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RESEARCH ARTICLE

Assessment of genetic diversity in rice [*Oryza sativa* L.] germplasm based on agro-morphology traits and zinc-iron content for crop improvement

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Abstract Genetic resources of landraces (84 cultivars) were collected from various agro-ecological regions of West Bengal and adjoining areas and characterized based on qualitative and quantitative agro-morphological descriptors along with zinc (Zn) and iron (Fe) content. The DUS protocol was employed to study 16 agro-morphological passport data such as: vegetative data (anthocyanin pigmentation, plant habit), reproductive data (flag leaf attitude, stigma colour, panicle attitude), including eight grain quality traits: grain length, grain width, 1000 grains weight, kernel length, kernel breadth etc. Highest seed weight was found in cultivar Khechri (32.04 g/1000 seeds), collected from Sundarban and least seed weight was 9.6 g/1000seeds in Katharibhog. Maturity duration was found very short (<100 days) in Jumla Marshi (97 days) collected from world's coldest rice growing area, Jumla, Nepal. Penultimate leaves breadth was observed broad (>2 cm) in one cultivar Jungli (2.3 cm). Seeds per panicle were 180 in Chinisakkar (medium range), 177 in Dudheswar, and 151 in Ladua. Flag leaf was found in erect condition in late observation in Dudheswar, Enda and Ghiosh. Seventeen cultivars were grouped in the aromatic rice category out of total 84 local landraces. Twenty-one cultivars were with awn, whose length ranges from 1.6 mm (Anandi) to 22.5 mm (Tulaipanji). Kernel colour varies from red, yellowish, brownish, creamy white to white. Kernel length varies from 4 mm to 8 mm and breadth 1.90 mm to 3 mm. Kernel length/breadth ration varied from 1.6 to 3.9. Highest ratio of L/B was found in Pusa Basmati 1(3.9) and lowest in Dudhey (1.6). Elongation ration was highest in Kalokure (2.07) and lowest in Phoolpakri (0.62). Nutritional values of mineral contents of iron (Fe) and zinc (Zn) were estimated in all cultivars by Atomic Absorption Spectrophotometic method. Iron concentration varies from 0.25 μg/g to 34.8 μg/g and zinc from 0.85 μg/g to 195.3 μg/g in the landraces. Highest iron containing rice was Swetonunia with 34.8 μg/g and highest amount of Zn was found in Nepali Kalam which was 195.3 μg/g. Anaerobic germination (AG) was observed in 18 cultivars among 84 land races (viz. Jungli, Kumrogore, Dudheshwar, Rambhog and Tulsi etc.), the trait is highly desired by the rice breeder for the introgression of this gene (QTL) to the HYV for direct seeding in the field for saving labour cost and reduced maturity time. Dendrogram showed genetic diversity among 84 landraces by grouping them into five major clusters. All the descriptors evaluated in this study have showed that there is enough genetic diversity among landraces and this information can be helpful to the breeders to choose the right parent for crop improvement.

Keywords Rice landraces · Aromatic rice · Genetic diversity · Agro-morpho-quality traits · Anaerobic germination · Micronutrient-Zinc and Iron

Introduction

Rice (*Oryza sativa* L.) is the most important staple food crop in the world and provides food and livelihood security to over half (~3.5 billion) of the global population. Approximately 150 million tons of rice is needed by 2030 to feed 1.378 billion Indian people (Goyal and Singh 2002) and ~850 million tons to feed 5 billion rice consumers in the world (at least 1.1 % yield increase is required every year) (Subudhi et al. 2006). Different breeding strategies such as introduction, selection, recombination breeding, heterosis breeding etc. were practiced during and after green revolution to increase the yield levels of rice. Although, yield was improved using these

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breeding strategies, the yield levels have stagnated subsequently. Breaking the yield ceiling through genetic improvement becomes the priority in rice research (Khush 2005). Aggressive introduction of modern high yielding varieties (HYV) has resulted in the loss of a large number of landraces especially from irrigated lands, which leads to narrowing down the gene pool of the rice diversity (Rana et al. 2000). The diversity of rice genetic resources is depleting. The diversity of rice has been well used in efforts to solve today's food problems (Khush and Virk 2000) since the green revolution. Rice landraces were collected over several decades to become 'parents' of the high-yielding, pest-resistant, and well-adapted varieties that resulted in unprecedented increase in rice yields. India is accomplished with a great diversity of rice germplasm in its vast territorial land areas (agro-ecological area). Any crop improvement program should aim at broadening the genetic base of the breeding stock (Collard et al. 2005). Wide genetic resources may be required to either increase the genepool for germplasm improvement or for the development of new cultivated varieties (Subudhi et al. 2006). Different agro-morphological traits (passport data) play very important roles for their characterization and varietal identification which ultimately helps rice breeder for its improvement (Laxuman et al. 2011). Agro-morphological properties of rice cultivars determine their yield potential, local agronomic suitability and ability to escape from or to tolerate biotic and abiotic stresses. As for example, one local rice cultivar, FR13A has revealed tolerance to submergence for 10 to 12 days and was used as donor to improve rice cultivars with tolerance to submergence (Xu and MacKill 1996). Rice is a poor source of essential micronutrients such as Fe and Zn (Bouis and Welch 2010). Diet deficient in minerals mainly Fe and Zn in staple food crops causes *'hidden hunger'* or micronutrient malnutrition in developing countries (Welch and Graham 2004). Some landrace may have high micronutrient content and can be screened from the germplasm. Recent trend in rice production is marked with the shift from transplanting to direct seeding notably in areas where there is scarcity of irrigation water and high cost of labor. It is important to choose varieties that have anaerobic germination and exceptional seedling vigor for use in direct seeding. Improving the productivity of rice cultivation shall involve screening of available germplasm for tolerance to biotic and abiotic stresses, and intensifying of research on the genetics and physiological mechanisms of tolerance to these stresses would be important. Thus accurate assessment of the levels and patterns of genetic diversity can be invaluable in crop breeding for diverse applications including introgression desirable genes from diverse germplasm into the available genetic base and for widening the narrow genetic base of the developed varieties. It is therefore, pertinent to capture and conserve the genetic diversity existing in farmers land either in ex situ, in the form of seed or on farm and also for protecting the sovereign rights.

Thus, the present investigation was intended to characterize the genetic resources of local landraces of rice [*Oryza sativa* L.] on the basis of agro-morpho-quality traits (passport data) for genetic diversity analysis including the genetic evaluation of micronutrient content and anaerobic germination.

Materials and methods

Experimental site

The present investigation was carried out during kharif 2010–2012 at Experimental Rice Field, Department of Botany, University of North Bengal, WB, India, representing the low land with sandy-loam soil, acidic pH which is located at latitude of 26° 84′ North and longitude of 88° 44′ East.

Experimental material

The experimental material for the present investigation comprised of 84 local landraces of rice (*Oryza sativa* L.) cultivars of West Bengal and adjoining areas. Landraces were collected from the farmer's field, threshing yard of West Bengal and adjoining areas. Relevant habitat information, unique features, common name and traditional knowledge about the landraces were gathered from local farmers during end of kharif season in 2010 and 2011.

Recording of observation

The data on morphological, physicochemical and other traits are recorded from three randomly selected representative plants in all the genotypes in each replication. The standard method of DUS test (Distinctiveness, Uniformity and Stability, Govt. of India) was used for recording observation for each of the character which includes the following- plant height, stem thickness, penultimate leaf length, penultimate leaf breadth, flag leaf length, flag leaf breadth, panicle length, panicle branching, seed/panicle, grain weight, grain length, awn length (Bioversity protocols: www.bioversity.org). The software STATISTICA 12 was run to construct the dendrogram based on morpho-quality traits of 84 landraces for their genetic diversity assessment.

Measurement of physico-chemical and cooking quality traits

The following physical and cooking quality characters viz., kernel length (KL), kernel breadth (KB), ratio of KL/KB, cooked kernel length (CKL), cooked kernel breadth (CKB), ratio of CKL/CKB, linear elongation ratio, cooking time was measured as per the descriptors suggested by IRRI.

