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Assessment of genetic diversity and DNA fingerprinting of aromatic rice varieties released by National Rice Research Institute (NRRI)

Pritesh S Roy*, Baisakhi Barada, SSC Patnaik, GJN Rao and Ashok Patnaik
Corresponding author: priteshroy1@gmail.com
Division of Crop Improvement, National Rice research Institute, Cuttack-753006, Odisha, India

Abstract: *Eastern India is one of the major producers and consumers of aromatic rice. Despite low yields, these aromatic rices are highly preferred by farmers and consumers due to their pleasant aroma and excellent cooking and eating quality. Over years, several new aromatic varieties have been released by different research organizations and private sector. The National Rice Research Institute, Cuttack has also been working on aromatic rice improvement and developed several breeding lines using different breeding approaches and released seven elite aromatic rice varieties. Understanding the variability and interrelationships among the released varieties will be of great help to us in planning the future aromatic rice improvement programs. The present study is an assessment of the diversity present in these aromatic rice varieties using microsatellite markers. The markers recorded high level of polymorphism with an average genetic diversity value (He) of 0.564. Also, these markers showed high discriminating power with an average polymorphism information content (PIC) of 0.734. Further, Ketekijoha and CR SugandhDhan 907 had lowest pair-wise genetic distance (0.425), whereas, Geetanjali and Nua Kalajeera were having highest genetic distance of 1.600. The Un-weighted Pair Group Method with Arithmetic Mean (UPGMA) dendrogram could group the purelines, mutants and varieties into separate groups. Besides, DNA fingerprints of these seven varieties were generated will be of great help in protecting the IPR of the varieties.*
Keywords: *Rice, SSR, marker, diversity, fingerprinting*

I. Introduction

Rice (*Oryza sativa* L.) is the primary food source for more than half of the world population and most of the Asian countries significantly rely on rice. In India, besides many rice varieties, aromatic rices are highly preferred in different occasions for their grain quality features and pleasant aroma. Both Basmati and non-basmati indigenous aromatic rices hold high market value at International and domestic markets, respectively (Ahuja, et al. 1995; Bhattacharjee, et al. 2002; Khush and Dela Cruz, 1998; Kumar, et al. 1996; Nene, 1998; Singh, et al. 2000). North-Eastern India, especially is the Basmati belt, whereas, other parts of the country grow and consume native short grain aromatic rices (Malik, et al. 1994; Shobha Rani and Singh, 2003). Odisha, an eastern Indian state, also holds tremendous significance for cultivation and consumption of these native aromatic rices and almost all districts of Odisha has its own set of aromatic rices according to their preferences and ethnic values (Das, 2012). Generally these native aromatic rices are long duration types and due to their tall, lodging prone stature and fewer number of panicles the yields are low. The National Rice Research Institute (NRRI) (former Central Rice Research Institute), established in Cuttack (Odisha) during 1946 is one of the pioneer Institute famous for its research work on rice. However, keeping in view of the importance of aromatic rices in the country NRRI has significantly contributed to aromatic rice improvement. Till date, seven high yielding aromatic rice varieties, developed through different breeding techniques, have already been released by NRRI, with a view to provide higher economic returns to the traditional, resource poor farmers of eastern India. In the present scenario of developing new high yielding aromatic rice genotypes, it is essential to understand the genetic base and relationship of released varieties before planning the strategy for the improvement of aromatic rices in future. The introduction of PCR technology and the abundant availability of molecular markers makes it possible to study the genetic variability present in these rice varieties at molecular level. SSR markers being co-dominant in nature and easy reproducibility, SSR based characterisation of aromatic rice varieties released by NRRI could provide unique DNA profiles to each genotype and help in the Intellectual Property Rights (IPR) issues.

II. Material and Methods

Ten seeds of the seven aromatic rice varieties released by NRRI (Table 1) were grown in pots under sterile condition. DNA was extracted from young leaves of each variety using the modified protocol of Dellaporta, et al. (1983). The PCR assays were conducted with rice microsatellite markers that were selected

from each chromosome representing the 12 chromosomes of rice. Polymerase chain reaction (PCR) was performed in 10 µl reaction volume with 1 µl of 10X buffer containing $MgCl_2$ (Promega), 0.5 µl of 10 mM dNTP, 1 µl of each 5 µM of primer pairs, 5.2 µl of nanopure water, 0.3 µl of 5U/µl Taq DNA polymerase (Geneaid) and 1µl of DNA template. A reference blank was loaded in every gel to avoid any contamination in the assays. Amplification was carried out in a thermal cycler (Applied Biosystems) at 94^0C for 4 minutes followed by 35 cycles of 94^0C for 30 seconds, $55/57^0$C for 45 seconds and 72^0C for 1 minute, with a final extension of 72^0C for 7 minutes. The PCR amplification products were visualized in 2.5% of ethidium bromide stained agarose gel using Typhoon FLA 7000 fluorescent image analyzer (GE Healthcare Bio-Sciences AB, Uppasala, Sweden).

