STATUS OF GENOMIC RESEARCH IN NON-BASMATI AROMATIC JOHA RICE OF ASSAM

ABSTRACT

Many natively adapted, aromatic, and high-quality rice landraces can be found in NE India. Among the different classes of cultivated rice varieties, Assam has a distinctive scented rice variety known locally as '*joha*,' which is particularly popular among the region's farmers. The aroma of *joha* rice is also thought to differ markedly from that of basmati rice. Genotype stability can be established through genetic variation. Thus, new disease resistant *joha* rice varieties are necessary to meet both domestic and worldwide demand. There is a need for conservation of this traditional aromatic joha rice germplasm which could be used in the future breeding programme for multiple disease resistance and grain and aroma quality traits improvement. The recent advancements in marker and genomics assisted breeding coupled with high-throughput genotyping and phenotyping enable the discovery of novel alleles and gene targets for the implementation of sustainable rice cultivars with improved grain aroma quality.

Keywords: Joha Rice, Aroma quality, Marker Assisted Breeding, Genomics Assisted Breeding

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1. INTRODUCTION

Among cereal crops, rice is a popular choice. Rice is a staple food for more than two-thirds of the world's population [1]. The genus Oryza and the tribe Oryzeae belong to the Gramineae family, which includes rice (Poaceae). The Oryza genus has 25 known species. There are 23 wild species, two of which are cultivated: *O. sativa* and *O. glaberrima*. It has been suggested that *O. sativa* is the more commonly cultivated of the two cultivated species of cannabis [1], [2], [3]. It is cultivated all over the world, including Asia, North and South America, the European Union, the Middle East, and Africa. *On* the other hand, *O. glaberrima* is only grown in West Africa.

2. ORIGIN

The origin and diversity of two cultivated species, *O. sativa* and *O. glaberrima*, have been documented combining genetic diversity, historical and archaeological evidence, and geographical distribution. The Himalayan foothills, Chhattisgarh, Orissa's Jeypore Tract, north-eastern India, northern Myanmar and Thailand, and Yunnan Province, China are among the Asian cultural hotspots. The Inner Niger Delta and the Guinean coast of Africa are regarded as the African species of *O. glaberrima* diversity hotspots [4]. The progenitors of O. sativa are *O. barthii* and *O. longistaminata*, and those of *O. glaberrima* are *O. barthii* and *O. longistaminata* [4], [5]. India is a major rice producer. *Indica* rice was believed to have been initially domesticated in the foothills of the Eastern Himalayas (north-eastern India), before being introduced to Japan. Assam and Nepal have perennial wild rice [6], [7].

3. RICE GENOME

Oryza has 12 basic chromosomes. O. sativa, *O. glaberrima*, and 14 wild species have 24 chromosomes each, whereas eight wild species have 48 chromosomes each. The nine different genomes A, B, C, D, E, F, G, H, and J were established through chromosome pairing behaviour and fertility in interspecific hybrids. Rice is the first cereal crop to have its whole genome sequenced. On average, the genome is 400-430 Mb in size [6].

4. CULTIVATION

Most people in the globe eat rice, which is one of the most important sources of energy. Globally, indica occupies between 85% and 90% of total land area [7]. For example, the Northeast is a rice biodiversity hotspot [8]. Rice is grown in upland, lowland, and deep water. This area has 10,000 indigenous rice cultivars [9]. With 44.6 million hectares of land dedicated to rice cultivation, India leads the world in terms of area. Rice yields around 90 million tonnes and yields 2086 kg/ha. Assam has a large rice cultivar collection. Rice is grown on 25.3 million hectares and produces 3.8 million tonnes and 1540 kg/ha. (Source: Directorate of Agriculture, Govt of Assam, and Guwahati 2009, unpublished data).

