Research article

Assessing the Genetic Variation of Swarna rice (*Oryza sativa* L.) Cultivars using SSR marker

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Abstract

Swarna rice cultivars (Indian cultivar) are widely grown in the northern part of Bangladesh because of their higher yield, drought tolerance, higher quality of grain and good plant appearance. Therefore, a study was conducted to observe genetic diversity among eight Swarna cultivars grown in Bangladesh. The genetic diversity of the cultivars was assessed by one hundred SSR (simple sequence repeat) markers. Among these, forty-four primers were polymorphic. In total, 188 alleles from eight rice genotypes with an average of 4.27 alleles per locus were identified. Allele numbers varied from 2 to 11. The average polymorphism information content (PIC) was 0.533, with a range of 0.195 to 0.889. The most suitable markers found were RM149, RM232, and RM3421, which provided the highest PIC value of 0.889. Genetic dissimilarity coefficient values between pairs indicated that the Guti Swarna and Nepali Swarna cultivars along with the Guti Swarna and Deshi Guti Swarna cultivars were the most genetically distant (0.818) cultivars whereas the Nepali Swarna and Nironjon Swarna cultivars were the closest (0.091). A dendrogram constructed based on polymorphism indicated five major groups. It was observed that the Guti Swarna and Sumon Swarna cultivars formed two different solitary groups, I and II whereas the Nepali Swarna and Nironjon Swarna formed group III, the Lal Swarna and Swarna5 constructed group IV, and the Deshi Guti Swarna and Tiger Indian Swarna were in group V. Our findings may help in designing appropriate cross combinations for further improvement of Swarna cultivars.

Keywords

diversity;

marker;

SSR;

Swarna;

rice

1. Introduction

Rice (*Oryza sativa* L.), a staple food for over half of the world's population [1], is the foundation of the food security in Bangladesh. This country ranks third position for both production and consumption of rice in the world [1]. In Bangladesh, there are three separate seasons for rice production: Aus (Kharif-I/Pre-Monsoon), Aman (Kharif-II/Monsoon), and Boro (Rabi/Dry). Aman

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rice is mainly rain-fed and highly dependent on monsoon precipitation, particularly at the beginning and the end of the monsoon [2]. Moreover, Aman season covers the highest rice growing areas [3]. Rice production reaches its maximum in the Boro season followed by Aman season where the average rice yield in Aman season has been estimated 4.13 tons per hectare for the financial year 2022-2023 [3]. Several domestic varieties and local cultivars are grown mainly in Aman season. In the recent years, the area under cultivation of domestic varieties grown in the wet season has decreased from 78% in 2011 to 58% in 2019 [4]. Despite growing seasonal indigenous varieties, farmers from the north-western area of Bangladesh also grow a unique kind of Indian rice named "Swarna". It was initially introduced into the Rangpur district in 2003 for the rainy season and soon gained popularity [4]. Farmers prefer Swarna cultivar more than domestic varieties due to its higher yield, drought tolerance, superior grain quality, good milling out turn, resistance to disease and pests, and higher price [4-6]. Farmers have adopted several kinds of this cultivar, including Guti Swarna (14.88%), Ronjit Swarna (1.32%), and other Indian Swarna (3.32%) of the total Aman rice growing areas [7]. Although the Bangladesh Rice Research Institute (BRRI) has released three varieties of Swarna rice [8], the development of better rice varieties with multiple desirable traits like 'Swarna' is needed to meet the farmer demand. To facilitate this process, genetic diversity analysis of the current cultivars is necessary.

Any crop improvement program needs stock genetic diversity because it facilitates the analysis and establishment of segregating progenies with high genetic variability through diverse parental combinations, and the potential for recombination to further select and introduce desirable genes from these diverse accessions [9, 10]. According to one estimation, only 15% of the available diversity has been used, meaning the majority of significant allelic variations which may contain desirable traits, remain unutilized [7]. Hybridization and subsequent selection are two major steps in rice breeding. The choice of suitable distant parents is an essential step in gaining genetic advantage, and is the first step in a hybridization program. Breeders can select effective tactics by using their grasp of diversity patterns [6].

