20.2d	675.0e
22	TopaBoro	5041	TPB22	26.6l	1.04bcd	20.7jk	3.1fgh	8ab	14.2j	61.0m	157d	50j	6i	7.10jk	3.10f	1.95ijk	21.8c	483.5l
23	TopaBoro	62	TPB23	43cdef	1.08bc	34.8cdef	3.6cd	9a	22.4f	110.2g	134q	82e	15bcdefg	7.35hi	2.64lm	2.40cd	18fg	830.9b
24	Tupa	1811	TPB24	51.2a	1.02cd	39.2a	3.6cd	9a	23.4e	123.0cd	142l	124c	11fgh	6.16m	2.72k	1.88kl	17.9fg	641.7fgh
25	Tupa	2257	TPB25	40.4ghi	1.06bcd	32.1fgh	3.4def	8ab	24.4d	125.6cd	140m	125bc	15bcdef	8.37c	2.82ij	2.42c	21.9c	496.8kl
Min				23.8	0.66	11.9	2.3	5	14.2	61	128	40	6	5.65	1.98	1.69	10	395.6
Mean				38.4	1.01	29.5	3.3	7	22.9	107.2	148	99	13	7.60	2.94	2.18	20.2	645.0
Max				51.2	1.24	39.6	4.8	9	30.0	138.6	176	153	20	10.26	3.42	3.53	26.8	930.2
Std, Dev.				8.3	0.1	8.2	0.6	1.4	3.3	21	11.7	33.2	3.6	0.9	0.4	0.4	4.2	146.9
SE				1.66	0.03	1.65	0.11	0.28	0.67	4.20	2.33	6.65	0.73	0.18	0.07	0.07	0.83	29.39
CV				21.5	14.2	27.9	16.8	19.1	14.5	19.6	7.9	33.7	27.4	11.7	12.0	16.8	20.7	22.8
LSD (0.05)				3.2	0.1	3.2	0.2	0.5	1.3	8.2	4.6	13.0	1.4	0.4	0.1	0.1	1.6	57.6

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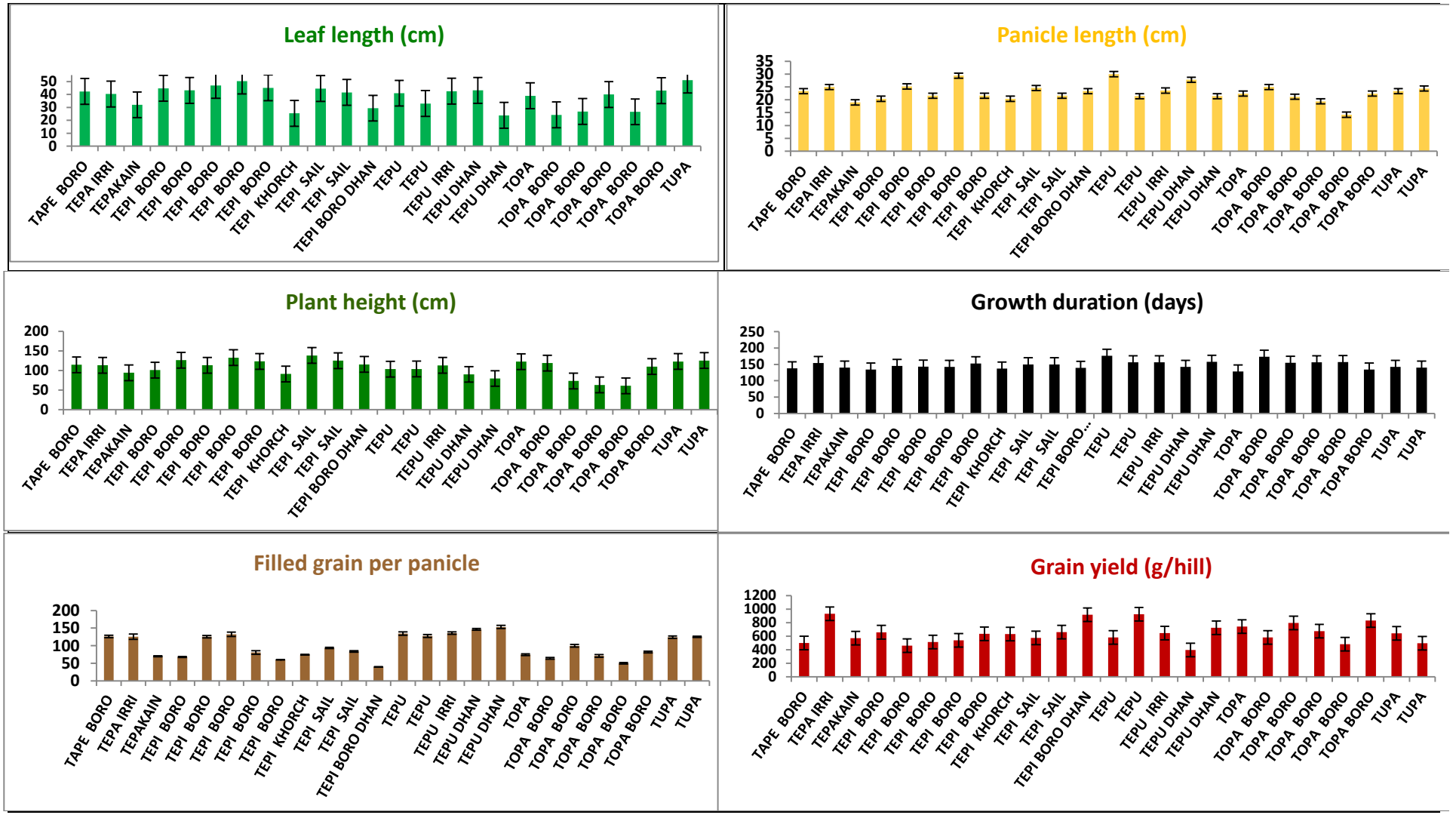