5. AROMATIC RICE OF ASSAM

"Specialy Rice" refers to a variety of rice genotypes with specific purposes around the world [8]. Basmati rice, aromatic non-basmati rice, non-aromatic basmati rice, and non-aromatic special purpose rice are all examples of specialty rice [11]. Assam is known for its aromatic, glutinous, and semi-glutenous joha, bora, and chokuwa rice varieties. Joha rice appears to be the most valuable of the bunch. The aromatic rice of Assam is a unique class of rice under Sali rice, traditionally known as 'Joha'. North-eastern (NE) India has a distinct group of locally cultivated non-basmati aromatic rice germplasm and quality rice land races [12]. For example, joha, chakhao, and tai are locally adapted cultivars grown in the states of Assam, Manipur, and Mizoram, respectively. Assam Joha rice is well-known for its distinct flavour, fine grain, easy cooking, and great taste, all of which make it a desirable commodity in society [7]. The smell of Joha rice is a key attribute that contributes to its widespread fame [13]. Aromatic rice is a type of rice that is believed to be of the highest quality and sells for a significantly greater price than non-aromatic rice [14]. So, Joha rice occupies a remarkable position in the local markets compared to other classes of rice. However, because of its scent, it is vulnerable to disease and insect onslaughts. The genetic diversity of joha rice is in jeopardy due to increased yields and the commercial value of longer grain forms [15]. Due to their low production, farmers rarely plant these cultivars for commercial purposes. Nearly 5% of the state's 17 lakh acres of sali rice is *joha* [16]. These photoperiod reactive, tall conventional joha cultivars yield 1.0 t/ha. Also, *joha* rice is farmed in marginal lands. They have a similar aroma and quality, but have not been extensively explored [17]. Few trials have been conducted to economically develop high-yielding fragrant rice suitable for this region. Assam Joha rice types were clearly unique from other Assam rice varieties, as evidenced by their distinctive grouping in a DNA diversity analysis based on molecular markers by Bhuyan *et al*. [18]. This mandates the use of DNA markers to identify these *joha* rice cultivars [19].

6. JOHA RICE CULTIVAR OF ASSAM

In terms of aroma, longevity, grain size and shape, production ability, and other traits, Assam's aromatic rice, locally known as "joha," differs from other aromatic rices. After transplanting seedlings late in the season, Joha rice is produced in water adjacent fields, hilly lands, or on Sali seed beds. Joha occupies around 5% of the Sali rice land; with an average yield of 1–1.5 t/ha [7]. Talukdar *et al.* cultivated 54 joha varieties. But after clustering, they got the results for 42 varieties, which came under the aromatic joha category.

Table 1: Some of the collected germplasm maintained at Assam Agricultural University (Talukdar *et al.,* 2017, [17])

Sl. No.	Variety Name	Sl. No.	Variety Name
4	Arab Joha	25	Tulsi Joha
3	Badshabhog	24	Ronga Joha-2
2	Bengoli Joha	23	Kamini Joha
8	Bhaboli Joha	30	Konbogi Joha
1	Boga Joha	22	Kunkuni Joha-1
9	Boga Joha-1	31	Goalporia Joha-2
6	Boga Maniki Modhuri	27	Kon Joha-3
7	Bogi Joha	28	Kola Joha-2
5	Bokul Joha	26	Monipuri Joha-2
18	Jeera Joha	39	Chufon Joha
20	Keteki Joha	41	Bor Sal Joha
10	Kharika Joha	32	Joha
17	Kolajoha new	38	Goalporia Joha-1
12	Koli Joha-2	33	Koli Joha
19	Kon Joha	40	Cheniguti Joha
14	Kunkuni Joha-2	35	Kon Joha-1
15	Maniki Madhuri Joha	36	Ronga Joha-1
16	Monipuri Joha-1	37	Ronga Joha-1
21	NDR6330	42	Boga Tulsi Joha
13	Siali Joha	34	Joha Bora

7. SPECIFICATION AND DESCRIPTION OF JOHA RICE

Physicochemical properties of the grains, growth patterns, habitat areas, and the shape and cooked grain elongation pattern of this particular aromatic rice class set it apart from Basmati rice. The major grain characteristics of Joha rice genotypes are given below:

Table 2: Physiochemical properties of grains of joha [17]

Grain length	5.2 - 10.0 mm
Grain breadth	1.9- 3.0 mm
Ratio between grain length and grain breadth	2.3- 4.5 (Short bold to medium
Decorticated grain colour:	Black, white
1000 grain weight:	7-26 g
Cooked kernel elongation ratio:	1.0- 1.4
Total milled rice	65-75%
Head rice recovery %)	60-65%
Gelatinization temperature:	Intermediate
Chalkiness	10-20%
Amylose content	20- 24%
Total soluble sugar	0.412- 0.773%
Reducing sugar	0.121 -0.264%
Gel consistency	Soft
Concentration of 2- acetyl-l-pyrroline	0.4 - 4.85 ppm
Aroma intensity	Strong
Amylose content	19-24%