Genetic diversity is mostly evaluated using the morphological variations of major quantitative traits. However, there are several drawbacks to this approach, in terms of labor cost, time, and space. In addition, this technique is unable to precisely quantify the amount of genetic diversity due to additive gene effect on the expression of the phenotypes (economically useful traits), which causes environmental influences to conceal their real phenotypic performance [11, 12]. It is tempting to select morphological traits since phenotypic expression is influenced by the environment [13, 14]. However, molecular markers help to recognize an organism more precisely and such markers have become a popular tool for accessing genetic diversity [15]. Among the PCR-based markers, simple sequence repeats (SSRs) are the most abundant markers [16]. They are very much powerful tools for assessment of genetic variation and the relationships among and within different species [17, 18]. SSR markers have been widely used in genetic diversity research, gene mapping and molecular map building [18, 19], fingerprint construction [18, 20] and genetic purity testing [18, 21] due to their characteristics like quick detection, simplicity, providing rich polymorphism and stability. Moreover, SSRs demonstrate lower repetition per locus at higher polymorphism level [22]. The high level of polymorphism is caused by the presence of different amounts of repetitions in microsatellite areas, and is easily detectable by PCR [23]. SSR markers have been used by many researchers [24-27] for diversity analysis of rice.

Diversity research on rice types such as Basmati, non-Basmati [28], pigmented [26, 29], aromatic [27, 30, 31], deep water [32], local landraces [26], stress resistant varieties [33], potential genotypes [33], and many other has been published so far. However, no precise data on 'Swarna' rice has been made available, which indicates that study on such potential cultivars is inadequate and rather fragmentary. In the present study, eight Swarna cultivars were analyzed using SSR markers to detect the existing genetic variation among the cultivars. This was needed to identify potential diverse genotypes that can be used as parents in future rice breeding programs.

2. Materials and Methods

2.1 Plant materials

The seeds of eight Swarna rice cultivars were collected from seven different regions of Bangladesh (Table 1). The dormancy of the seeds was first broken by putting them at 50°C for 72 h in an oven. Then, the seeds were placed on a moistened paper in petri dish and allowed to germinate. After that, the germinated seeds were sown in earthen pots as fresh seedlings.

Table 1. Name, collection point and origin of eight Swarna cultivars

Sl No.	Name of the Cultivar	Collection Point	Year	Origin
01	Guti Swarna	Rangpur, Bangladesh	2019	India
02	Lal Swarna	Dinajpur, Bangladesh		
03	Swarna 5	Rangpur, Bangladesh		
04	Sumon Swarna	Thakurgaon, Bangladesh		
05	Deshi guti Swarna	Panchagarh, Bangladesh		
06	Nironjon Swarna	Rajshahi, Bangladesh		
07	Nepali Swarna	Kurigram, Bangladesh		
08	Tiger Indian Swarna	Nilphamari, Bangladesh		

2.2 DNA extraction

DNA was extracted from fresh 21-days-old-seedling leaf tissue using a specific procedure as suggested by Ferdous *et al*. [34]. Briefly, 800 µL of extraction buffer was added to leaf tissue and the mixture was ground with a mortar and pestle. The obtained liquid was dispensed into a 2 mL Eppendorf tube. After that, 400 µL 2x CTAB solution and 400 µL CIP (chloroform: isoamyl alcohol: phenol = 24:1:5) were added to the tube. At room temperature, the mixture was well vortexed and centrifuged at 8,400 g for 10 min. A new tube was used to collect the supernatant. After adding isopropanol in a ratio of two thirds, the Eppendorf tube was gently turned upside down to mix the content. Each tube was centrifuged at 8400 g for 5 min after incubating the tube at room temperature for 15 min. After the removal of the supernatant, DNA pellet left was cleaned with ethanol (70%). Each air-dried pallet was re-suspended in 10 µL TE buffer. DNA quality was confirmed using agarose gel electrophoresis and quantified with a spectrophotometer (Nano drop 1000 V3.6, USA).

2.3 Marker selection

Initial testing consisted of 117 markers [35], 44 of which showed polymorphism. Thus, these polymorphic markers were chosen (dispersed across the 12 chromosomes) for genetic diversity analysis. Detailed information of the 44 markers is given in Table 2.