Alkali spreading value/gelatinization temperature

Gelatinization temperature (GT) was estimated based on alkali spreading value (ASV). The alkali spreading value (ASV) was determined by the method of Little et al. (1958). Two sets of seven polished rice kernels of each cultivar was placed in Petri plates containing 10 ml of freshly prepared 1.7 % KOH solution. The kernels were arranged in such a way to provide space between kernels for spreading. The plates were covered and incubated at 30 °C for 23 h. The appearance and disintegration of kernels were rated visually as follows- Score 1: kernels unaffected, score 2: kernel swollen, score 3: kernel swollen with collar incomplete and narrow, score 4: kernel swollen with collar complete and wide, score 5: kernel split or segmented with collar complete and wide, score 6: kernel dispersed, merging with collar and score 7: kernels were dispersed, intermingled and disappeared completely. A low AVS corresponds to a high GT, conversely, a high ASV indicates a low GT. The aroma of polished rice grains was determined by a sensory evaluation protocol according to the method of Sood and Siddiq (1978). Ten milled rice grains were placed in a 50 mm Petri plate containing 10 ml of 1.7 % KOH and incubated at room temperature for 10 min with lids on. The lids were then opened one by one and samples were smelt and rated for aroma by sensory evaluation in a scale of 0 to 3, where 0 means non-aromatic and 3 means highly aromatic. Two blind checks, Pusa Basmati 1(moderately aromatic) and Ranjit (non aromatic) were included in this observation.

Gel consistency

The GC was measured according to the method of Cagampang et al. (1973). Briefly, 100 mg rice flour was weighted in duplicate into 13 mm×100 mm culture tubes, to which 200 μl of ethyl alcohol (95 %) containing 0.025 % thymol blue was added to prevent clumping of the powder during gelatinization. Two milliliter of 1 N KOH was added. The contents of the tube were mixed thoroughly using a Vortex Genie mixer. The test tubes were placed in a vigorously boiling water bath for 8 min. Tubes were removed from the water bath and left at room temperature for 5 min. Tubes were cooled in an ice-water bath for 15 min then laid down horizontally on a table surface. The blue gel length was measured 1 h later as the distance from the bottom of the tube to the front of the gel migration. The gel length thus obtained provides a measurement of the gel consistency (GC): the longer the distance, softer the gel and classified into three categories: hard GC (30–40 mm length), medium GC (40–60 mm length), and soft GC (61–100 mm length).

Amylose estimation by spectrophotometer

The simplified method of Juliano (1971) was used for estimating the amylose content. One hundred (100) mg flour was transferred to volumetric flask and homogenized with 1 ml of 95 % ethanol and 9 ml of 1 N NaOH. The samples were heated for 10 min in the water bath to gelatinize the starch. After cooling, it was made up 100 ml with distilled water. Half ml aliquots of each test solution were separately placed in two test tubes. Five ml of water, 0.10 ml of acetic acid and 0.20 ml of iodine solution were added. An additional 4.20 ml of water was added into each tube to make the total volume of reaction mixture to 10 ml. Absorbance was measured using a spectrophotometer (Systronic, India) at a wavelength of 620 nm. The amylsoe content was determined using a standard curve developed from known quantities of purified potato amylose from Sigma, USA.

Fe and Zn content estimation

Iron (Fe) and Zinc (Zn) content of 84 local cultivars were estimated according to standard method (Lindsey and Norwell 1969) by Atomic Absorption Spectrophotometer (Varian Spectra AA50B). Seeds from 84 cultivars were dehusked gently using a palm dehusker. One gram oven dried ground dehusked seed samples were placed in a 150 ml conical flask. To this 25–30 ml diacidic mixture (HNO_3:$HClO_4$; 5:1 v/v) was added and kept overnight. Next day, it was digested by heating till clear white precipitates settled down at the bottom. The crystals were dissolved by diluting in double distilled water. The contents were filtered through Whatman No. 42 filter paper. The filtrates were made to 50 ml with double distilled water and used for the determination of iron and zinc contents. Concentration was expressed in μg/g of rice against standard curve.

Anaerobic germination method

Seed dormancy was broken by incubating the seeds at 50 °C for 5 days. The seeds were subjected to imbibitions overnight by soaking them in water and incubate for synchronous germination in an incubator at 28 ° C in the dark (Boamfa et al. 2003). Germinating seeds at the pigeon breast stage (pre-germinated seeds of about 3 days old) were seeded for anaerobic experiment (Manangkil et al. 2008).

i. Test Tube Method

In this method pre- germinated seeds were kept in a glass test tube (25 mm in diameter and 150 mm in height) filled with 10 cm deep distilled water. At the 7th day of incubation, shoot (coleoptiles) lengths were measured.

ii. Water logged soil Method

Pre- germinated seeds were sown in paddled soil filled in plastic cup of 4×5 cm at the depth of 3 cm from the soil

Table 1 Characterization of 84local rice landraces based on 16 agro-morphological traits

Agro-morphological characters	Colour pattern/type	Frequency	Percent
1. Coleoptile colour on tenth days	Colourless	83	98.81
	Green	–	–
	Purple	1	1.19
2. Basal leaf sheath colour on booting stage	Uniform purple	3	3.57
	Green	81	96.43
	Light purple	–	–
	Purple lines	–	
3. Leaf intensity of green colour on booting stage	Light	14	16.6
	Medium	2	2.38
	Dark	68	80.95
4. Colour of ligule on booting stage	White	87	97.71
	Light purple	1	2.29
	Purple	–	
5. Leaf sheath: Intensity of anthocyanin colouration	Very weak	81	96.43
	Weak	2	2.38
	Medium	–	–
	Strong	1	1.19
	Very strong	–	
6. Spikelet colour of stigma	White	80	95.24
	Light green	–	4.76
	Yellow	–	
	Light purple	–	
	Purple	4	4.76
7. Anthocyanin colouration of auricles	Colourless	83	98.8
	Light purple	–	–
	Purple	1	1.19
8. Spikelet colour of tip of lemma	White	72	85.71
	Yellowish	–	–
	Brown	7	8.33
	Red	5	5.95
	Purple	–	–
	Black	–	–
9. Time of heading (50 % of plants with panicles)	Very early (<71 days)	5	5.95
	Early (71–90 days)	22	26.19
	Medium (91–110 days)	42	50.00
	Late (111–130 days)	13	15.47
	Very late (>131 days)	2	2.38
10. Spikelet density of pubescence	Absent	18	21.42
	Weak	42	50.00
	Medium	24	28.57
	Strong	–	–
	Very strong		
11. Stem length	Very short (<91 cm)	38	45.23
	Short (91–110 cm)	32	38.09
	Medium (111–130 cm)	9	10.71
	Long (131–150 cm)	5	5.95
	Very long (>150 cm)	–	
12. Leaf: Pubescence of blade surface on booting stage	Weak	43	51.19
	Absent	37	44.04
	Medium	4	4.7
13. Panicle curvature of main axis	Straight	25	29.76
	Semi-straight	24	28.57
	Deflexed	28	33.33
	Dropping	7	8.33
14. Leaf collar present/Absent on booting stage	Absent	0	0
	Present	85	100
15. Culm: attitude	Erect	15	17.85
	Semi-erect	64	76.19
	Opened	–	–
	Spreading	5	5.95
16. Flag leaf attitude in late observation	Erect	44	52.38
	Semi-erect	28	33.33
	Horizontal	2	2.38
	Deflexed	10	11.90

surface. Each cup then immersed inside a plastic flat tray filled with 3 cm deep water above the soil surface and placed in a green house. To obtain comparable data with those from the test tube method, seedlings were removed from the soil at the 5th day and their shoot lengths were measured.

Results and discussion

Morpho-metric analysis

Rice germplasm were evaluated to access genetic variability on the basis of 16 important agro- morphological parameters, and eight quality characters. All the (84 cultivars) local land-races of rice under study showed wide range of genetic vari-ation in respect to these traits. These traits belong to three categories viz., qualitative - measured through visual obser-vation like anthocyanin coloration (present or absent), quantitative- measurable traits (leaf length, leaf width etc.), pseudo-qualitative- characters whose range of expression is at least partly continuous but may vary in one measurement