Fig. 1 Bar diagram with error bars from mean values of six morphological traits for 25 TepiBoro rice landraces.

Principal Component and Cluster analysis for Grouping: The similar and duplicate named TepiBoro rice germplasms were grouped into five clusters on the basis of cluster analysis (Table 4). The maximum genotypes (11) were grouped into cluster III and the minimum (1) in cluster I. Table 5 indicates the variations among the intra- and inter-cluster distances. The highest intra-cluster distance was recorded for cluster V (1.29) and the lowest for cluster I (0.0). The inter-cluster D^2 values ranged from 11.77 to 43.13 which indicate wide range of diversity. The lowest inter-cluster distance was observed between cluster IV and V and the highest between clusters I and V. Table 6 indicates the cluster means of studied TepiBoro. Cluster I showed the highest leaf length (43.2 cm), leaf area (33.6 cm^2), effective tiller number (8), panicle length (27.8 cm), filled grains per panicle (146), grain length (7.83 mm) and the lowest plant height (90.2 cm), days to maturity (142 days). Similarly, cluster V showed the highest grain yield (923.5 g) and moderate LB ratio (2.32) and the lowest un-filled grains per panicle (11) and cluster IV showed the highest DG LBR (2.57) and TGW (23.32 g). Therefore, the genotypes under clusters V may be selected for crossing with the Tepu Dhan (TPB16) genotype of cluster I and genotypes of cluster IV for developing improved TepiBoro rice variety with high yielding and others good agronomic traits. Similar trend of findings were also reported by Ahmed *et al.* (2024) and Islam *et al.* (2017).

Sensory Test of Leaf Aroma: The combined result of the sensory test for leaf aroma by five expert persons revealed that all of the evaluators of the sensor test found aroma from the leaf of TepiBoro (Acc. 4526), TepuDhan (Acc. 7931) and TopaBoro (Acc. 4373), followed by two persons from Tepu IRRI (Acc. 6213) and Tupa (Acc. 2257) etc (Table 7). Hien *et al.* (2006b) also evaluated 40 rice varieties collected from seven Asian countries through sensory test. Ndikuryayo *et al.* (2023) screened aroma in 215 rice genotypes with diverse origins (Africa and Asia) through sensory test. Hien *et al.* (2006a) tested 70 rice cultivars originating from nine Asian countries and found a strong correspondence of the aroma character.