III. Statistical analysis

The sizes of the amplified fragments were determined using 100 bp DNA ladder (BR Biochem) as the size standard reference. Markers were scored for the presence (1) or absence (0) of the corresponding band among the genotypes. The polymorphism information content (PIC) for each of the SSR markers was calculated using the formula: $\mathbf{PICi} = 1 - \sum_{j=1}^{n} (Pij)^2$, where n is the number of marker alleles for marker i and P_{ij} is the frequency of the j^{th} allele of marker I (Anderson, et al. 1993). Genetic diversity parameters like *Na* (number of alleles), *He* (Nei's genetic diversity) (Nei, 1973), *I* (Shannon's information index) and Nei's unbiased pair wise genetic distance was evaluated using POPGENE v 1.32 (http://www.ualberta.ca/fyeh). The neighbour-joining dendrogram was constructed based on unbiased pair wise genetic distances by MEGA 6 to establish the relationship among genotypes (Tamura, et al. 2013).

IV. Results and discussion

Of 36 tested marker (3 per chromosome), 29 could detect polymorphism in the genotypes (Table 2). The 29 tested loci cumulatively could detect 96.5% polymorphism in the studied rice varieties. Polymorphism pattern of some of the loci discriminating the studied genotypes is given in Fig 1. The *PIC* value in this study ranged from 0.296 (RM463) to 0.934 (RM444) with a mean of 0.734 (Table 2). Polymorphism information content (*PIC*) is the measure which reflects the discriminating power of the markers. The PIC value detected in or study is higher than the earlier reports of Agrawal, et al. (2002), Joshi and Behera, (2006) and Siwach, et al. (2004). Of 29 markers, 21 showed a high *PIC* value of more than 0.7. All the 29 markers could amplify a total of 84 alleles in the 7 of the genotypes studied with a mean of 2.897 alleles per marker, which is higher than the reports of Sivaranjani, et al. (2010) who could detect 61 alleles in 46 aromatic cultivars with 26 SSR markers. A highest of 5 alleles were detected for the marker RM8060 followed by 4 alleles each for RM10864, RM6378, RM480, RM510, RM253 and RM444. RM495, RM71, RM423, RM422, RM3866, RM413, RM336, RM8020, RM447, RM206 and RM286 could amplify 3 alleles each. Similarly, RM580, RM207, RM81B, RM335, RM261, RM245, RM311, RM3472 and RM463 were detected with 2 alleles. The number of effective alleles (*Ne*) ranged between 1.000 for RM244 to 4.667 for RM8060. Wide genetic diversity was detected for all the loci except RM244. The genetic diversity value (*He*) ranged from 0.245 (RM335) to 0.786 (RM8060) with an average of 0.564. Nineteen of the markers could detect a diversity value of more than 0.5. The average *He* value of the present study is slightly lower than the earlier studies of Wei, et al. (2009) and Yuan, et al. (2007). This showed that these released aromatic rice varieties possesses moderate level of genetic diversity.

From the genetic distance analysis, it is observed that the pair-wise genetic distance ranged from 0.425 between Ketekijoha and CR Sugandh Dhan 907 to 1.600 between Geetanjali and Nua Kalajeera (Table 3). The average genetic distance in the study is very high at 0.933. An UPGMA dendrogram based on pair-wise genetic distance measures was constructed to understand the relationship pattern of the released aromatic rice varieties. The dendrogram grouped the 7 varieties in to two major clusters at 0.6 level (Fig 2). Five varieties i.e. Nua Kalajeera, Nua Dhusara, Nua Chinikamini, CR Sugandh Dhan 907 and Ketekijoha were grouped in cluster I, whereas, Poornabhog and Geetanjali were grouped in cluster II. The genotypes selected from native landraces through pureline approach were in close association with the varieties developed by hybridization where also native landraces were utilized. Further, at 0.4 level the 7 varieties were divided in to 3 different groups (A, B and C). Group A contained improved traditional land races developed by Pureline breeding, group B represented the varieties developed through hybridization process involving a non aromatic parent while group C contain Geetanjali and Poornabhog, the varieties having Basmati background. This observation suggests that the short grain aromatic rices are distinct from Basmati and help in designing future programs.