Table 2 Characterization of 84 landraces of rice based on grain quality traits

Sl No.	Name of cultivars	Grain length (GL) mm	Grain breadth (GB) mm	GL/GB ratio	1000Grain wt.(gm)	Awn length (mm)	Bran color	Shape of grain
1	Anandi	7.7	2.9	2.6:1	28.52	1.6	White	Medium
2	Attey	8.1	2.0	3.2:1	21.53	–	White	Medium
3	Aichung	7.6	2.4	3.1:1	16.24	–	White	Medium
4	Swetonunia	7.1	3.0	2.3:1	21.89	–	White	Medium
5	Bharlang	7.2	2.8	2.5:1	24.49	–	White	Medium
6	Badshabhog	6.0	3.0	3:1	25.09	–	White	Medium
7	Bhangeri	8.0	2.9	2.7:1	21.87	–	White	Medium
8	Bagrakalam	7.6	2.0	3.8:1	26.05	–	White	Long
9	Bhadoi	7.7	3.0	2.5:1	21.27	–	Red	Medium
10	Chinisakkar	5.7	2.0	2.8:1	24.00	–	White	Medium
11	Champa	9.0	3.0	3:1	24.10	2.2	White	Medium
12	Champsali	8.7	3.0	2.9:1	21.27	4	Red	Medium
13	Charinagrey	8.8	2.0	4.4:1	17.11	3.4	White	Long
14	Chunakathi	8.7	2.5	3.4:1	18.39	–	CW	Long
15	Chirakhey	6.2	3.0	2.06:1	22.08	–	White	Short
16	Chiniatop	6.1	2.0	2.4:1	10.43	–	White	Medium
17	Chunia	6.2	2.5	2.4:1	16.26	–	YW	Medium
18	Dhanraj	7.8	3.0	2.6:1	21.26	–	White	Medium
19	Dudheswar	7.8	2.0	3.9:1	18.12	–	White	Long
20	Dangimarua	6.8	3.0	2.2:1	19.28	3	CW	Medium
21	Dharia	7.8	3.0	2.6:1	21.19	15.3	White/ red	Medium
22	Dasnunia	7.8	2.5	3.1:1	25.37	9	White	Medium
23	Dudhey	6.6	2.5	2.6:1	17.44	–	White	Medium
24	Dudheykalam	8.6	2.5	3.4:1	26.15	8	CW	Long
25	Enda	7.5	3.0	2.5:1	25.88	12.5	Red	Medium
26	Ghiosh	6.8	2.8	2.4:1	26.98	–	CW	Medium
27	Gokhraj	7.03	2.4	2.9:1	22.07	–	White	Medium
28	Gobindobhog	6.03	2.5	2.4:1	10.68	–	CW	Medium
29	Begunbeej	7	2.0	2.5:1	9.75	–	CW	Medium
30	IR64	8.6	2.5	3.4:1	24.12	–	White	Long
31	Jumla Marsi	8.2	3.0	2.7:1	26.22	–	CW	Medium
32	Phoolpakri	7.4	3.2	4:1	22.94	–	CW	Medium
33	Jungli	7.6	2.5	3.04:1	20.24	–	CW	Medium
34	Jhulur	8.2	3.0	2.7:1	20.01	–	CW	Medium
35	Jeerasari	7.1	2.5	2.8:1	18.16	–	White	Medium
36	Kalonunia	7.5	2.0	3.7:1	21.21	12.5	Red/ white	Long
37	Kattaka	6.9	2.33	3:1	21.14	–	White	Medium
38	Katharibhog	6.3	2.0	3.1:1	9.34	–	White	Medium
39	Kaberi	9.7	3.0	3.2:1	26.44	–	White	Medium
40	Koshia binni	8.5	2.5	3.4:1	21.05	–	Red	Long
41	Kumrogore	7.5	3.0	2.5:1	21.02	–	Red	Medium
42	Kabiraj	9.2	2.0	4.6:1	25.86	22.2	CW	Long
43	Khasha	6.3	2.0	3.1:1	12.35	–	CW	Medium
44	Khamtilai	7.9	3.4	2.8:1	21.73	–	White	Medium
45	Kalojera	6.1	2.5	2.4:1	24.65	–	Red	Medium
46	Kalobhog	6.5	2.0	3.2:1	13.17	–	White	Medium
47	Khechri	7.8	3.0	2.6:1	32.49	18	CW	Medium

Table 2 (continued)

Sl No.	Name of cultivars	Grain length (GL) mm	Grain breadth (GB) mm	GL/GB ratio	1000Grain wt.(gm)	Awn length (mm)	Bran color	Shape of grain
48	Kantarangi	9.06	3.0	3.02:1	30.48	–	Red	Medium
49	Khal khajara	7.4	2.6	2.8:1	20.43	–	White	Medium
50	Kantajinghasal	9.2	2.5	3.6:1	26.08	4.5	Red/CW	Long
51	Kholako dhan	9.2	4.1	2.2:1	28.12	4.8	White	Medium
52	Kalokure	7.7	2.3	2.3:1	20.87	–	White	Medium
53	Laxmansal	8.5	3.4	2.5:1	24.08	–	White	Medium
54	Laxmikajol	9.3	3.0	3.1:1	29.86	–	CW	Medium
55	Ladua	7.6	3.0	2.5:1	22.53	2.4	White	Medium
56	Malsira	7.2	2.5	2.8:1	20.95	15.2	CW	Medium
57	Metung	8.2	2.8	2.9:1	17.37	–	White	Medium
58	Marshi	7.03	2.5	2.8:1	26.05	–	CW	Medium
59	Maipali	8.1	2.5	3.2:1	19.3	–	White	Medium
60	Mahukal	7.9	3.0	2.6:1	29.27	–	Red	Medium
61	Mala dhan	8.1	2.1	3.8:1	20.60	16.6	CW	Long
62	Magursal	7.4	2.5	2.9:1	23.95	–	Brown	Medium
63	Nav dhan	6.0	2.1	2.8:1	14.21	–	White	Medium
64	Newlo dhan	8.1	2.6	3.1:1	27.05	–	CW	Medium
65	Nageswari	7.7	3.0	2.5:1	25.95	12	Red	Medium
66	Nepali Kalam	5.5	2.1	2.6:1	25.49	–	White	Medium
67	Nagra	7.7	2.5	3.08:1	24.00	–	White	Medium
68	Pusa Basmati 1	9.5	2.0	4.7:1	22.29	7.8	CW	Long
69	Pijam	8.3	2.5	3.2:1	16.82	–	White	Medium
70	Pahalman	9.9	2.5	3.9:1	26.95	–	CW	Long
71	Radhunipagal	5.7	2.0	2.8:1	11.72	–	CW	Medium
72	Ranjit	8.2	2.5	3.2:1	14.69	–	White	Medium
73	Sanu Addey	5.3	2.5	2.1:1	21.64	–	CW	Short
74	Solapatnai	10.4	2.5	4.1:1	28.12	–	Red/ white	Long
75	Sukha dhan	7.7	2.5	3.08:1	12.25	–	Red	Medium
76	Sita bhog	5.4	2.0	2.7:1	22.45	–	CW	Medium
77	Sarulalat	9.3	2.5	3.7:1	21.24	4	CW	Medium
78	Tulaipanji	9.0	2.0	4.5:1	15.44	22.25	White	Long
79	Tulsi	5.7	2.5	2.2:1	16.24	–	White	Medium
80	Thulo addey	6.7	2.7	2.4:1	23.86	–	CW	Medium
81	Tangrey	8.0	2.5	3.2:1	20.83	–	White	Medium
82	Thakur binni	8.1	2.5	3.2:1	17.66	–	CW	Medium
83	Birimphole	7.8	2.5	3.1:1	18.77	–	White	Medium
84	Rambhog	5.3	2.5	2.1:1	21.64	–	CW	Short
	Mean	7.69	2.59	0.13	21.96			
	SE	0.12	0.04	0.01	0.529			
	SD	1.120	0.41	0.02	4.848			
	Variance	1.255	0.174	0.004	23.508			
	CD	0.039	0.012	–	0.255			

CW creamy white, *YW* yellowish white

(intensity of green coloration: light, medium and dark). The frequency distributions of 16 agro-morphological characters showed variable range of characters (Table 1). Out of 16 morphological characters, basal leaf sheath colour, leaf blade colour, ligule colour, and plant habit showed more frequent variation among the rice landraces (genotypes). Majority of

