



## Research article

## Genetic characterization and diversity analysis of indigenous aromatic rice

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## ABSTRACT

Gangetic old alluvial zone in India has conserved many locally adapted aromatic rice landraces. In order to determine the extent of genetic divergence of ten morphological characters, the study was conducted to examine forty-eight aromatic rice genotypes for six *Kharif* seasons (2016–2021) at the Instructional Farm of Regional Research Station (Old Alluvial Zone), Uttar Banga Krishi Viswavidyalaya, Majhian, West Bengal, India. The experiment was laid out in Randomized Complete Block Design (RCBD) with three replications. A considerable degree of variation was noted for all the traits being investigated. It was found that the total number of tillers per plant, panicle numbers per plant, number of grains per panicle, fertility percentage, test weight, and grain length/breadth ratio had significantly positive correlated with seed yield per plant. Based on D<sup>2</sup> analysis values, all the genotypes were grouped into six clusters. Cluster III (Tulaipanji, Patnai, Basmati 1121, Jugal, and Bahurupi) and Cluster VI (Kanakchur), containing genotypes were found most divergent with maximum inter-cluster distance (6941.51). According to the cluster means, Cluster II had the largest intra-cluster distance (1937.52), and important attributes including test weight, number of grains per panicle, seed yield per plant, and fertility percentage made remarkably significant contributions to this cluster. In terms of principal component analysis, maximum variability was found in PC1 (23.88 %), with high positive loading values for tillers per plant (0.459), panicle number per plant (0.441), seed yield per plant (0.408), fertility percentage (0.364), test weight (0.264), and grain length/breadth (L/B) ratio (0.263). On the basis of biplot analysis, four genotypes, namely Shakhbhati, Sugandhi, Bahurupi and Kanakchur, were identified as the most divergent types for the yield-attributing traits of aromatic rice. The diverse genotypes could be used as potential donors in future breeding programmes.

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## 1. Introduction

Rice is a cheap, adaptable, and nutrient-dense grain that is primarily consumed in Asia. Rice is regarded as the “global grain” and is

### Abbreviations

PCA	Principal component analysis
PCV	Phenotypic coefficient of variation
GCV	Genotypic coefficient of variation
GI	Geographical indication
PH	Plant height
DF	Days to 50 % flowering
TPP	Total number of tillers per plant
PNP	Panicle number per plant
PL	Panicle length
NGP	Number of grains per panicle
FP	Fertility percentage
TW	Test weight
LBR	Length/breadth ratio
SYP	Seed yield per plant
WAT	Week after transplanting

a staple food for over 50 % of the world’s population [1]. Since it provides 20 % of the world’s dietary energy, it is considered as most significant strategic crop for global food and nutritional security, followed by wheat (19 %) and maize (5 %) [2]. This commodity ranks third on the global production list, accounting for 8 percent of all agricultural output, following sugarcane and maize [3]. Rice is grown under diverse ecosystems in India viz., deep water, rainfed lowland, rainfed upland, and irrigated. For centuries, the old alluvial zone of the Gangetic plains has preserved a high level of agro-biodiversity in rice germplasm, providing both genetic and nutritional diversity [4]. Having an abundance of indigenous aromatic rice landraces, this area is considered one of the pioneers in rice cultivation in India. From this region two cultivars, namely Tulaipanji [5], and Kalonunia, have garnered significant interest on the global stage and have been granted geographical indication status (GI).

From Southeast Asia, the demand for premium rice has expanded to Africa, Europe, and the United States. India has witnessed an increase in the demand for premium rice production, driven by a focus on exports and a rise in living standards. Aromatic or scented rice has long been considered auspicious in Indian society mainly for its excellent quality and fragrance. Due to its ‘pop-corn’ like aroma and its peculiar fragrance they are much preferred by foreign consumers [6], thus increasing India’s foreign exchange earnings from the export of this commodity. It produces a fine, appealing flavour at the time of cooking and shows well separability and non-stickiness with absolutely no hardcore after cooking. So far, the compounds like alkanals, alk-2-enals, 4-dienals, 2-pentylfuran, 2-pentyl ethanol and 2-acetyl-1-pyrroline have been identified for its aroma [7]. Out of these, 2-acetyl-1-pyrroline is the primary aroma compound that gives its characteristic flavour. Thus, this compound is of prime importance in the food industry [8].

Historically, aromatic rice has only been grown in a few areas of eastern India, including West Bengal, Orissa, Western Uttar Pradesh, and Assam. However, many farmers have lost interest in growing aromatic rice because of their lodging problem and low yield potential. It is, therefore, essential to develop high-yielding, aromatic rice varieties. A high level of genetic variability and distinctive qualities, such as resistance to biotic and abiotic stresses, are also present in traditional aromatic rice. In the selection process of crops, variability plays a dynamic role in identifying superior varieties [9]. The knowledge of genotypic and phenotypic variances [10] and their coefficient of variation [11] can help to know the influence of genetic makeup, environments and their interaction on traits which ultimately direct us to know the status of selection criterion of traits for the crop improvement programs [12]. Success in crop improvement generally depends on the magnitude of genetic variability and/or diversity [13,14] and the extent to which the desirable characteristics are heritable [15,16]. The degree of variation of heritable components is very important because it would guide the selection of parents [17]. A complex trait like grain yield is controlled by many genes as well as influenced by the environment [18,19]. It is determined by the magnitude and nature of genetic variability [20,21]. In addition, grain yield is related to other traits such as plant types, growth duration, and other yield components [22,23]. These traits are also correlated among themselves [24,25]. Therefore, to incorporate locally grown aromatic rice varieties into the hybridization program, it is imperative to evaluate their genetic diversity [26]. Evaluation of the genetic diversity and variability present in base populations is a prerequisite for the success of any breeding programme [27,28]. This variability can be estimated by applying suitable biometrical techniques. Multivariate analysis is one method for assessing this variability. Principal component analysis (PCA) and cluster analysis ( $D^2$ ) are two multivariate statistical methods that are primarily used to eliminate repetitiveness in phenotypic data [29] and reveal genetic diversity [30]. The present study, therefore, aimed to estimate the genetic diversity of aromatic rice genotypes in the Gangetic old alluvial plain region of India.

