Morphological, Biochemical and Molecular Characterization of Indigenous *Aromatic* Rice of Assam

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Abstract

We carried out the morphological, biochemical, and molecular characterization of twenty cultivars of the least studied *Joha* (aromatic) rice indigenous to Assam. Unweighted Neighbour Joining (UNJ) clustering based on usual Euclidean distances for thirty-seven polymorphic morphological markers grouped the cultivars into three clusters with eight, eleven, and one genotype. The *Joha* rice cultivars showed highly significant differences for all the quantitative traits except for panicle length. The genotypic and phenotypic coefficients of variability (GCV & PCV) were high for grain yield ha^{-1} (24.62 & 24.85%) and filled grains $panicle^{-1}$ (23.69 & 25.02%). All the traits except days to flowering and maturity, flag leaf breadth, and spikelet fertility exhibited high heritability along with high to moderate genetic advance, indicating the predominant role of additive gene action. Mahalanobis D^2 analysis revealed three multi-genotypic and four mono-genotypic clusters of the cultivars. The cultivars' average polyunsaturated fatty acids were 37.9% oleic acid, 39.22% linoleic acid, and 0.5% linolenic acid. The fatty acid profile of *Local Joha* was superior to the other cultivars as it showed a high level of linoleic and linolenic acid and low saturated fatty acid content. *Kon Joha 4* and *Ronga Joha* contained the highest iron (82.88 mg kg^{-1}) and zinc (47.39 mg kg^{-1}), respectively, while protein content of *Kon Joha-1 and* amylose content of *Harinarayan* were the highest. *Joha (Bihpuria)* showed the highest gel consistency of 140.50 mm. *Kalijeera, Kunkuni Joha, Kon Joha-5, Manimuni Joha* and *Kon Joha-2 accorded a strong aroma*. PCR amplified 174 alleles with a mean value of 2.64 across the 66 polymorphic SSR markers. PIC values ranged from 0.091 to 0.698, with an average of 0.326. The highly informative (PIC>0.50) markers were RM316, RM283, RM585, RM1388, RM3562, RM171, R1M30, RM118, RM11and RM29 for identification of the twenty aromatic rice cultivars. The UNJ clustering based on Jaccard's coefficients classified the cultivars into three distinct clusters with eight, ten, and two genotypes. Our study revealed the nutritional richness of these specialty *Joha* rice cultivars and sufficient scope for yield enhancement through their interbreeding to keep quality intact.

Introduction

Globally rice is planted to about 162 million ha and 755 million tonnes of produce harvested annually (FAO, 2020). Ninety percent of the production and consumption of the world's rice occur in Asia. India has the world's largest area under rice with 44.0 million ha and is the second-largest producer (96.0 million tonnes in 2010), next only to China. It contributes 21.5 percent of global rice production. The FAO (2009) estimated a requirement of 70 percent more food for over nine billion people expected to inhabit planet earth by 2050. As the staple food for most Indians, rice's future demand would increase with the growing population, projected at 1.378 billion by 2030 (Goyal and Singh, 2002). Assam has a wide range of variation of cultivated rice along with several new wild varieties. In Assam, rice cultivation occupies about 70% of the total cropped area that dominates the state's agriculture scenario (Singh et al., 2009). Rice, being the single primary source of agricultural GDP, plays a significant role in the state economy.

Aromatic rice is a significantly small but significant sub-group of rice gained in general popularity for consumers. The fragrance of Joha rice is the crucial feature, which increases its popularity in the international market (Nayak et al., 2002). Aromatic rice constitutes a particular group, which is considered the best in quality and fetches a much higher price than non-aromatic rice (Hien et al., 2007). A unique class of aromatic rice grown as winter rice in Assam is very popular and highly valued due to its quality, popularly known as Joha rice in Assam. Joha rice possesses a superfine kernel, unique aroma, better cooking properties, and excellent palatability (Das et al., 2010).

Despite domestic aromatic rice, there are large numbers of wild varieties, which are still unexplored. The agricultural productivity of Joha rice is the lowest with the demand for production, and the gap is increasing over time. Agro-morphological characterization of rice germplasm is fundamental to provide information for plant breeding programs (Lin, 1991). There are several agro-morphological markers used in the description and study of rice germplasm diversity. The nutritional status of rice is becoming increasingly important among consumers because of deficiency disorders. There is limited knowledge regarding the nutritional composition of different rice varieties available in Assam. However, the characterization of the rice cultivars in terms of composition and physicochemical aspects is relatively meager. Rice is a leading food crop with a different nutritional status that helps alleviate poverty (Otegbayo et al., 2001). The proximate analysis is an essential parameter for the routine description of foodstuffs. This analysis consists of six fractions viz., amylose, crude protein, iron, zinc, and fatty acid content. The proximate composition, sensory, gelatinization temperature, and alkali digestion analysis of rice help identify nutritionally complete rice varieties (Dong et al., 2007).

Rice showed a genetically greater extent of diversity. DNA-based molecular markers play essential roles in assessing genetic variation and the elucidation of gene relationships within and among species. Molecular markers have been extensively used in crop improvement research throughout the globe as an appropriate and effective tool addressing biological parameters in agricultural productivity (Jones et al., 1997). Among PCR-based markers, SSR or microsatellite markers are excellent due to their locus of identity, high polymorphisms, and multi-allelic nature. Moreover, SSR markers are tandemly interspersed repeats throughout the genome that get amplified through primers that flank these regions. Studies to characterize and improve the aromatic rice of Assam are limited, even though this region of the country stores enormous rice diversity. The present study aimed at characterizing the aromatic rice of Assam using morphological, biochemical, and molecular markers for their usage in breeding manipulation.

Material And Methods

Phenotypic characterization

The experiments were carried out during *the Sali* season of 2018 and 2019 at Instruction-cum-Research (ICR) Farm, Assam Agricultural University. The site is located at 26°45′ north latitude and 94°12′ east longitude having an elevation of 86.6 m above the mean sea level. The experimental area was relatively uniform in respect of topography and fertility. All the molecular works, including DNA extraction, PCR, and gel electrophoresis, were done in Mutation Breeding

Section-I Laboratory of Nuclear Agriculture and Biotechnology Division (NA&BTD), Bhabha Atomic Research Centre, Trombay. The twenty aromatic *Joha* rice cultivars (**Table 1**) were grown in a randomized complete block design with three replications.

The seedlings' age was 30 days at transplanting in the main field. Each genotype constituted ten rows of 2.5 m long spaced 20 cm apart with one seedling per hill. Gap filling was done in the first week of transplanting to maintain a uniform plant population. A fertilizer dose of 60 kg N, 20 kg P_2O_5, and 40 kg K_2O was applied as per *Sali* rice recommendation for Assam. The entire quantity of P_2O_5 and K_2O and a half dose of N were applied as basal dose at the time of final field preparation; the remaining nitrogen was applied in two equal splits at maximum tillering and booting stage. The standard agronomic practices recommended for the state of Assam were adopted in both experiments. Observations were recorded according to the National Test Guidelines for DUS test in rice developed by the Directorate of Rice Research, Hyderabad (Shobha Rani *et al*., 2004). The yield attributing traits were based on five random plants per replication, while days to flowering and maturity were recorded on a per plot basis. The characteristics observed were days to first flowering, days to 50% flowering, days to maturity, number of productive tillers plant^{-1}, plant height (cm), panicle length (cm), spikelet fertility (%), 1000-grain weights (g), grain yield plant^{-1} (g), biological yield plant^{-1} (g), harvest index (%), protein content (Kjeldahl's method), iron (Fe) and zinc (Zn) content (Atomic Absorption Spectrophotometer), fatty acid profiling in rice bran.

Genomic DNA extraction

Seeds of cultivars were germinated and grown in a growth chamber, maintaining a temperature of 30^{o}C, 10 hours of light, and 85% relative humidity. At the three-leaf stages, the leaves were harvested by liquid nitrogen and taken for DNA isolation. The genomic DNA was then isolated by the CTAB (Cetyl Trimethyl Ammonium Bromide) method (Murray and Thompson, 1980).

DNA quantification

The concentration and quality of genomic DNA samples were estimated on a 260, 280 nm, and 230 nm spectrophotometer, the samples with a 260/280 ratio exceeding 1.8 were considered good quality DNA. Quality of DNA fragment was also confirmed by 0.8% agarose gel electrophoresis using 1XTBE buffer at 100V for 90 min.

Molecular marker selection

Seventy-one SSR markers were used for the genotyping (**Supplementary Table S1**). The markers were selected from various published literature and the Gramene database (www.gramene.org), maintaining the genome-wide distribution of markers.