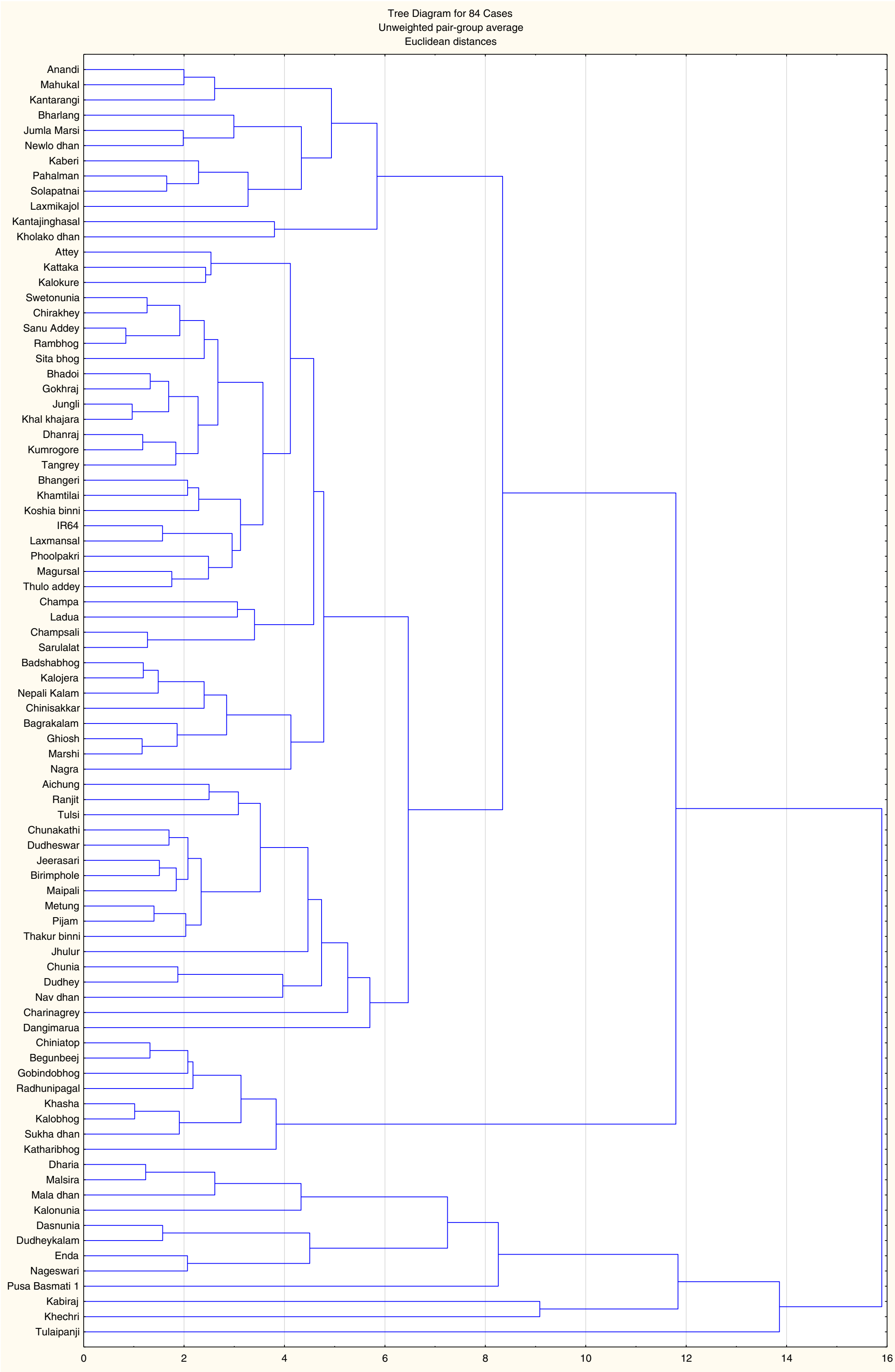

Fig. 1 Dendrogram of 84 local rice landraces constructed based on agro-morphology traits

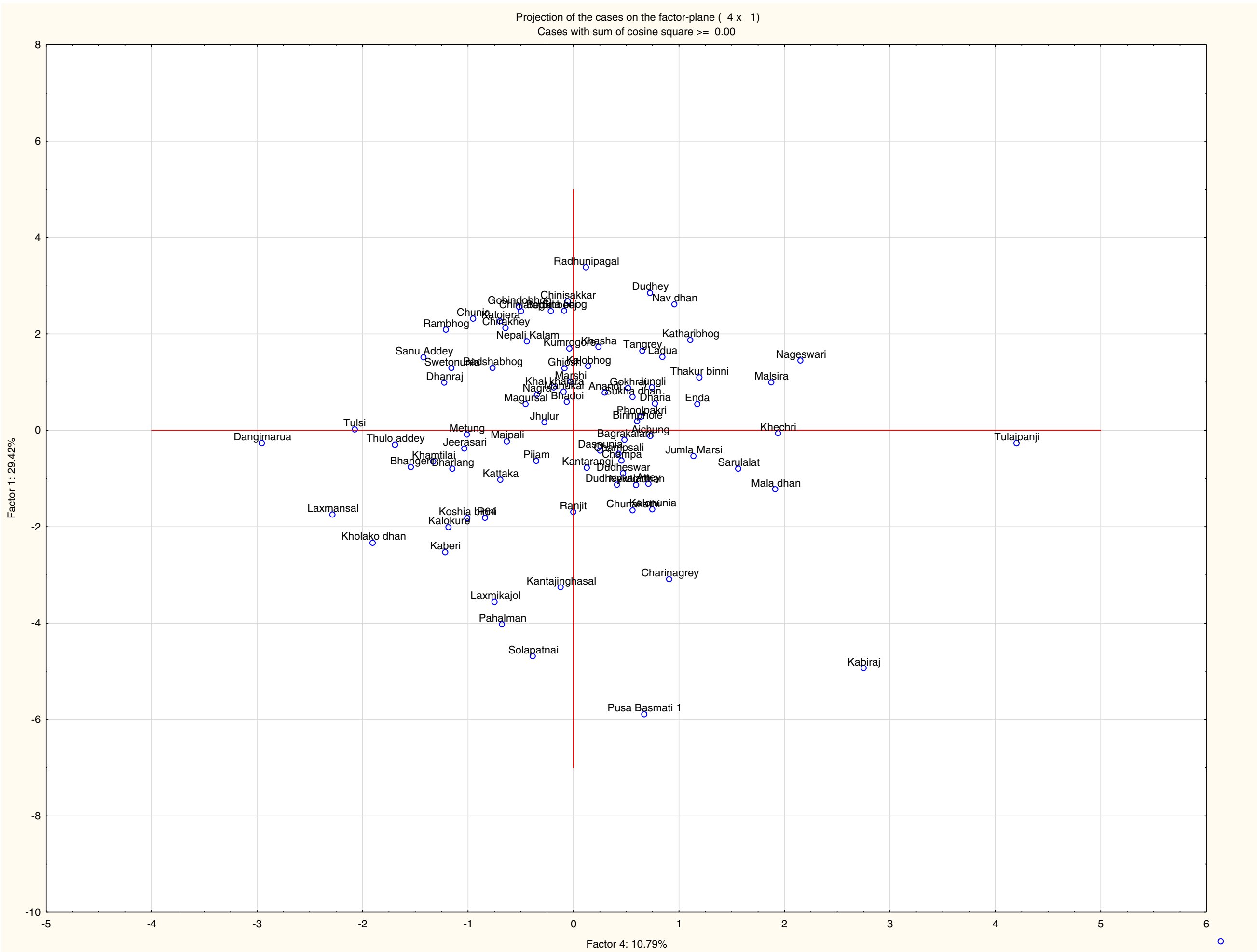

Fig. 2 The principal component analysis (PCA) of the 84 local rice landraces showing genetic relationship in a graphical representation scatter plot

landraces were found to possess colourless coleoptiles (98.81 %) on tenth days in coleoptile colour character category, basal leaf sheath colour on booting stage was green (96.6 %), intensity of leaf green colour on booting stage was dark (98.02 %), colour of ligule on booting stage was white (97.7 %), in leaf sheath character: intensity of anthocyanin

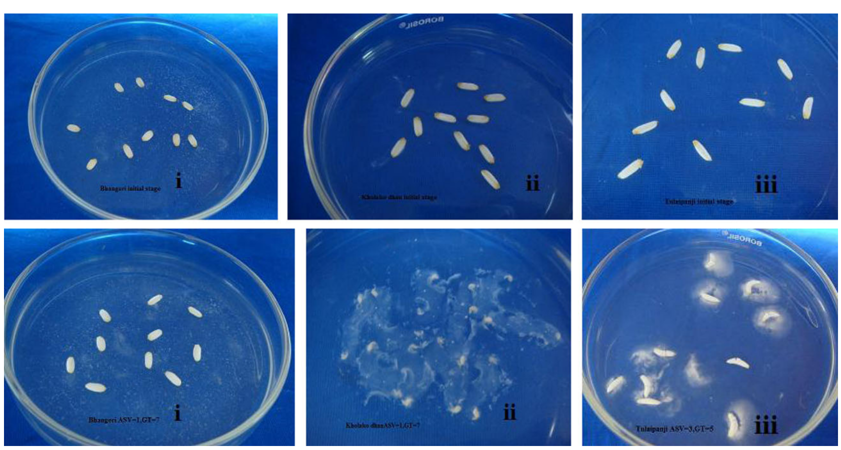

Fig. 3 Some example of ASV and GT experiment. *First row* showing (i–iii above) the initial stage of Alkali spreading value (ASV) and Gelatinization Temperature (GT) and *second row* showing (i–iii below) after 23 h stage of Alkali spreading value (ASV) and Gelatinization Temperature (GT). *i.* High gelatinization: No dispersion (Alkali spreading value-1). *ii.* Low gelatinization: almost complete dispersion (Alkali spreading value-7). *iii.* Intermediate gelatinization: moderate dispersion (alkali spreading value-4)