Table 3: Morphological characters of joha rice

Plant height	121- 174 cm

Number of grain bearing effective tiller per plant	8 - 13
Days from sowing to maturity	135— 165 days
Panicle length	24 — 33 cm
Flag leaf length	27 — 41 cm
Flag leaf width	0.8 — 1.3
Flag leaf area	30 -44 cm2
Number of grains per panicle	108 -303
Spikelet sterility percentage	18— 30%

Joha rice is a cross between Indica and Japonica. It belongs to the moderate slim or medium wide class with an L: B of less than 3. A grain's length can grow 1.4 times but ripen when cooked. In many ways, Joha is unlike Basmati rice (the other scented rice of India). Basmati has refined grains (greater than 6.5 mm in length), 1.8 times elongation, a rich scent, and a delicate texture. However, Joha grains are smaller (4.7 mm) and have less elongation (1.1 times). Even though they don't have a distinct aroma and aren't soft after cooking [7]. They deliberated on 37 indigenous Joha cultivars, using "Pusa Basmati" and "Kernel local" as check varieties. Some of the features they looked at were husking recovery, milling recovery, head rice recovery, as well as kernel length and breadth. High diversity in head rice recovery, kernel length, breadth, and L/B ratio, elongation, and alkali value. Each Joha cultivar had a different kernel length and different breath measurements, but the L/B ratio was always 3. The highest elongation percentage (110%) was recorded in Koli Joha, followed by Manipuri Joha (107%) and Ronga Joha (Ronga) [7]. Das *et al*. compared Joha rice to basmati and kasturi. They discovered joha had higher crude protein content than basmati and kasturi (9.17–11.67%). They found genetic variation and environmental influences in the protein, amylase, amylopectin, and mineral content of the grains. A higher concentration of albumin, globulin, and amylase was detected in indigenous Joha cultivars compared to Basmati and Kasturi. According to their research, Kola Joha, Badshabhog, and Keteki Joha have the strongest aromas. The main reason why fragrant rices are studied is their exquisite aroma, which is attributed to 2-acetyl-1-pyrroline. This molecule is more potent than other rice volatile components [20]. Ahmed *et al*. classified Joha cultivars based on scent strength and pyrroline content [21].

8. GENETICS OF AROMA IN AROMATIC RICE

Among the various grain quality parameters, aroma is the most instantly detectable sensory trait. This is why farmers have prioritized it over other traits for decades. The chewing test has been used to pick up aromas for a long time. Following the introduction of the KOH test, more complex tests were developed using gas chromatography mass spectrometry (GCMS) and other sensitive devices to accurately assess fragrance and identify the volatile components that cause it [22]. Sensory modalities and functional markers correlated with polymorphisms that define the fragrance genes can be used to accomplish accurate phenotyping and genotyping [23]. Aromatic hydrocarbons, aldehydes, phenols, alcohols, ketones, and esters are among the more than 500 volatile aromatic compounds

(VACs) found in cooked aromatic rice. 2 acetyl-1-pyrroline is the main VAC contributing to scent and is found in aerial regions of plants and deposits throughout seed maturity [24]. It was determined that a single recessive gene (fgr) was responsible for producing aroma in rice plants, which was connected to the RFLP clone RG28 on chromosome 8 (4.5cM genetic distance) [61], [62]. Kovach *et al.* identified the betaine aldehyde dehydrogenase gene (BADH2) linked with the aromatic phenotype through fine mapping and sequence [36]. Genetically, aroma in rice is investigated to be restricted by a major gene, *osBadh2* (also documented as *fgr/badh2/os2AP/osbadh2*, *LOC_Os08g0424500*), which is found as a homozygous recessive gene at chromosome 8 expressed under mutation of an eight-base-pair (8-bp) deletion ‘‘GATTAGGC’’ in exon 7 codes for betaine aldehyde hydrogenase (*osBadh2*) results in a premature stop codon, leading to the lack of gene's of activity and a buildup of 2- Acetyl-1- Pyrroline (2AP) in an allele at the fragrance locus in aromatic genotypes [7], [25], [26], [27], [28], [29]. Bradbury *et al*. also found 3 SNPs in the 7th exon of the Badh2 gene [25]. Using aromatic rice and non-fragrant rice as experimental materials, Chen *et al*. were able to position the aroma gene (*osBadh2)* at a distance of 252 KB between 20175367 bp and 20386172 bp on chromosome 8 [30]. Later analysis by Shan *et al*. revealed that Badh2 is 1509 bp long, with 15 exons and 14 introns [31]. On the other hand, distinct aromatic rice types have 7 bp deletions in exon 2 of the Badh2 gene, as well as an 803 bp loss between exon 4 and exon 5 of the Badh2 gene [29], [32]. Furthermore, the Badh2 gene's 5'UTR region and promoter region feature insertion/deletion/single nucleotide mutation sites [32]. Thus, the Badh2 gene allelic variation affects rice grain fragrance. In addition to the Badh2 gene, two QTLs were discovered on the 3rd and 4th chromosomes that affect rice aromatic traits [33]. The Badh1 gene is homologous to the Badh2 gene and may be associated with QTL on the 4th chromosome.