Table 2. Polymorphic markers name, chromosome no., sequence and repeat motif

Marker	Chromosome No.	Primer Forward	Primer Reverse	Repeat Motif
RM243	1	GATCTGCAGACTGCAGTTGC	AGCTGCAACGATGTTGTCC	(CT)18
RM312	1	GTATGCATATTTGATAAGAG	AAGTCACCGAGTTTACCTTC	(ATTT)4(GT)9
RM5365	1	TCTGTTCGATGTTCCCATCG	TAAACTCAAACAGGCTGGGC	(TC)13
RM7075	1	TATGGACTGGAGCAAACCTC	GGCACAGCACCAATGTCTC	(ACAT)13
RM211	2	CCGATCTCATCAACCAACTG	CTTCACGAGGATCTCAAAGG	(TC)3A(TC)18
RM3421	2	AACTCTCCTAAACCTCCCCG	ACCACGTATGTATTGCACGC	(CT)18
RM482	2	TCTGAAAGCCTGACTCATCG	GTCAATTGCAGTGCCCTTTC	(AT)9
RM6	2	GTCCCCTCCACCCAATTC	TCGTCTACTGTTGGCTGCAC	(AG)16
RM7355	2	GAACCCACCCAAACAAACAC	GCTTCGGTTTTTCGTGAGAG	(CTAC)7
RM232	3	CCGGTATCCTTCGATATTGC	CCGACTTTTCCTCCTGACG	(CT)24
RM3867	3	TTGACTGGAACATCGAGCTC	ATCCCCTCTACACCGTACCC	(GA)30
RM489	3	ACTTGAGACGATCGGACACC	TCACCCATGGATGTTGTCAG	(ATA)8
RM565	3	AGTAACGAGCATAGCAGGCG	GCAAAGCCTTCAGGAATCAG	(GA)11
RM131	4	TCCTCCCTCCCTTCGCCCACTG	CGATGTTCGCCATGGCTGCTCC	(CT)9
RM261	4	CTACTTCTCCCCTTGTGTCG	TGTACCATCGCCAAATCTCC	C9(CT)8
RM335	4	GTACACACCCACATCGAGAAG	GCTCTATGCGAGTATCCATGG	(CTT)25
RM122	5	GAGTCGATGTAATGTCATCAGTGC	GAAGGAGGTATCGCTTTGTTGGAC	(GA)7A(GA)2A(GA)11
RM13	5	TCCAACATGGCAAGAGAGAG	GGTGGCATTCGATTCCAG	(GA)6-(GA)16
RM204	6	GTGACTGACTTGGTCATAGGG	GCTAGCCATGCTCTCGTACC	(CT)44
RM469	6	AGCTGAACAAGCCCTGAAAG	GACTTGGGCAGTGTGACATG	(AG)15
RM214	7	CTGATGATAGAAACCTCTTCTC	AAGAACAGCTGACTTCACAA	(CT)14
RM248	7	TCCTTGTGAAATCTGGTCCC	GTAGCCTAGCATGGTGCATG	(CT)25

Table 2. Polymorphic markers name, chromosome no., sequence and repeat motif (continued)