PCR amplification using SSR primers

PCR reaction was performed in 25 µL mixture containing 1 µL (25 ng/ µL) template DNA, 2.5 µL of 10x PCR buffer with 25 mM MgCl2, 1.0 µL 5 mM of each forward and reverse SSR primers, 1.0 µL of 10 mM dNTPs and 0.2 µL of *Taq* DNA polymerase. PCR reactions were performed in a thermal-cycler (Eppendorf, Hamburg, Germany). The amplification profile consisted of initial denaturation for 2 min at 95^{o}C, 35-40 cycles of denaturation at 95^{o}C, annealing at 50-60^{o}C, and extension at 72^{o}C. After that, the final extension was carried out at 72^{o}C for 7 min.

Gel electrophoresis

PCR products of SSR markers were resolved on capillary electrophoresis system (Qiagen Pvt. Ltd., Hamburg, Germany).

Scoring of SSR data

The molecular weight of the PCR products for each SSR primer was determined from a ladder of known molecular weights. During band scoring, faint bands and bands with smeared backgrounds were avoided, and only intense bands were scored based on their product size. The presence of a product in a particular genotype was designated as '1', and the absence was designated as '0'. Only the specific PCR products showing consistency in the successive amplifications were selected to minimize the possibility of mis-scoring markers.

Statistical analysis

A pooled ANOVA for the traits over the two years was done considering replication, genotype, and environment as fixed effects (Singh and Chaudhary, 1985) in MS Excel 2007. Genetic parameters were estimated by the formulae given by Burton (1952) for GCV and PCV, Hanson, Robinson, and Comstock (1956) for heritability, and Allard (1960) for expected genetic advance in MS Excel 2007. Mahalanobis D^2 analysis was done in Windostat version 9.2 (http://www.windostat.org). Usual Euclidian distances between the cultivars were worked out from the standardized data matrix in DARwin version 6.0.021 (Perrier and Jacquemoud-Collet, 2006) and represented through cluster analysis using the algorithm of Unweighted Neighbour-joining (UNJ) method by feeding the distance matrix as input data. Genetic relatedness among the genotypes was computed by using Jaccard's coefficient of similarity (Jaccard, 1908) and a dendrogram was constructed illustrating the genetic relationship among the rice genotypes using the UNJ method as proposed by Gascuel (1997), which uses a criterion of weighted average, in DARwin 6 (Perrier and Jacquemoud-Collet, 2006). The number of different alleles amplified per locus (*Na*), major allele frequency (*MAF*), number of effective alleles (*Ne*), Shannon's information index (*I*), observed heterozygosity (*Ho*), expected heterozygosity (*He*), and polymorphism information content (*PIC*) values were calculated using GenAlEx version 6.5 (Peakall and Smouse, 2012).

Results And Discussion

Morphological characterization of the cultivars

A total of sixty-two qualitative and quantitative traits characterized the distinctiveness of the twenty Joha rice cultivars. Qualitative characteristics are considered morphological markers for identifying rice landraces because environmental changes least influence these traits (Raut, 2003). The stable morphological traits serve as reliable morphological markers for the identification of a cultivar. Each cultivar must have certain novel diagnostic features which will distinguish a variety from the others. Such diagnostic characters should be uniformly present in the population and inherit in the next generation, and then only the character is supposed to be stable and can be used as morphological marker traits to distinguish that variety. Among the traits, the spectrum of variability in the twenty cultivars revealed 22 monomorphic (Table 2) and 37 polymorphic characteristics (Table 3), suggesting their potential for cultivar characterization and distinctiveness. Earlier studies of Bisne and Sarawgi (2008), Mathure et al. (2011), Subudhi et al. (2012), Subba Rao et al. (2013), Sarawgi et al. (2014), Parikh et al. (2012), and Sinha et al. (2015) corroborated the above findings.

Cluster analysis of the cultivars based on polymorphic DUS characteristics

Clustering by unweighted neighbor-joining (UNJ) of usual Euclidean distance matrix (Supplementary Table S2) based on thirty-seven polymorphic traits grouped the twenty indigenous aromatic rice cultivars into two multi-genotypic clusters and one mono-genotypic cluster G3 (Fig. 1). The clusters were further subdivided into sub-clusters and had an unequal distribution of the cultivars. The nine cultivars viz., Local Joha, Keteki Joha, Harinarayan, Kon Joha-5, Kon Joha (Moran), Jeera Joha, Kon Joha-3, and Ronga Joha belonged to G1. At the same time, G2 had eleven cultivars viz., Kon Joha-1, Manimuni Joha, Kon Joha-4, Kalijeera, Kola Joha, Joha (Bihpuria), Soru Joha (Tinsukia), Kon Joha-Bongaigaon, Joha (Golaghat), Kon Joha (Teok) and Kunkuni Joha whereas G3 having Kon Joha-2 alone. The observed group constellation pattern proved the existence of a substantial amount of variability among the indigenous Joha rice cultivars. The present findings agree with Mondal et al. (2014) and Sripunitha and Sivasubramaniam (2014).

Pooled analysis of variance

The pooled ANOVA over the two years (Supplementary Table S3) revealed that the mean squares due to years were significant for fifteen traits viz., days to first flowering, flag leaf length, flag leaf breadth, flag leaf area, days to 50% flowering, days to maturity, plant height, productive tillers plant-1, filled grains panicle-1, spikelet fertility, 1000-grain weights, biological yield plant-1, grain yield plant-1, harvest index and grain yield kg ha-1, suggesting a significant influence of the years on the phenotypic expression of these traits. The yearly variation was mainly due to the difference in the 2018 and 2019 crops' sowing times. The crops were sown on 11th July and 17th June in 2018 and 2019, respectively. The June planted crop in 2019 exhibited higher mean performances for most of the traits mentioned above. Delaying the sowing time decreased the number of days to flowering and maturity for most of the cultivars. A similar observation was reported by Song et al. (1996) for days to heading reduced in different rice cultivars due to delayed sowing. Nahar et al. (2009) observed a significant decrease in filled grain production consequent upon delayed transplanting, attributed to low temperature at anthesis and spikelet primordial formation. Khalifa (2009) noted the early date of sowing the best time for maximizing morpho-physiological traits such as tillering, panicle initiation, chlorophyll content, leaf area index, sink capacity, panicle length, panicle number, and grain yield. Delayed sowing significantly reduces the number of filled grains, panicles, and test weight, finally lowering rice cultivars' grain yield (Patel et al., 2019). The cultivar differences for all the traits except for panicle length were also evident from highly significant mean squares. The Years x Cultivars interaction component was substantial for days to first flowering, days to 50% flowering, days to maturity, filled grains panicle-1, and spikelet fertility, suggesting differential behavior of the cultivars early and late sown crops. The findings of Ganesan et al. (1995), Karim et al. (2007), Padmaja et al. (2008), Sahidullah et al. (2009), Singh et al. (2010), Khan et al. (2012), Gangashetty et al. (2013) and Verma et al. (2014) further supported the presence of significant cultivar differences. Mahapatra et al. (1996), Reddy et al. (1998), and Panwar et al. (2008) also reported substantial Years x Cultivars interaction for days to 50% flowering and days to maturity.

Mean performance of the cultivars

The variation among the various traits under study revealed free variability in different cultivars populations, reflecting the unforeseen impact of potential variability on yield. The cultivar mean performance for the observed traits (Supplementary Table S4) identified the top-ranking cultivars Kon Joha-2 for stem thickness; Joha (Bihpuria), Joha (Golaghat) and Kola Joha for the earliest days to first flowering; Kon Joha (Teok) for flag leaf length; Joha (Bihpuria), Ronga Joha and Soru Joha (Tinsukia) for flag leaf breadth; Ronga Joha for flag leaf area; Kola Joha for the earliest days to 50% flowering and maturity; Keteki Joha for the shortest height and the highest number of productive tillers; Manimuni Joha, Kon Joha (Moran), Kon Joha-2, Jeera Joha and Kon Joha (Teok) for filled grains panicle-1; Jeera Joha and Kon Joha (Teok) for spikelet fertility; Kola Joha for thousand-grain weights; Soru Joha (Tinsukia) for the most extended rice length and the highest rice length/breadth ratio; Kon Joha-2 for the broadest rice breadth; Ronga Joha and Kola Joha for biological yield; Ronga Joha for grain yield per plant; Joha (Bihpuria) for harvest index; and Soru Joha (Tinsukia) for grain yield kg ha-1 (Table 4). The majority of the Joha rice cultivars showed low to medium tillering, whereas none of the cultivars showed high tillering ability, as also supported by Ogunbayo et al. (2005), Singh et al. (2010), Mathure et al. (2011), and Sarawgi et al. (2014). The presence of considerable genetic variability for yield components observed in the present study was also similar to the findings of Marchenzan et al. (2005) and Singh et al. (2010). The variations in the grain characteristics of the Joha rice cultivars was in tune with the findings of Singh et al. (2010), Singh et al. (2012), Pachauri et al. (2013), Sarawgi et al. (2014), Semwal et al. (2014) and Sarhadi et al. (2015). The findings of Koutroubas et al. (2004), Vanaja and Babu (2006), Bajpai and Singh (2010), and Srivastava and Jaiswal (2013) further corroborated the present results on grain physical quality characteristics.

