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RESEARCH ARTICLE

Morphological and Molecular Analyses of Grain Traits in Aromatic Rice Landrace Accessions from Indo-Gangetic Plain Region of India

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Abstract

Information on genetic variation in rice grain's morphological traits is vital, affecting its yield and quality parameters. In present study, 92 accessions of aromatic rice landrace germplasm from the Indo-Gangetic plain region of India have assessed for grain morphometric traits as-well-as for genotypic mutation in *exon* 3 of grain size (*GS3*) gene. Significant variability was detected for the grain traits, namely rough rice length and breadth (RRL, RRB), rough rice length/breadth ratio (RRLB), 1000-grain weight (TGW), brown rice length and breadth (BRL, BRB), and brown rice length/breadth ratio (BRLB). The highest values of the phenotypic coefficient of variation and genotypic coefficient of variation were observed for TGW (40.56; 38.38%) and the lowest for BRB (14.96; 15.02%). For all the grain traits, high heritability (broad-sense) was observed, which ranged from 85.67 (BRLB) to 99.13% (BRB). Genetic advances ranged from 0.58 (BRB) to 9.13 (TGW). Principal component (PC) analysis revealed that first two PCs explained 86.04% of phenotypic variation. A positive association found between all the grain traits except grain breadth (RRB and BRB), which showed a negative correlation with the grain length-breadth ratio. The variation revealed for C/A single nucleotide polymorphism in *GS*3 gene. The superior accessions for grain traits, namely RRB (Jeera Sail/IC419047), TGW (Hansraj/IC0419006) and BRL (Kala Jira/IC0419052) identified. The genetically variable germplasm of aromatic rice with superior grain traits could be utilized for genetic enhancement of grain size in aromatic rices.

Keywords: Aromatic rice landraces, Characterization, Grain size, GS3 gene, Indo-Gangetic Plains.

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Introduction

Rice is known as Global grain because it is the primary source of food for half of the global population (Brar and Khush, 2018). It fulfils nearly 19% of the global population's calorie requirements per capita and 13% of their protein needs. In terms of global rice production, India ranks second. Indian region represents the center of origin and diversity for aromatic rice. Aromatic rice possesses superior grain and cooking quality and has aroma mainly due to the presence of 2-acetyl pyrroline (Buttery *et al*., 1983; Verma and Srivastav, 2022). These rices have better nutritional qualities and hence fetch premium price than the non-fragrant types. The Indian subcontinent is gifted with an enormous diversity of aromatic rice landraces; presently, there are hundreds of locally adapted genotypes of aromatic rices (Khush, 2000). Indian aromatic rices exhibit variation for grain length from small, medium to long grain types having mild to strong aroma (Singh *et al*., 2000). In rice, crop yield is primarily determined by three characters: number of panicles per plant, the number of grains per panicle and 1000 grain weight (TGW). TGW is positively associated with grain size. Grain size is positively correlated with grain length, width, and thickness (Xu *et al*., 2002; Zeng *et al*., 2017). Large seeds are favored during domestication due to ease of harvesting and better seedling vigour (Harlan *et al*., 1973). Rice exhibits substantial phenotypic diversity for seed size and shape unlike other cereal crops. In rice, cultivated varieties showed greater seed length variation than wild rice, probably due to human selection (Takano-Kai *et al*., 2009). In rice breeding, grain size is an important agronomic trait. In rice, breeders tend to select large-seeded plants for better yield and appropriate grain-sized plants for milling and market preferences (Wang *et al*., 2011). Market value and consumer preference in rice are mainly driven by rice seed shape and appearance. Grains with long and slender shape are preferred by peoples of India, Pakistan, Thailand, China and the United States, while short and bold-grained rice varieties are consumed by peoples of Japan, South Korea and Sri Lanka (Juliano and Villareal, 1993; Unnevehr *et al*., 1992). Grain size also plays a pivotal role in the adoption of new varieties (Champagne *et al*., 1999). However, breeding for enhanced grain size is a tedious task, because of its quantitative inheritance (McKenzie and Rutger, 1983). In rice, several QTLs governing seed size have been identified. Few of them have been successfully cloned and functionally validated *viz*., *GS3* (Fan *et al*., 2006), *GW2* (Song *et al*., 2007), *GS5* (Li *et al*., 2011), *GS7* (Shao *et al*., 2012), *qGS*10 (Zhu *et al*., 2019) and *qTGW*2 (Ruan *et al*., 2020). Among these genes, *GS*3 is the major gene governing the grain size in rice. One common non-sense mutation (C/A SNP) detected at the second exon of the *GS3* gene results in change of cysteine codon (TGC) to termination codon (TGA). This premature termination resulted in a 178-aa truncation in the C-terminus of the predicted protein in the large-grain size group of rice varieties. *GS3* act as a negative regulator of grain size. The wild allele is present in small to medium grain rice genotypes and the mutant allele in large grain size genotypes (Fan *et al*., 2006). Till date most of the breeding efforts were focused on Basmati-type aromatic rice. But Indian region is blessed with hundreds of short to medium grained indigenous aromatic landraces, adapted to different regions' local climatic conditions. Basmati cultivation is confined to northern states of India, while these aromatic landraces are under cultivated throughout the country. Although aromatic rice landraces possess rich diversity with respect to grain dimension traits, there have been limited efforts to characterize them for grain traits. In context of this, the current study was designed to characterize 92 accessions of aromatic rice landraces for grain features. This study revealed the high extent of variability for grain traits and allelic variation in *GS*3 gene, which could be useful for mining novel alleles for grain quality traits.

Materials and Methods

Germplasm Materials and Field Experimentation

A panel of 92 aromatic rice landrace accessions (Table 1), representing 37 districts and 10 states of Indian Union were selected for the study (Table 2). Among these, 86 accessions were from the Indo-Gangetic plains of India, representing centre of diversity for aromatic rices. The seeds of aromatic rice landraces were obtained from Indian National Genebank housed at ICAR-NBPGR, New Delhi. Geo-referencing of the aromatic rice landrace accessions was performed using passport data information using 'DIVA-GIS' software version 7.5 (Hijmans *et al*., 2001; Figure 1). The rice accessions were grown under normal sown irrigated conditions at Experimental Farm of Division of Genetics, ICAR-IARI, New Delhi during *Kharif* 2017. The seeds were sown in a flat-bed nursery. After 25 days, the seedlings were transplanted under Augmented Block Design with four standard checks: Badshahbhog, Kalanamak, P1176, and Taraori Basmati using six replications. The plant-to-plant and row to row distances were 15 and 20 cm, respectively. Standard package of practices were used to raise a healthy crop of rice landraces.