Table 3 Characterization of 84 local rice landraces based on grain quality

Sl no.	Cultivar name	Kernel length (KL) mm	Kernel breadth (KB) mm	KL/KB ratio	Cooked kernel length (CKL) mm	Cooked kernel breadth (CKB) mm	CKL/ CKB ratio	Linear elongation ratio (LER)	Breadth wise elongation ratio
1	Anandi	6.0	2.6	2.3:1	7.6	3.5	2.1:1	1.2	1.3
2	Attey	5.5	2.3	2.3:1	10.2	3.0	3.4:1	1.8	1.3
3	Aichung	5.4	2.5	2.1:1	8.9	3.0	2.9:1	1.6	1.2
4	Swetonunia	5.03	2.03	2.4:1	7.2	3.0	2.4:1	1.4	1.4
5	Bharlang	5.2	2.03	2.5:1	10.3	2.7	3.8:1	1.9	1.3
6	Badshabhog	4.5	2.0	2.2:1	6.8	2.5	2.7:1	1.5	1.2
7	Bhangeri	6.3	2.0	3.1:1	7.03	2.3	3.05:1	1.1	1.1
8	Bhagrakalam	5.4	2.0	2.7:1	7.8	3.0	2.6:1	1.4	1.5
9	Bhadoi	5.2	2.5	2.08:1	8.2	3.0	2.7:1	1.5	1.2
10	Chinisakkar	4.2	2.0	2.1:1	5.1	2.5	2.04:1	1.2	1.2
11	Champa	5.9	2.5	2.3:1	8.1	3.0	2.7:1	1.3	1.2
12	Champsali	6.1	2.5	2.4:1	7.8	3.0	2.6:1	1.2	1.2
13	Charinagrey	6.3	2.0	3.1:1	9.5	2.5	3.8:1	1.5	1.2
14	Chunakathi	6.07	2.5	2.4:1	8.8	2.5	3.5:1	1.4	1.0
15	Chirakhey	5.2	2.5	2.08:1	6.8	3.0	2.2:1	1.3	1.2
16	Chiniatop	4.6	2.0	2.3:1	5.2	2.5	2.08:1	1.1	1.2
17	Chunia	4.8	2.0	2.4:1	5.03	2.5	2.01:1	1.04	1.2
18	Dhanraj	5.7	2.0	2.8:1	6.1	3.0	2.03:1	1.07	1.5
19	Dudheswar	5.6	2.0	2.8:1	8.7	3.0	2.9:1	1.5	1.5
20	Dangimarua	6.03	2.0	5.7:1	6.8	3.0	2.2:1	1.1	1.5
21	Dharia	5.6	2.0	2.08	7.1	3.0	2.3:1	1.2	1.5
22	Dasnunia	6.03	2.0	3.01:1	7.6	3.0	2.5:1	1.2	1.5
23	Dudhey	4.2	2.5	1.6:1	5.6	3.0	1.8	1.3	1.2
24	Dudheykalam	6.2	2.0	3.1:1	7.9	3.0	2.6:1	1.2	1.5
25	Enda	5.2	2.5	2.08:1	7.9	3.0	2.6:1	1.2	1.2
26	Ghiosh	5.1	2.5	2.04:1	7.7	3.0	2.5:1	1.5	1.2
27	Gokhraj	5.0	2.4	2.08:1	7.9	3.0	2.6:1	1.5	1.2
28	Gobindobhog	4.03	2.0	2.01:1	6.7	3.0	2.2:1	1.6	1.5
29	Begunbeej	4.0	1.90	2.1	5.1	2.5	2.04:1	1.28	1.32
30	IR64	7.1	2.0	3.5:1	8.6	3.0	2.8:1	1.2	1.5
31	Jumla Marsi	5.7	3.0	1.9:1	11.17	3.8	2.9:1	1.9	1.2
32	Phoolpakri	5.5	2.5	2.2:1	7.1	3.0	2.3:1	0.62	1.2
33	Jungli	5.1	2.5	2.04:1	7.2	3.0	2.4:1	1.4	1.2
34	Jhulur	6.9	2.5	2.7:1	9.0	3.0	3:1	1.3	1.2
35	Jeerasari	5.8	2.0	2.9:1	7.9	2.5	3.1:1	1.5	1.2
36	Kalonunia	7.5	2.0	3.7:1	7.6	3.0	2.5:1	1.01	1.5
37	Kattaka	5.1	2.0	2.5:1	8.7	2.0	4.3:1	1.7	1.0
38	Kataribhog	4.0	2.0	2:1	6.6	2.0	3.3:1	1.6	1.0
39	Kaberi	7.1	2.0	3.5:1	9.3	3.0	3.1:1	1.3	1.5
40	Koshia binni	7.4	2.0	3.7:1	8.2	3.0	2.7:1	1.1	1.5
41	Kumrogore	5.2	2.5	2.08:1	6.07	3.0	2.02:1	1.1	1.2
42	Kabiraj	7.1	2.03	3.4:1	10.8	2.5	4.3:1	1.5	1.2
43	Khashadhan	4.4	2.0	2.2:1	7.0	3.0	2.3	1.5	1.5
44	Khamtilai	6.0	2.1	2.8:1	8.8	3.0	2.9:1	1.4	1.4
45	Kalojera	4.4	2.0	2.2:1	6.8	3.0	2.2:1	1.5	1.5
46	Kalobhog	4.9	2.0	2.4:1	6.9	3.0	2.3:1	1.4	1.5
47	Khechri	5.1	2.5	2.04:1	8.5	3.0	2.8:1	1.6	1.2

Table 3 (continued)

Sl no.	Cultivar name	Kernel length (KL) mm	Kernel breadth (KB) mm	KL/KB ratio	Cooked kernel length (CKL) mm	Cooked kernel breadth (CKB) mm	CKL/ CKB ratio	Linear elongation ratio (LER)	Breadth wise elongation ratio
48	Kantarangi	6.2	2.5	2.4:1	8.1	3.0	2.7:1	1.3	1.2
49	Khal khajara	4.7	2.1	2.2:1	7.8	3.0	2.6:1	1.5	1.4
50	Katajinghasal	7.6	2.0	3.8:1	9.4	3.0	3.1:1	1.2	1.0
51	Kholako dhan	6.3	2.2	2.8:1	8.7	2.0	4.3:1	1.3	0.9
52	Kalokure	5.1	2.0	2.5:1	10.6	2.0	5.3:1	2.07	1.0
53	Laxmansal	7.1	2.0	3.5:1	8.05	2.5	3.2:1	1.4	1.2
54	Laxmikajol	7.8	2.5	3.1:1	10.3	2.5	4.1:1	1.3	1.0
55	Ladua	4.9	2.8	1.7:1	6.4	2.8	2.2:1	1.3	1.0
56	Malsira	4.8	2.3	2.08:1	7.1	3.0	2.3:1	1.4	1.3
57	Metung	6.06	2.0	3.03:1	7.2	3.0	2.4:1	1.1	1.5
58	Marshi	5.0	2.2	2.2:1	7.5	3.0	2.5:1	1.5	1.3
59	Maipali	6.2	2.0	3.1:1	7.1	3.0	2.3:1	1.1	1.5
60	Mahukal	5.5	2.5	2.2:1	7.1	3.0	2.3:1	1.2	1.2
61	Mala dhan	6.1	2.0	3.0:1	7.8	3.0	2.6:1	1.2	1.5
62	Magursal	5.1	2.0	2.5:1	7.7	3.0	2.7:1	1.5	1.5
63	Nav dhan	4.0	2.0	2:1	6.9	2.5	2.7:1	1.7	1.2
64	Newlo dhan	6.0	2.6	2.2:1	9.9	3.0	3.3:1	1.5	1.1
65	Nageswari	5.2	3.0	1.7:1	6.1	3.0	2.03:1	1.1	1.0
66	Nepali Kalam	5.0	1.98	2.1:1	7.1	2.75	2.3:1	1.0	1.1
67	Nagra	5.7	2.0	2.8	9.0	3.0	3:1	1.5	1.5
68	Pusa Basmati	8.0	2.0	3.9	10.4	2.0	5.2:1	1.3	1.0
69	Pijam	5.4	2.0	2.7:1	7.6	2.5	3.04:1	1.4	1.2
70	Pahalman	7.8	2.0	3.8:1	10.6	3.0	3.5:1	1.3	1.5
71	Radhunipagal	4.1	2.0	2.05:1	4.9	3.0	1.6:1	1.1	1.5
72	Ranjit	6.9	2.5	2.7:1	8.5	2.5	3.4:1	1.2	1.0
73	Sanu Addey	4.8	2.0	2.4:1	7.6	2.5	3.04:1	1.5	1.2
74	Solapatnai	7.4	2.0	3.7:1	10.4	2.5	4.2:1	1.4	1.2
75	Rambhog	5.5	2.5	2.2:1	7.1	3.0	2.3:1	1.2	1.2
76	Sukha dhan	5.6	2.2	2.5:1	6.1	3.5	1.7:1	1.08	1.5
77	Birimphole	5.7	2.5	2.2:1	8.0	3.0	2.6:1	1.4	1.5
78	Sita bhog	4.1	2.0	2.05:1	5.6	2.5	2.2:1	1.3	1.2
79	Sarulalat	6.6	2.0	3.3:1	9.1	3.0	3.03:1	1.3	1.5
80	Tulaipanji	6.0	1.96	2.7:1	7.7	2.2	3.5:1	1.4	1.1
81	Tulsi	4.1	2.1	1.9:1	6.1	3.0	2.03:1	1.4	1.4
82	Thulo addey	4.7	2.5	1.8:1	6.7	3.0	2.2:1	1.4	1.2
83	Tangrey	5.6	2.5	2.2:1	7.8	3.0	2.6:1	1.3	1.2
84	Thakur binni	4.5	2.0	2.2:1	6.9	2.5	2.7:1	1.5	1.2
	Mean	5.63	2.21	1.78	7.84	2.84	1.27	1.36	1.36
	SE	0.11	0.03	0.75	0.15	0.03	0.47	0.02	0.01
	SD	1.018	0.279	1.686	1.415	0.359	1.059	0.210	0.175
	Variance	1.038	0.078	2.844	2.003	0.129	1.123	0.044	0.030
	CD	0.038	0.012	–	0.041	0.006	–	–	–