Genetic variability parameters

Grain yield kg ha-1 showed the highest range of variation followed by filled grains panicle-1, 1000-grain weights, grain length/breadth ratio, grain yield plant-1, productive tillers plant-1, rice length, stem thickness, and biological yield plant-1. Flag leaf breadth showed minor variability among the cultivars.

The magnitude of the genotypic and phenotypic coefficient of variation (Table 5) was the highest for grain yield kg ha-1 (24.62 & 24.85%), followed by filled grains panicle-1 (23.69 & 25.02%). The genotypic and phenotypic coefficient of variation ranked moderate for rice length/breadth ratio (19.61 & 19.63%) followed by 1000-grain weights (19.41 & 19.47%), grain length/breadth ratio (18.86 & 18.90%), rice length (16.99 & 17.01%), grain length (16.55 & 16.56%),

productive tillers plant-1 (13.15 & 17.27%), flag leaf area (10.62 & 10.66%) and stem thickness (10.23 & 10.37%). The phenotypic variations for all the above traits except productive tillers plant-1 were determined largely by the genotypes. Therefore, phenotype-based selection would be effective for these traits. These findings were in tune with Singh and Choudhary (1996), DebChoudhary and Das (1998), Kavitha and Reddy (2002), Karim et al. (2007), Osman et al. (2012), Babu et al. (2012), and Karuppaiyan et al. (2013). High GCV and PCV for the number of filled grains panicle-1 agreed with Pandey and Anurag (2010) and Mazid et al. (2013). Kavitha and Reddy (2002) and Gangashetty et al. (2013) also reported moderate GCV and PCV for grain breadth and panicle length.

Heritability estimates ranged from 37.36 percent for grain yield per plant to 99.99 percent for flag leaf length (Table 5). Most of the traits exhibited high heritability values (82.64 to 99.99%); the exceptions were productive tillers (58.00%), biological yield (41.19%), and harvest index (45.26%) with average estimates and grain yield plant-1 were low heritable. The genetic advances as a percent of mean were high (>20%) for grain yield kg ha-1 (50.24%), filled grain panicle-1 (46.22%), rice length/breadth ratio (40.33%), thousand-grain weights (39.85%), grain length/breadth ratio (38.77%), rice length (34.98%), grain length (34.06%), flag leaf area (21.79%), stem thickness (20.79% and productive tillers plant-1 (20.64%). The traits excluding days to first/50 percent flowering and maturity, flag leaf breadth, and spikelet fertility exhibited high heritability in conjunction with high to moderate genetic advance, indicating the most likely role of additive gene action and effectiveness of simple selection for the traits. Similar results on days to 50 percent flowering were in agreement with Bihari et al. (2004), Sankar et al. (2006), and Karthikeyan et al. (2009). High heritability and low genetic advance for days to 50% flowering agreed with Chaurasia et al. (2012). Plant height registered high heritability coupled with the moderate genetic advancement in conformity with the findings of Mishra and Verma (2002) and Chaurasia et al. (2012). Expression of moderate heritability coupled with high genetic advance for productive tillers plant-1 was in tune with Mishra and Verma (2002), Kumari et al. (2003), Jaiswal et al. (2007), and Nandan et al. (2010). For filled grains panicle-1, a high heritability concomitant with high genetic advance was in agreement with the results of Hasib et al. (2004) and Panwar (2005). High heritability in concurrence with high genetic advance for 1000-grain weights was in accord with the findings of Reddy et al. (1997), Murthy et al. (1999), Rao (2000), Mishra and Verma (2002), and Nandan et al. (2010). The grain quality traits viz., grain length, breadth and length-breadth ratio, rice length, breadth, and length-breadth ratio registered high heritability coupled with the high genetic advance in consonance with the findings of Jaiswal et al. (2007), Reddy et al. (2013), Fatema et al. (2011) for grain length; Murthy et al. (1999) and Jaiswal et al. (2007) for grain breadth; and Jaiswal et al. (2007) and Fatema et al. (2011) for length-breadth ratio. A low heritability coupled with moderate genetic advance for grain yield plant-1 was in agreement with Adjah et al. (2020). Similarly, Islam et al. (2015) observed high estimates of both heritability and genetic advance for grain yield kg ha-1 as obtained in the present study.

Genetic divergence among the twenty Joha rice cultivars

The twenty aromatic Joha rice cultivars were assessed for the nature and magnitude of genetic divergence (Singh and Chaudhary, 1985) based on the twenty-three quantitative traits following Mahalanobis D2 statistics. The V statistics and the analysis of dispersion (Supplementary Table S5) showed that the mean differences for the pooled effect of p characters between the cultivars were highly significant. Mahalanobis distances (D2) distinguished the twenty Joha rice cultivars into seven clusters, showing three multi- and four mono-genotypic collections (Table 6); group I was the largest with eight cultivars, followed by IV and II with five and three cultivars, respectively. The average intra- and inter-cluster distances (Table 7) showed cluster I to have the maximum intra-cluster distance (331.08) followed by cluster IV (307.84) and cluster II (223.91). The rest clusters were mono-genotypic, consisting of one cultivar each. The inter-cluster D2 values were the least between the clusters V and VI (353.94), while it was the maximum between clusters IV and VI (7303.31). Cluster I, the largest group comprising eight cultivars, was distantly apart from cluster IV and cluster VI by 2281.86 and 1815.30 units, respectively, and was nearest to cluster III (490.87), followed by cluster V (854.97). Cluster II, with three cultivars, had the lowest inter-cluster distance from cluster III (564.34), followed by cluster V (892.56), while the highest inter-cluster distance was from cluster IV (4709.44). Cluster III consisted of one aromatic Joha rice cultivar and was nearest to cluster VII (434.10) whereas distantly placed from cluster IV (2323.71). Cluster IV was a multi-genotypic cluster having four aromatic Joha rice cultivars and was nearest to cluster VII (1256.85) while most distantly related to cluster VI (7303.31). Mono-genotypic cluster V was closest to cluster VI (353.94) and separated from cluster IV by 5187.67 units. Cluster VII having one aromatic Joha rice cultivar was distantly placed from cluster VI (4073.37). Hybridization between parents from the widely divergent cluster pairs viz., IV-VI, IV-V, II-IV, VI-VII, V-VI, and III-IV would be likely to produce a broad spectrum of variability and transgressive segregations with high heterotic effects as also suggested by Shahidullah et al. (2009), Patil et al. (2012), Ali et al. (2012), Allam et al. (2014) and Patel et al. (2015).

The cluster mean performances for the various traits showed a wide range of variations among the clusters (Table 8). Cluster III registered the earliest days to first and 50 percent flowering (108 & 114) and maturity (147) and the highest mean performance for flag leaf breadth (0.84 cm), grain yield per plant (14.83 g), and harvest index (36.40%). Cluster VII showed superiority for spikelet fertility (90.92%), thousand-grain weights (22.50 g), grain and rice length (9.04 &6.97 mm), rice length-breadth ratio (3.32), and grain yield kg ha-1 (3012 kg). The highest mean performances for flag leaf length (54.21 cm), flag leaf area (32.42 cm2), panicle length (27.26 cm), and biological yield plant-1 (36.73 g) characterized cluster IV while cluster VI exhibited superiority for stem thickness (4.62 mm), filled grains panicle-1 (252), grain and rice breadth (2.82 & 2.33 mm). Cluster II was superior to other clusters in having the highest grain length-breadth ratio (3.89) and the shortest plant height (111 cm).

Of the twenty-three traits observed, only eight contributed to the genetic divergence. The contribution towards the total variation was the maximum for flag leaf length (72.11%) followed by rice length (13.68%), grain length (6.84%), rice breadth (3.16%), grain yield kg ha-1 (1.58%), 1000-grain weights (1.05%), grain breadth (1.05%) and rice length-breadth ratio (0.53%). These results agreed with the findings of Allam et al. (2014) for rice length, Nayak et al. (2004), Chandra et al. (2007), Singh et al. (2008), and Shahidullah et al. (2009) for grain length.

The clustering pattern of the twenty Joha rice cultivars also confirmed the quantum of diversity present in the indigenous aromatic rice of Assam and offer scope for its exploitation through breeding for yield improvement. Previous studies reported different numbers of clusters in fragrant rice, e.g., two clusters by Das et al. (2012), four clusters by Kole (2000), five clusters by Patel et al. (2015), six clusters by Singh et al. (1996) and Awasthi et al. (2005) and Shahidullah et al. (2009), Patil et al. (2012) and Allam et al. (2014), ten clusters by Pradhan and Mani (2005) and Ali et al. (2012).