Morphological Characterization

After harvesting individual aromatic rice accessions, grains were shade dried to reduce the moisture content to 12% level and kept in zip-lock bags at room temperature. Grain's phenotypic observations were recorded for seven quantitative and two qualitative traits. Ten grains from each accession were measured for data recording of seven quantitative traits namely rough rice length (RRL), rough rice breadth (RRB), rough rice length to breadth ratio (RRLB), thousand grain weight (TGW), brown rice length (BRL), brown rice breadth (BRB) and brown rice length to breadth ratio (BRLB). The lemma and palea (husk) color and caryopsis pericarp color were recorded for all the landrace materials.

Molecular Analysis

Genomic DNA extracted from 30 random grains of each accession to access the allelic polymorphism in aromatic rice landrace accessions. The grains were germinated in folds of germination paper and DNA was extracted from two weeks old seedlings using cetyl trimethyl ammonium bromide (CTAB) method (Murray and Thompson, 1980). The quality and quantity of extracted DNA was checked in 0.8% agarose gel using standard uncut λ phage DNA as a weight marker. The DNA samples of 96 accessions of aromatic rices were diluted in Tris-EDTA (ethylene diamine tetra acetic acid) buffer to obtain a working concentration of 25 ng/µL. The rice landrace accessions were genotyped for grain length gene '*GS3*' located on chromosome 3 of the rice genome, using SF28 primer-pair (Fan *et al*., 2008; Figure 2). PCR amplification was carried out in 10 µL reaction mixture containing DNA template 25 ng, GoTaq Green (1X; Promega) containing optimized concentration of PCR buffer, dNTPs and enzyme Taq DNA polymerase, primers (20 pmol) and DNase free H_2O (Promega). The sequences of SF28 forward and reverse primers are 5'-TGCCCATCTCCCTCGTTTAC-3' and 5'-GAAACAGC AGGCTGGCTTAC-3'. The PCR was performed

Table 1: Details of aromatic rice landraces from Indo-Gangetic plains of India used in the assessment of genetic variation and allele mining of quality traits

S. No.	*Acc. Code*	*IC Number*	*Name of Landrace*	*Geographical Coordinates*	*District*	*State*
1.	ARG01	IC0086170	Adamchini	25° 1′ N 82° 33′ E	Mirzapur	Uttar Pradesh
2.	ARG02	IC0553809	Adamchini	25° 1′ N 82° 33′ E	Mirzapur	Uttar Pradesh
3.	ARG03	IC0553863	Adamchini	24° 27′ N 82° 59′ E	Sonbhadra	Uttar Pradesh
4.	ARG04	IC0553848	Adamchini	24° 27′ N 82° 59′ E	Sonbhadra	Uttar Pradesh
5.	ARG05	IC0553871	Adamchini	35° 0′ N 80° 0′ E	Chandauli	Uttar Pradesh
6.	ARG06	IC0553887	Adamchini	35° 0′ N 80° 0′ E	Chandauli	Uttar Pradesh
7.	ARG07	IC0554649	Kalanamak	26° 53′ N 83° 58′ E	Kushinagar	Uttar Pradesh
8.	ARG08	IC0574862	Kalanamak-1	25° 8′ N 83° 15′ E	Chandauli	Uttar Pradesh
9.	ARG09	IC0553882	Badshahbhog	35° 0′ N 80° 0′ E	Chandauli	Uttar Pradesh
10.	ARG10	IC0574865	Kalanamak-3	26° 39′ N 82° 0′ E	Ayodhya	Uttar Pradesh
11.	ARG11	IC0554631	Kalanamak	27° 18′ N 83° 43′ E	Maharajganj	Uttar Pradesh
12.	ARG12	IC0554622	Kalanamak	27° 16′ N 82° 49′ E	Siddharthnagar	Uttar Pradesh
13.	ARG13	IC0554644	Kalanamak	27° 8′ N 83° 33′ E	Maharajganj	Uttar Pradesh
14.	ARG14	IC0553819	Kalanamak	25° 1′ N 82° 33′ E	Mirzapur	Uttar Pradesh
15.	ARG15	IC0554657	Sakkarchini	27° 8′ N 81° 57′ E	Gonda	Uttar Pradesh
16.	ARG16	IC0554618	Kalanamak	28° 39′ N 79° 22′ E	Bareilly	Uttar Pradesh
17.	ARG17	IC0554624	Kalanamak	26° 48′ N 82° 45′ E	Basti	Uttar Pradesh
18.	ARG18	IC0553830	Badshahbhog	25° 8′ N 82° 33′ E	Mirzapur	Uttar Pradesh
19.	ARG19	IC0553833	Badshahbhog	24° 27′ N 82° 59′ E	Sonbhadra	Uttar Pradesh
20.	ARG20	IC0554639	Sakkarchini	27° 25′ N 82° 10′ E	Balrampur	Uttar Pradesh
21.	ARG21	IC0554641	Sakkarchini	27° 34′ N 81° 35′ E	Bahraich	Uttar Pradesh
22.	ARG22	IC0553836	Sonachur	25° 8′ N 82° 33′ E	Mirzapur	Uttar Pradesh
23.	ARG23	IC0370895	Sonachur	25° 18′ N 81° 57′ E	Prayagraj	Uttar Pradesh
24.	ARG24	IC0553875	Sonachur	35° 0′ N 80° 0′ E	Chandauli	Uttar Pradesh
25.	ARG25	IC0370882	Thakurbhog	24° 27′ N 82° 59′ E	Sonbhadra	Uttar Pradesh
26.	ARG26	IC0554652	Badshah Pasand	27° 34′ N 81° 35′ E	Bahraich	Uttar Pradesh
27.	ARG27	IC0554638	Badshah Pasand	26° 28′ N 81° 35′ E	Ambedkar Nagar	Uttar Pradesh
28.	ARG28	IC0554627	Lal Mati	26° 46′ N 81° 8′ E	Ayodhya	Uttar Pradesh
29.	ARG29	IC0554633	Lal Mati	26° 55′ N 81° 11′ E	Barabanki	Uttar Pradesh
30.	ARG30	IC0553883	Tulsi Manjari	24° 27′ N 82° 59′ E	Sonbhadra	Uttar Pradesh
31.	ARG31	IC0553844	Tulsi Manjari	24° 27′ N 82° 59′ E	Sonbhadra	Uttar Pradesh
32.	ARG32	IC0554629	Dubraj	26° 55′ N 81° 11′ E	Raibarelly	Uttar Pradesh
33.	ARG33	IC0553879	Shyam Jeera	24° 27′ N 82° 59′ E	Sonbhadra	Uttar Pradesh
34.	ARG34	IC0554647	Kanak Jeera	27° 16′ N 82° 49′ E	Siddharthnagar	Uttar Pradesh
35.	ARG35	IC0554654	Gopalbhog	26° 46′ N 83° 2′ E	Sant Kabir Nagar	Uttar Pradesh
36.	ARG36	IC0553839	Govindbhog	25° 8′ N 82° 33′ E	Mirzapur	Uttar Pradesh
37.	ARG37	IC0554646	Juhi Bengal	27° 17′ N 83° 5′ E	Siddharthnagar	Uttar Pradesh
38.	ARG38	IC0574876	Dhania 32	26° 39′ N 82° 0′ E	Ayodhya	Uttar Pradesh
39.	ARG39	IC0574922	Tulsi Joha	27° 12′ N 94° 9′ E	Lakhimpur	Assam
40.	ARG40	IC0574875	Juhi Bengal	26° 39′ N 82° 0′ E	Ayodhya	Uttar Pradesh
41.	ARG41	IC0419241	Tulsi Prasad	30° 54′ N 75° 48′ E	PAU, Ludhiana*	Punjab
42.	ARG42	IC0419239	Tulsi Joha-b	30° 54′ N 75° 48′ E	PAU, Ludhiana	Punjab
43.	ARG43	IC0419238	Tulsi Joha-a	30° 54′ N 75° 48′ E	PAU, Ludhiana	Punjab