## 2. Materials and methods

### 2.1. Description of experimental sites

The experiment was conducted at Instructional Farm, Regional Research Station, Uttar Banga Krishi Viswavidyalaya, Majhian, Dakshin Dinajpur, West Bengal, India. The study site is situated at longitude 88.7635° E and latitude 25.3118° N in the old alluvial agro climatic zone of West Bengal, close to the Bangladesh border at an elevation of 15 m from the mean sea level, with an annual rainfall of 1690 mm. This alluvial soil is composed primarily of clay and silt, which originated from the Ganges river. The meteorological data (average rainfall, minimum and maximum temperatures) recorded throughout the rice cultivation period, is illustrated in Fig. 1.

### 2.2. Experimental materials

A total of forty-eight aromatic rice (*Oryza sativa* var. indica) genotypes were collected for the study from different parts of the Gangetic old alluvial region (Fig. 2). The source and kernel colour characteristics for each genotype are shown in Table 1.

### 2.3. Evaluation and cultural practices

The investigation was conducted utilising replicated pooled data and a six-year evaluation of forty-eight aromatic rice genotypes during the *Kharif* season from 2016 to 21. Twenty-five-day-old seedlings of each genotype were transplanted in the main field and sown in a plot size of 4 m<sup>2</sup> with a spacing of 20 × 20 cm. A randomized complete block design (RCBD) was followed with three replications for each genotypes. Before rice seedlings were transplanted, *Sesbania rostrata* was planted in the main field and its vegetative portion was well mixed with the puddled soil. It exhibits notable biomass production and rapid growth within a short period of time, serving as a green manure that effectively fixes a substantial proportion of nitrogen in rice fields. This green manure also increased the amount of water-stable macro aggregates and the capacity of microbes to mineralize organic nitrogen [31]. Additionally, inorganic fertilizer was applied with very limited N: P: K at the rate of 40:30:20 kg/ha. Notably, no herbicide was used in the aromatic rice field. Manual weeding was done twice: first at two weeks after transplanting (WAT) and second at five weeks after transplanting. Neem oil at a rate of 2 mL per litre of water was sprayed three times (25 days after transplanting, 35 days after transplanting, and flowering stage) to suppress the insect infestation. The two applications of Tricyclazole at the rate of 0.5 g/lit was done to suppress the blast disease. Even though the rice cultivars were planted as rain-fed crops, they were given irrigation two to three times till reproductive stage. Birds perched at 35–40 numbers per hectare were implied to avoid rodent attacks during the pre-harvest stage.

### 2.4. Observation recorded

A total of ten plants were selected at random for observation in each replication. Biometrical observations on plant height in cm (PH), days to 50 % flowering (DF), total number of tillers per plant (TPP) inclusive of effective and ineffective tillers per plant, panicle number per plant (PNP), panicle length in cm (PL), number of grains per panicle (NGP), fertility percentage (FP), test weight in gram (TW), length/breadth ratio (LBR) and seed yield per plant in gram (SYP) were recorded. Grain length and grain width were measured using Vernier callipers.

### 2.5. Statistical analysis

The mean pooled data obtained from six *kharif* seasons (2016–21) was used for biometrical analysis. The genotypic and phenotypic

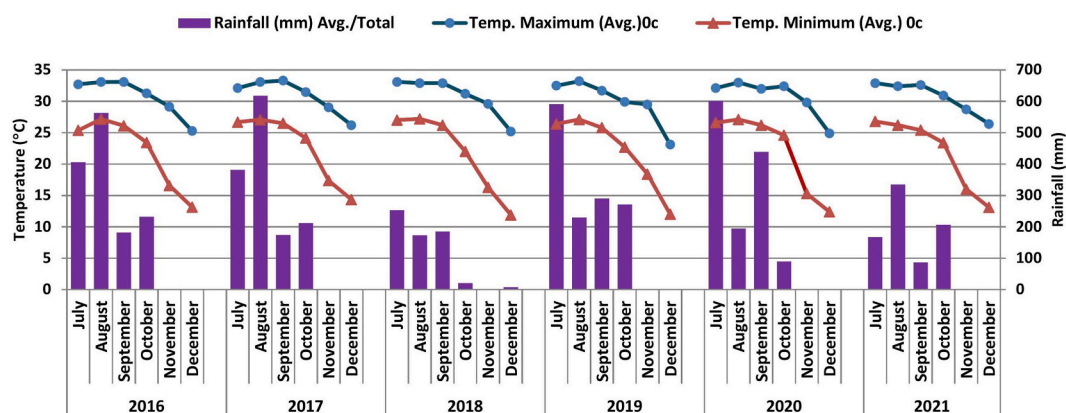


Fig. 1. Meteorological parameters observation during growing period of rice. The average rainfall (mm), maximum and minimum temperatures for the six-year (2016–21) *Kharif* season from July to December were recorded.

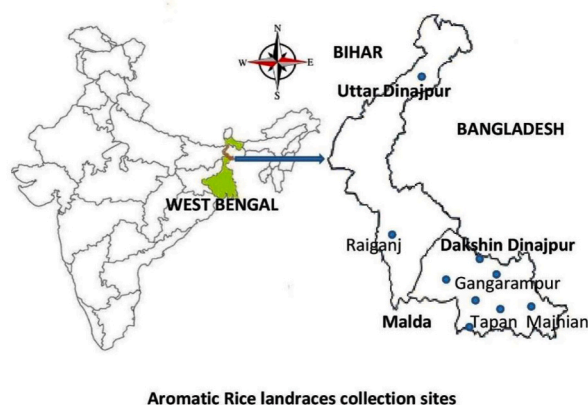


Fig. 2. Aromatic Rice germplasm collection sites from Gangetic Old Alluvial Region, India.

variation, broad sense heritability, genetic advance and genotypic and phenotypic correlation coefficients were estimated [32,33]. Path coefficients analysis was done according to the method suggested by Dewey and Lu [34]. Mahalanobis  $D^2$  statistics [35] were calculated to estimate the level of genetic divergence between the genotypes. By using Tocher's principle, non-hierarchical Euclidean cluster analysis was performed, and genotypes were categorized into distinct clusters based on the inter-cluster and intra-cluster generalized distances ( $D^2$ ) [36]. The multivariate principal component analysis (PCA) method used in this study was developed by Hotelling [37] based on an original concept presented by Pearson [38]. Principal component analysis and PCA-biplot were performed using R package version 4.2.3. The dendrogram was prepared using Past software version 4.11.