Biochemical characterization of the Joha rice cultivars

Table 9 shows the biochemical characterization of the twenty Joha rice cultivars based on fatty acid profile, Fe and Zn content, crude protein, amylose, gel consistency, and aroma, a cultivar's mean value was considered desirable for all other biochemical traits except for polyunsaturated fatty acids when it exceeded the cultivars' mean plus the standard deviation. A low mean less than the cultivars' mean minus the standard deviation was desirable for polyunsaturated fatty acids.

Fatty acid profile

Palmitic acid content ranged from 17.98% (Local Joha) to 20.57% (Kon Joha-Teok), with an average of 19.01%. Stearic acid content was the lowest (0.90%) in Harinarayan and the highest (1.86%) in Kalijeera; the average was 1.40 percent. The range of oleic acid was from 33.53% in Kunkuni Joha to 41.32% in Joha (Golaghat), having an average of 37.90 percent. The linoleic acid content varied from 36.02% in Joha (Golaghat) to 44.61% in Local Joha, with an average of 39.22 percent. Local Joha recorded the highest linolenic acid content (2.14%), whereas the Kola Joha had the lowest estimate (1.06%). Arachidic acid content ranged from 0.28% (Local Joha) to 0.61% (Kon Joha-3), with an average of 0.48 percent. Fatty acids are vital components of food and human health. Fatty acids are the major constituents of cell membrane structure and play important biological, structural, and functional roles in the human body (Nagy and Tiuca 2017). They act as modulators of gene transcription, cytokine precursors, and energy sources in complex interconnected systems (Glick and Fischer 2013) by producing a vast ATP quantity during their metabolism (Nagy and Tiuca 2017). The role of dietary fatty acids in human health is strongly evident for their influence on cardiovascular disease and mental health (Glick and Fischer 2013). Besides, rice is a dietary consumption; rice fats have unique health benefits (Jennings and Akoh, 2009). In the present investigation, the contents of oleic, linoleic, and palmitic acids were the primary fatty acids, and those of stearic, linolenic, and arachidic acids were minor in the aromatic rice cultivars. Palmitic, stearic, and arachidic acids were the saturated fatty acids present in rice bran, increasing the health risk such as atherosclerosis, a disease associated with a heart attack (Oluremi et al. 2013). Linoleic acid gets absorbed as a predominant unsaturated fatty acid, followed by oleic and linolenic acid. The high contents of polyunsaturated fatty acids are desirable for human health as their consumption minimizes the risk of heart-related diseases (Law, 2000). The mean polyunsaturated fatty acid (PUFA) contents of the aromatic cultivars were 37.9% for oleic acid, 39.22% for linoleic acid, and 0.5% for linolenic acid, whereas the contents of saturated fatty acids (SFA) accorded 1.40% for stearic acid and 19.01% for palmitic acid. These estimates were comparable or even better than the values of 38.4% oleic acid, 34.4% linoleic acid, and 2.2% α-linolenic acid of PUFA; 2.9% stearic acid and 21.5% palmitic acid of SFA (Sayre and Saunders, 1990). The present results were also comparable with those reported by Resurreccion and Juliano (1975) and Taira and Itani (1988).

Similarly, the variations in the fatty acid profile of the present study proved better in having lower maximum limits for SFA and higher maximum limits for linoleic and linolenic acid than those reported by Goffman et al. (2003), who obtained 13.9-22.1% for palmitic, 1.5-2.7% for stearic, 35.9-49.2% for oleic, 27.3-41.0% for linoleic and 1.0-1.9% for linolenic acid in rice bran. Stearic acid and arachidic acid were present in trace amounts in all the studied aromatic rice cultivars. Comparatively, the fatty acid profile of Local Joha was better than the rest cultivars as it possessed a high level of linoleic and linolenic acid and low saturated fatty acid content. In general, the Joha rice cultivars' fatty acid profile qualified for extraction of quality bran oil for consumption.

Iron and Zn content of the cultivars

The cultivars' iron content varied from 21.33 (Keteki Joha) to 82.88 mg kg-1 in Kon Joha-4 with an average of 43.57 mg kg-1. The zinc content ranged from 12.26 (Keteki Joha) to 47.39 mg kg-1 in Ronga Joha, showing an average of 28.43 mg kg-1. Iron and Zinc deficiency is a severe nutritional problem for humans and is particularly prevalent among children and pregnant women, especially in developing countries. The Joha rice cultivars with very high iron and zinc contents in brown rice could play an essential role in the developing world's nutritional upliftment. Therefore, increasing the iron and zinc content and bioavailability in rice grains is an essential target for breeders and offers potential benefits to a large proportion of the human population. Substantial variations in iron and zinc content of brown rice agreed with Banerjee et al. (2010), Nachimuthu et al. (2014), and Chowdhury et al. (2016).

Protein content, amylose content, gel consistency, and aroma score of the cultivars

The range of variation in the cultivars' protein content was from 7.51 percent in Ronga Joha to 10.32 percent in Kon Joha-1 with an average of 9.09 percent. The variation in protein content was in agreement with Banerjee et al. (2011), who reported 4.91 to 12.08% protein in 258 diverse rice landraces with a mean of 6.63 percent. The amylose content of the cultivars varied from 15.20% in Jeera Joha to 24.40% in Harinarayan, with an average of 19.86%. The cultivars exhibited two classes of amylose content - medium (20-25%) and low (10-20%), as also noted by Bajpai and Singh (2010). The lowest and the highest gel consistency in the cultivars were 61.50 mm in Kon Joha 5 and 140.50 mm in Joha (Bihpuria), respectively, with an average of 100.25 mm. Joha (Golaghat) (129.50 mm), Kunkuni Joha (123.00 mm), and Manimuni Joha (121.50 mm) followed Joha-Bihpuria. The cultivars viz., Kalijeera, Kunkuni Joha, Kon Joha-5, Manimuni Joha and Kon Joha-2 showed strong aroma, Joha (Bihpuria), Keteki Joha, Kon Joha-3, Kon Joha (Moran), Joha (Golaghat), Kola Joha, Jeera Joha, Kon Joha (Bongaigaon), Local Joha, Harinarayan, Kon Joha-1, Soru Joha (Tinsukia) and Kon Joha-4 had a mild aroma. Ronga Joha and Kon Joha (Teok) possessed a light aroma. In aromatic rice, Bajpai and Singh (2010), Mia et al. (2010), Golam et al. (2010), Mathure et al. (2011), Pachauri et al. (2013), Semwal et al. (2014), and Sarhadi et al. (2015) also observed variation in aroma and accordingly classified the genotypes.

Molecular characterization

Among the seventy-one SSR markers, sixty-six showed polymorphism. The analysis excluded the markers with monomorphic banding patterns. Tables 10 summarize the results obtained on the analysis of 20 aromatic rice cultivars using the polymorphic SSR loci. Figure 1 depicts representative gel pictures of the PCR products.

The 66 polymorphic SSR loci amplified a total of 174 alleles. The allelic richness per locus ranged from 2 to 4 alleles, with an average of 2.64 alleles. Among the polymorphic markers, 30 markers produced two alleles each, 30 produced three alleles each, and 6 generated four alleles. The markers RM 283, RM 118,