Table 4. Distribution of 25 similar and duplicate named TepiBoro group of rice germplasm into five clusters for 15 morphological characters.

Cluster	Genotypes		Local name (Code) (BRRI Genebank accession no.)
	Number	%	
I	1	4	Tepu Dhan (TPB16)(7931)
II	6	24	Tape Boro (TPB1)(7297), Tepi Boro (TPB5)(930), Tepi Boro (TPB6)(3998), Tepi Boro(TPB7)(4526), Topa Boro(TPB22)(5041), Tupa (TPB25)(2257)
III	11	44	Tepa Kain (TPB3)(1532), Tepi Boro (TPB4)(258), Tepi Boro (TPB8)(4959), Tepi Khorch (TPB9)(931), Tepi Sail (TPB10) (1712), Tepi Sail (TPB11)(1724), Tepu (TPB13)(7382), Tepu IRRI (TPB15)(6213), Topa Boro (TPB19)(4373), Topa Boro (TPB21)(4983), Tupa (TPB24)(1811)
IV	4	16	Tepu Dhan (TPB17)(7986), Topa (TPB18)(2256), Topa Boro (TPB20)(4981), Topa Boro (TPB23)(62)
V	3	12	Tepa IRRI (TPB2)(7855), Tepi Boro Dhan (Kalo)(TPB12)(8058), Tepu (TPB14)(7473)

Table 5. Average intra-(bold) and inter-cluster distances (D^2) for 15 morphological characters of 25 similar and duplicate named TepiBoro group of rice germplasm.

Cluster	I	II	III	IV	V
I	0				
II	13.33	0.97			
III	20.85	13.84	0.95		
IV	35.92	28.2	15.91	1.03	
V	43.13	36.92	23.84	11.77	1.29

Table 6. Cluster means of 25 similar and duplicate named TepiBoro group of rice germplasm for 15 morphological and yield contributing characters.

Cluster	LL (cm)	LW (cm)	LA (cm^2)	CD (mm)	ETN	PL (cm)	PH (cm)	DM (Days)	FGPP	UFGPP	GL (mm)	GW (mm)	DG LBR	TGW (g)	GY (g/plot)
I	43.2	1.00	33.6	2.5	8	27.8	90.2	142	146	19	7.83	3.26	1.92	10	395.6
II	41.67	1.00	31.12	3.57	7.5	23.03	112.37	144.17	106.67	12.17	7.5	2.93	2.07	20.32	499.82
III	39.33	1.00	30.53	3.32	7.27	22.64	108.71	151.55	89	13.45	7.6	3.06	2.07	19.22	624.22
IV	33.15	1.00	24.2	3.2	7	21.85	96.4	143.75	102.5	14.75	7.63	2.61	2.57	23.32	773.7
V	34.27	1.07	28.27	3.07	8	23.27	111.07	149.67	98	11	7.71	2.86	2.32	22.5	923.53

Table 7. Combined result of leaf aroma response of 25 TepiBoro rice cultivars based on sensory test by using 1.7% KOH solution

Sl. no.	Genotype name	Acc. No.	Sensory test (Smelling)*					Combined Status
			P1**	P2	P3	P4	P5	
1	Tape Boro	7297						
2	Tepa IRRI	7855						
3	Tepakain	1532						
4	TepiBoro	258						
5	TepiBoro	930	+					+
6	TepiBoro	3998			+			+
7	TepiBoro	4526	+	++	++	++	++	+/++
8	TepiBoro	4959	+					+
9	TepiKhorch	931						
10	Tepi Sail	1712	+					+
11	Tepi Sail	1724				+		+
12	TepiBoroDhan (Kalo)	8058						
13	Tepu	7382						
14	Tepu	7473						
15	Tepu IRRI	6213	+				+	+
16	TepuDhan	7931	+	++	++	++	++	+/++
17	TepuDhan	7986	+					+
18	Topa	2256						
19	TopaBoro	4373	+	+	++	++	++	+/++
20	TopaBoro	4981				+		+
21	TopaBoro	4983						
22	TopaBoro	5041						
23	TopaBoro	62						
24	Tupa	1811	+					+
25	Tupa	2257	+			+		+