Marker	Chromosome No.	Primer Forward	Primer Reverse	Repeat Motif
RM3753	7	GAATGAGCTAAGAACACGCC	CTGATGGCCCAAGACTTTTG	(GA)17
RM149	8	AAGTTTTTTAATTGATACTT	GCGATGTGCAGTTGAACCT	(AT)10
RM407	8	GATTGAGGAGACGAGCCATC	CTTTTTCAGATCTGCGCTCC	(AG)13
RM5556	8	ATCTCCCTCCCTCTCCTCAC	TCCACACCTTCACAGTTGAC	(TG)15
RM6878	8	GAGCTCCTAAAGCTGCCTCC	CTCCTCTCTCTCTCACCCCC	(TGG)9
RM205	9	CTGGTTCTGTATGGGAGCAG	CTGGCCCTTCACGTTTCAGTG	(CT)25
RM219	9	CGTCGGATGATGTAAAGCCT	CATATCGGCATTCGCCTG	(CT)17
RM278	9	GTAGTGAGCCTAACAATAATC	TCAACTCAGCATCTCTGTCC	(GA)17
RM228	10	CTGGCCATTAGTCCTTGG	GCTTGCGGCTCTGCTTAC	(CA)6(GA)36
RM271	10	TCAGATCTACAATTCCATCC	TCGGTGAGACCTAGAGAGCC	(GA)15
RM474	10	AAGATGTACGGGTGGCATTC	TATGAGCTGGTGAGCAATGG	(AT)13
RM5608	10	GTATCTTTGATCGCGCGC	ACTGGTAGAGAGCCCTGCTG	(AAG)9
RM590	10	CATCTCCGCTCTCCATGC	GGAGTTGGGGTCTTGTTCG	(TCT)10
RM6142	10	TCTTCCTCACCTGCTTCTCC	TACAGAGGCTACTACCACGACG	(CGC)9
RM1233	11	TTCGTTTTCCTTGGTTAGTG	ATTGGCTCCTGAAGAAGG	(AG)15
RM206	11	CCCATGCGTTTAACTATTCT	CGTTCCATCGATCCGTATGG	(CT)21
RM330	11	CAATGAAGTGGATCTCGGAG	CATCAATCAGCGAAGGTCC	(CAT)5
RM7283	11	GGGGCATATAACGCAAACAC	ATTTTAGGAGGCTCACGTGG	(ATCT)13
RM260	12	ACTCCACTATGACCCAGAG	GAACAATCCCTTCTACGATCG	(CT)34
RM277	12	CGGTCAAATCATCACCTGAC	CAAGGCTTGCAAGGGAAG	(GA)11
RM309	12	GTAGATCACGCACCTTTCTGG	AGAAGGCCTCCGGTGAAG	(GT)13
RM5338	12	TGCACTCACCAGTTTTACCG	TGGCATGAGAGCTAGCACTG	(TC)12

2.4 PCR amplification

Polymerase chain reaction (PCR) was processed using a 'G-Storm GS1 Thermal Cycler' [36] PCR machine and PCR components with a quantity of 10 µL was used (Table 3). Conditions for the PCR are listed below (Table 3). However, the annealing temperatures were modified in accordance with the unique requirements of each primer combination. For manual genotyping, a vertical polyacrylamide gel (8% denatured polyacrylamide gel with 19:1 acrylamide: bhisacrylamide) was used to electrophorese PCR samples after they had been stained with the gel filling dye (bromophenol blue, xylene cyanol, and sucrose). In the gel, 4 µL of amplified compounds were run at approximately 90 volts and 500 mA of electricity in a 1 TBE buffer for 40 to 90 min (depending on the size of the allele). Using gel documentation equipment, the gels were documented after staining with 1 µg/mL ethidium bromide.

Table 3. Components of 10 µL reaction along with the criteria followed for PCR

Components of PCR		**Criteria followed for PCR**			
Name of the Component	**Amount (µL)**	**Stages**	**Temperature (°C)**	**Time**	**Cycle Completed**
DNA (10, ng)	3.0	Initial denaturation	94	5 min	-
dNTPs (10 mM)	0.2	Denaturation	94	30 s	35
Taq DNA polymerase (5U/µL)	0.2	Annealing	55	30 s	-
PCR buffer (10X)	2.0	Extension	72	30 s	-
Forward Primer (10 µM)	0.5	Final extension	72	7 min	-
Reverse Primer (10 µM)	0.5				
Water	3.6				

Allele size (in base pair) was calculated via Alpha-Ease FC 4.0 software (Alpha Infotech, USA). Version 3.25 'Power Marker' was used to determine the summary statistics including polymorphism information content (PIC) values, allele number per locus, frequency of major alleles, and gene diversity [37]. The PIC values were calculated using the formula by De Riek *et al*. [38]

$$\mathrm{PIC} = 1 - \sum_{i=1}^{K} \mathrm{P}_i^2$$

Where, P is the frequency of the present alleles.

The genetic similarity of eight cultivars was determined using the data matrix and Jaccard's similarity coefficients, and a dendrogram which show the relationship between the cultivars was created using the un-weighted pair group method with arithmetic mean (UPGMA).

3. Results and Discussion

The genetic diversity of the eight Swarna rice cultivars was evaluated in the current study. These cultivars were gathered from seven different areas of Bangladesh. One hundred and seventeen SSR markers were used in the evaluation, 44 of which were found to be polymorphic and therefore suitable for diversity analysis.