RM316, RM29, RM 585, and RM 26063 amplified the maximum number of alleles. The result revealed that all the markers showed distinct polymorphisms among the cultivars studied, indicating the robust nature of microsatellites revealing polymorphism. The number of alleles per locus (2.64) obtained in the present study was comparable with the earlier reports by Joshi and Behera (2007), Shah et al. (2013), and Venkatesan and Bhat (2015), who reported 2.6, 2.75, and 2.3 alleles per locus, respectively. The mean allele number (2.64) obtained in the present study was higher than the result of Meti et al. (2013), who detected 2.08 alleles per locus using 48 traditional indigenous aromatic rice germplasm grown under the Eastern part of India through 12 polymorphic SSR loci. In contrast, the mean alleles (2.64) detected was markedly lower than the average number of alleles reported in previous diversity studies by Pervaiz et al. (2010) and Rahman et al. (2012), who reported an average of 4.4 and 4.18 alleles per locus, respectively. The variability in the number of alleles detected per locus might be due to the use of diverse genotypes and the selection of different SSR primers with scorable alleles. Among the sixty-six markers, the highest major allele frequency was 0.950 in 4 markers (RM 237, RM 215, RM6641, RM3481 & RM21), followed by 0.900 in 9 markers and 0.875 in 2 markers. However, RM 316 recorded the lowest major allele frequency of 0.300. The mean value of major allele frequency among the markers was 0.727, and it ranged from 0.300 to 0.950 among the sixty-six markers scored against a set of twenty aromatic Joha rice cultivars. Similarly, Sajib et al. (2012) reported major allele frequency ranging from 0.41 to 0.91; Shah et al. (2013) noted a range of 0.425 to 0.975 with an average of 0.647, and Kumar et al. (2015) observed it to vary from 0.510 to 0.970 averaging 0.74. The number of effective alleles was from 1.105 (RM 237) to 3.922 (RM 316), with a mean value of 1.759. A greater number of alleles generated by SSR markers suggest this marker system's usefulness for detecting genetic polymorphisms. Aljumaili et al. (2018) detected 1.48 effective alleles per SSR locus among 53 rice cultivars. On the contrary, the effective allele number detected in the present study was lower than the average number of effective alleles (5.51) reported by Yelome et al. (2018) among West African rice accessions. Shannon's information index ranged from 0.199 (RM 237) to 1.376 (RM 316), with a mean value of 0.640. Aljumaili et al. (2018) reported a similar Shannon's informative index by evaluating fifty-three aromatic rice accession using 32 SSR markers, and they reported a mean value of 0.580. The high value of Shannon's information index indicated the presence of high genetic diversity in the rice germplasm under consideration (Kibria et al. 2008). The gene diversity varied from 0.095 to 0.745, with a mean value of 0.377. RM 316 showed the highest gene diversity (0.745), followed by RM 283 (0.715) and RM 1388 (0.645). In contrast, Shah et al. (2013) recorded an average gene diversity of 0.448, ranging from 0.049 to 0.664, whereas Kumar et al. (2015) reported gene diversity ranging from 0.045 to 0.588 with a mean of 0.340. A set of four SSRs viz., RM 271, RM 279, RM 7434, and RM501 represented heterozygosity among sixty-six SSRs. All four markers showed the same value, 0.050, with a mean value of 0.003. The rest of the markers showed no heterozygosity. Similarly, Choudhury et al. (2014), Nachimuthu et al. (2014), and Yelome et al. (2018) reported low observed heterozygosity. The autogamous mode of reproduction in rice could be the reason for the low level of observed heterozygosity. The polymorphism information content (PIC) values, a reflection of allele diversity and frequency among the genotypes, also varied from one locus to another. PIC values varied from 0.091 to 0.698, with an average of 0.326, indicating that not all the SSR markers used were highly informative. The highest PIC value 0.698 was obtained for RM 316 followed by RM 283 (0.665), RM 585 (0.573), RM 1388 (0.572), RM 3562 (0.559), RM 171 (0.559), R1M30 (0.534), RM118 (0.531), RM11 (0.513) and RM29 (0.509), suggesting that these ten markers were highly informative (PIC>0.50) for identification of the twenty aromatic rice cultivars. Among the remaining polymorphic SSR markers, 30 markers were informative (0.50 < PIC > 0.25) and 26 markers were slightly informative (PIC < 0.25). The level of polymorphism detected in the present study was consistent with the reported mean PIC values in previous works (Pal et al., 2004; Hossain et al., 2007; Sajib et al., 2012). However, Nadia et al. (2014) reported an average PIC value of 0.84, markedly higher than the present average PIC value. The presence of sufficient polymorphism by the 66 SSR markers among the twenty indigenous Joha rice cultivars justifies their proper classification and use in the genetic improvement program, based on the extent of genetic variation for desirable alleles.

Molecular diversity among the aromatic rice cultivars

An unweighted neighbor-joining (UNJ) cluster analysis based on Jaccard's similarity coefficients (Supplementary Table S6) resolved the phylogenetic relationships among the aromatic rice cultivars collected from different parts of Assam. The UNJ cluster diagram showed three major clusters (G1, G2 & G3) with additional sub-clusters (Fig. 2). This dendrogram revealed that the cultivars derived from a genetically similar type clustered together. Cluster I comprised eight cultivars, whereas cluster II had ten cultivars, forming the most significant cluster. Cluster III had only two cultivars (Local Joha & Kunkuni Joha). Cluster I included Kon Joha-3, Jeera Joha, Kon Joha-4, Keteki Joha, Joha (Bihpuria), Joha (Golaghat), Kon Joha-5, and Kon Joha-2. Kon Joha (Bongaigaon), Kon Joha (Teok), Soru Joha (Tinsukia), Ronga Joha, Kola Joha, Kon Joha (Moran), Kalijeera, Kon Joha-1, Manimuni Joha and Harinarayan belonged to cluster II. The cluster I sub-divided into two clusters, IA and IB, consisting of six and two cultivars. Cluster II had two sub-clusters, IIA and IIB, consisting of nine and one cultivars, respectively. Similar to the present investigation's clustering pattern, Meti et al. (2013) obtained two major clusters for 48 aromatic rice genotypes from Odisha using 12 SSR markers at 49 percent genetic similarity. Shah et al. (2013) effectively differentiated the basmati cultivars from non-basmati ones based on the cluster analysis using 24 microsatellite loci, classifying 40 rice cultivars into three groups. Pervaiz et al. (2010) reported four significant clusters of 75 rice landraces using 35 SSR markers at 0.40 similarity coefficients. Thus, SSR markers provided an adequate resolution to discriminate between aromatic rice accessions, and it could serve as a potential tool in the identification and characterization of genetically distant accessions from various sources. The microsatellite assay generated genotype-specific alleles in some of the cultivars evaluated for DNA fingerprints for cultivar identification and differentiation of aromatic rice. DNA fingerprints would be enormous assistance for establishing and defending the proprietary rights and maintaining the cultivar purity.

Conclusion

The present investigation on morpho-molecular and biochemical profiling of a panel of popular indigenous Joha cultivars of Assam has been a step forward for exploiting variability in this unique rice class to improve its inherently low yield potential breeding. In light of the results on diversity analyses, it is evident that the Joha rice cultivars are highly diverse regarding yield and quality traits, and utilization of these diverse traits specific genotypes to develop crop varieties with a broad genetic base would prove beneficial for aromatic Joha rice improvement program. The low to a high degree of dissimilarity among the aromatic rice accessions observed in the present study exemplified the high level of diversity at the molecular level among the aromatic rice cultivars used and indicated their possible utilization in breeding programs targeted at developing elite aromatic rice varieties. Besides, marker-based identification and differentiation of aromatic rice cultivars might help maintain this high-quality product's integrity to benefit both farmers and consumers. The Joha rice

cultivar Soru Joha (Tinsukia) with the highest yield (3012 kg ha-1), high spikelet fertility (90.9%), and high Fe content (61.09 mg kg-1) could serve as an immediate recommendation in the state of Assam. The Joha rice cultivars' fatty acid profile qualified for extraction of quality bran oil for consumption. Our study opened the scope for value addition through nutritional profiling and yield enhancement through interbreeding in these specialty Joha rice cultivars without compromising quality characteristics.

Declarations

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CONFLICT OF INTEREST

The authors declare no competing interest.

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Tables

Table 1 List of indigenous *Joha* rice cultivars used in the investigation

S No	Cultivar name	Pedigree	Origin
1	Joha (Bihpuria)	Landrace	Assam
2	Kalijeera	Landrace	Assam
3	Ronga Joha	Landrace	Assam
4	Joha (Golaghat)	Landrace	Assam
5	Manimuni Joha	Landrace	Assam
6	Kon Joha (Moran)	Landrace	Assam
7	Keteki Joha	Savitri/Badsabhog	Assam
8	Kon Joha-1	Landrace	Assam
9	Soru Joha (Tinsukia)	Landrace	Assam
10	Kon Joha-2	Landrace	Assam
11	Jeera Joha	Landrace	Assam
12	Kon Joha-3	Landrace	Assam
13	Kon Joha-4	Landrace	Assam
14	Kunkuni Joha	Landrace	Assam
15	Kon Joha-5	Landrace	Assam
16	Local Joha	Landrace	Assam
17	Harinarayan	Landrace	Assam
18	Kon Joha (Teok)	Landrace	Assam
19	Kola Joha	Landrace	Assam
20	Kon Joha (Bongaigaon)	Landrace	Assam

Table 2 Distribution of the *Joha* rice cultivars based on monomorphic characteristics

S No*	Characteristics	States	Notes
1	Coleoptile: colour	Green	2
3	Leaf: intensity of green colour	Medium	5
4	Leaf anthocyanin colouration	Present	9
6	Leaf sheath: anthocyanin colouration	Absent	9
9	Leaf: auricles	Present	9
10	Leaf: anthocyanin colouration of auricles	Colourless	1
11	Leaf: collar	Present	9
12	Leaf: anthocyanin colouration of collar	Absent	1
13	Leaf: ligule	Present	9
14	Leaf: shape of ligule	Split	3
15	Leaf: colour of ligule	White	1
17	Leaf: width of blade	Narrow	3
19	Culm: attitude	Erect	1
20	Time of heading (% of plants with panicles) (days)	Late	7
23	Male sterility	Absent	1
35	Panicle curvature of main axis	Dropping	7
43	Panicle: presence of secondary branch	Present	9
46	Panicle: exsertion	Well exerted	7
57	Decorticated grain: colour	Light brown	2
58	Endosperm presence of amylose	Present	9
61	Gelatinization temperature through alkali spreading value	Low	1
62	Decorticated grain: aroma	Present	9

*Serial numbers are as per DUS descriptor.

