International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2017; 5(4): 97-101

Received: 19-05-2017
Accepted: 20-06-2017

Lokesh Gour
Department of Plant Breeding & Genetics, J.N.K.V.V Krishi Nagar, Adhartal, Jabalpur Madhya Pradesh India

GK Koutu
Department of Plant Breeding & Genetics, J.N.K.V.V Krishi Nagar, Adhartal, Jabalpur Madhya Pradesh India

SK Singh
Department of Plant Breeding & Genetics, J.N.K.V.V Krishi Nagar, Adhartal, Jabalpur Madhya Pradesh India

DK Mishra
Department of Plant Breeding & Genetics, J.N.K.V.V Krishi Nagar, Adhartal, Jabalpur Madhya Pradesh India

Correspondence
Lokesh Gour
Department of Plant Breeding & Genetics, J.N.K.V.V Krishi Nagar, Adhartal, Jabalpur Madhya Pradesh India

Genetic potency identification of indigenous rice (*Oryza sativa* L.) lines using quality assessment and SSR marker analysis

Lokesh Gour, GK Koutu, SK Singh and DK Mishra

Abstract
To understand the genetic wealth of rice, a total of 83 indigenous rice germplasm were collected from rice core collection of Madhya Pradesh and screened for different yield and quality traits. Less difference between GCV and PCV was observed for the characters viz., grain length, decorticated grain length and amylose content. On the basis of trait differences in the materials for quality aspect, 19 rice lines were selected for molecular analysis. A total of 12 SSR markers were applied from which only nine markers were found polymorphic. The polymorphic markers RM276, RM256, RM259, RM234, RM142, RM7, RM201, RM42, and BADEX 7-5 were associated with amylose content, panicle length per plant, grain length, grain protein, grain elongation ratio, 1000 grain weight, drought tolerance, amylose and aroma content respectively. The average percentage of major allele frequency ranged between 42.11 % (RM256) to 100.00% (RM341). Based on dendrogram generated by neighbor-joining method implemented in Power Maker version 3.25 genotypes Karnal Basmati and Tarrori Basmati were highly diverse with other experimental genotypes. Marker RM234 amplified 2 unique alleles (190 bp) in Tarrori basmati and Karnal basmati. The unique alleles found in Tarrori basmati and Karnal basmati which were highly diverse genotypes would help to search new alleles for the concern traits and might be utilized in DNA finger printing of rice genotypes.

Keywords: SSR, polymorphic, quality, genetic diversity, indigenous rice, DNA fingerprinting

1. Introduction
Rice is a major source of food for more than half of the world population. In recent years due to rise in living standard, people started demanding quality rice with good eating and cooking quality attributes with varied preferences across different regions. Breeding efforts are more focused on improving the quality of rice to meet out the specific type of quality rice demand of consumers as well as potential market demand for various commercial uses. Grain quality can be considered as physical, chemical, cooking and nutritional quality groups (Verma *et al*. 2015) [52].

Furthermore, it is the main determinant of the eating and cooking quality (ECQ) of rice as amylose content (AC) are main parameter that significantly contribute to the ECQ (Fernando *et al*. 2015) [9]. AC is responsible for texture and appearance in rice hence; regulating AC in rice has been a major concern of rice breeders. To facilitate the development of new varieties with high cooking and eating qualities, it is necessary to understand the genetic bases of such traits (Sabouri H, 2009) [34]. Gelatinization temperature is used in varietal development as an indicator of the cooking time (Cuevas *et al*., 2010) [7]. It is an economically important indicator of quality, because selecting for varieties with shorter cooking time can lead to savings in fuel costs (Fitzgerald *et al*., 2009) [10].

Traditionally used morphological and physico-chemical parameter does not provide more precise techniques (Nagaraju *et al*., 2002) [27]. Therefore, foolproof instant safeguards are required to confirm the concern quality trait/traits. In parallel, reliability and precision of such discriminatory functions will apparently depend upon adequacy of molecular markers used and characters contributing towards estimates of genetic diversity. Molecular markers are a useful tool for assaying genetic variation, determining cultivar identity and have greatly enhanced the genetic analysis of crop plants and also an indispensable tool for characterizing genetic resources and providing breeders with more detailed information to assist in selecting parents. Among different classes of molecular markers, SSRs loci are most commonly used molecular markers, as they are relatively abundant, evenly distributed over the genome and are easily

assayed by PCR. These loci typically exhibit variation for allele frequency distribution within a species. SSRs have power to detect high level of allelic diversity and have been extensively used to identify genetic variation, analyze genetic structure among rice species and within the cultivated rice (Garris *et al*., 2005) [11]. Alternatively, it is needed to find out characteristics/determinants contributing to genetic diversity (Singh, 2010) [40] which is the basis of classification/clustering of genotypes.