44.	ARG44	IC0418945	Chinnisakkar	30° 54’ N 75° 48’ E	PAU, Ludhiana	Punjab
45.	ARG45	IC0419046	Jeera Sail-a	30° 54’ N 75° 48’ E	PAU, Ludhiana	Punjab
46.	ARG46	IC0419047	Jeera Sail-b	30° 54’ N 75° 48’ E	PAU, Ludhiana	Punjab
47.	ARG47	IC0419219	Soribhog	30° 54’ N 75° 48’ E	PAU, Ludhiana	Punjab
48.	ARG48	IC0418906	Begami 1-T-1-A	30° 54’ N 75° 48’ E	PAU, Ludhiana	Punjab
49.	ARG49	IC0419006	Hansraj-a	30° 54’ N 75° 48’ E	PAU, Ludhiana	Punjab
50.	ARG50	IC0418918	Bindli sd mutant-a	30° 54’ N 75° 48’ E	PAU, Ludhiana	Punjab
51.	ARG51	IC0418919	Bindli sd mutant-b	30° 54’ N 75° 48’ E	PAU, Ludhiana	Punjab
52.	ARG52	IC0418920	Bindli sd mutant-c	30° 54’ N 75° 48’ E	PAU, Ludhiana	Punjab
53.	ARG53	IC0419052	Kala Jira-245	30° 54’ N 75° 48’ E	PAU, Ludhiana	Punjab
54.	ARG54	IC0419055	Kala Jira 449-b	30° 54’ N 75° 48’ E	PAU, Ludhiana	Punjab
55.	ARG55	IC0419050	Kala Jira-8-1-a	30° 54’ N 75° 48’ E	PAU, Ludhiana	Punjab
56.	ARG56	IC0419053	Kala Jira-286	30° 54’ N 75° 48’ E	PAU, Ludhiana	Punjab
57.	ARG57	IC0419051	Kala Jira-8-1-b	30° 54’ N 75° 48’ E	PAU, Ludhiana	Punjab
58.	ARG58	IC0419054	Kala Jira 449-a	30° 54’ N 75° 48’ E	PAU, Ludhiana	Punjab
59.	ARG59	IC0418979	Dulhabhog	30° 54’ N 75° 48’ E	PAU, Ludhiana	Punjab
60.	ARG60	IC0574897	Malbhog Sataria	25° 46’ N 85° 52’ E	Samastipur	Bihar
61.	ARG61	IC0569188	Champaran Basmati	27° 9’ N 84° 21’ E	West Champaran	Bihar
62.	ARG62	IC0574775	Tulsimanjari I	25° 20’ N 86° 58’ E	Bhagalpur	Bihar
63.	ARG63	IC0574776	Tulsimanjari II	25° 20’ N 86° 58’ E	Bhagalpur	Bihar
64.	ARG64	IC0574843	Kalanamak	25° 46’ N 85° 52’ E	Samastipur	Bihar
65.	ARG65	IC0574883	Malbhog Sataria-B	25° 46’ N 85° 52’ E	Samastipur	Bihar
66.	ARG66	IC0574927	Sonachur	25° 46’ N 85° 52’ E	Samastipur	Bihar
67.	ARG67	IC0574793	Badshahbhog	25° 20’ N 86° 58’ E	Bhagalpur	Bihar
68.	ARG68	IC0574830	Badshahbhog	25° 46’ N 85° 52’ E	Samastipur	Bihar
69.	ARG69	IC0569410	Kalonuniya	26° 32’ N 88° 43’ E	Jalpaiguri	West Bengal
70.	ARG70	IC0470970	Badshahbhog	22° 8’ N 88° 24’ E	South 24 Parganas	West Bengal
71.	ARG71	IC0569444	Tulsibhog	25° 58’ N 88° 3’ E	North Dinajpur	West Bengal
72.	ARG72	IC0569416	Lal Badshahbhog	23° 16’ N 86° 25’ E	Puruliya	West Bengal
73.	ARG73	IC0584173	Hari Bhog	23° 28’ N 88° 31’ E	Nadia	West Bengal
74.	ARG74	IC0594002	Kanakchur	22° 11’ N 88° 41’ E	South 24 Parganas	West Bengal
75.	ARG75	IC0594012	Sitabhog	22° 8’ N 88° 24’ E	South 24 Parganas	West Bengal
76.	ARG76	IC0594017	Tulaipanji	22° 11’ N 88° 48’ E	South 24 Parganas	West Bengal
77.	ARG77	IC0569443	Tulaipanji	26° 20’ N 89° 26’ E	Cooch Behar	West Bengal
78.	ARG78	IC0596840	Kalonunia	22° 13’ N 88° 46’ E	South 24 Parganas	West Bengal
79.	ARG79	IC0596834	Gobindabhog	22° 9’ N 88° 49’ E	South 24 Parganas	West Bengal
80.	ARG80	IC0596837	Gobindabhog	22° 20’ N 88° 41’ E	South 24 Parganas	West Bengal
81.	ARG81	IC0470934	Govindbhog	22° 8’ N 88° 24’ E	South 24 Parganas	West Bengal
82.	ARG82	IC0611186	Lal Basmati Local	31° 35’ N 76° 55’ E	Mandi	H.P.
83.	ARG83	IC0611190	Begami	32° 33’ N 76° 7’ E	Chamba	H.P.
84.	ARG84	IC0085729	Gandhasalai	12° 46’ N 75° 12’ E	Dakshina Kannada	Karnataka
85.	ARG85	IC0280808	Jeerakachampav	9° 51’ N 76° 57’ E	Idukki	Kerala
86.	ARG86	IC0591553	Wayanad Jeerakasala	11° 22’ N 76° 7’ E	Wayanad	Kerala
87.	ARG87	IC0520332	Jeerakasala	11° 51’ N 75° 21’ E	Kannur	Kerala
88.	ARG88	IC0121907	Ambemohar	21° 8’ N 79° 5’ E	Nagpur	Maharashtra
89.	ARG89	IC0574923	Tulsiamrit	17° 40’ N 74° 10’ E	Satara	Maharashtra