3.1 Band size and allelic diversity

From the 117 SSR markers tested, 44 (37.61%) showed clear and repeatable polymorphic bands and were chosen for analysis as shown in Table 4 and Figure 1. The largest amplicon size was formed by RM149 (333 bp) and the smallest by RM 5338 (73 bp). Band size range was highest for RM149 (275-333 bp) followed by RM204 (127-180 bp) and RM330 (180-235 bp), respectively (Table 4). The number of alleles indicates the richness of the population. A total of 188 alleles were revealed using 44 microsatellite markers for the cultivars tested (Table 4). Allele number per locus ranged from 2 (RM6, RM206, RM214, RM228, RM261, RM474, RM656, RM1233, RM3867, RM5338, RM5608, RM6142, RM7075, RM7283, RM7355) to 11 (RM149, RM 232 and RM 3421), and the average was 4 alleles across 44 loci. RM149, RM232 and RM3421 markers detected maximum number of alleles (11 alleles) which could be used for characterization of other cultivars. At each locus, the most prominent allele frequency ranged from 12% (RM149, RM232, RM3421 and RM5365) to 87% (RM228, RM261, RM1233, RM3867 and RM7283). On average, at any given locus, a common major allele was shared by 50.28% of the eight rice genotypes. Previously, Singh *et al.* [39] reported 112 alleles with an average of 3.11 alleles per locus were amplified while observing genetic diversity of 729 Indian rice varieties using 36 SSR markers. During the characterization of 140 Basmati rice varieties through SSR markers, Salgotra *et al.* [40] found 114 alleles with frequency ranging was from 5 to 2. Siwach *et al.* [28] reported 2-8 alleles and 4.58 alleles in average which was similar to the findings of Hossain *et al.* [30], Hossain *et al.* [31], Upadhay *et al.*[41] and Pervaiz *et al*. [42]. However, 31 alleles with an average of 2.6 were also reported by Wong *et al.* [43] in a diversity study using eight rice cultivars and 12 SSR primers which produced a distinctly lower number of alleles than our study. This difference might be due to the use of different genotypes and SSR markers.

3.2 Polymorphism of SSR markers

Values of polymorphic information content (PIC) reflect allelic diversity and frequency across genotypes. In this study, the values of PIC ranged from 0.19 to 0.89, with a mean of 0.53. This result was consistent with previously reported works by other researchers including Siwach *et al.* [28] (range from 0.0 to 0.78, mean= 0.62); Lu *et al.* [44] (range from 0.028 to 0.881, mean=0.463); Shakil *et al.* [45] (range from 0.37 to 0.87, mean=0.65) and Wong *et al.* [43] (range from 0.375 to 0.656, mean=0.52). The moderate mean PIC value (0.53) could have been attributable to the following factors: (1) only a handful of genotypes were well adapted to the local environment; (2) fewer differences in the contribution of the marker DNA regions; (3) a gene pool with a narrow genetic base; and (4) a lower mutation rate in the di/tri repeat [29, 46]. A review of the literature on the SSR primers employed in the current study revealed that RM206 had been used by many rice researchers. In this study, the PIC value of RM206 was 0.38, which was close to the findings of Islam *et al*. [26]. Again, moderate PIC values for RM 206 was also reported by Syed *et al*. [47]. Ashraf *et al.* [29] and Shah *et al.* [48] reported a PIC value for RM206 of 0.57. Matin *et al.* [32] reported 0.62 and Suvi *et al.* [49] reported 0.83 in other diversity studies. The values for RM204 (0.68) and RM205 (0.61) were close to the findings of Matin *et al.* [32] (0.62), but their value for RM211 (0.54) was considerably lower than our findings (0.81). The PIC value for RM335 was 0.37 which was similar to the findings of Rashmi *et al*. [24]. Again, Aljumaili *et al.* [27] reported a PIC value for RM 469 of 0.70, which was close to our findings (0.64). Since RM206 showed polymorphism in several study and genotype sets, it might be employed in subsequent diversity studies. A comparison of the PIC values reported by several researchers reveals that each SSR marker's ability to discriminate across genotypes changed with genotype variation. These changes could have resulted from differences in the DNA length between these primer binding sites and allele-specific DNA sequence