So, the present study was undertaken with the objective to fingerprint/characterize varied of local rice germplasm& released varieties adapted to different agro-ecological systems by using SSR (Singh *et al*., 2000a, 2000b) [44, 45] and biochemical analysis to examine the results in order to work out efficient determinants for molecular marker and biochemical based classification

2. Materials and Methods

2.1 Evaluation of grain and quality

The material used in the present study comprised of 83 germplasm lines & released varieties received from rice core collection of Madhya Pradesh (India). For determination of the length breadth (L/B) ratio and decorticated L/B ratio, 10 grain length & breadth of whole grain and decorticated grain, 10 fully developed wholesome and randomly selected milled rice grain were measured. The grain elongation ratio (ER) was estimated as ratio of length of the cooked grain (CGL) to that of uncooked grain (GL).

Amylose content of each rice variety was estimated using the method described by Juliano (1971) [16]. For this analysis in 100 mg of rice flour 1 ml of 95% ethanol and 10 ml of 1.0 N NaOH was added and leaved for overnight. Samples were diluted to 100 ml with distilled water. From this suspension, 2.5 ml of the extract were taken and about 20 ml of distilled water and 3 drops of phenolphthalein were added. Then 0.1 N HCL added drop by drop until the pink colour just disappears. After this 1 ml of iodine reagent were added and the volume made up 50 ml. Theabsorbance was measured at 590 nm using spectrophotometer. For this programme 0.2, 0.4, 0.6, 0.8 and 1 ml of the standard amylose solution were made and used. Amylose was calculated by the following formula:

Absorbance corresponds to 2.5 ml of the test solution = x mg amylose

$$100 \text{ ml. contains} = \frac{x}{2.5} \times 100$$

2.3 Molecular markers assessment

The genomic DNA of 19 selected rice germplasm after selection on the basis of various quality assessments was isolated using CTAB method (Moller *et al*., 1992) [25]. A total of 12 SSR markers (Table No. 1) were utilized for molecular analysis. The Thermo Hybrid (*Px2*) PCR Machine was used to enhance the speed of sensitivity of detection of molecular markers. The 5'- 3' anchored SSR primers synthesized by IDT USA (Promega) were used. Each reaction mixture (20 ul) that used SSR primers for amplification consisted of 10x assay and approximately 25 ng of genomic DNA for SSR respectively.

The PCR amplification conditions for SSR analysis were applied as initial extended step of denaturation at 94 °C for 4 min, followed by 35 cycles of denaturation at 94 °C for 30 second, primer annealing at 55 °C for 30 second, elongation at 72 °C for 30 second followed by extension step at 72 °C for 5 minutes. PCR products were mixed with 5 µl of gel loading dye. The amplification products were electrophoresed on 1.5 % SFR agrose gel at 3 to 5 V/cm in 1x TAE buffer. Genomic DNA was quantified by UV absorbance at 260 nm using UV syngene gel documentation system.

2.4 Data analysis and detection of genetic diversity

Polymorphic products from SSR-PCR assays were calculated qualitatively for presence (1) or absence (0). The proportion of bands that have been shared between any of the two varieties averaged over loci SSR primers were used as the measure of similarity. The calculation was based on the number of bands per primer for SSR. Cluster pattern was based on distance matrices by using neighbor-joining method implemented in Power Maker version 3.25. The diversity or similarities between varieties were given in the form of dendrogram.

3. Results & Discussion

3.1 Evolutionary result of grain and quality

Table 1.1

Genetic Variability

Analysis of variance indicated that the mean sum of squares due to genotypes were significant for all the characters which revealed that there was considerable genetic variability amongst the material under study. The performance of the varieties was evaluated by the mean performance of the observed traits. It compares the varieties for specific characters. Maximum variability and highest mean was observed for amylose content and lowest for grain elongation ratio.