90.	ARG90	IC0618897	Jeeragasamba	9° 13′ N 78° 25′ E	Ramanathapuram	Tamil Nadu
91.	ARG91	IC0454228	Kamod	30° 54′ N 75° 48′ E	PAU, Ludhiana	Punjab
92.	ARG92	IC0553842	Tinsukhiya	25° 8′ N 82° 33′ E	Mirzapur	Uttar Pradesh
93.	ARG93	Check01	Badshahbhog	28° 38′ N 77° 10′ E	Genetics, IARI*	Delhi
94.	ARG94	Check02	Kalanamak	28° 38′ N 77° 10′ E	Genetics, IARI	Delhi
95.	ARG95	Check03	P1176	28° 38′ N 77° 10′ E	Genetics, IARI	Delhi
96.	ARG96	Check04	Taraori Basmati	28° 38′ N 77° 10′ E	Genetics, IARI	Delhi

*Abbreviations: PAU = Punjab agricultural University, Ludhiana; H.P. = Himachal Pradesh; Genetics IARI = Genetics Division of ICAR - Indian Agricultural Research Institute, New Delhi.

Table 2: Indian states, districts and number of aromatic rice landrace accessions characterized

S. No.	*Name of the state*	*Name of district and number of accessions*	*Number of collections*
(a).	*Indo-Gangetic Plain region (27 Districts)*		
1.	Uttar Pradesh	Mirzapur (7), Sonbhadra (7), Chandauli (5), Kushinagar (1), Ayodhya (4), Maharajganj (2), Siddharthnagar (3), Gonda (1), Bareilly (1), Basti (1), Balrampur (1), Bahraich (2), Ambedkar Nagar (1), Barabanki (1), Raibarelly (1), Sant Kabir Nagar (1) and Prayagraj (1)	40
2.	Punjab	Ludhiana (20)	20
3.	Bihar	Samastipur (5), Bhagalpur (3) and West Champaran (1)	9
4.	West Bengal	South 24 Parganas (8), Jalpaiguri (1), Uttar Dinajpur (1), Puruliya (1), Nadia (1) and Cooch Behar (1)	13
(b).	*Other States of India (10 Districts)*		
5.	Assam	Lakhimpur (1)	1
6.	Himachal Pradesh	Mandi (1) and Chamba (1)	2
7.	Karnataka	Dakshina Kannada (1)	1
8.	Kerala	Idukki (1), Wayanad (1) and Kannur (1)	3
9.	Maharashtra	Nagpur (1), Satara (1)	2
10.	Tamil Nadu	Ramanathapuram (1)	1

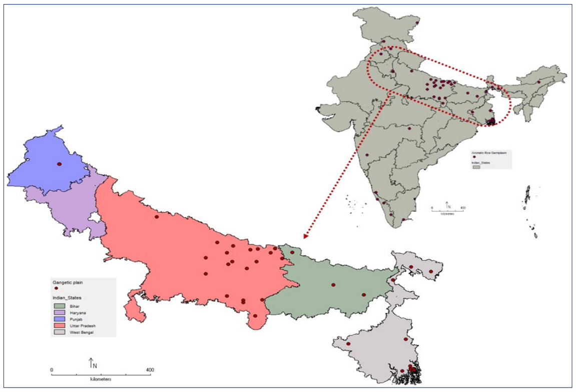

Figure 1: Geo-referencing of 92 accessions of aromatic rice landrace collection sites located in 37 districts of 10 states of India.