Kernel shape (USDA): long means KL/KB ratio 3.0:1 and more; medium – 2.0:1 to 2.9:1; short- 1.9:1 and less

colouration was very weak (96.5 %), in spikelet character: colour of stigma was white (95.4 %), colour of tip of lemma white (86.36 %), density of pubescence medium (52.27 %); auricle was without anthocyanin colouration (97.7 %); leaf: pubescence of blade surface on booting stage was weak (51.3 %), leaf collar was present on booting stage (100 %),

Table 4 Correlation between eight physico-chemical parameters of rice grains

Characters	KL	KB	KL/KB	CKL	CKB	CKL/CKB	LER	BWER
KL	1	0.23	0.56	0.52	−0.02	0.37	−0.55	−0.17
KB		1	−0.64	0.06	0.53	−0.33	0.05	0.53
KL/KB			1	0.35	−0.41	0.57	−0.36	0.35
CKL				1	0.26	0.61	0.40	0.14
CKB					1	−0.58	0.30	0.35
CKL/CKB						1	0.03	−0.29
LER							1	0.29
BWER								1

stem length varies from very short (45.97 %), short (39.08 %), to medium (5.74 %). Heading time varies from (50 % of plants with panicles) very early (28.73 %), early (26.43 %), medium (48.27 %), late (17.24 %), and very late (2.29 %). On late observation it was noticed that flag leaf attitude was erect (54.4 %), semi-erect (31.81 %), horizontal (2.27 %), and deflexed (13.63 %) (Table 1).

Parameters pertaining to grain characters were recorded (Table 2) using standard method (www.bioversity.org) for genetic variation analysis. Grain weight, grain length, grain breadth, awn length, bran colour, and grain shape were measured. Out of 84 local landraces highest grain weight was found in cultivar, Khechri (32.49 mg) collected from Sundarban, and lowest grain weight was found in Kataribhog (9.34 mg). According to length/breadth ratio, rice grains have been grouped into three categories (USDA protocol; Adair 1972), short grains L/B ratio is ≤2.2:1, medium grain L/B ratio is 2.3:1 to 3.3:1 and long grain L/B ratio is ≥3.4:1 (Table 2). Seventeen cultivars were grouped in the aromatic rice category out of total 84 local landraces. Among these categories, Tulaipamji cultivar of North Dinajpur district was considered as best quality and preferred by consumers for its fragrance and soft fluffy grain quality. One cultivar Chinisakkar showed highest spikelet branching (11), seeds per panicle (180), and panicle length (23.48 cm) among other aromatic rices. Twenty-one cultivars were with awn, whose lengths were ranges from 1.6 mm (Anandi) to 22.25 mm (Tulaipanji). Bran colour ranges from red, yellowish, brownish, creamy white to white (Table 2). Some landraces Newlodhan, Dudheswar, and Bhangeri showed stout stem and remain in green condition for long periods. Dendrogram was constructed based on agro-morpho-quality characters of 84 local landraces of rice which showed five clusters with genetic variation (Fig. 1). This genetic variation is the inherent characteristic of the individual landrace. The principal component analysis (PCA) showed the scattered plot of the dendrogram as graphical representation with genetic relationship among 84 local rice landraces (Fig. 2).

Physico-chemical analysis

Kernel length (KL) was measured according to Govt. of India notification (No 67, 23 Jan, 2003, Ministry of Commerce). Grain was considered as medium slender A grade basmati rice with 7 mm minimum length, while its minimum LBR is 3.5, kernel breadth (KB) 1.95 mm and KL/KB ratio is 3.07. Other

Table 5 Seedling performance of some rice landraces in submergence anaerobic conditions

Name of varieties	Test tube (on 7th day) (cm)		Waterlogged soil method			
			Surface (on 14th day)		Sub-surface (on 14th day)	
	Plumule	Radicle	Plumule	Radicle	Plumule	Radicle
Nageswari	2.1	7.7	5.26	4.14	6.78	3.98
Ghiosh	2.5	Nil	8.02	2.48	9.92	5.12
Dudheswar	1.3	1.3	6.88	2.34	4.78	4.28
Sitabhog	1.1	Nil	3.38	1.52	Nil	Nil
Tulshi	2.3	1.2	7.32	3.38	7.36	4.52
Chunia	1.2	Nil	4.38	3.28	5.28	1.78
Rambhog	2.9	1.6	7.4	3.32	4.04	2.26
SD	0.71	3.17	1.76	0.86	1.68	1.46
SE	0.27	1.20	0.66	0.33	0.63	0.55

Mean ± SE (Three replicates)

Table 6 Correlation among three parameters obtained by two bioassay methods

Characters	Test tube	Waterlogged soil method		Vigor index
		Surface	Sub-surface	
Test tube	1	0.54	0.29	0.98
Surface		1	0.43	0.57
Sub-surface			1	0.37
Vigor index				1

R values ranging from 0.29 to 0.98

quality parameters were recorded for basmati rice as per DUS protocol. Knowledge accumulated in the past decades indicates that the poor cooking and eating quality is directly related to three attributes of the physico-chemical characteristics of the starch in the endosperm; namely, amylose content (AC) (Juliano 1971), gel consistency (GC) (Cagampang et al. 1973) and gelatinization temperature (GT). Alkali spreading value (ASV) was showed distinct genetic variability among the land races (Fig. 3). Cultivar Kholakodhan showed low ASV (1), and GT (7), this character is preferred for grain quality improvement (IRRI) by the breeders. The ASV depends on the nature of the amylopectin molecules and is reported to be dependent on soluble starch synthase gene on the chromosome 6. Thirty-six rice grains were found with higher length elongation ratio (LER) greater than 1.32 which is desirable as good quality grains (Table 3). Among collected cultivars highest linear elongation ratio (LER) was found in Kalokure (2.07), lowest in Phoolpakri (0.62). Breadth wise highest elongation ratio (BWER) was found in Birimphole (2.17) and lowest in Magursal (0.89). The cultivar Tulaipanji showed a significantly lower ELR of 1.40 with KL 6 mm and width 1.96 mm (Table 3). Kernel length was found to have positive correlation with KB, KL/KB, CKL, CKL/CKB and negatively correlated with CKB and LER (Table 4). Cooking time for rice is the time when 90 % of the starch in the grain no longer show opaque center when pressed between two glass plates. Rice differs in optimum cooking time in excess water between 15 and 25 min without pre-soaking. Landraces like Govindobhog, Tulaipanji, Chiniatap, Begunbeej, Chanachur, Bhadaore, Bhangeri takes 5 min to cook in excess water and Ghiosh takes 25 min to cook. The lower the cooking time better is the quality in terms of fuel and energy consumption during cooking. Amylose content of the rice grain determines whether it will be firm and fluffy on cooking, or it will turn sticky and glutinous. Apparent amylose content (ACC) falls into the following four categories: glutinous = 0 to 5 %, Low = 5 to 19 %, Intermediate = 19 to 23 %, and High >23 %. Glutinous category was absent in this study. Basmati type varieties have intermediate amylose content (AC) of 20–25 % and their grains remain firm and separated after cooking, at the same time they give a soft mouth feel while eating. Tulaipanji grain showed the same amylose content (20 %) and giving the same mouth feel as Basmati with aroma. Both the traits (GT and ASV) are known to be governed by the enzymes of granule bound starch synthase I (GBSSI) encoded by the *Waxy* gene locus on short arm of chromosome 6 (Umemoto et al. 2002 and 2004). The GBSSI, is responsible for amylose biosynthesis and also has a role in the elongation of long chains in amylopectin. Mutations in the *Waxy* locus leading to loss of GBSSI activity result in amylose free (waxy) starch.