Several tasty and healthy rice cultivars from South and Southeast Asia lack the 8-bp deletion, yet 2AP was identified in both raw and cooked rice. Fitzgerald *et al*. claimed that another mutation causes 2AP accumulation and not the fgr 8-bp deletion [28]. Most uniform fgr genotypes had 2AP levels similar to aromatic nfgr genotypes, while certain South Asian genotypes had high levels. Chakraborty *et al*. studied whether any locus other than osBadh2 controls scent in 84 indica rice landraces [34]. The *osBadh2* deletion was detected in practically all landraces. But eleven aromatic genotypes, including wild progenitors, lacked the functional characteristic allele. This revealed a second gene, or allele, controlling fragrant rice. Not all aroma-responsible genes have been identified [35], [36], [37].

Rice aroma eminence is also heavily influenced by the farming process as well as environmental factors such as temperature, soil type, abiotic stress, water, CO2, light, salinity, and shading [38], [39]. According to a study, the basmati aromatic rice variety becomes more aromatic and expresses a super aroma while cultivating in the Punjab of India and Pakistan, but is found to be less aromatic in other regions or countries [6]. The grain articulates a super fine aroma when cultivation is done with humidity of about 70%–80% and a relatively cool temperature in the afternoon (25 ºC–32 ºC) and night (20 ºC–25 ºC) at the primal and grain filling stages [40]. The jasmine rice (KDML105, a popular aromatic rice variety) is found to be higher in aroma while growing in various dry land areas in the north and north-eastern parts of Thailand than in other countries [41]. Some studies have concluded that the aromatic rice varieties are typically prone to numerous diseases and pests, abiotic stress, and extremely effective against photoperiodism [42], [43], [44]. The internal mechanism of divergence in aroma development at the molecular level in different environmental conditions has not been studied and elucidated completely. They are used as morphological markers to tell rice landraces apart because changes in the environment have the least effect on the quality of the rice.