Table 3 Distribution of the *Joha* rice cultivars based on polymorphic characteristics

Characteristics	States	%
Basal leaf: sheath colour	Green (1)	95
	Purple lines (3)	5
Leaf: distribution of anthocyanin colouration	On margins only (1)	40
	On tips only (2)	60
Leaf pubescence of blade surface	Weak (3)	85
	Medium (5)	15
Leaf: length of blade	Medium (5)	25
	Long (7)	75
Flag leaf: attitude of blade (early observation)	Semi-erect (3)	85
	Horizontal (5)	15
Spikelet: density of pubescence of lemma	Medium (5)	85
	Strong (7)	15
Lemma: anthocyanin colouration of keel	Absent (1)	30
	Strong (7)	20
	Very strong (9)	50
Lemma: anthocyanin colouration of area below apex	Absent (1)	30
	Strong (7)	20
	Very strong (9)	50
Lemma: anthocyanin colouration of apex	Absent (1)	30
	Strong (7)	25
	Medium (5)	5
	Very strong (9)	40
Spikelet: colour of stigma	White (1)	80
	Light green (2)	10
	Yellow (3)	5
	Purple (5)	5
Stem thickness	Thin (3)	50
	Medium (5)	50
Stem: length (excluding panicle; excluding floating rice)	Very short (1)	10
	Short (3)	90
Stem: anthocyanin colouration of nodes	Absent (1)	90
	Present (9)	10
Stem: intensity of anthocyanin colouration of nodes	Weak (3)	5
	Medium (5)	5
Stem: anthocyanin colouration of internodes	Absent (1)	90
	Present (9)	10
Panicle: length of main axis	Medium (5)	40
	Long (7)	55
	Very long (9)	5
Lemma and palea: colour	Straw (1)	5
	Brown spot on straw (3)	10
	Brown furrows on straw (4)	10
	Brown (tawny) (5)	5
	Black (9)	45
Panicle: awns	Absent (1)	70
	Present (9)	30
Panicle: colour of awns (late observation)	Yellowish white (1)	10
	Brown (3)	5
	Purple (8)	5
	Black (9)	10
Panicle: length of the longest awn	Very short (1)	10
	Short (3)	10
	Very long (9)	10
Panicle: distribution of awns	Tip only (1)	5
	Upper half only (3)	10
	Whole length (5)	15
Panicle: secondary branching	Strong (2)	80
	Clustered (3)	20
Panicle attitude of branches	Semi-erect (5)	75
	Semi-erect to spreading (7)	25
Time maturity (days)	Late (7)	85
	Very late (9)	15
Leaf senescence	Early (3)	20
	Medium (5)	15
	Late (7)	65
Sterile lemma: colour	Straw (1)	45
	Purple (4)	55
Grain weight of 1000 fully developed grains	Very low (1)	5
	Low (3)	75
	Medium (5)	15
	High (7)	5
Grain: length	Short (3)	80
	Medium (5)	20
Grain: width	Narrow (3)	70
	Medium (5)	30
Grain: Phenol reaction of lemma	Absent (1)	80
	Present (9)	20
Decorticated grain: length	Short (1)	75

Flag leaf: attitude of blade (late observation)	Semi-erect (3)	40		Medium (3)	25
	Horizontal (5)	60	Decorticated grain: width	Narrow (3)	10
Panicle: number per plant	Few (3)	65		Medium (5)	85
	Medium (5)	35		Broad (7)	5
Spikelet: colour of tip of lemma	Yellowish (2)	25	Decorticated grain: shape (in lateral view)	Short bold (2)	70
				Long bold (4)	10
	Brown (3)	15		Long Slender (5)	15
			Endosperm content of amylose	Low	50
				Medium (5)	50
	Black (6)	60			

Table 4 Top ranking cultivars with desirable characteristics

Cultivar	Desirable characteristics
Kola Joha	Days to 1st flowering*, days to 50% flowering*, days to maturity*, 1000-grain weights (g), biological yield (g Plant^{-1})
Soru Joha-Tinsukia	Flag leaf breath (cm), rice length (mm), rice length/breadth ratio, grain yield (kg ha^{-1})
Ronga Joha	Flag leaf area (cm^2), grain yield (g Plant^{-1})
Keteki Joha	Plant height (cm), productive tillers Plant^{-1}
Kon Joha-5	Filled grains Panicle^{-1}, grain breadth (mm)
Kon Joha-2	Stem thickness (mm), rice breadth (mm)
Kon Joha-Teok	Flag leaf length (cm)
Jeera Joha	Spikelet fertility (%)
Kon Joha-3	Grain length (mm)
Local Joha	Grain length/breadth ratio
Joha-Bihpuria	Harvest index (%)

*Earliness is desirable.

Table 5 Genetic variability parameters for the traits of the twenty indigenous *Joha* rice cultivars of Assam evaluated during *Sali* season of 2018 and 2019

Character	Range	Mean ± SE	GCV (%)	PCV (%)	h^2_{bs} (%)	GA (5%) As % of mean
Stem thickness (mm)	3.00 – 4.96	4.09 ± 0.07	10.23	10.37	97.34	20.79
Days to 1st flowering	108.00 – 123.33	114.51 ± 0.52	3.83	3.86	98.62	7.84
Flag leaf length (cm)	40.84 – 54.82	47.96 ± 0.05	9.08	9.08	99.99	18.71
Flag leaf breath (cm)	0.75 – 0.84	0.79 ± 0.01	3.61	3.73	93.80	7.21
Flag leaf area (cm^2)	24.21 – 34.0	28.29 ± 0.26	10.62	10.66	99.23	21.79
Days to 50% flowering	112.50 – 128.50	119.80 ± 0.56	3.93	3.96	98.59	8.05
Days to maturity	144.50 – 162.03	152.16 ± 0.69	3.35	3.38	98.18	6.85
Plant height (cm)	98.90 – 142.90	127.80 ± 3.74	7.83	8.36	87.75	15.11
Productive tillers Plant^{-1}	7.80 – 13.80	10.42 ± 1.17	13.15	17.27	58.00	20.64
Filled grains Panicle^{-1}	101.73 – 252.07	177.21 ± 14.24	23.69	25.02	89.69	46.22
Spikelet fertility (%)	78.97 – 93.03	87.04 ± 1.88	4.72	5.19	82.64	8.83
1000-grain weights (g)	13.02 – 26.69	17.60 ± 0.27	19.41	19.47	99.38	39.85
Grain length (mm)	5.95 – 9.30	7.28 ± 0.05	16.55	16.56	99.84	34.06
Grain breadth (mm)	2.09 – 2.87	2.41 ± 0.02	9.68	9.73	99.00	19.84
Grain length/breadth ratio	2.08 – 4.30	3.04 ± 0.04	18.86	18.90	99.58	38.77
Rice length (mm)	4.13 – 6.97	5.18 ± 0.04	16.99	17.01	99.83	34.98
Rice breadth (mm)	1.89 – 2.57	2.20 ± 0.02	8.54	8.57	99.16	17.51
Rice length/breadth ratio	1.79 – 2.32	2.38 ± 0.03	19.61	19.63	99.70	40.33
Biological yield (g Plant^{-1})	28.98 – 44.97	33.56 ± 3.30	8.23	12.83	41.19	10.88
Grain yield (g Plant^{-1})	8.64 – 17.20	12.94 ± 1.65	9.83	16.09	37.36	12.38
Harvest index (%)	22.99 – 36.40	31.65 ± 3.16	9.08	13.49	45.26	12.58
Grain yield (kg ha^{-1})	1000.00 – 3011.67	2012.08 ± 68.16	24.62	24.85	98.14	50.24

Table 6 Composition of the Tocher's clusters based on Mahanobis D^2 analysis

Cluster identity	No. of cultivars	Name of the cultivars
I	8	*Kon Joha-Moran, Jeera Joha, Manimuni Joha, Kon Joha-1, Kon Joha-Bongaigaon, Kon Joha-4, Kalijeera, Kon Joha-2*
II	3	*Keteki Joha, Local Joha, Kon Joha-3*
III	1	*Joha-Bihpuria*
IV	5	*Ronga Joha, Kola Joha, Joha-Golaghat, Kon Joha-Teok, Harinarayan*
V	1	*Kunkuni Joha*
VI	1	*Kon Joha-5*
VII	1	*Soru Joha-Tinsukia*