### 3. Results

#### 3.1. Genetic variability and character association for yield and its contributing traits of aromatic rice

Analysis of variance revealed significant differences ( $P < 0.05$ ) among the rice genotypes. The genetic parameters pertaining to the degree of variability among the aromatic rice genotypes were estimated (Table 2) using the genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), broad sense heritability (H), and genetic advance (GA). For the majority of characters, the magnitude of PCV was either significantly or marginally greater than that of GCV. The difference between PCV and GCV was noted to be maximum for the characters PL, PNP, and TPP, and these characters are suggested to be substantially influenced by the environment. In contrast, the maximum value for both GCV and PCV was noted in SYP, TPP, and NGP. Broad sense heritability was high (>80%) in all the characters studied except PL (41.74%), which indicates little environmental influence. It was observed that SYP, NGP, TPP, PNP, and TW had high genetic advances as a percentage of the mean.

At the genotypic ( $r_g$ ) and phenotypic ( $r_p$ ) levels, it was found that the characters TPP, PNP, NGP, FP, TW, and LBR had significant positive correlation with SYP (Table 3). A significant negative correlations were found between PH, PNP and TPP. The FF exhibited a notable positive correlation with PL and NGP and conversely a strong negative correlation with FP and TW. Moreover, TPP revealed significant positive correlations with PNP, PL, FP, and SYP. Similarly, PNP showed positive significant correlations with both SYP and FP. NGP exhibited positive significant correlations with SYP and negative correlations with TW and LBR. Positive correlations were found between FP and TW, LBR, and SYP.

The outcomes of the path coefficient (PC) analysis conducted on yield and yield component traits for a total of 48 rice genotypes are presented in Table 4. Maximum direct path coefficient effect towards seed yield per plant (SYP) was expressed for NGP (0.5807), TW (0.5319), PNP (0.3046), TPP (0.2534), LBR (0.1875), PH (0.1739), PL (0.1107), FP (0.0497), and FF (0.004). Direct PC in a positive direction (0.1739) was shown by PH on SYP, with the exception of LBR (0.0184), all traits showed a negative indirect PC. On SYP, the TPP (0.2534) had a direct positive effect and indirect positive PC via PNP, PL, NGP, FP, TW and LBR. PNP exhibited a direct positive impact on the following variables: TPP (0.153), LBR (0.0238), PL (0.0152), FP (0.0114) and FF (0.0006). Similarly, NGP showed direct PC on SYP in a positive direction (0.5807); except for FF, all of the traits had negative indirect PCs. FP had a significant positive direct effect on SYP. TW (0.5319) displayed a positive direct PC on SYP, and a positive indirect PC via PL, FP and LBR. Conversely, LBR exhibited a noteworthy positive direct PC effect on SYP, while it had a positive indirect effect on the following variables: PH (0.0171), TPP (0.0219), PNP (0.0386), PL (0.018), FP (0.0095), and TW (0.0815). Therefore, it would be feasible to select directly on this trait in order to increase yield potential effectively.

#### 3.2. $D^2$ analysis of yield contributing traits of indigenous aromatic rice germplasm

From the  $D^2$  analysis results of the experimental population, six clusters were identified (Table 5), and Fig. 3 shows the hierarchical dendrogram. Based on the distribution pattern, the 48 genotypes were divided into six clusters of which Cluster I had the most genotypes (28), followed by Cluster II (8) and Cluster III (5), with further 3 genotypes each in Clusters IV and V. However, Cluster VI had

**Table 1**  
Source and kernel colour of aromatic rice genotypes.

Serial No	Genotypes	IC No	Source	Kernel colour
1	Dudheswar	IC421216	Majhian, Dakshin Dinajpur	White
2	Kalojera	–	Raiganj, Uttar Dinajpur	Brown
3	Lal Badsha Bhog	IC569416	Gangarampur, Dakshin Dinajpur	Greyed yellow
4	Pakistani Basmati	–	Raiganj, Uttar Dinajpur	Greyed yellow
5	Indurshal	IC646599	Gangarampur, Dakshin Dinajpur	White
6	Chamarmoni	IC421324	Tapan, Dakshin Dinajpur	Light yellow
7	Tulsimukul	–	Majhian, Dakshin Dinajpur	Off white
8	Komal	–	Majhian, Dakshin Dinajpur	Greyed yellow
9	Khara	–	Majhian, Dakshin Dinajpur	White
10	Kanak Chur	IC594002	Raiganj, Uttar Dinajpur	Greyed yellow
11	Balam	IC618970	Gangarampur, Dakshin Dinajpur	Greyed yellow
12	Baskathi	–	Gangarampur, Dakshin Dinajpur	Creamy white
13	Kalolahi	–	Gangarampur, Dakshin Dinajpur	Greyed yellow
14	Shakbhati	IC646592	Raiganj, Uttar Dinajpur	White
15	Kalomih	–	Gangarampur, Dakshin Dinajpur	White
16	Kalo Aush	–	Gangarampur, Dakshin Dinajpur	Greyed yellow
17	Baran Shal	–	Majhian, Dakshin Dinajpur	White
18	Gopalbhog	IC593988	Majhian, Dakshin Dinajpur	Greyed yellow
19	Basmati 1121	–	Raiganj, Uttar Dinajpur	White
20	Kalabati	IC646586	Raiganj, Uttar Dinajpur	Purple
21	Mohanbhog	IC99455	Gangarampur, Dakshin Dinajpur	Greyed yellow
22	Jhara	IC330656	Gangarampur, Dakshin Dinajpur	White
23	Beto Buna	–	Gangarampur, Dakshin Dinajpur	White
24	Sugandhi	–	Gangarampur, Dakshin Dinajpur	White
25	JP-120	–	Gangarampur, Dakshin Dinajpur	White
26	Jugal	IC646601	Majhian, Dakshin Dinajpur	White
27	Chamatkar	IC646598	Majhian, Dakshin Dinajpur	Greyed yellow
28	Khaliifulo	–	Majhian, Dakshin Dinajpur	White
29	Krishnabhog	IC283244	Majhian, Dakshin Dinajpur	Greyed yellow
30	Kerela Sundari	IC646585	Majhian, Dakshin Dinajpur	White
31	Radhatilok	IC646594	Majhian, Dakshin Dinajpur	White
32	Dehradun Gandheswary	IC399089	Majhian, Dakshin Dinajpur	White
33	Patnai	IC623276	Majhian, Dakshin Dinajpur	White
34	Ramachandra Bhog	–	Tapan, Dakshin Dinajpur	White
35	JP-90	–	Tapan, Dakshin Dinajpur	Greyed yellow
36	Kakri	IC310422	Tapan, Dakshin Dinajpur	Greyed yellow
37	Halde Patnai	–	Tapan, Dakshin Dinajpur	Greyed yellow
38	Bahurupi	IC646580	Raiganj, Uttar Dinajpur	Greyed yellow
39	Chiniatop	IC598081	Majhian, Dakshin Dinajpur	–
40	Malsira	–	Raiganj, Uttar Dinajpur	Greyed yellow
41	Katharibhog	IC640647	Tapan, Dakshin Dinajpur	White
42	Kalonunia	IC596840	Tapan, Dakshin Dinajpur	Brown
43	Tulaipanji	IC569443	Raiganj, Uttar Dinajpur	Greyed yellow
44	Gobindabhog	IC637607	Majhian, Dakshin Dinajpur	Greyed yellow
45	Radhunipagal	IC619255	Majhian, Dakshin Dinajpur	White
46	Sitabhog	IC594012	Majhian, Dakshin Dinajpur	White
47	Chinikamini	IC455641	Tapan, Dakshin Dinajpur	Greyed yellow
48	Khejurphuli	IC646595	Tapan, Dakshin Dinajpur	White