Anaerobic germination (AG)

Some cultivars showed significant difference in anaerobic germination under submergence condition (Table 5). Correlation analysis was conducted among the cultivars using three parameters by two bioassay methods and R values ranging from 0.29 to 0.98 (Table 6). The test tube method showed significant correlations with all other parameters (i.e. water logged soil method-surface and subsurface; and vigor index) (Figs. 4, 5 and 6). Based on this correlation study, the Test tube method was considered to be the most simple, rapid and

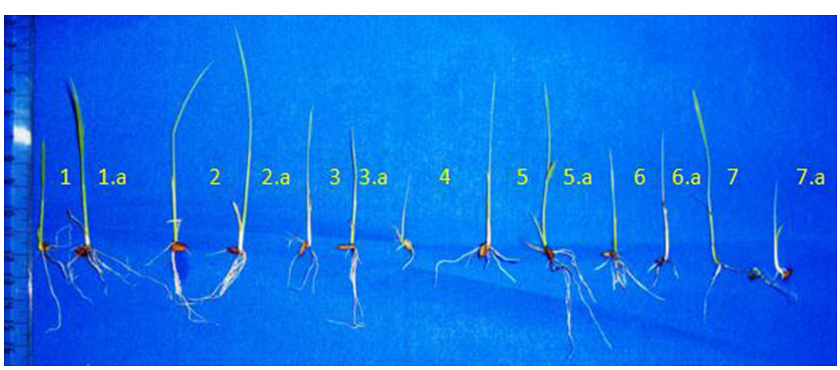

Fig. 4 Measurement of seedling length under anaerobic condition. [*1.* Nageswari- surface, *1.a.* Nageswari- subsurface, *2.* Ghiosh- suface, *2.a.* Ghiosh-subsuface, *3.* Dudheswar-surface, *3.a.* Dudheswar- subsurface, *4.* Sitabhog-surface, *5.* Tulshi- surface, *5.a.* Tulshi-subsurface, *6.* Chunia-surface, *6.a.* Chunia- subsurface, *7.* Rambhog-surface, *7.a.* Rambhog-subsurface]

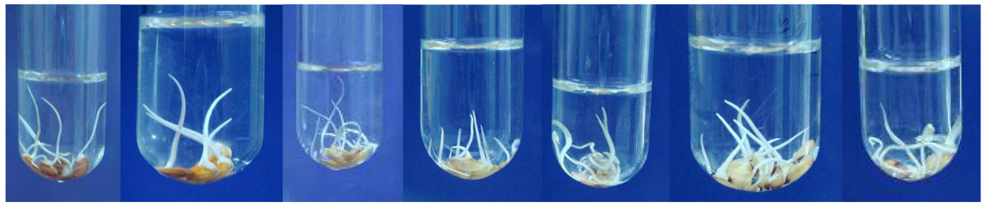

Fig. 5 Test tube bioassay method for evaluating seedling vigor under anaerobic germination. [*1*-Nageswari, *2*- Ghiosh, *3*-Dudheswar, *4*-Sitabhog, *5*- Tulshi, *6*-Chunia, *7*-Rambhog]

reliable bioassay for the anaerobic germination (AG). The seed vigor index (SVI) was calculated by multiplying seed germination percentage with seedling length (in mm). The seed lot showing the higher seed vigour index (SVI) is considered to be more vigorous. The SVI values vary among the cultivars which were as follows - Nageswari (80×98=7840), Ghiosh (98×25=2450), Dudheswar (60×25=1500), Sitabhog (90×23=2070), Tulsi (99×35=3465), Chunia (50×12=600), and Rambhog (90×74=6660). Seedling vigor plays a key role in the submergence avoidance mechanism (Manangkil et al. 2008). Because, low oxygen (O_2) level enhances coleoptile elongation during the germination of rice seeds (Redona and MacKill 1996). Several other investigators have taken this as evidence and tried to relate coleoptile elongation or biochemical changes that are initiated by water uptake with tolerance to anaerobic conditions. Rice is well-known for its ability to germinate without oxygen. In the present study it was observed that the cultivar Ghiosh collected from Sundarban showed anaerobically better germinating performance without an anchoring rootlet followed by cultivars Tulsi and Nageswari (Tables 5 and 6). They showed some kind of submergence avoidance mechanism. Compared to transplanted culture, direct seeding method can reduce labor input by as much as 90 % and can shorten crop growth duration up to 14 days. Submergence is a major stress causing yield loss particularly in the direct seeded rice cultivation system. High germination rate and fast shoot and root elongation are the major traits that are closely related to seedling vigor that is a determinant of the optimum crop establishment in flood prone areas.

Fig. 6 An overview of Water-logged soil method under anaerobic germination

Iron and zinc content in 84 local rice landraces

Iron content varied between 0.25 μg/g to 34.8 μg/g and Zinc content varied from 0.85 μg/g to 195.3 μg/g (Table 7). Local cultivar Swetonunia had highest iron content of 34.8 μg/g followed by the other cultivars Gobindobhog 3.1 μg/g, and Attey 2.05 μg/g. Nepali Kalam had the highest Zinc content 195.3 μg/g followed by Govindobhog 138.6 μg/g, Begunbeej 20.4 μg/g and Ghiosh16.15 μg/g. Iron content in all the local landraces were very poor but Zinc content in some of the landraces was promising containing 195.3 μg/g in Nepali Kalam and 138.6 μg/g in Gobindobhog. Most of the commercially cultivated indica and japonica rice cultivars are deficient in iron and zinc compared to the other staple food crops such as wheat and maize (Gregorio et al. 2000). Zinc deficiency is probably the most widespread micronutrient deficiency in cereals. Since rice is the principal food of the Asian continent (Developing world), a lot of efforts are being made to develop nutritionally improved genotypes of rice. Breeding for enhancing bio-available minerals in edible portions through increasing the concentrations of metal-binding proteins have been studied by many researchers (Zhang et al. 2005). The first pre-requisite for initiating a breeding program to develop micronutrient-rich genotypes, is to screen the available germplasm and identify the source of genetic variation for the target trait, which can be used in crosses, genetic studies, molecular marker development and to understand the basis of enhanced micronutrient accumulation. Iron and Zinc contents in edible portions also depend on the efficiency of translocation of minerals from root tissues to edible plant organs and accumulation thereof. Mineral-rich and mineral poor rice genotypes identified in this study may be used in breeding program for introgression of high Fe and Zn content gene or QTLs in the improved varieties. Notably, there was about many fold difference in Fe and Zn content suggesting the existence of genetic potential to increase the concentration