DNA fingerprinting is one of the widely adapted approaches for discrimination of rice genotypes based on the allelic variation at different loci. The DNA fingerprint of the 7 released aromatic rice varieties were generated based on the 84 detected alleles with 29 microsatellite markers (Fig 3). A highest of 38 alleles was detected in Nua Chinikamini whereas in Nua Dhusara, the number of alleles were minimum (32).

V. Conclusions

The present study demonstrates the efficient utilization of molecular markers for varietal discrimination and diagnostic purposes. The DNA fingerprints of the rice genotypes could provide uniqueness to individual variety and could be useful for molecular level identification of these released aromatic rice varieties and help in their description for Intellectual Property Rights (IPR) issues.

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Table 1. Details of released aromatic rice varieties used in this study

Variety	Development Method	Parentage	Year of release	Released states
Geetanjali	Mutation	Basmati 370	2005	Odisha
Ketekijoha	Pedigree	Badshahbhog/Savitri	2005	Odisha
Nua Dhusara	Pureline	Dhusara	2008	Odisha
Nua Kalajeera	Pureline	Kalajeera	2008	Odisha
NuaChinikamini	Pureline	Chinikamini	2010	Odisha
Poornabhog	Mutation	Poornabhog	2012	Odisha
CR SugandhDhan 907	Pedigree	Pusa 44/Dubraj	2013	Odisha, Chhattisgarh, A. Pradesh and Gujarat

Table 2. SSR markers used in the present study and their genetic diversity parameters

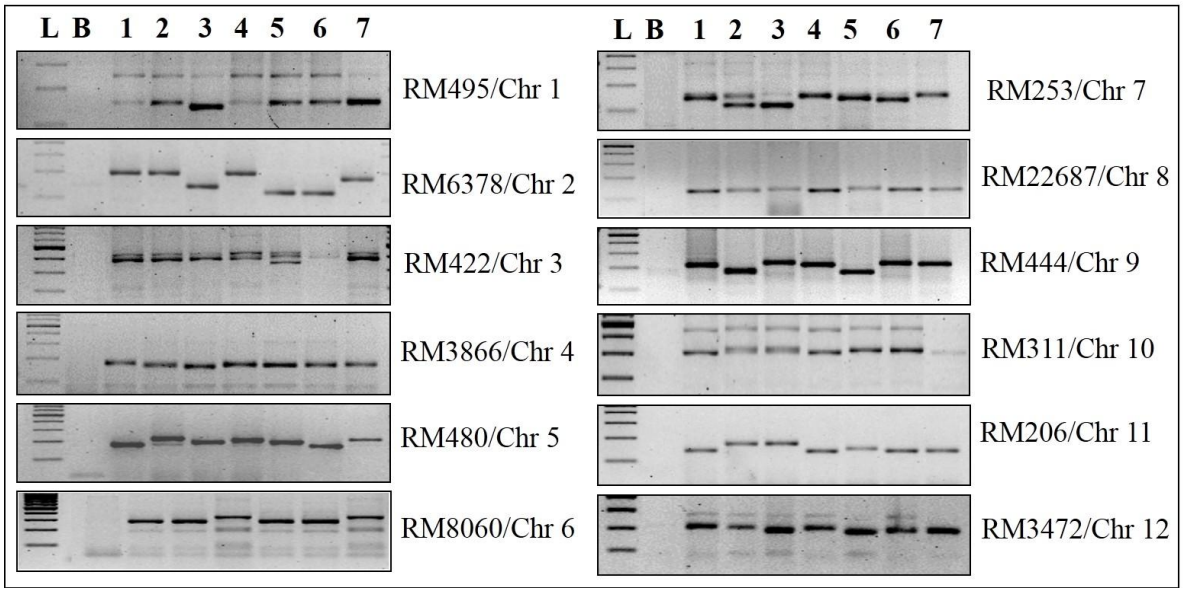
Marker	**Chr.**	**Repeat Motif**	**Annealing temp.**	***Na***	***PIC***	***Ne***	***He***
RM495	1	(CTG)7	55	3	0.415	2.279	0.561
RM580	1	(CTT)19	55	2	0.745	1.960	0.490
RM10864	1	(GT)27	57	4	0.801	3.267	0.694
RM71	1	(ATT)10T(ATT)4	55	3	0.558	2.800	0.643
RM207	2	(CT)25	55	2	0.704	1.690	0.408
RM6378	2	(GAA)19	55	4	0.923	3.267	0.694
RM423	2	(TTC)9	55	3	0.531	2.333	0.571
RM422	3	(AG)30	55	3	0.415	2.279	0.561
RM81B	3	(TCT)10	55	2	0.745	1.960	0.490
RM3866	4	(GA)29	55	3	0.857	2.333	0.571
RM335	4	(CTT)25	55	2	0.622	1.324	0.245
RM261	4	C9(CT)8	55	2	0.745	1.960	0.490
RM480	5	(AC)30	55	4	0.908	3.379	0.704
RM413	5	(AG)11	55	3	0.871	2.579	0.612
RM8060	6	(AT)27	55	5	0.878	4.667	0.786
RM510	6	(AG)11	55	4	0.643	2.800	0.643
RM336	7	(CTT)18	55	3	0.823	2.279	0.561
RM253	7	(GA)25	55	4	0.908	3.630	0.725
RM8020	8	(TA)20(GA)19	55	3	0.857	2.333	0.571
RM447	8	(CTT)8	55	3	0.857	2.333	0.571
RM22687	8	(AT)34	55	3	0.857	2.333	0.571
RM444	9	(AT)12	55	4	0.934	3.769	0.735
RM245	9	(CT)14	55	2	0.704	1.690	0.408
RM244	10	(CT)4(CG)3C(CT)6	55	1	0.490	1.000	0.000
RM311	10	(GT)3(GTAT)8(GT)5	55	2	0.745	1.960	0.490
RM206	11	(CT)21	55	3	0.857	2.333	0.571
RM287	11	(GA)21	55	3	0.857	2.333	0.571
RM3472	12	(CT)21	55	2	0.745	1.960	0.490
RM463	12	(TTAT)5	55	2	0.296	1.690	0.408
Mean	-	-	-	**2.897**	**0.734**	**2.432**	**0.546**
St. Dev.	-	-	-	**0.900**	**-**	**0.779**	**0.156**