\*IC= Indigenous collection, Database- National Bureau of Plant Genetic Resources (NBPGR), New Delhi, India.

only one genotype. Fig. 4 displayed the intra and inter-cluster distances within the six clusters, indicating the degree of divergence within and between the groups. The largest inter-cluster distances were found between Clusters III and VI (6941.50), IV and VI (6782.64), and V and VI (4482.53), respectively. Cluster II had the greatest intra-cluster distance (1397.52), followed by Cluster IV (1383.34) and Cluster III (1207.90).

There was a significant difference in the average performance of six clusters based on various attributes, as shown in Table 6. Based on the cluster mean, Cluster III had the highest values for PNP (16.28), PL (27.97 cm), FP (88.53 %), TW (21.60 g), and grain L/B ratio (3.25). The highest mean value for PH (180.76 cm) was found in Cluster IV. Cluster VI had the highest mean value for FF (121.37), while Cluster V had the highest TPP (25.94). Cluster II had the highest SYP (61.40 g) with maximum contributions from NGP, FP, and TW. Moreover, SYP benefited most from Clusters VI (48.97 g) and IV (48.97 g). However, Table 6 also displayed each trait's contribution to the overall divergence. The maximum contribution to the divergence was made by NGP (24.47 %), which was followed in order of contribution by PH (24.29 %), SYP (23.00 %), grain L/B ratio (18.80 %), FF (6.64 %), TW (2.30 %), and TPP (0.54 %). PNP, PL, and FP made no discernible contribution to the divergence of the clusters.

**Table 2**  
Genetic variability parameters for different yield component characters of aromatic rice germplasm.

Genetic parameter	PH	FF	TPP	PNP	PL	NGP	FP	TW	LBR	SYP
Mean $\pm$ S.E	155.39 $\pm$ 0.742	112.94 $\pm$ 0.487	19.76 $\pm$ 0.738	15 $\pm$ 0.686	23.52 $\pm$ 1.88	164.29 $\pm$ 1.857	83.99 $\pm$ 1.399	17.84 $\pm$ 0.334	2.719 $\pm$ 0.017	42.001 $\pm$ 0.684
Range	114.71–186.84	95.99–122.99	8.28–39.33	8.83–25.83	15.14–37.84	86–340.22	72.55–92.46	10.88–26.76	2.14–3.87	22.68–81.73
GCV (%)	10.85	6.14	27.3	22.2	11.72	27.58	6.12	21.14	13.92	35.02
PCV (%)	10.89	6.19	28.06	23.57	18.14	27.65	6.77	21.38	13.96	35.13
Heritability (%)	99.42	98.54	94.68	88.71	41.74	99.5	81.82	97.7	99.4	99.36
Genetic Advance (GA)	34.64	14.19	10.81	6.46	3.67	93.1	9.58	7.68	0.78	30.21
Genetic Advance (% of Mean)	22.29	12.57	54.72	43.07	15.6	56.67	11.41	43.04	28.58	71.91

PH= Plant height (cm), DF = 50 % flowering, TPP=Tillers per plant, PNP=Panicle number per plant, PL=Panicle length (cm), NGP=Number of grains per panicle, FP=Fertility percentage, TW=Test weight (g), LBR = Grain L/B ratio, SYP=Seed yield per plant (g); GCV = genotypic coefficient of variation, PCV = phenotypic coefficient variation.

**Table 3**  
Genotypic and phenotypic correlation of yield component traits of aromatic rice.

Characters	Coefficient type	PH	FF	TPP	PNP	PL	NGP	FP	TW	LBR
FF	$r_g$	-0.052								
	$r_p$	-0.052								
TPP	$r_g$	-0.288**	-0.066							
	$r_p$	-0.279**	-0.061							
PNP	$r_g$	-0.228**	0.141	0.604**						
	$r_p$	-0.216**	0.132	0.570**						
PL	$r_g$	-0.164 <sup>a</sup>	0.285**	0.196 <sup>a</sup>	0.137					
	$r_p$	-0.103	0.181 <sup>a</sup>	0.128	0.095					
NGP	$r_g$	-0.055	0.199 <sup>a</sup>	-0.049	-0.094	-0.268**				
	$r_p$	-0.054	0.198 <sup>a</sup>	-0.045	-0.088	-0.178 <sup>a</sup>				
FP	$r_g$	-0.059	-0.206 <sup>a</sup>	0.249**	0.230**	0.144	-0.140			
	$r_p$	-0.050	-0.186 <sup>a</sup>	0.219**	0.203 <sup>a</sup>	0.085	-0.128			
TW	$r_g$	-0.096	-0.180 <sup>a</sup>	-0.027	-0.065	0.175 <sup>a</sup>	-0.454**	0.247**		
	$r_p$	-0.093	-0.177 <sup>a</sup>	-0.025	-0.053	0.126	-0.450**	0.222**		
LBR	$r_g$	0.098	-0.079	0.086	0.127	0.163	-0.326**	0.192 <sup>a</sup>	0.153	
	$r_p$	0.098	-0.079	0.083	0.120	0.108	-0.324**	0.172 <sup>a</sup>	0.151	
SYP	$r_g$	-0.055	0.048	0.394**	0.380**	0.150	0.191 <sup>a</sup>	0.274**	0.285**	0.185 <sup>a</sup>
	$r_p$	-0.054	0.046	0.388**	0.364**	0.102	0.191 <sup>a</sup>	0.250**	0.279**	0.185 <sup>a</sup>

PH= Plant height (cm), DF = 50 % flowering, TPP=Tillers per plant, PNP=Panicle number per plant, PL=Panicle length (cm), NGP=Number of grain per panicle, FP=Fertility percentage, TW=Test weight (g), LBR = Grain L/B ratio, SYP=Seed yield per plant (g);  $r_g$  = Genotypic correlation coefficient,  $r_p$  = Phenotypic correlation co-efficient.