Table 7 Intra- (bold) and inter-cluster distances

Cluster	I	II	III	IV	V	VI	VII
I	**331.08**	1293.16	490.87	2281.86	854.97	1815.30	1167.98
II	1293.16	**223.91**	564.34	4709.44	892.56	1187.69	1560.50
III	490.87	564.34	**0.00**	2323.71	1046.91	1930.59	434.10
IV	2281.86	4709.44	2323.71	**307.84**	5187.67	7303.31	1256.85
V	854.97	892.56	1046.91	5187.67	**0.00**	353.94	2733.52
VI	1815.30	1187.69	1930.59	7303.31	353.94	**0.00**	4073.37
VII	1167.98	1560.50	434.10	1256.85	2733.52	4073.37	**0.00**

Table 8 Biochemical characteristics of the twenty *Joha* rice cultivars

Cultivars	Palmitic acid (16:0)	Stearic acid (18:0)	Oleic acid (18:1)	Linoleic acid (18:2)	Linolenic acid (18:3)	Arachidic acid (20:0)	Fe (mg kg^{-1})	Zn (mg kg^{-1})	Protein content (%)	Amylose content (%)	Gel consistency (mm)	Aroma (KOH test score)
Joha-Bihpuria	19.45	1.29	37.93	37.91	1.09	0.38	23.41	26.73	8.94	20.10	**140.50**	2
Kalijeera	18.50	1.86	**40.70**	36.77	1.23	0.61	31.19	23.49	9.69	17.40	84.50	**3**
Ronga Joha	**18.22**	1.25	38.55	39.62	1.33	0.54	47.00	**47.39**	7.51	19.10	113.00	1
Joha-Golaghat	19.16	1.50	**41.32**	36.02	1.11	0.51	28.58	32.60	7.93	19.42	**129.50**	2
Manimuni Joha	18.97	1.59	**40.56**	36.51	1.36	0.57	36.23	26.71	9.37	**23.20**	**121.50**	**3**
Kon Joha-Moran	19.19	1.48	36.90	39.83	1.66	0.54	25.61	28.56	**9.87**	17.30	98.00	2
Keteki Joha	**18.08**	1.20	37.37	40.90	1.77	**0.37**	21.33	12.26	9.07	22.60	82.00	2
Kon Joha 1	**18.19**	1.70	38.66	38.92	1.50	0.61	**61.00**	17.50	**10.32**	16.20	105.50	2
Soru Joha-Tinsukia	18.78	1.59	40.23	37.32	1.09	0.59	**61.09**	22.98	8.20	19.50	94.50	2
Kon Joha-2	19.21	1.56	38.07	38.23	**2.10**	0.51	**62.50**	23.00	**9.89**	22.60	86.00	**3**
Jeera Joha	18.86	1.19	35.71	**41.82**	1.63	0.43	43.07	**37.95**	8.59	15.20	118.50	2
Kon Joha-3	19.18	1.40	40.19	36.30	1.86	0.61	24.05	**45.32**	8.65	21.40	79.50	2
Kon Joha-4	20.11	1.50	37.91	38.26	1.24	0.55	**82.88**	**38.25**	9.23	21.40	71.00	2
Kunkuni Joha	19.78	**1.04**	33.53	**43.13**	1.78	0.40	34.61	28.76	9.17	15.70	**123.00**	**3**
Kon Joha-5	18.94	1.72	39.84	36.72	**1.93**	0.50	34.91	24.45	8.41	18.92	61.50	**3**
Local Joha	**17.98**	**1.08**	33.66	**44.61**	**2.14**	**0.28**	51.91	20.13	9.38	**23.84**	85.50	2
Harinarayan	19.94	**0.90**	35.56	41.14	1.81	**0.29**	53.83	31.30	9.18	**24.40**	91.00	2
Kon Joha-Teok	20.57	**1.10**	34.92	41.41	1.34	**0.34**	**63.66**	31.80	8.66	18.00	113.50	1
Kola Joha	18.76	1.63	40.26	37.36	1.06	0.53	37.31	25.86	**10.20**	17.20	95.50	2
Kon Joha-Bongaigaon	18.38	1.48	36.10	41.58	1.63	0.49	47.21	23.62	9.65	**23.80**	111.00	2
Lowest mean	17.98	0.90	33.53	36.02	1.06	0.28	21.33	12.26	7.51	15.20	61.50	1.00
Highest mean	20.57	1.86	41.32	44.61	2.14	0.61	82.88	47.31	10.32	24.40	140.50	3.00
Mean	19.01	1.40	37.90	39.22	1.53	0.48	43.57	28.43	9.09	19.86	100.25	2.15
SE	0.16	0.06	0.53	0.56	0.08	0.02	3.75	1.96	0.35	0.56	3.23	0.13
CV (%)	3.71	18.49	6.30	6.35	22.53	22.01	38.51	30.76	8.22	14.62	20.72	27.30

Aroma sore: 0 – No aroma; 1 - Slight aroma; 2 - Mild aroma; 3 - Strong aroma; 4 - Very strong aroma. Values > (Mean + SD) are considered as desirable for all the traits except PUFA; For PUFA, values < (Mean - SD) were desirable (bold figures).

Table 9 SSR markers with their number of different alleles amplified (*Na*), major allele frequency (*MAF*), number of effective alleles (*Ne*), Shannon's informative index (*I*), observed heterozygosity (*Ho*), expected heterozygosity (*He*) and polymorphic information content (PIC) in the twenty indigenous *Joha* rice cultivars.

Locus	*Na*	*MAF*	*Ne*	*I*	*Ho*	*He*	*PIC*	Locus	*Na*	*MAF*	*Ne*	*I*	*Ho*	*He*	*PIC*	Locus	*Na*	*MAF*	*Ne*
RM495	2	0.90	1.22	0.33	0.00	0.18	0.16	RM489	3	0.70	1.80	0.75	0.00	0.46	0.38	RM 20236	2	0.90	1.22
RM 283	4	0.40	3.51	1.32	0.00	0.72	0.67	RM55	3	0.85	1.36	0.52	0.00	0.27	0.25	RM 7434	3	0.78	1.58
RM 237	2	0.95	1.11	0.20	0.00	0.10	0.09	RM 510	2	0.55	1.98	0.69	0.00	0.51	0.37	RM2126	2	0.90	1.22
RM431	3	0.70	1.80	0.75	0.00	0.45	0.38	RM474	3	0.60	2.25	0.94	0.00	0.57	0.49	RM 253	3	0.55	2.15
RM154	3	0.70	1.80	0.75	0.00	0.45	0.38	RM 171	3	0.45	2.74	1.05	0.00	0.65	0.56	RM217	2	0.90	1.22
OSR 13	3	0.80	1.50	0.61	0.00	0.34	0.30	RM212	2	0.90	1.22	0.33	0.00	0.19	0.16	RM7434	3	0.85	1.36
RM338	2	0.65	1.84	0.65	0.00	0.46	0.35	RM23	3	0.60	2.06	0.82	0.00	0.53	0.42	RM 481	3	0.70	1.85
RM514	3	0.60	2.06	0.82	0.00	0.52	0.35	RM 229	2	0.70	1.72	0.61	0.00	0.43	0.33	RM11	3	0.55	2.41
RM124	2	0.90	1.22	0.33	0.00	0.18	0.16	RM1003	2	0.70	1.72	0.61	0.00	0.43	0.33	RM 505	3	0.65	1.94
RM 161	2	0.55	1.98	0.69	0.00	0.50	0.37	RM29	4	0.60	2.30	1.03	0.00	0.58	0.51	RM501	3	0.73	1.75
RM133	3	0.75	1.68	0.73	0.00	0.41	0.37	RM 221	2	0.80	1.47	0.50	0.00	0.33	0.27	RM25	3	0.85	1.36
RM 125	3	0.85	1.36	0.52	0.00	0.27	0.25	RM6641	2	0.95	1.11	0.20	0.00	0.10	0.09	RM407	3	0.70	1.85
RM 118	4	0.55	2.47	1.07	0.00	0.60	0.53	RM 279	2	0.88	1.28	0.38	0.05	0.22	0.09	RM3481	2	0.95	1.11
RM 152	2	0.85	1.34	0.42	0.00	0.26	0.35	RM3562	3	0.45	2.74	1.05	0.00	0.65	0.56	RM3395	3	0.90	1.23
RM284	3	0.85	1.36	0.52	0.00	0.27	0.25	RM60	2	0.65	1.84	0.65	0.00	0.47	0.35	RM228	3	0.85	1.36
RM316	4	0.30	3.92	1.38	0.00	0.75	0.70	RM1388	3	0.45	2.82	1.07	0.00	0.66	0.57	RM590	2	0.80	1.47
RM 215	2	0.95	1.11	0.20	0.00	0.10	0.09	RM471	2	0.85	1.34	0.42	0.00	0.26	0.22	RM591	3	0.70	1.85
RM 271	2	0.88	1.28	0.38	0.05	0.22	0.16	RM17467	3	0.70	1.87	0.82	0.00	0.48	0.42	RM 26063	4	0.60	2.38
RM484	2	0.85	1.34	0.42	0.00	0.26	0.35	RM 317	2	0.85	1.34	0.42	0.00	0.26	0.22	RM21	2	0.95	1.11
RM 536	2	0.50	2.00	0.69	0.00	0.50	0.38	RM 187	2	0.85	1.34	0.42	0.00	0.26	0.22	RM27601	3	0.90	1.23
RM 227	2	0.60	1.92	0.67	0.00	0.48	0.37	RM 3322	2	0.90	1.22	0.33	0.00	0.19	0.16	R1M7	2	0.85	1.34
RM 29	3	0.45	2.41	0.95	0.00	0.59	0.50	RM 585	4	0.50	2.74	1.14	0.00	0.65	0.57	R1M30	3	0.45	2.60
Mean	**2.64**	**0.73**	**1.76**	**0.64**	**0.00**	**0.38**	**0.33**	**Mean**	**2.64**	**0.73**	**1.76**	**0.64**	**0.00**	**0.39**	**0.33**	**Mean**	**2.64**	**0.73**	**1.76**
SE	**0.08**	**0.02**	**0.07**	**0.04**	**0.00**	**0.02**	**0.02**	**SE**	**0.08**	**0.02**	**0.07**	**0.04**	**0.00**	**0.02**	**0.02**	**SE**	**0.08**	**0.02**	**0.07**