variations at the locations of primer binding. The highest PIC value (0.89) was attained for RM149, RM232 and RM3421 on chromosomes 8, 3 and 2 followed by RM5365 (0.87), RM590 (0.83) and RM211 (0.81) on chromosomes 1, 10 and 2, respectively. Higher PIC values compared to our study were also reported by Upadhay *et al*. [41] and Borba *et al*. [50]. Hence, it can be inferred that these markers were highly informative and capable of distinguishing among these cultivars. Moreover, markers with high PIC values can be useful for linkage analysis [31] . For better understanding and a clear view, the DNA profile of markers RM13 and RM7075 for all 8 cultivars are shown in Figure 1.

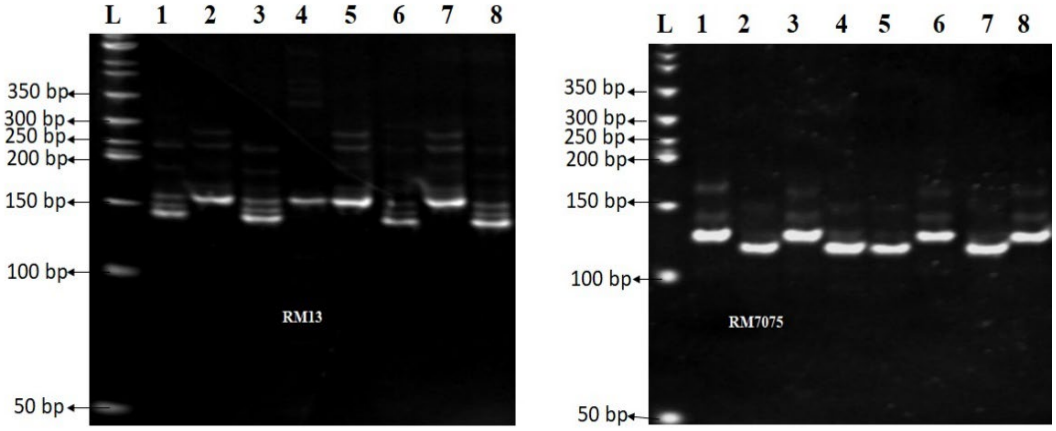

Figure 1. Allele separation of eight Swarna rice cultivars. PCR products were amplified with rice microsatellite primers RM13 (left) and RM7075 (right). Lane L: 50 bp ladder, lane 1: Guti Swarna, lane 2: Lal Swarna, lane 3: Swarna 5, lane 4: Sumon Swarna, lane 5: Deshi Guti Swarna, lane 6: Nironjon Swarna, lane 7: Nepali Swarna, lane 8: Tiger Indian Swarna

3.3 Dissimilarity co-efficient between cultivars

The extent of closeness between each pair of cultivars was determined using a dissimilarity coefficient matrix based on the fractions of shared SSR alleles. The dissimilarity values fluctuated from 0.818 to 0.091, respectively (Table 5). Two pairs (Guti Swarna and Nepali Swarna) and (Deshi Guti Swarna and Guti Swarna) produced highest co-efficient values of 0.818, indicating that they were genetically distant from each other. Crossing between these two pairs would involve a high possibility of producing heterozygous population. Breeders will therefore have more options to choose progeny with desired qualities like 'Swarna'. On the other hand, Nepali Swarna and Nironjon Swarna displayed the lowest value (0.091), which revealed that they were very close and may have arisen from same parent. Rashmi *et al.* [24] examined 65 rice genotypes using twenty polymorphic SSR markers, and found an average dissimilarity coefficient ranging from 0.77 to 0.51, with the highest and lowest coefficients being 0.94 and 0.19, respectively. Shakil *et al.* [45] found a highest dissimilarity coefficient was 0.85 and the lowest was 0.24 using 19 SSR markers to identify genetic variation among 26 promising genotypes of rice. Suvi *et al.* [49] used 24 SSR markers for diversity analysis of 54 rice genotypes, and the pair-wise estimations of similarity were between 0.04 and 0.68, with an average similarity of 0.39.