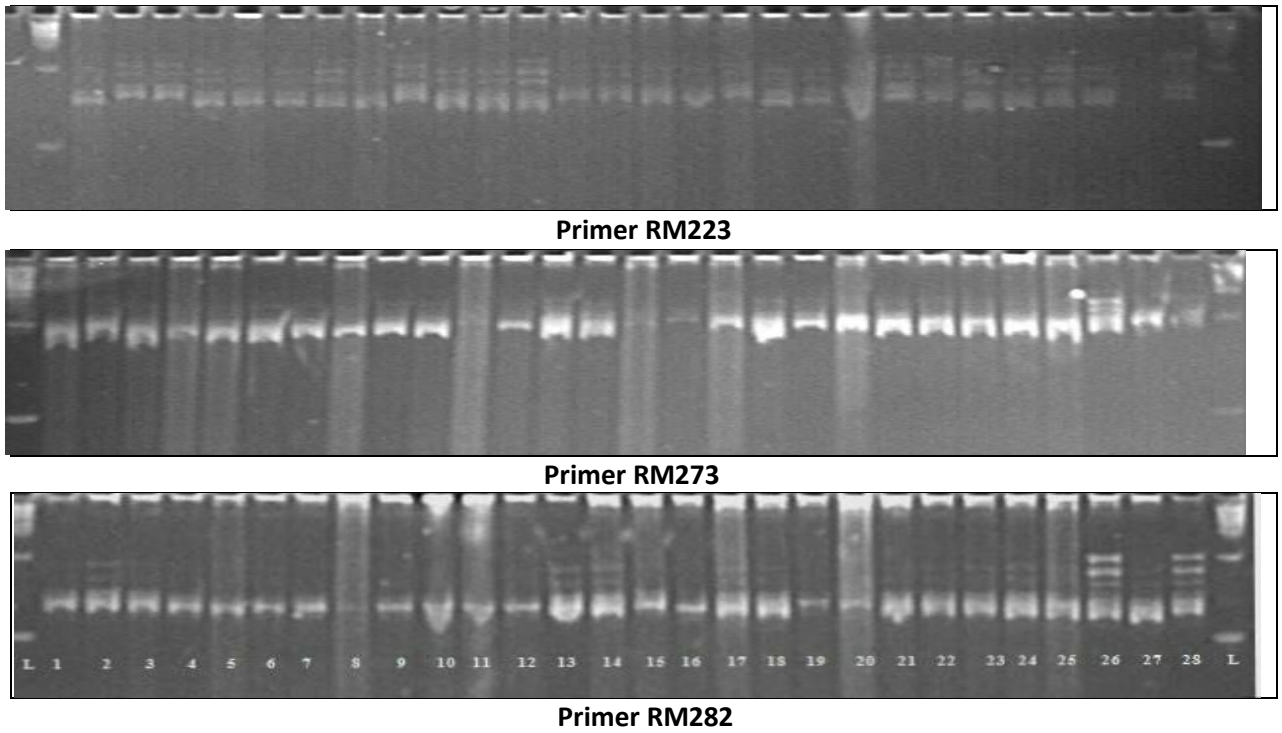
*Aroma present= **+**, Aroma more moderately present=++, Aroma absent= **–**. **P=Person.