Figures

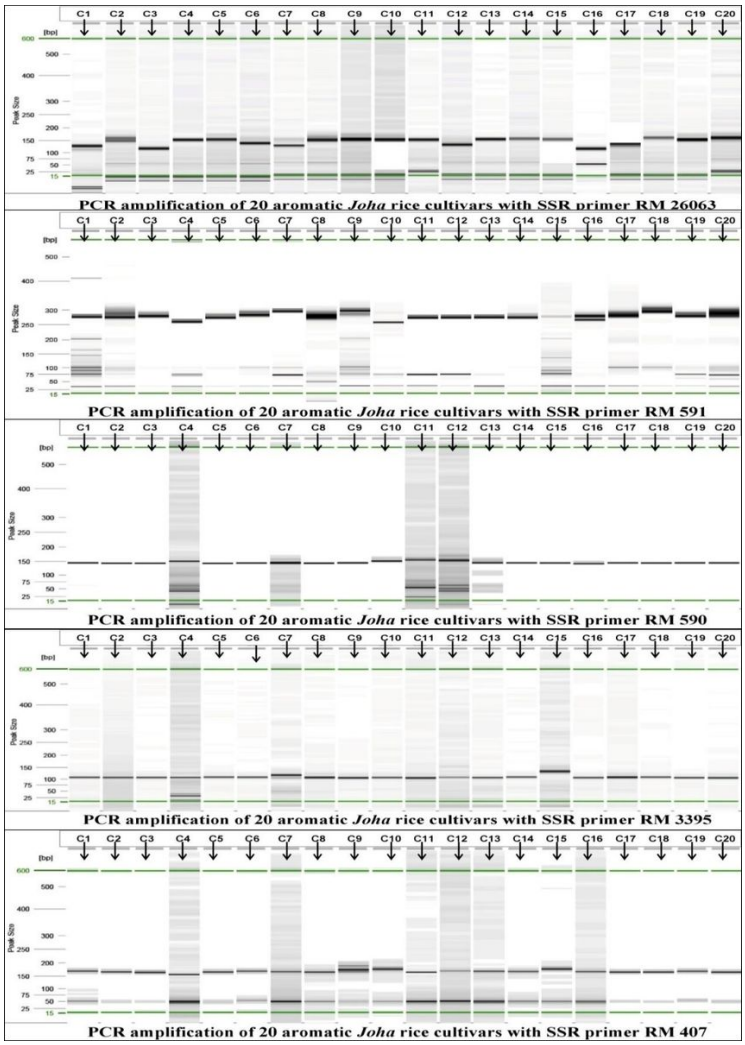

Figure 1

Representative gel pictures showing the PCR products

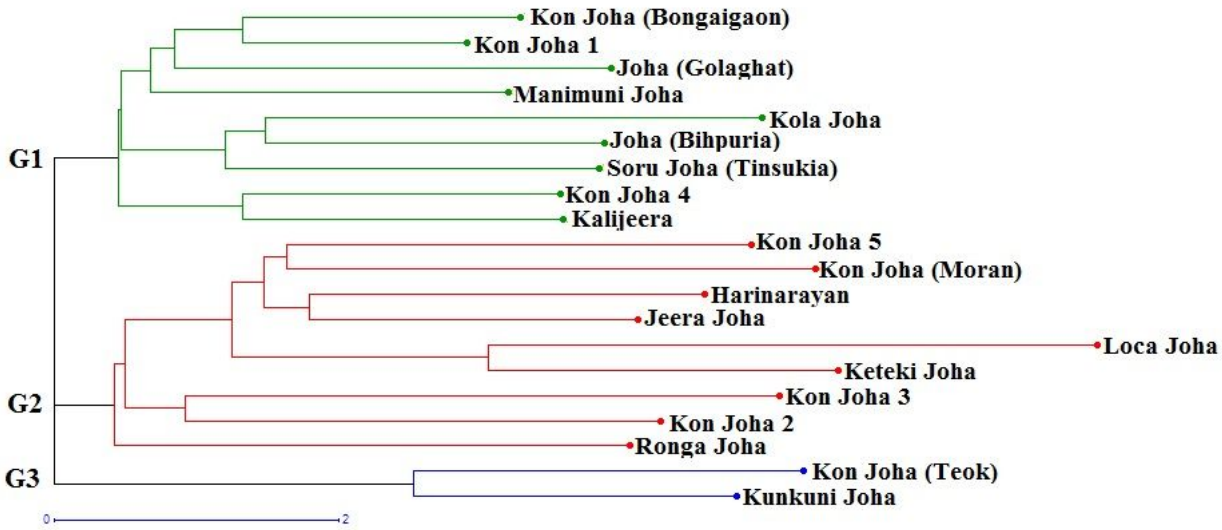

Figure 2

Hierarchical horizontal clustering of the 20 Joha rice cultivars using Unweighted Neighbour-Joining (UNJ) method based on usual Euclidean distances estimated from 37 polymorphic morphological markers

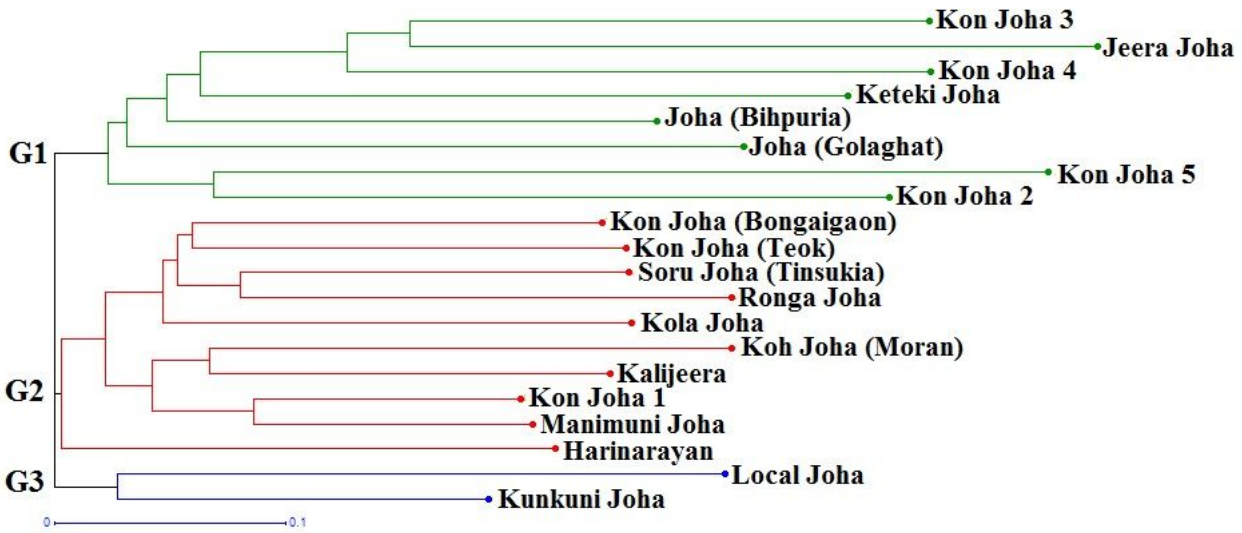

Figure 3

Hierarchical horizontal clustering of the 20 Joha rice cultivars using Unweighted Neighbour-Joining (UNJ) method based on Jaccard's coefficients of similarity estimated from 66 polymorphic SSR markers

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementaryfile1.xlsx
- Tables.docx