<sup>a</sup> Indicates significant at 5 % level and \*\* Indicates significant at 1 % level.

**Table 4**  
Path Coefficient (PC) analysis of yield component traits among 48 rice genotypes.

Characters	PH	FF	TPP	PNP	PL	NGP	FP	TW	LBR	SYP
PH	<b>0.1739</b>	-0.0002	-0.0729	-0.0696	-0.0182	-0.0321	-0.0029	-0.051	0.0184	-0.054
FF	-0.0091	<b>0.004</b>	-0.0167	0.0431	0.0315	0.1156	-0.0102	-0.0956	0.0148	0.047
TPP	-0.0500	-0.0003	<b>0.2534</b>	0.1839	0.0217	-0.0287	0.0124	-0.0143	0.0162	0.394**
PNP	-0.0397	0.0006	0.153	<b>0.3046</b>	0.0152	-0.0548	0.0114	-0.0344	0.0238	0.379**
PL	-0.0286	0.0011	0.0497	0.0418	<b>0.1107</b>	-0.1554	0.0072	0.0929	0.0305	0.149
NGP	-0.0096	0.0008	-0.0125	-0.0288	-0.0296	<b>0.5807</b>	-0.0069	-0.2417	-0.0611	0.191**
FP	-0.0102	-0.0008	0.0631	0.07	0.0159	-0.0811	<b>0.0497</b>	0.1314	0.036	0.273**
TW	-0.0167	-0.0007	-0.0068	-0.0197	0.0193	-0.2638	0.0123	<b>0.5319</b>	0.0287	0.284**
LBR	0.0171	-0.0003	0.0219	0.0386	0.018	-0.1893	0.0095	0.0815	<b>0.1875</b>	0.184*

Residual effect = 0.38; \* Indicates significant at 5 % level and \*\* Indicates significant at 1 % level; Partitioning of genotypic correlation with SYP into direct (bold) and indirect path coefficients.

PH= Plant height (cm), DF = 50 % flowering, TPP=Tillers per plant, PNP=Panicle number per plant, PL=Panicle length (cm), NGP=Number of grain per panicle, FP=Fertility percentage, TW=Test weight (g), LBR = Grain L/B ratio, SYP=Seed yield per plant (g).

**Table 5**  
Grouping of 48 aromatic rice genotypes in different clusters.

Cluster No.	No. of Genotypes	Name of Genotypes
I	28	Dudheswar, Radhunipagal, Kalojera, Lal Badsha Bhog, Ramachandra Bhog, Kakri, Indurshal, Sitabhog, Mohanbhog, Krishnabhog, Kalomihi, Kalolahi, Chinikamini, Tulsimukul, Dehradun Gandheswary, Halde Patnai, Pakistani Basmati, Jhara, Gobindabhog, Radhatilok, Khaliifulo, Balam, Shakhbhati, Malsira, Gopalbhog, Chiniatop, Baskathi, Kalonunia
II	8	Khara, Baran Shal, Chamarmoni, Sugandhi, JP-90, Kerela Sundari, Katharibhog, Khejurphuli
III	5	Tulaipanji, Patnai, Basmati 1121, Jugal, Bahurupi,
IV	3	Kalabati, Beto Buna, Komal
V	3	Kalo Aush, JP-120, Chamatkar
VI	1	Kanak Chur

### 3.3. Principle component analysis of aromatic rice germplasm

Principal component analysis (PCA) was performed using phenotypic diversity based on morphological traits from the 48 aromatic rice germplasm. The variance proportion, cumulative proportion, and Eigen values are presented in Table 7. The results of the principal component analysis showed that the first six components contributes 81.78 % of the variability. In terms of overall variability, PC1 (23.88 %) was followed by PC2 (17.67 %), PC3 (12.35 %), PC4 (10.63 %), PC5 (9.81 %), and PC4 (7.44 %). According to the scree plot illustrated in Fig. 5, the scaling of the Eigen vectors of the initial four principal components exceeded 1. The plot showed

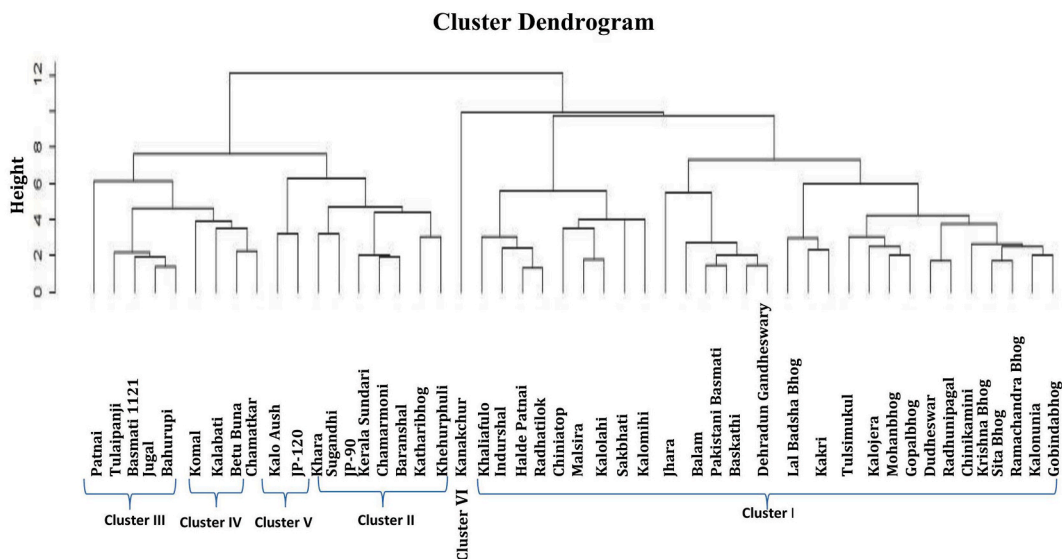


Fig. 3. Hierarchical Wards methods dendrogram of 48 rice genotypes. Genotype displaying various cluster groups.