To get a clear picture of variability among the lines under study, coefficient of variation was calculated. Very less difference between GCV and PCV was observed for the characters viz., grain length, decorticated grain length and amylose content. Lingaiah *et al*. (2014) [22] was partly in agreement with these findings. High phenotypic and genotypic coefficients of variation were observed for amylose content and decorticated length breadth ratio. This was in agreement with the findings reported by Nachimuthu *et al*. 2015 [26]. High heritability was recorded for grain length and decorticated grain length (mm). The result was in agreement with that reported by Selvaraj *et al*. (2011) [35] and Jha *et al*. (2014) [14]. In this study very high genetic advance with high heritability recorded for amylose content and decorticated length breadth ratio (Table: 2).

3.2 Assessment of polymorphism from SSR profiles

In this study 12 SSR markers linked with different quality traits were chosen for the identification of genetic architecture of studied material. SSR markers even in less number can give a batter genetic diversity spectrum due their multi allelic and highly polymorphic nature. Several previous works have shown the power of such markers for differentiating individual germplasm accessions, particularly when they are closely related (Singh *et al*. 2004, Singh *et al* 2014) [43, 47]. In a total of 12 SSR markers, 9 polymorphic markers showed consistent banding patterns and were ultimately chosen for assessing genetic diversity. Four SSR markers RM259, RM234, RM142 and RM7 were associated with important quality traits i.e. grain length, grain protein, grain elongation and 1000 grain weight. Two markers RM276 and RM42 linked with amylose content while again three markers RM201, RM256 and BADEX 7-5 related with drought,

panicle number per plant and aroma content (Lang *et al*. 2004a, Lang *et al*. 2004b) [18, 19]. The markers RM223, RM236 and RM341 associated with aroma content, panicle number and culm length produced monomorphic allelic pattern in all the studied rice germplasm.

The total numbers of alleles amplified were 26 with a mean value of 2.17. The maximum number of alleles was 3 (Table: 3) amplified by marker RM256 (100,120 and 180 bp), RM259 (120,150 and 350 bp) and BADEX 7-5 (150,195 and 210 bp). Similar observations were also reported; 3.11 alleles per locus with SSR markers during characterization of 729 rice varieties (Singh *et al*. 2016) [42] and 3.02 alleles per locus in a set of 25 Indian rice hybrids characterization (Anand *et al*. 2012) [2].

Highest allele size produced by RM259 (350 bp) whereas lowest in RM276, RM256 (Table 3 & Fig: 2) and RM236 (100 bp). Average percentage of major allele frequency ranged between 42.11 % (RM256) to 100.00% (RM341). The mean of major allele frequency was 66.89 %. Genetic diversity values varied from 0.000 (RM 341) to 0.655 (RM256) with an average of 0.391. Similar findings ranged from 0.04 (HvSSR06-16) to 0.66 (HvSSR03-37) with an average of 0.33 reported by Singh *et al*. 2016. Gene diversity obtained in the present study was quite low as compared to 0.52 (Nachimuthu *et al*. 2015) [26] and 0.54 (Choudhary *et al*. 2013) [6] reported in rice germplasm lines and varieties, respectively.

The heterozygosity was moderate with an average of 0.386 and varied from 0.000 to 1.000. Highest heterozygosity was reported with marker RM 223 (1.000) (Table: 3). The low level of heterozygosity has also been reported in other studies on rice (Nachimuthu *et al*. 2015 and Singh *et al*. 2016) [26, 42] and this could be attributed to its self pollination behavior.