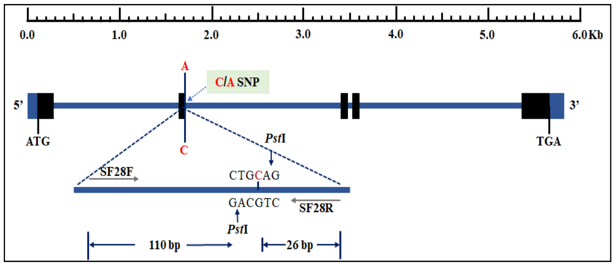

Figure 2: Structure of GS3 gene. Black boxes indicate exons, while Blue boxes indicate 5′ and 3′ Untranslated Regions. Functional mutation responsible for grain length is C/A SNP in the second exon (modified from Fan *et al.*, 2008).

in Eppendorf's thermocycler using the following thermal cycling parameters: initial DNA denaturation at 95°C for 4 minutes, followed by 35 cycles of 94°C for 30 seconds, 55°C for 30 seconds, 72°C for 30 seconds and finally a primer extension cycle of 5 minutes at 72°C. Restriction digestion was performed in 15 µL reaction volume containing buffer (O), *Pst*I enzyme, and H_2O (Promega) to digest *GS*3 gene PCR amplicons. The reaction mixture was incubated for 60 minutes at 37°C for complete digestion. The enzyme-cleaved products were separated in 3.5% agarose gel pre-stained with ethidium bromide. The resolved products were visualized under UV light and documented using the syngene gel documentation system. The gel profiles were scored for the presence of domesticated and mutated alleles of *GS*3 gene.

Statistical Analysis

The data of seven quantitative grain traits were subjected to analysis of variance (ANOVA). Descriptive statistics and genetic parameters were calculated. The adjusted mean values obtained from ANOVA were used for principal component analysis (PCA) and for correlation studies. Pearson correlation coefficient values were used to determine the relationship between different traits. The R statistical environment (base package) has been used for the analyses. For two qualitative qualities, frequency distribution bar diagrams were generated. Sivasubramanian and Madhavamenon (1973) categorization was used to classify the PCV and GCV values, whereas Robinson (1966) categorization of heritability values was followed. Genetic advance was categorized in accordance with Johnson *et al*. (1955).

Results

Phenotypic Diversity for Quantitative Grain Traits

ANOVA showed significant differences for all the grain traits and revealed the presence of high extent of morphological variability in the aromatic rice landraces (Supplementary Table 1). The descriptive statistics for seven grain morphometric characters revealed that ample variability exists in aromatic rice landrace germplasm (Table 3 Figure 3). The significant variations were observed for RRL, which ranged from 5.04 (IC0553830/ Badshahbhog) to 10.77 mm (Taraori Basmati) with mean value of 6.91 mm, and RRB, which varied from 1.61 (IC0554622/ Kalanamak) to 3.45 mm (IC419047/Jeera Sail-b) with mean value of 2.18 mm. RRLB ranged from 2.07 (IC0419219/ Soribhog) to 5.25 mm (Taraori Basmati) with average of 3.12 mm. TGW ranged from 4.98 (IC0569444/Tulsibhog) to 27.68 g (IC0419006/ Hansraj-a) with an average of 12.18 g. BRL varied from 3.5 (IC0553809/ Adamchini) to 7.69 mm (IC0419052/ Kala Jira-245) with mean value of 4.92 mm, whereas BRB ranged from 1.49 (IC0553819/ Kalanamak) to 3.13 mm (IC0419047/Jeera Sail) with mean value of 1.88 mm. For BRLB, the mean value was 2.63 on a scale of 1.72 (IC0419047/Jeera sail) to 4.52 (Taraori Basmati). Medium values of GCV and PCV were observed for five traits *viz*., RRL, RRB, BRL, BRB and BRLB, while high GCV and PCV values were recorded for TGW and RRLB. All the traits under study had comparable PCV and GCV values, indicating lesser influence of the environment on the expression of these traits. The broad-sense heritability estimates ranged from 85.7 (BRLB) to 99.1% (BRB) with all traits showing high heritability. The genetic advance ranged from 30.72 (BRB) to 74.92% (TGW) revealing high GA for all the traits.

Ward's method of agglomerative cluster analysis grouped 92 aromatic rice landrace accessions into three clusters based on Euclidean distances (Figure 4). The clusters I, II and III comprised 26, 37 and 33 aromatic rice landrace accessions. The cophenetic value of 0.497 was obtained for the dendrogram.

Principal Component Analysis

Principal component analysis (PCA) revealed that first two PCs accounted for 86.04% variance. PCA bi-plot illustrated the distribution and diversity type for grain morphometric traits and the aromatic rice landrace accessions (Figure 5). The diagram depicts that all grain morphometric traits had substantial diversity. On the basis of PCA bi-plot aromatic rice landrace accessions could be selected for various grain traits like accession IC0419006 (Hansraj) for TGW and Taraori Basmati for BRL and BRLB ratio. PC1 contributed to 55.34% of the total variation with large loadings in BRL (0.4954), RRL (0.4916), TGW (0.4394) and BRLB (0.3753). The PC2 contributed 30.70% of the overall variation with large contributions from RRB (0.5505), BRB (0.5493), RRLB (0.4135) and BRLB (0.3962). The PC3, PC4 and PC5 contributed 8.06, 3.05 and 1.88% of the total variance, respectively (Supplementary Table 2). In the present study, total variance existing among aromatic rice landraces represented by seven PCs and the first three PCs contributed to 94.10% of the total variance, indicating a high correlation among the traits being investigated. Scree plot depicted the percentage proportion of variance associated with each PC (Figure 6). PC1 contributed to 55.34% variability of total data set. The variance is gradually declining up to PC3. After

Table 3: Descriptive statistics of seven quantitative grain traits analysed in 96 accessions of aromatic rices

Trait	Range		Mean ± SE	SD	CV	GCV	PCV	ECV	Hbs (%)	GA	GAM
	Min.	Max.									
RRL	5.04	10.77	6.91 ± 0.13	1.32	2.41	18.1	18.27	2.48	98.16	2.56	36.99
RRB	1.61	3.45	2.18 ± 0.04	0.36	3.50	16.41	16.77	3.46	95.75	0.72	33.13
RRLB	2.07	5.25	3.12 ± 0.08	0.77	5.11	23.02	23.63	5.34	94.89	1.45	46.26
TGW	4.98	27.68	12.18 ± 0.49	4.84	12.8	38.38	40.56	13.12	89.54	9.13	74.92
BRL	3.50	7.69	4.92 ± 0.10	1.02	5.69	19.63	20.47	5.80	91.97	1.91	38.85
BRB	1.49	3.13	1.88 ± 0.03	0.28	1.43	14.96	15.02	1.40	99.13	0.58	30.72
BRLB	1.72	4.52	2.63 ± 0.05	0.53	6.85	17.44	18.85	7.13	85.67	0.88	33.31

Abbreviations: RRL: Rough rice length, RRB: Rough rice breadth, RRLB: Rough rice length to breadth ratio, TGW: Thousand grain weight, BRL: Brown rice length BRB: Brown rice Breadth; BRB: Brown rice length to breadth ratio PCV: Phenotypic coefficient of variation, GCV: Genotypic coefficient of variation, PCV: Phenotypic coefficient of variation, Hbs: Heritability (broad sense), GA: Genetic advance, GAM: Genetic advance as per cent of mean.