Molecular Characterization of Leaf Aroma: All the three microsatellite primers revealed the polymorphism among the all studied landraces and a total of ten alleles were identified (Table 8). The average number of alleles per locus was 3.33, ranging from 3 (RM273 and RM282) to 4 (RM223). The gene diversity ranged from 0.31 (RM282) to 0.56 (RM223) with an average of 0.41. The PIC for the SSR loci ranged from 0.29 (RM282) to 0.47 (RM223). Primer RM223 had the highest PIC value as well as Gene diversity value and was considered to be the best marker to evaluate the studied 25 TepiBoro rice landraces. The frequency of the most common allele at each locus ranged from 50.0% (RM223) to 82.14% (RM282). On average, 70.24% of the 25 TepiBoro rice landraces shared a common major allele at any given locus. Figure 2 presents the DNA profiles of 25 TepiBoro rice landraces with SSR marker RM282. The SSR primer RM223 produced a distinct band at 142 bp in both the highly scented check varieties BRRI dhan34 and Bangladesh Kalijira (Acc. no. 247), as well as in Tepu Dhan (Acc. 7931) and Topa Boro (Acc. 4373), which also exhibited leaf aroma in sensory tests (Fig. 2). Additionally, another scented check, Kalizira (Acc. no. 1589) displayed a similar band at 132 bp, which was also found in Tepi Boro (Acc. 4526), which showed leaf aroma in the sensory test. Again, the SSR primer RM282 revealed a distinct band at 105 bp in Kalizira (Acc. no. 1589) with similar results in Tepi Boro (Acc. 4526) and Tepu Dhan (Acc. 7931), both showing leaf aroma in the sensory test. BRRI dhan34 also showed a 110 bp band, similar to Topa Boro (Acc. 4373), which was found to have leaf aroma in sensory tests. Additionally, 10 lightly

scented accessions were identified through sensory tests, showing distinct or nearly identical band patterns compared to the check varieties. Nanoukon *et. al.* (2024) also identified aromatic rice by RM223 in 72 Benin rice. The genetic distance-based unrooted neighbour-joining tree revealed that the studied germplasm were grouped into three major clusters using Mega software (Fig. 3). The highest numbers of germplasm (19) were found in cluster III, followed by cluster I (7). The cluster II contained the lowest (2) number of germplasm. Earlier, Hien *et al.* (2006a); Singh *et al.* (2007); Sarhadi *et al.* (2009) and Smritia *et al.* (2016) reported similar or comparable trend of results in aromatic rice.

Table 8. Allele and gene diversity with polymorphism information content (PIC) found among 25Tepi Boro rice germplasm for three microsatellite markers.

SL. No.	Marker	Chro. No.	Position (cM)	Motif	Allele No.	Unique allele	Size range (bp)	Size (bp)	Freq (%)	Gene diversity	PIC
1	RM223	8	80.5	(CT)25	4	2	125-148	148	50.0	0.56	0.47
2	RM273	4	94.4	(GA)11	3	-	199-223	199	78.57	0.36	0.33
3	RM282	3	100.6	(GA)15	3	-	98-110	110	82.14	0.31	0.29
				Max.	4			199	82.14	0.56	0.47
				Min.	3			110	50.0	0.31	0.29
				Total	10	2			210.78	1.23	1.09
				Mean	3.33				70.24	0.41	0.36



Legend: 1. Tape Boro (Acc. 7297), 2. Tepa IRRI (Acc. 7855), 3. Tepakain (Acc. 1532), 4. TepiBoro (Acc. 258), 5. TepiBoro (Acc. 930), 6. TepiBoro (Acc. 3998), 7. TepiBoro (Acc. 4526), 8. TepiBoro (Acc. 4959), 9. TepiKhorch (Acc. 931), 10. Tepi Sail (Acc. 1712), 11. Tepi Sail (Acc. 1724), 12. TepiBoroDhan (Kalo) (Acc. 8058), 13. Tepu (Acc. 7382), 14. Tepu (Acc. 7473), 15. Tepu IRRI (Acc. 6213), 16. TepuDhan (Acc. 7931), 17. TepuDhan (Acc. 7986), 18. Topa (Acc. 2256), 19. TopaBoro (Acc. 4373), 20. TopaBoro (Acc. 4981), 21. TopaBoro (Acc. 4983), 22. TopaBoro (Acc. 5041), 23. TopaBoro (Acc. 62), 24. Tupa (Acc. 1811), 25. Tupa (Acc. 2257), 26. Kalizira (Acc. no. 1589), 27. Bangladesh Kalijira (Acc. 247), 28. BRRI dhan34 and Ladder=1Kb.

Fig. 2. DNA profile of 25 TepiBoro rice germplasm with primer RM223, RM273 and RM282.