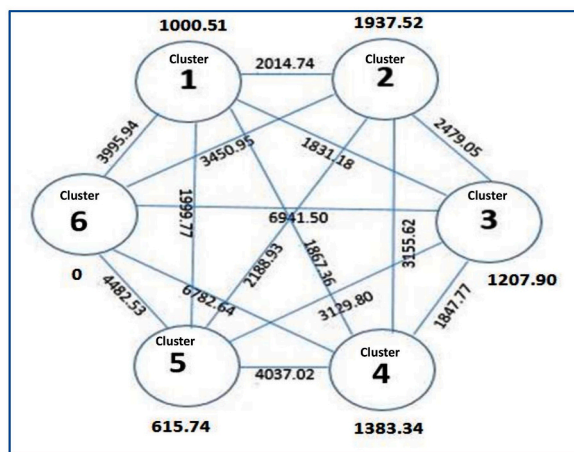


Fig. 4. Average inter and intra-cluster distances ( $D^2$ ) among 48 rice genotypes in six clusters based on Euclidean values.

Table 6

Cluster mean of individual characters and their percentage contribution.

Characters	CLUSTER I	CLUSTER II	CLUSTER III	CLUSTER IV	CLUSTER V	CLUSTER VI	% Contribution
PH	159.19	143.96	154.38	180.76	122.33	168.59	24.29
FF	113.98	114.70	114.27	104.43	102.08	121.37	6.64
TPP	18.47	20.84	21.96	20.57	25.94	15.17	0.54
PNP	14.72	16.24	16.28	15.50	13.28	10.44	0
PL	23.03	23.80	27.97	22.01	22.48	20.07	0
NGP	165.91	185.94	106.54	112.51	180.83	340.22	24.47
FP	83.41	85.18	88.53	81.83	83.10	76.92	0
TW	16.77	19.15	21.60	19.87	17.10	14.52	2.30
LBR	2.64	2.75	3.25	2.78	2.54	2.37	18.80
SYP	35.56	61.40	47.09	48.97	32.78	48.97	23.00

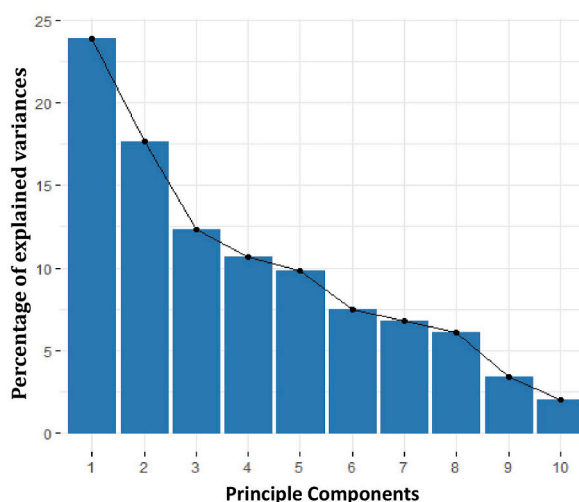
PH= Plant height (cm), DF = 50 % flowering, TPP=Tillers per plant, PNP=Panicle number per plant, PL=Panicle length (cm), NGP=Number of grain per panicle, FP=Fertility percentage, TW=Test weight (g), LBR = Grain L/B ratio, SYP=Seed yield per plant (g).



**Table 7**  
Contribution of different traits towards total variance in aromatic rice germplasm.

Parameter	PC1	PC2	PC3	PC4	PC5	PC6
PH	-0.210	0.239	-0.118	0.706	0.079	0.126
FF	-0.043	-0.363	0.603	0.264	-0.155	-0.059
TPP	0.459	-0.284	-0.152	-0.112	0.281	-0.041
PNP	0.441	-0.314	-0.025	0.052	0.342	-0.145
PL	0.259	0.030	0.643	-0.006	-0.101	0.420
NGP	-0.212	-0.531	-0.253	0.167	-0.372	0.134
FP	0.364	0.151	-0.278	0.036	-0.149	0.742
TW	0.264	0.452	0.062	-0.202	-0.516	-0.327
LBR	0.263	0.303	0.081	0.479	0.281	-0.197
SYP	0.408	-0.173	-0.183	0.342	-0.507	-0.259
Eigen values	2.388	1.767	1.235	1.063	0.981	0.743
Total variance (%)	23.88	17.67	12.35	10.63	9.812	7.438
Cumulative variance %	23.88	41.55	53.9	64.53	74.345	81.782

PH= Plant height (cm), DF = 50 % flowering, TPP=Tillers per plant, PNP=Panicle number per plant, PL=Panicle length (cm), NGP=Number of grain per panicle, FP=Fertility percentage, TW=Test weight (g), LBR = Grain L/B ratio, SYP=Seed yield per plant (g).



**Fig. 5.** Scree plots in aromatic rice germplasm accessions for various PCs. Scree plot displaying the ten PCs' eigenvalues.

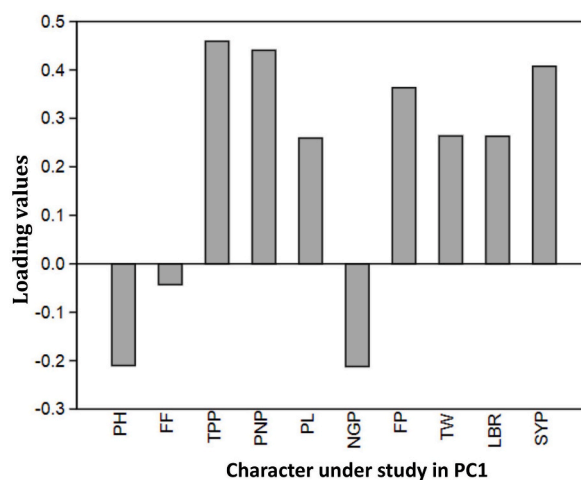
the relative weight of each variable in each component (Fig. 5). Significant variables were indicated by high positive loading values (Table 7). PC1, which included the grain L/B ratio (0.263), FP (0.364), SYP (0.408), TPP (0.459), PNP (0.441), and TW (0.264), accounted for 23.88 % of the variation as a whole. These characters had a positive variance contribution, while the negatively loaded characters were PH (-0.210), FF (-0.043), and NGP (-0.212) (Table 7, Fig. 6). The PC2 accounted positive loading traits were TW (0.452), grain L/B ratio (0.303), PH (0.239), and FP (0.151), with 17.67 % of the variability. The third principal component exhibited a high positive value to PL (0.643), FF (0.603) and grain L/B ratio (0.081), whereas PC4 with PH (0.706), grain L/B ratio (0.479), SYP (0.342), FF (0.264) and NGP (0.167). The grain L/B ratio displayed positive values throughout the first five principal components. In the first two principal components, FF and NGP exhibited negative values.