Figure 3: Variability of husk color in representative accessions of aromatic rice landraces a): Adamchini (ARG06), b): Badshahbhog (ARG18), c): Sakkarchini (ARG 21), d): Lal Mati (ARG29), e): Kanak Jeera (ARG34), f): Gopalbhog (ARG35), g): Kalanamak (ARG39), h): Tulsi Prasad (ARG41), i): Tulsi Joha (ARG42), j): Jeera Sail (ARG46), k): Soribhog (ARG47) and l): Bindli (ARG50). Below panel: variability for brown color of dehusked grains (caryopsis pericarp).

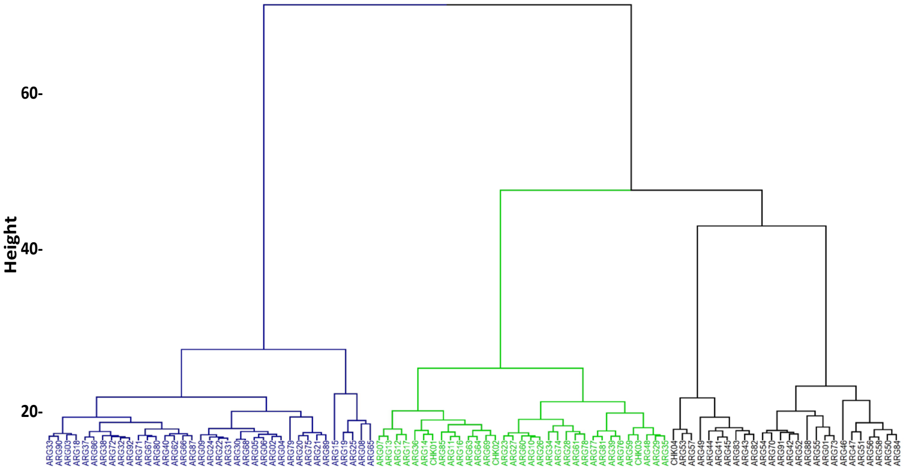

Figure 4: Cluster dendrogram constructed using Ward.D2 method of hierarchical clustering showing inter-relationships amongst 96 accessions of aromatic rice accessions. Three clusters formed.

that straight line with little variance was observed in each PC. From the graph, we could conclude that maximum variation was included in PC1 and PC2; hence, the genotypes could be selected from these PCs for further breeding programmes.

Correlation Studies

Understanding the relationship between grain traits is important for improving yield and quality of rice. To comprehend the type and extent of the association between the seven quantitative grain traits, Pearson's correlation coefficients were calculated (Supplementary Table 3; Figure 7). RRL had highly significant positive correlation with BRL (0.94), BRLB (0.76) and RRLB (0.61). RRB showed highly significant positive association with BRB (0.63). RRLB ratio

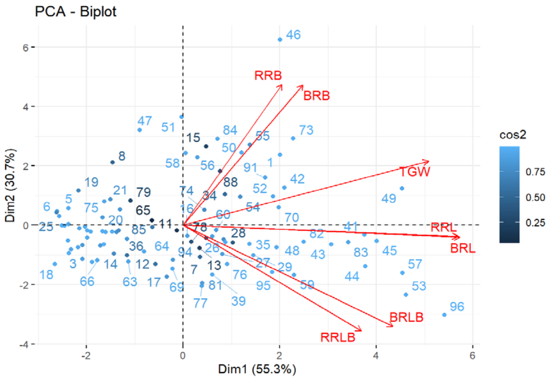

Figure 5: Principal Component-Biplot depicting two dimensional alignment of aromatic rice landrace accessions for seven quantitative grain traits for first two principal components (PC1 and PC1).

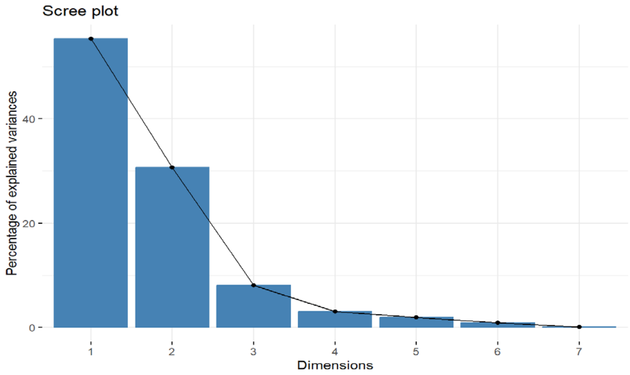

Figure 6: Scree plot for different PCs in aromatic rice germplasm accessions.

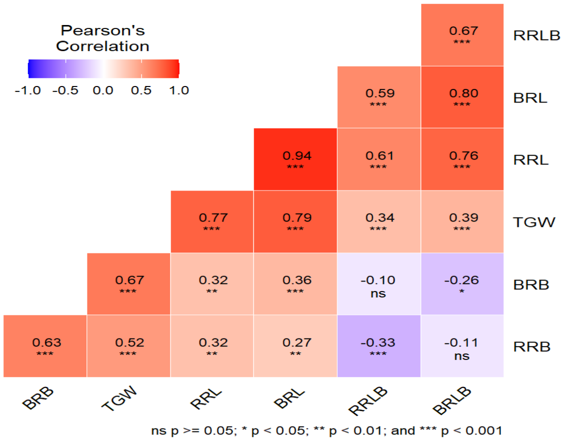

Figure 7: Correlation between rice grain dimension traits. Rough rice length (RRL), rough rice breadth (RRB), rough rice length to breadth ratio (RRLB), brown rice length (BRL), brown rice breadth (BRB), brown rice length to breadth ratio (BRLB) and thousand grain weight (TGW).