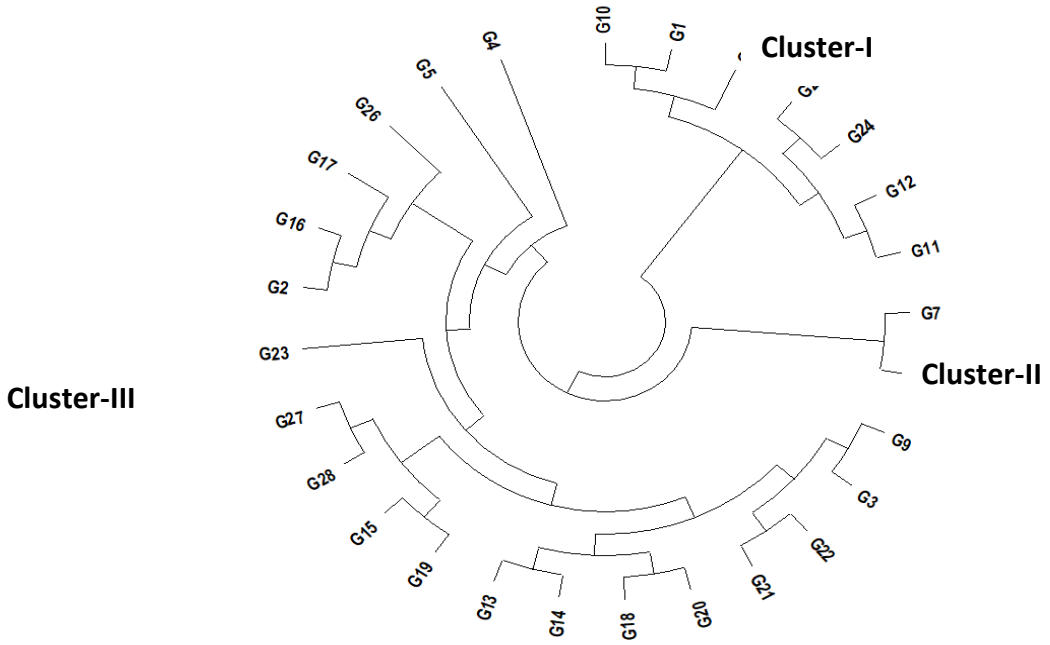


Fig. 3. Unrooted neighbour-joining tree showing the genetic relationships among 25 TepiBoro rice along with Kalizira, Bangladesh Kalijira and BRRI dhan34 by using three SSR markers

Conclusion

Characterization of germplasm is very important as it reveals the variability and potentiality. Moreover, it ensures effective collection, evaluation, utilization and conservation management of Genebank. Considerable ranges of useful genetic variations were observed among the studied 25 TepiBoro rice landraces for all 15 quantitative Agronomical traits though they have the similar or duplicate names. It is revealed that studied landraces offer valuable genes or traits, which needs to be utilized for TepiBoro rice improvement or new aromatic Boro rice variety development. For PCA, the studied TepiBoro genotypes were grouped into five clusters. The genotypes under clusters V may be selected for crossing with the genotypes of cluster I and cluster IV for developing improved TepiBoro rice variety. On the other hand, Primer RM223 was considered to be the best marker to evaluate the studied 25 TepiBoro rice landraces. The unrooted neighbour-joining tree revealed that the studied germplasm were grouped into three major clusters. The presence of aroma in TepiBoro rice germplasm need to be validated with the combination of sensory test, gas chromatography-mass spectrometry-selected ion monitoring (GC-MS-SIM) and molecular analysis with sufficient aroma linked SSR markers, since aroma in rice is affected by genetic composition and as well as the environment conditions. The identified superior genotypes were Tepa IRRI (Acc. no. 7855), TepiBoro Dhan (Kalo) (Acc. 8058), Tepu (Acc. 7473) and TopaBoro (Acc. 4981) for different studied Agronomical traits, TepiBoro (Acc. 4526), TepuDhan (Acc. 7931) and TopaBoro (Acc. 4373) by sensory test for leaf aroma and Topa Boro (Acc. 4373), Tepi Boro (4526) and Tepu Dhan (7931) by SSR primers for grain aroma. Finally, this study would be useful for breeders to choose and identify the potential pre-breeding materials for developing aromatic TepiBoro rice variety. Moreover, the candidate genes for rice grain aroma could be identified by combining QTL mapping and transcriptome profiling approaches.

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