The PCA-biplot analysis indicated the comparative distance between different genotypes and phenotypic characteristics by employing the first two principal components along the X and Y axes (Fig. 7). The PC analysis of this experiment ultimately revealed the degree of trait variability among genotypes that could be useful for improvement of aromatic rice. In accordance with the scatter diagram's most distant genotypes, Shakbhati, Sugandhi, Bahurupi, and Kanakchur exhibited a statistically significant degree of divergence with respect to the assessed traits.

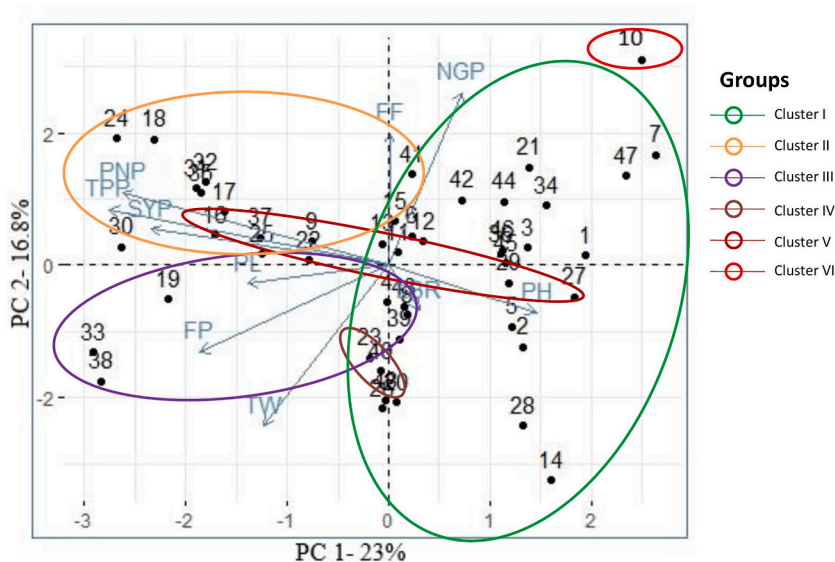
## 4. Discussion

### 4.1. Genetic variability and character association of yield component traits

The aromatic rice genotypes in eastern India, specifically in West Bengal, are diverse due to their confined cultivation within their geographical adaptation and consumer preference. Moreover, there are variations in grain morphology, length/breadth ratio, and strength of aroma. The observed means of the assessed traits varied significantly, indicating that the genotypes were distinguished



**Fig. 6.** Loading values of different yield component parameters of PC1. The up-ward direction indicates values that load positively, and the down-ward direction indicates values that load negatively.



**Fig. 7.** PCA-Biplot. The biplot shows rice genotype clusters in PC1 and PC2. The biplot displays the individual and vector PCA scores for each of the six clusters (different colours), which are the explanatory variables. The strength of each vector's contribution to each PC is indicated by its magnitude. Positively correlated variables are indicated by vectors pointing in the same direction, negatively correlated variables are indicated by vectors pointing in the opposite direction, and low or no correlation is indicated by vectors at proximately right angles. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

using these variables (Table 2). Highly significant differences in rice for yield component traits were shown in previous studies [39,40]. This variation was beneficial in the initial breeding material because it allows for phenotypic selection for desired traits. In addition to being a useful tool for studying variability, morphological traits could be used to identify different parental combinations that would aid in further selection and the introgression of desirable genes [41,42]. In this study, except for panicle length, all the parameters showed high heritability values (>80 %), suggesting that these traits can be used as selection criteria for aromatic rice yield improvement [43]. The success of a crop improvement program depends on the wide genetic variability and high heritability of the traits [44]. High GCV and PCV for yield and yield-contributing traits of rice were noted by earlier researchers [45–47] which corroborated the results of the present study. Meanwhile, FF and FP traits with low PCV and GCV values illustrated a low level of diversity [48]. In this study, NGP, SYP and PH contributed high genetic advance to high heritability. Thus, these characters were mainly influenced by additive and non-additive gene action [49]. Estimation of genetic advance increases the efficacy of heritability by indicating the degree of gain in a trait obtained under particular selection pressure. As desirable selection criteria, traits exhibiting high genetic advance, GCV, and heritability were reported to be preferred [50,51].

The association of phenotypic traits unveiled the mutual relationship among yield component traits, thereby presenting an opportunity to employ selection strategies that facilitate the genetic enhancement of aromatic rice. The correlation between grain yield and tiller per plant, panicle numbers per plant, number of grains per plant, fertility percentage, test weight and grain length/breadth ratio yield-related traits indicated an indirect selection of these traits for yield improvement in aromatic rice. The correlation between grain yield and yield-related traits indicated an indirect selection of these traits for yield improvement in aromatic rice. In previous studies [42,52], the positive correlation was observed between grain yield and different yield component traits of indigenous aromatic rice in West Bengal. According to a recent study, grain yield was found to be significantly positively correlated with the harvest index, plant height, panicle length, number of filled spikelet panicles per hill, flag leaf length, and number of effective tillers per hill [45]. Similar to previous investigations, our study found a negative correlation between SYP and PH [53]. This may be attributed to the weaker stem characteristics and taller plants of these cultivars, both of which exacerbate to lodging problems and reduced yield. Using path coefficient analysis, the selection of direct and indirect effects for each trait related to the other traits was assessed (Table 4). Direct selection amongst NGP, TW, and PNP with the highest positive direct attributes will be advantageous since there is a correlation between a causal component and a direct impact that is about equal in magnitude, confirming the real and perfect relationship between the traits.

#### 4.2. Genetic diversity studies

Cluster analysis was employed to identify six distinct clusters within the experimental population. Notably, no correlation was observed between the collection sites and the clusters of genotypes (Table 4). In the previous two studies, 43 and 25 amaranth genotypes were grouped in six clusters [54,55], which support our current clustering pattern.