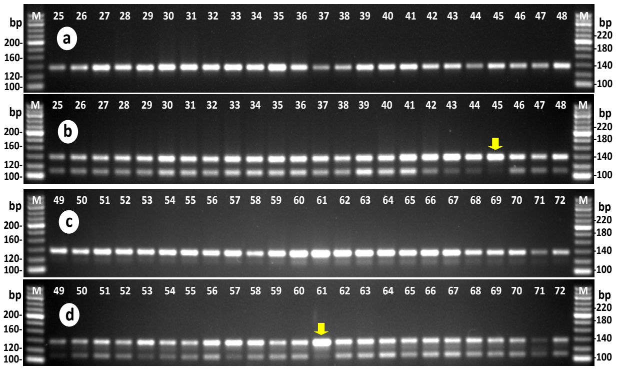

Figure 8: Gel profile of GS3 gene generated by SF28 primers in 96 aromatic rice accessions. (a) Undigested amplicons of 136 bp in accessions 25-48, (b) DNA fragments of 136 bp and 110 bp after digestion with PstI restriction enzyme in accessions 25-48, (c) Undigested amplicons of 136 bp in accessions 49-72, and (d) DNA fragments of 136 bp and 110 bp after digestion with PstI restriction enzyme in accessions 49-49. Arrow indicates the undigested amplicon showing in accessions 45 (Jeera Sail-a) and 61 (Champaran Basmati).

also showed highly significant and positive association with BRLB (0.67) and BRL (0.59). However, it revealed negative correlation with RRB (-0.33). BRL showed highly significant positive association with BRLB (0.80). TGW showed highly significant positive correlation with BRL (0.79), RRL (0.77), BRB (0.67) and RRB (0.52).

Phenotypic Diversity in Qualitative Traits

The grain color (lemma-palea color) was recorded in 96 accessions of aromatic rices. Straw colored hull (lemma and palea) was present in 65.96% accessions followed by gold furrows on straw colored hull in 15.62% and black colored in 14.58% of accessions. However, two accessions each showed brown spots on straw and brown tawny hull color. Husk was removed and observations were recorded for caryopsis pericarp color (Figure 3b). White colored caryopsis pericarp was observed in 84 genotypes (87.50%), followed by brown colored (10.42%) and light brown colored (2.08%). On the basis of brown rice length to width ratio aromatic rice landraces were classified into three size categories namely slender (21.88%), medium (73.96%) and bold (4.17%). On the basis of brown rice length, the germplasm under study is categorized in to three groups *viz.*, short (76.04%), medium (16.67%) and long grained (7.29%) accessions.

GS3 C/A Allele Genotyping

The SF28 primers generated a 136-bp fragment from second exonic region of the gene '*GS3*' in all aromatic rice landrace accessions (Figure 8). *PstI*, a restriction enzyme (hexacutter), was used to digest the PCR amplicons. *Pst*I recognised the restriction site CTGCAG and cleaved the amplicons into two fragments of 110 and 26 bp. The restriction site of *PstI* is reported to be present in short to medium grained rice cultivars. This represents the wild type allele, whereas the amplicon remains undigested by pstI enzyme in domesticated allele. Because these varieties contain a C to A SNP mutation, which results in elimination of the enzyme restriction site (CTGAAG). The rice landraces with an undigested fragment of 136 bp were scored as presence (+) of C/A SNP mutation and those with digested fragment of 110 and 26 bp size were scored as absence (-) of C/A SNP mutation. In panel of 96 aromatic rice accessions, only two landrace accessions, IC419046 (Jeera Sail-a) and IC569188 (Champaran Basmati) possessed (2.08%) the domesticated allele (Figure 8). Remaining landrace accessions had both fragment of size 136 and 110 bp after restriction digestion. These accessions were scored as heterozygous with respect to this allele.

Discussion

Grain dimension traits are important for rice quality and consumers' acceptability. We have analyzed seven quantitative and two qualitative traits in 92 aromatic rice landraces accessions and found high extent of variability for these traits. The earlier studies from India and abroad also reported high variability for grain morphological traits (Karim *et al*., 2007; Islam *et al*., 2018; Prasad *et al*., 2020; Dixit *et al*., 2022). In a 208 indigenous aromatic rice germplasm study, Prasad *et al.* (2020) reported a dehusked grain length of 3.4 to 7.2 mm with a mean of 4.7 mm, whereas the grain breadth was 1.3 to 4.4 mm, with a mean value of 2.3 mm. The grain length/ breadth ratio was between 1.3 and 4.4 with a mean value of 2.3. They also observed that 41% of rice germplasm showed very low (<15 g) TGW. Jaiswal *et al.* (2007) reported high heritability (98.0%) for grain length, breadth and L/B ratio, and TGW (10.90 – 24.00 g with a mean of 16.40 g) in a set of 25 aromatic rice landrace accessions from eastern Uttar Pradesh that included accessions of Kalanamak, Juhi Bengal, Kanak Jeera and Dhania. However, Karim *et al*. (2007) recorded 5.90 to 30.72 g TGW with high heritability of 98.5% in 41 aromatic rices. The variation for grain length (3.7 to 6.8 mm), width (1.2 to 2.3 mm) and L/B ratio (1.82 to 3.25) was reported by Bisne and Sarawgi (2008) in 32 accessions of Badshahbhog landrace.

The earlier studies also reported high heritability (88.0–99%) for grain length, grain breadth, grain L:B ratio and TGW (Nirmaladevi *et al*. 2015; Roy *et al*., 2021) and support our results. Medium range of GCV and PCV values for grain length (11.19 and 11.20%), breadth (10.05 and 10.09%) and length/breadth ratio (16.81 and 16.83%) was reported by Nirmaladevi *et al*. (2015). In another study of 213 traditional rice varieties from Chhattisgarh, India, Sahu *et al*. (2017) reported moderate levels of GCV and PCV for the grain traits *viz.,* brown rice length (12.60 and 12.85%), brown rice width (14.78 and 16.44%) and brown rice L/B ratio (13.89 and 16.10%). They also observed high genetic advance (24.68–27.36%) for these traits. However, in our study a higher range of genetic advance (30.72–46.26%) observed for all the traits except TGW, which showed the highest GA (74.92%). The minor variations in the findings are due to difference in genetic makeup of landrace materials analyzed in these studies.