Table 4. The number of alleles, their sizes, major allele frequencies, and polymorphism information content (PIC) were identified using 44 microsatellite markers among eight rice cultivars.

Marker	Chromosome No.	Allele No.	Allele Size (bp)	Major Allele Size (bp)	Allele Frequency	PIC
RM312	1	5	105-119	109	0.313	0.717
RM5365	1	9	154-171	168	0.125	0.871
RM7075	1	2	119-126	126	0.500	0.375
RM243	1	4	116-135	116	0.375	0.630
RM6	2	2	152-156	156	0.625	0.359
RM7355	2	2	208-213	213	0.875	0.195
RM482	2	6	185-209	186	0.250	0.766
RM211	2	8	156-189	176	0.250	0.807
RM3421	2	11	152-167	162	0.125	0.889
RM3867	3	2	106-109	106	0.875	0.195
RM565	3	2	155-167	155	0.750	0.305
RM489	3	3	261-267	261	0.500	0.511
RM232	3	11	142-162	149	0.125	0.889
RM335	4	3	99-111	111	0.750	0.371
RM261	4	2	119-126	126	0.875	0.195
RM131	4	4	224-228	224	0.500	0.605
RM122	5	3	155-160	155	0.500	0.511
RM13	5	5	131-153	153	0.375	0.712
RM204	6	5	127-180	127	0.375	0.682
RM469	6	5	170-192	186	0.375	0.642
RM214	7	2	105-118	105	0.750	0.305
RM248	7	6	85-115	92	0.250	0.766
RM3753	7	5	153-166	161	0.375	0.712
RM5556	8	6	103-135	122	0.375	0.655
RM149	8	11	275-333	310	0.125	0.889
RM407	8	5	180-191	180	0.375	0.712
RM6878	8	4	100-104	103	0.375	0.630
RM219	9	4	105-226	207	0.625	0.524
RM278	9	4	149-154	154	0.500	0.605
RM205	9	4	115-122	120	0.500	0.605

Table 4. The number of alleles, their sizes, major allele frequencies, and polymorphism information content (PIC) were identified using 44 microsatellite markers among eight rice cultivars. (continued)

Marker	Chromosome No.	Allele No.	Allele Size (bp)	Major Allele Size (bp)	Allele Frequency	PIC
RM228	10	2	110-139	139	0.875	0.195
RM6142	10	2	86-95	95	0.625	0.359
RM5608	10	2	151-161	151	0.750	0.305
RM590	10	9	145-165	154	0.250	0.835
RM474	10	2	116-121	121	0.750	0.305
RM271	10	3	100-102	100	0.500	0.511
RM1233	11	2	167-175	175	0.875	0.195
RM206	11	2	107-128	128	0.500	0.375
RM7283	11	2	154-160	160	0.875	0.195
RM330	11	5	180-235	227	0.375	0.722
RM5338	12	2	73-76	76	0.500	0.375
RM277	12	3	120-131	131	0.500	0.456
RM260	12	3	96-117	96	0.625	0.468
RM309	12	4	164-183	164	0.438	0.530
Mean		**4**			**0.503**	**0.533**

Table 5. Microsatellite marker study results revealing the genetic distances by dissimilarity values between pairs of eight rice cultivars

Cultivars	Deshi Guti Swarna	Guti Swarna	Lal Swarna	Nepali Swarna	Nironjon Swarna	Sumon Swarna	Swarna5	Tiger Indian Swarna
Deshi Guti Swarna	0.000							
Guti Swarna	0.818	0.000						
Lal Swarna	0.489	0.750	0.000					
Nepali Swarna	0.500	0.818	0.705	0.000				
Nironjon Swarna	0.500	0.795	0.693	0.091	0.000			
Sumon Swarna	0.636	0.773	0.693	0.591	0.591	0.000		
Swarna5	0.409	0.807	0.295	0.682	0.670	0.545	0.000	
Tiger Indian Swarna	0.318	0.795	0.511	0.455	0.466	0.636	0.466	0.000