The initial assessment of aromatic rice cultivars and the analysis of their diversity benefit from the assessment of distinguishable morphological traits [56]. The average inter-cluster distances were observed to be greater than the average intra-cluster distances, suggesting that the 48 rice genotypes possess a greater degree of genetic diversity (Fig. 4) [57]. Higher inter-cluster distances are also explained by other researchers [58–60]. Maximum heterosis would result from a cross between genotypes from Clusters III and VI that had the greatest genetic distance [61,62]. Therefore, hybridizing Tulaipanji, Patnai, Basmati 1121, Jugal, Bahurupi (cluster III) and Kanakchur (Cluster VI) could be very advantageous, but crossing genotypes within a cluster would not produce superior hybrids [63]. In the event of a future breeding program, genotypes belonging to distinct clusters may be selected as parents based on highly correlated traits [64]. Moreover, the genotypes with the largest genetic distance in yield-attributing parameters would result in the complementation of gene effects in the hybridization program [65]. Grain morphological traits may reveal the ideal genetic diversity of indigenous rice varieties [66]. The main characteristics that helped our study's diversity to be expressed were NGP, PH, SYP, FF, LBR, and TW (Table 6). Therefore, when implementing a breeding program for heterotic F1, these traits should be prioritized. A number of researchers have documented the relative significance of a subset of these characters in divergence [67–69].

A distinct group of rice landraces from West Bengal have also been reported to possess a pleasant scent and exhibit tolerant against both biotic and abiotic stresses [42,67,70,71].

#### 4.3. Principal component analysis

Principal component analysis was utilised to categorise all yield-attributing traits into distinct principal components, thereby illuminating the individual traits' contributions to genetic divergence. The first four components in our study were considered the primary principal component since they showed the greatest variability with Eigen values greater than 1 [71,72]. Prior to this, the first five components [73] and the first eight out of thirteen [74] reported significant (Eigen value > 1) variability in rice germplasms. The higher coefficients of grain LBR, PNP, SYP, and TW would be more useful in genotype differentiation, regardless of direction. These results were consistent with another report [75], which concluded that grain length, kernel length, grain weight, and grain length/breadth ratio are responsible for the greatest phenotypic diversity in a set of Indian rice. On the other hand, characteristics such as harvest index, days to maturity, field grain per panicle, 50 % flowering, and biological yield each contributed the most variability among Assamese native aromatic rice genotypes [50]. Breeders could use selection to influence such significant traits in the divergence analysis of aromatic rice. In this study, only the grain L/B ratio exhibited positive values in the first five principal components associated with divergence. When determining the quality and appeal of aromatic rice to consumers, the grain L/B ratio was a crucial factor. In our experimental genotypes, grain L/B ratio was found to vary from 2.17 in Sitabhog to 3.35 in Basmati 1121. The variation in the L/B ratio of kernel and grain was previously reported in aromatic rice [76]. PC1 and PC2 in the PCA-biplot diagram explained the dispersion and nature of diversity for both variables and genotypes in this study by forming six divergent groups (Fig. 7). Similar to our findings, the biplot analysis of 98 genotypes and morphological characteristics revealed high variability [77]. The most diverse genotypes, Shakhbhati, Sugandhi, Bahurupi, and Kanakchur, could be used as donors in future breeding efforts to improve the yield and quality attributes of aromatic rice. In order to increase the yield and quality of traits in upcoming breeding programs, they might be utilised as donors. Based on their biplot analysis, a previous study showed that the most divergent genotypes were Dhaur, Dhaura, Badalphool, Jaya, Parra, Beo-I, and Dhamna panda [74]. The genetic diversity of rice genotypes was clarified and component traits contributing to variability were broken down through the combination of principal component analysis and cluster analysis; this could provide the framework for a well-run hybridization program [50].

The study on aromatic rice genotypes effectively employed various statistical methods, revealing significant genetic variability and character associations among yield component traits. High heritability values were identified for most parameters, implying their potential as selection criteria for yield enhancement. Additionally, positive correlations between grain yield and related traits were

observed, supporting indirect selection strategies. The identification of six distinct clusters underscored the population's genetic diversity and highlighted key characteristics for breeding programs. However, limitations included the lack of correlation between collection sites and genotype clusters, low diversity levels for certain traits, negative correlations potentially exacerbating lodging issues, and the study's regional specificity, which could hinder broader applicability. Moreover, the reliance solely on statistical analyses might overlook environmental influences and unmeasured variables, warranting cautious interpretation of the findings.

## 5. Conclusions

With a focus on yield improvement in aromatic rice breeding programs, the current study offers the best guidelines for selecting parents based on multivariate analysis of agronomic traits. The phenotypic data showed a broad genetic variability within the rice germplasm, resulting in six clusters with significant genetic distances in the diversity analysis. From the multivariate approaches, agronomic variables NGP, PH, SYP, LBR, and TW showed most significant divergence. In order to increase the production of aromatic rice, breeding efforts should give priority to SYP, which was found to have a significant positive correlation with TPP, PNP, NGP, FP, TW, and LBR. The genotypes of aromatic rice that exhibited the greatest divergence were Tulaipanja, Patnai, Shakhbhati, Sugandhi, Bahurupi, and Kanakchur. Cluster II, which represents a significant improvement in seed yield, comprises the genotypes Khara, Baran Shal, Chamaroni, Sugandhi, JP-90, Kerela Sundari, Katharibhog, and Khejurphuli. Nevertheless, these genotypes may be utilised in a future breeding programme to improve yield and quality characteristics of aromatic rice.

## Data availability statement

Data will be made available on request.

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## CRediT authorship contribution statement

**Sourav Mondal:** Methodology, Formal analysis, Data curation. **Prajwal Pradhan:** Validation, Methodology, Data curation. **Bimal Das:** Writing – original draft, Software, Methodology, Formal analysis, Conceptualization. **Deepak Kumar:** Writing – original draft, Visualization, Validation. **Bappa Paramanik:** Writing – review & editing, Writing – original draft. **Rakesh Yonzone:** Writing – review & editing, Resources. **Ratul Barman:** Resources, Methodology, Data curation. **Debraj Saha:** Resources, Methodology, Data curation. **Jyotirmay Karforma:** Writing – original draft, Resources. **Achyuta Basak:** Writing – review & editing, Data curation. **Prithwiraj Dey:** Writing – review & editing. **Mahmoud F. Seleiman:** Writing – review & editing.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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