Na: Number of alleles; *PIC*: Polymorphism information content; *Ne*: Number of effective alleles; *He*: Nei's genetic diversity

Table 3. Nei's pair-wise genetic distance among 7 released aromatic rice varieties

Geotypes	**1**	**2**	**3**	**4**	**5**	**6**	**7**
NuaKalajeera	****						
NuaChinikamini	0.607	****					
CR Sugandh Dhan 907	0.674	0.854	****				
NuaDhusara	0.627	0.635	0.723	****			
Poornabhog	1.179	0.786	1.466	0.743	****		
Ketekijoha	0.723	1.253	0.425	0.713	1.479	****	
Geetanjali	1.600	1.004	1.386	0.851	0.753	1.118	****

Fig 1. Agarose gel showing polymorphic pattern of the SSR markers detected in 7 released aromatic rice varieties



L: 100bp ladder; B: Blank; 1: Nua Kalajeera; 2: Nua Chinikamini; 3: CR Sugandh Dhan 907; 4: Nua Dhusara; 5: Poornabhog; 6: Geetanjali; 7: Ketekijoha

Fig 2. UPGMA dendrogram showing genetic relationship among the 7 released aromatic rice varieties

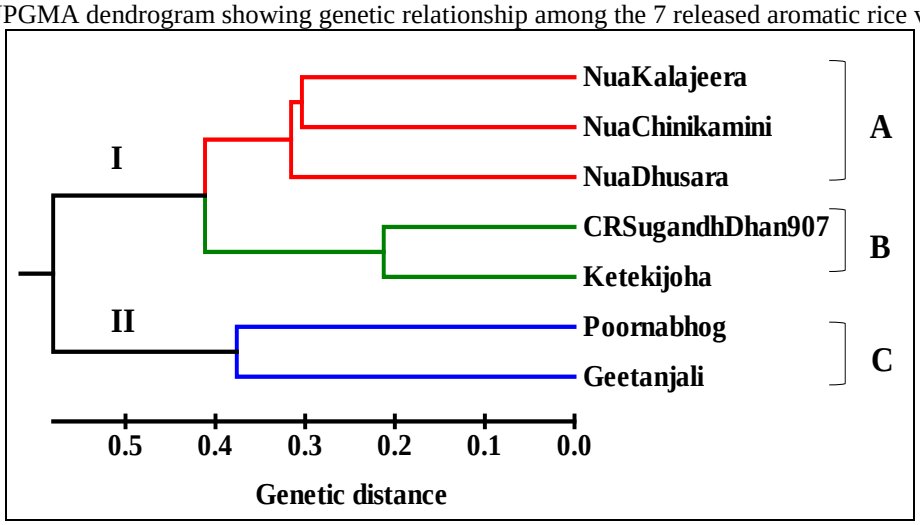


Fig 3. DNA fingerprints of 7 released aromatic rice varieties generated based on 29 polymorphic markers