Table 7 Characterization of 84 landraces of rice based on Iron and Zinc content

Rice landraces	Fe (µg/g)	Zn (µg/g)
Bhagrakalam	2.05±0.011	9.77±0.011
Aichung	4.3±0.003	3.8±0.029
Anandi	0.75±0.025	2.55±0.003
Dharia	0.7±0.029	3.4±0.003
Attey	7.9±0.006	4.1±0.002
Badsabhog	6.2±0.001	2.1±0.008
Solapatnai	4.3±0.003	3.8±0.029
Begunbeej	7.0±0.002	2.1±0.008
Jumla Marshi	1.6±0.021	20.4±0.020
Champa	3.25±0.068	0.85±0.28
Champasali	0.6±0.024	2.55±0.21
Charinagrey	6.8±0.004	1.5±0.001
Chiniatop	0.33±0.032	3.4±0.24
Chinisakkar	13.4±0.008	4.4±0.003
Chirakhey	6.0±0.000	2.3±0.001
Chunakathi	7.0±0.000	3.4±0.004
Chunia	7.1±0.003	2.1 ±0.003
Dangimarua	7.3±0.001	2.7±0.002
Rambhog	6.7±0.002	2.5±0.009
Dhanraj	4.7±0.011	2.7±0.000
Sitabhog	9.7±0.002	6.3±0.14
Sarulalat	11.9±0.001	3.2±0.006
Tulaipanji	0.45±0.04	2.21±0.14
Khamtilai	4.6±0.002	1.7±0.13
Thulo addey	5.1±0.001	1.4±0.006
Kalobhog	7.1±0.001	2.4±0.12
Kalojera	6.9±0.001	1.6±0.003
Kalokure	10.7±0.002	1.9±0.16
Kalonunia	6.0±0.005	2.55±0.266
Kantarangi	4.9±0.002	2.6±0.009
Kantajinghasal	3.8±0.003	3.9±0.003
Katharibhog	5.5±0.001	2.6±0.009
Kattaka	5.1±0.003	2.5±0.006
Khalkhajara	6.9±0.001	2.5±0.009
Khasa dhan	6.9±0.004	2.2±0.001
Sanu addey	6.9±0.002	2.2±0.004
Kholako Dhan	0.25±0.07	2.12±0.385
Khechri	1.73±0.014	4.67±0.137
Koshia Binni	4.3±0.001	2.1±0.017
Kumrogore	7.2±0.001	1.6±0.007
Ladua	8.0±0.001	2.9±0.006
Sukhadhan	12.1±0.001	6.7±0.001
Bhadoi	0.5±0.038	1.7±0.002
Bhangeri	6.7±0.000	1.7±0.001
Bharlang	6.7±0.001	1.8±0.003
Birimphole	0.4±0.026	0.85±0.70
Tulshi	7.0±0.002	2.1±0.008
Dasnunia	6.9±0.004	3.8±0.001
Dudheykalam	4.4±0.001	2.0±0.008
Dudheswar	5.8±0.005	2.4±0.005
Dudhey	65.4±0.055	8.5±0.008
Enda	0.4±0.021	0.85±0.35
Ghiosh	0.55±0.13	8.5±0.177
Gokhraj	8.6±0.002	2.0±0.006
Gobindobhog	6.2±0.002	138.6±0.071
IR64	5.00±0.003	2.0±0.12
Ranjit	0.5±0.029	1.27±0.199
Metung	7.0±0.002	3.2±0.006
Jeerasari	4.4±0.001	2.0±0.006
Maipali	4.4±0.004	1.9±0.11
Mahukal	5.2±0.003	2.1±0.002
Jhulur	6.5±0.002	4.6±0.14
Jungli	1.8±0.023	15.4±0.077
Kaberi	7.1±0.002	1.8±0.11
Kabiraj	0.35±0.049	0.85±0.99
Pijam	8.3±0.009	3.2±0.008
Laxmansal	6.8±0.004	1.3±0.003
Laxmikajal	7.3±0.001	2.5±0.008
Magursal	7.0±0.003	2.9±0.007
Maladhan	6.6±0.003	4.2±0.007
Malsira	7.0±0.003	1.7±0.004
Marshi	9.8±0.003	1.5±0.006
Nageswari	6.6±0.004	2.2±0.11
Nagra	6.3±0.003	2.4±0.003
Nav dhan	6.6±00.03	1.7±0.006
Nepali Kalam	12.3±0.001	195.3±0.3
Pahalman	6.7±0.001	2.0±0.005
Newlodhan	4.8±0.006	2.8±0.009
Phoolpakri	7.4±0.003	3.4±0.001
Pusa Basmati	6.2±0.004	1.9.009
Radhunipagal	6.2±0.003	2.0±0.005
Swetonunia	34.8±0.003	35.2±o.oo2
Tangrey	0.43±0.06	1.27±0.34
Thakurbinni	6.4±0.008	3.0±0.001

of these micronutrients in rice grain. Identified cultivar Nepali Kalam (195.3 µg/g Zinc) and Gobindobhog (138.6 µg/g Zinc) may be used as zinc donor in future.

Key observations of this study are summarized in Table 8. Agronomically important traits were identified which can be used by plant breeders for introgression into the elite cultivar for widening the gene pool as well as their improvement. Landraces such as, Newlodhan, Dudheswar and Bhangeri have stout stems and remain in green condition for long days, so breeders can consider them to be included in breeding program. Anaerobic germination traits can be transferred to

Table 8 Genetic diversity observed in the 84 local landraces of rice for specific traits preferred by breeders

Characteristics/specific traits	Breeders choice	Name of local rice (*Oryza sativa* L.) cultivars
Culm attitude	Erect	Champa, Chunia, Chiniatop, Dharia, Kalobhog, Khechri, Gobindobhog, Kumrogore,
Time of heading (50 % of plants with panicles)	Very early (<71 days)	Jumla marshi, Dudhey kalam
Stem thickness	>0.55 cm	Anandi, Bharlang, Bhangeri, Chinisakkar, Champa, Champasali, Charinagrey, Chunakathi, Chirakhey, Dudheswar, Enda, Ghiosh, Jumla marshi, Jungli, Jhulur, Jeerasari, Kalonunia, Kattaka, Koshiabinni, Kumrogore, Kabiraj, Sarulalat, Tulaipanji, Tulshi, Newlodhan
Stem length	Very short <91 cm	Badshabhog, Bhadoi, Dhanraj, Gobindobhog, Jumla marshi, Jhulur, Jeerasari, Kattaka, Kaberi, Khechri, Laxmansal, Kalojera, Khashadhan
Panicle: Length of main axis	Medium (21–25 cm)	Chunia (22.98 cm), Bhangeri (23.88 cm), Chinisakkar (25.26 cm), Dudheswar (25.94), Newodhan (22.64 cm), Ladua (22.6 cm), Sukhadhan (22.4 cm), Sarulalat (25.28 cm).
	Long (26–30 cm)	Not available in the collected germplasm
	Very long (>30 cm)	Not available in the collected germplasm
Panicle curvature of main axis	Straight	Chiniatop, Dudheswar, Dharia, Dasnunia, Kalonunia, Kattaka, and Kataribhog
Panicle number per plant	Many >20	Not available in the collected germplasm
	Medium (11–20)	Dudheswar (14.8), Chinisakkar (11), Newlodhan (11.2), Ladua (11.4),
No. of seeds per panicle	240	Chinisakkar (180), Dudheswar (177), Jungli (129), Chirakhey (101), Koshiabinni (101.8), Kabiraj (117), Ladua (151), Newlodhan (116), Maipali (131), Sukha dhan (110), Sarulalat (111)
Penultimate leaf width	Broad >2 cm	Jungli (2.3 cm)
	Medium (1-2 cm)	Anandi (1.36 cm), Bharlang (1.02 cm), Bhangeri (1.12 cm), Dudheswar (1.14 cm), Kantajingasal (1.18 cm), Laxmikajol (1.04 cm), Maipali (1.12 cm), Newlodhan (1.34 cm), Sarulalat (1.02 cm),
Penaltimate leaf blade length	Medium (30–45 cm)	Attey (35.74 cm), Jumla marshi (34 cm), Jhulur (42.46 cm), Jeerasari (47.94 cm), Katharibhog (18.54 cm), Khechri (44.2 cm), Kholako dhan (40.7 cm), Tulshi (43.6 cm)
Time of maturation	Very early (<100)	Jumla marshi black (97 days)
Flag leaf in early and late observation	Erect	Anandi, Aichung, Chiniatop, Dudheswar, Enda, Ghiosh, Jungli, Jhulur, Kantarangi, Laxmansal
Disease resistance	Bacterial blight (based on frequency observation in the field)	Dudheswar, Bharlang, Anandi, Aichung, Kholakodhan, Jungli, Newlodhan (These are least affected by diseases)
Abiotic stress tolerance		Cold tolerance-Jumla Marshi black
i) cold tolerance		Jumla Marshi (White)
ii) salinity tolerance		Salinity tolerance- Ghiosh
iii) Anaerobic germination		Anaerobic germination- Tulshi, Nageswari, Ghiosh, Dudheswar
Root character		Long and highly branched in Jungli, Dudheswar

elite HYV from Nageswary, Ghios, Dudheswar, and Tulshi for high seedling vigor. Root characters for long and highly branched traits can be accumulating into HYV from Jungli and Dudheswar. Disease tolerance traits (based on field observation data) mainly bacterial blight may be taken from Kholakodhan, Bharlang, Jungli, Newlodhan, and Dudheswar. Flag leaf erect trait was observed in Chiniatop, Ghios, Kantarangi, and Laxmansal which can be utilized by breeder for physiological efficiency. Most important traits identified in this investigation: very early maturity (<100 days) plant, Jumla Marshi (Black) collected from Jumla, Nepal which was also cold resistant cultivar. Broad penultimate leaf width was found in Jungli (2.3 cm). Time of heading very early (<71 days) was observed in Jumla marshi black, and Dudheykalam. High amount of micronutrients were found in some landraces as such Nepali Kalam contained highest amount of Zinc (195.3 μg/g) and highest amount of Iron was found in Swetonunia (34.8 μg/g) those can be considered by the breeder to alleviate hidden hunger. The cultivar Tulaipanji can be considered for grain quality trait with aroma. Thus, the present study gives detail characterization of 84 local landraces of rice with agronomic passport data so that breeders can choose donor parent according to their genetic improvement program as well as for conservation of the genetic resources.

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