In the present study, grain length showed positive association with grain breadth (0.32), which is lower than the value of correlation (0.67) reported for these traits by Lakhar and Tanti (2017) in aromatic rice germplasm of Assam, India. Ngangkham *et al*. (2018) found highly significant and strong positive correlation of grain length with grain length/ breadth ratio (GBLR; 0.91) and with TGW (0.58). Grain length contributes more to TGW as compared to grain breadth, while grain breadth negatively affects the GBLR (-0.54). Dixit *et al*. (2022) also reported highly significant positive correlation of brown rice (dehusked rice) grain L/B ratio with grain length (0.88) and significantly negative association with grain breadth (-0.508) and corroborates our findings.

In rice, *GS*3 is the major gene located on chromosome 3 that plays a pivotal role in regulation of grain length (Fan *et al*., 2006). In the present study, allele 'A' found in two accessions (2.1%), while in 94 accessions (97.9%) the SNP was in heterozygous form after molecular analysis of C/A SNP in *GS*3 gene in 96 aromatic rice accessions. Fan *et al.* (2008) reported the presence of 'C' allele in 142 rice varieties having grain length of 6.4 to 8.8 mm, whereas 38 varieties possessed allele 'A' with long grains (8.8 to 10.7 mm). Similarly, Ngangkham *et al*. (2018) also studied allelic polymorphism in 89 rice genotypes with respect to C/A SNP in *exon* 2 of *GS3* gene. They reported allele 'A' in 37.07% of the genotypes. Rasheed *et al*. (2022) screened 17 rice genotypes for the presence of C/A SNP in *exon* 2 of *GS3* gene and reported that domesticated allele was present in 15 rice accessions.

Conclusion

The current study revealed that accessions of aromatic rice landraces from India's Indo-Gangetic Plain region have significant genetic variation for grain morphometric traits. The genotypic and phenotypic coefficients of variation for grain phenotypic traits in the rice crop were found to be comparable. It suggests that these traits are quite stable in nature. In our germplasm grain length contributed more to 1000-grain weight as compared to grain breadth. The polymorphism revealed for C/A SNP in grain size gene *GS*3, which could be utilized for genetic enhancement of grain size in aromatic rices.

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Supplementary Table 1: ANOVA for seven quantitative grain traits studied in aromatic rice germplasm

Source	*D.F.*	*RRL*	*RRB*	*RRLB*	*TGW*	*BRL*	*BRB*	*BRLB*
Treatment (ignoring Blocks)	95	2.56 **	0.13 **	0.85 **	26.61 **	1.55 **	0.09 **	0.49 **
Treatment: Check	3	24.33 **	0.02 *	5.94 **	78.69 **	15.79 **	0.01 **	5.27 **
Treatment: Test vs. Check	1	24.65 **	0.37 **	13.09 **	70.27 **	7.07 **	0.86 **	8.54 **
Treatment: Test	91	1.59 **	0.13 **	0.55 **	22.41 **	1.02 **	0.08 **	0.25 **
Block (eliminating Treatments)	5	0.01 ns	0.004 ns	0.03 ns	4.51 ns	0.05 ns	0.00025 ns	0.02 ns
Residuals	15	0.03	0.01	0.03	2.55	0.08	0.00068	0.04

*, ** Significant at 0.05 and 0.001 level of probability; Abbreviations: ns: non-significant; RRL: Rough rice length, RRB: Rough rice breadth, RRLB: Rough rice length to breadth ratio, TGW: Thousand grain weight, BRL: Brown rice length BRB: Brown rice Breadth; BRB: Brown rice length to breadth ratio.

Supplementary Table 2: The Eigen vectors, proportion of variance, cumulative proportion of variance and Eigen values for principal components for seven grain traits

Parameter	*PC1*	*PC2*	*PC3*	*PC4*	*PC5*	*PC6*	*PC7*
RRL	-0.4916	0.0432	-0.1279	-0.1110	-0.2099	0.8268	-0.0416
RRB	-0.1769	-0.5505	-0.5163	-0.5879	0.0326	-0.2252	0.0411
RRLB	-0.3190	0.4135	0.5061	-0.6522	0.0512	-0.2068	0.0188
TGW	-0.4394	-0.2514	0.1760	0.2495	0.8057	0.0156	-0.0322
BRL	-0.4954	0.0477	-0.0682	0.3133	-0.3043	-0.3090	0.6792
BRB	-0.2142	-0.5493	0.4990	0.1404	-0.4539	-0.1380	-0.3984
BRLB	-0.3753	0.3962	-0.4199	0.1912	-0.0666	-0.3313	-0.6125
Eigenvalues	3.8737	2.1490	0.5643	0.2136	0.1317	0.0617	0.0059
Proportion	0.5534	0.3070	0.0806	0.0305	0.0188	0.0088	0.008
Cumulative Proportion	0.5534	0.8604	0.9410	0.9715	0.9903	0.9991	1.000

Abbreviation: RRL: Rough rice length, RRB: Rough rice breadth, RRLB: Rough rice length to breadth ratio, TGW: Thousand grain weight, BRL: Brown rice length BRB: Brown rice Breadth; BRB: Brown rice length to breadth ratio

Supplementary Table 3: Pearson correlation coefficients values between 7 quantitative grain traits studied in 96 aromatic rice landraces accessions

	RRL	*RRB*	*RRLB*	*TGW*	*BRL*	*BRB*	*BRLB*
RRL	---	0.325 (0.0012)	0.613 (0.0000)	0.773 (0.0000)	0.938 (0.0000)	0.323 (0.0013)	0.762 (0.0000)
RRB		---	-0.333 (0.0009)	0.519 (0.0000)	0.267 (0.0086)	0.633 (0.0000)	-0.109 (0.2908)
RRLB			---	0.340 (0.0007)	0.593 (0.0000)	-0.102 (0.3237)	0.673 (0.0000)
TGW				---	0.795 (0.0000)	0.670 (0.0000)	0.386 (0.0001)
BRL					---	0.364 (0.0003)	0.796 (0.0000)
BRB						---	-0.261 (0.0103)
BRLB							---

Data in parentheses means the p-values.