3.4 Cluster analysis

Cluster analysis was performed for grouping the cultivars and building a dendrogram. Applying this approach, genotypes were classified into five major genetic clusters by genetic similarity analysis using the UPGMA clustering method (Figure 2). It was observed that the Guti Swarna and Sumon Swarna cultivars formed separate solitary groups I and II. The Nepali Swarna and Nironjon Swarna were grouped as cluster III, the Lal Swarna and Swarna 5 were in group IV, the Deshi Guti Swarna and Tiger Indian Swarna belonged to group V. Six cluster were reported by Ashraf *et al.* [29] in the diversity analysis of 16 pigmented rice genotypes using 24 SSR markers. Suvi *et al.* [49] analyzed the diversity of 54 rice genotypes through 14 SSR markers, and three major clusters were found. Two major clusters with four sub-clusters were reported by Gaballah *et al.* [33] when assessing the genetic diversity of 15 rice genotypes with 10 SSR markers . According to dendogram, Nepali and Nironjon Swarna were found in same group, supporting the dissimilarity coefficient findings and further suggesting that they may arise from similar parents. However, the Guti Swarna and Deshi Guti Swarna and the Guti Swarna and Tiger Indian Swarna were the most distant cultivars. Again, first pair (Guti Swarna and Deshi Guti Swarna) was also supported by dissimilarity coefficient. Thus, this pair could be used as parents to develop 'Swarna"- like varieties.

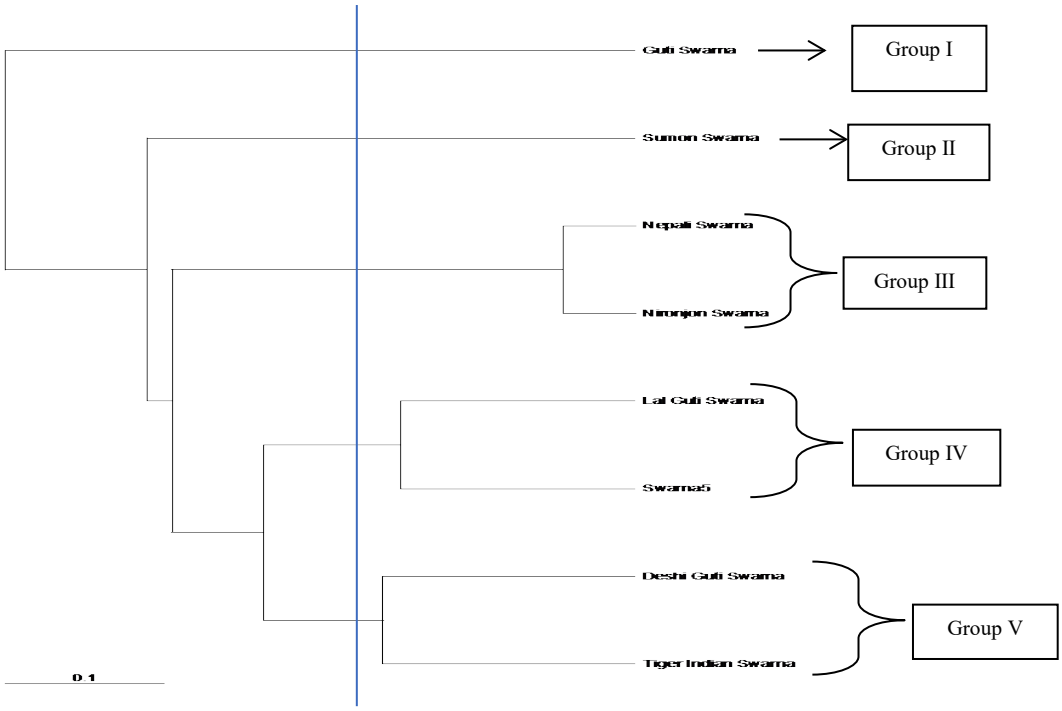

Figure 2. A UPGMA cluster dendrogram based on alleles discovered by 44 SSR markers demonstrating the genetic links between eight rice cultivars

4. Conclusions

This study provides precise information about the genetic diversity and relationship between these Swarna cultivars. Breeders will benefit from the knowledge of genetic variation by choosing appropriate parents for breeding programs as well as development of Swarna type rice varieties. Moreover, it has been demonstrated conclusively that SSR markers are effective tools for determining genetic diversity. Therefore, the PIC and allele related information of the microsatellite markers used could be helpful for similar studies.

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