9. GENOMIC STUDY IN JOHA RICE FOR AROMA AND YIELD

The study of genetic diversity is an important factor for breeders to explore variability. Rice taxonomists prefer to do rapid classification of different taxonomic groups, while breeders are interested in finding important agronomic variation in breeding programmes [46]. In addition to the two main subspecies, Indica and Japonica, five genetically characterised groupings, indica, aus, aromatic, temperate japonica, and tropical japonica, have been discovered with genetic markers [35], [47], [48], [49], [50]. The genetic structure of O. sativa cultivars around the world has been examined a few times, but few studies have been associated with a specific region [47], [51]. An abundance of genetic differences among the native rice cultivars of northeastern India has been discovered by previous studies based on morphology and other agronomic variables [52], [53], [54]; as well as DNA profiling (isozyme, RAPD, ISSR) [18], [55], [56]. The foothills of the Himalayas, which encompass Uttar Pradesh, Bihar, and Nepal's Tarai area, are the hub of scented rice varieties in India [57]. Aromatic rice germplasm spread from this centre of diversity to other Indian states and neighbouring nations, where it was suited to local conditions. Previous research examined the genomic structure of Indian aromatic rice [58], [59], [60]. However, these studies place less emphasis on NE state fragrant rice accessions. Molecular markers with high polymorphism can be used to map genes/QTLs used in molecular breeding for aroma and yield improvement of Joha rice [17]. It smells stronger than other rice volatiles [20]. AAU investigated the inheritance of morphological traits. Some studies also claim that the aroma is caused by duplicate factors. Roy *et al.* classified the 107 numbers of aromatic rice of the NE region into three genetically distinct population clusters: P1, joha rice accessions from Assam, P2, tai rice from Mizoram and Sikkim; P3, chakhao rice germplasm from Manipur; and, aromatic rice accessions from Nagaland. Pair-wise FST was found between three groups and ranged from 0.223 (P1 vs P2) to 0.453 (P2 vs P3). A total of 322 alleles were amplified through 40 simple sequence repeat (SSR) markers with an average of 8.03 alleles per locus, with an average gene diversity of 0.67 revealed to understand the diversification of aroma and quality of rice germplasm for exploiting the genetic diversity for rice improvement in N-E India [12]. Talukdar and co-workers screened 155 simple sequence repeat (SSR) markers for genome wide distribution in rice based on the available map information [15], [63]. They found 136 primers were highly polymorphic and spread across 12 chromosomes. They selected seven aromatic primers linked to the *BAD2* locus for studying the genetic variability. A total of 143 simple sequence repeat markers were analysed to study genetic variations among the 40 Joha and 14 non-Joha rice genotypes. The polymorphism information content of these markers ranged from 0.17 to 0.88 and found an average of 3.7 alleles. They found three distinct groups among 54 rice accessions through the model-based population assignment and dendrogram analysis, leading to a clear distinction of Joha accessions from the Basmati accession. They detected ten characters through trait association of 29 probable markers with a P value of 0.05. This result in high diversity and a strong population structure in Joha rice. The study of QTLs for yield and grain quality can be further utilised for excellent mapping and validation of specific genes to develop DNA-based molecular markers in rice breeding and better allele mining in Joha rice. By crossing Ranjit and Kola Joha, Talukdar *et al*. discovered quantitative trait loci (QTLs) that contribute to 12 phenotypic features in rice [17]. They employed 102 SSR markers as well as a few fragrance related markers in their study, creating a 1387.9cM linkage map and discovering 24 QTLs, two of which were for grain aroma on chromosome 5 and chromosome 8, respectively. The investigation also revealed that the QTLs of Aro1-BAD2 were detected in an analogous place to the aroma gene of Basmati rice between the two QTLs. The majority of QTLs in this analysis show a variety of partial to over-dominance effects, suggesting that the characteristics are complex. These QTL-linked markers might be exploited in future marker-assisted breeding research. Saikia *et al*. investigated the genetic link between fifteen fragrant Joha rice landraces unique to Assam's Upper Brahmaputra Valley and found a total of 110 polymorphic alleles across all landraces using 34 markers, with an average of 3.25 per locus [64]. For each marker, the Polymorphic Information Content (PIC) varied from 0.24 to 0.83, with an average of 0.5. Another study was done by taking 20 Assam indigenous Joha (aromatic) rice cultivars morphologically, biochemically, and molecularly and finding the aromas of Kalijeera, Kunkuni Joha, Kon Joha-5, Manimuni

Joha, and Kon Joha-2 were powerful [65]. Across the 66 polymorphic SSR markers, PCR amplified 174 alleles with a mean value of 2.64, and the average PIC value was 0.326, with values ranging from 0.091 to 0.698. Applying UNJ clustering depending on Jaccard's coefficients, the 20 cultivars were classified into three groups, each with eight, ten, and two entries. Chetia *et al*., (2019) used SNP analysis to investigate the activity of the Badh2 gene in 39 Joha accessions and discovered a link between Badh2 gene fragrances in the majority of the Joha germplasm [66]. Furthermore, the aroma of some aromatic Joha germplasms isn't linked to the Badh2 gene, which could be because of other factors [65], [66].

10. CONCLUSION

Genetic variation can be helpful in establishing a stable and biologically potential genotype. Therefore, new potential disease resistant Joha rice varieties need to be developed to satisfy the demand at both the domestic and global level. Organic farming has now been proven to improve the quality of fragrant rice on a global scale. Along with quality, increasing grain output per unit area is critical to meeting the growing population's food production requirement. The study of the wide genetic diversity of Joha rice varieties can be further utilised for future breeding programmes. The use of association mapping can be helpful for identifying the molecular marker for stacking the QTL responsible for aroma and agronomic traits for varietal development. Novel markers can be used for future validation. Interspecific crosses with high yielding varieties have a chance to give a desirable variety with high yield and aroma. Precise genomic and proteomic tools can be used to analyse the biochemical and qualitative characteristics of joha rice. Proper preservation of Joha rice’s germplasm is essential for future breeding programmes. Through the use of bioinformatics tools and sequenced data information, speed breeding can be possible. By focusing more attention on the research area of joha rice, it can be beneficial.

COMPETING INTERESTS

The authors declare no conflict of interest.

AUTHORS’ CONTRIBUTIONS

All Authors are equally contributed for the preparation of the manuscript. All authors read and approved the final manuscript.

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