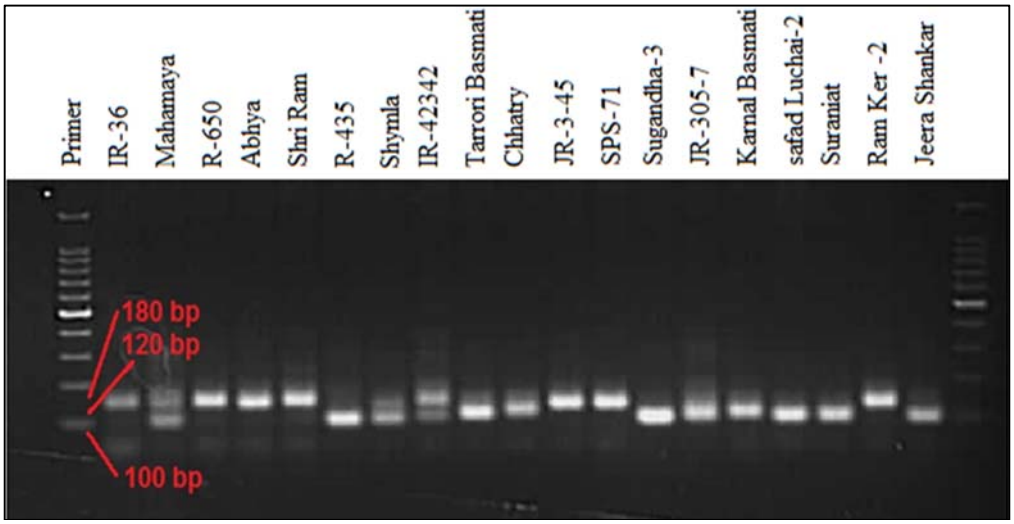

Fig 1: Allele profiles of a subset of 19 rice genotypes for SSR locus RM 256 on 2% agarose gel

3.3 Specific locus/marker based genetic diversity evaluation

The genetic diversity for a specific locus/marker can be evaluated by the Polymorphic Information Content (PIC) value. The overall PIC value ranged between 0.000 (RM341) to 0.582 (RM256) with a mean value of 0.324. PIC value ranged from 0.54 to 0.96 by Singh *et al*. (2011) [41], 0.74 to 0.92 by Shah *et al*. (2012) [36] whereas, mean value of PIC was 0.44 reported by Mahalingam *et al*. (2013) [23]. Shah *et al*. (2013) [37] and Pachauri *et al*. (2013) [30] were reported mean PIC values 0.37 and 0.38, respectively in different sets of rice varieties which were closer to our result. The highest PIC value was reported for marker RM256 (0.582) amplified with three alleles that was agreement with the findings of XiLan *et al*. 2010 [54] and Singh *et al*. 2016 [42].

3.4 Unique Alleles found from SSR on elite lines

Marker RM256, RM259 and BADEX 7-5 amplified highest number of allele (three), out of which no one amplified any unique allele (Table: 3). Marker RM201 amplified a total of three unique alleles in the genotypes Tarrori Basmati, Sugandha-3 and Karnal Basmati with the allele size of 170 bp each. RM234 amplified 2 unique alleles in Tarrori Basmati and Karnal Basmati (190 bp).

Table 1: List of twelve SSR markers with their sequences

Marker	Forward sequence	Reverse sequence	Character associated	Chromosome No
RM276	5'-CTCAACGTTGACACCTCGTG-3'	5'-TCCTCCATCGAGCAGTATCA-3'	Amylose content	6
RM256	5'-GACAGGGAGTGATTGAAGGC-3'	5'-GTTGATTTCGCCAAGGGC-3'	Panicle length	8
RM259	5'-TGGAGTTTGAGAGGAGGG-3'	5'-CTTGTTGCATGGTGCCATGT-3'	Grain length	1
RM223	5'-GAGTGAGCTTGGGCTGAAAC-3'	5'-GAAGGCAAGTCTTGGCACTG-3'	Aroma content	8
RM236	5'-GCGCTGGTGGAAAATGAG-3'	5'-GGCATCCCTCTTTGATTCCTC-3'	Panicle number per plan	11
RM234	5'-ACAGTATCCAAGGCCCTGG-3'	5'-CACGTGAGACAAAGACGGAG-3'	Grain protein	7
RM142	5'-CTCGCTATCGCCATCGCCATCG-3'	5'-TCGAGCCATCGCTGGATGGAGG-3'	Grain elongation	4
RM7	5'-TTCGCCATGAAGTCTCTCG-3'	5'-CCTCCCATCATTTCGTTGTT-3'	1000 grain weight	3
RM201	5'-CTCGTTTATTACCTACAGTACC-3'	5'-CTACCTCCTTTCTAGACCGATA-3'	Drought tolerance	9
RM341	5'-CAAGAAACCTCAATCCGAGC-3'	5'-CTCCTCCCGATCCCAATC-3'	Culm thickness	2
RM42	5'-ATCCTACCGCTGACCATGAG-3'	5'-TTTGGTCTACGTGGCGTACA-3'	Amylose content	6
ADEX7-	5'-TGTTTTCTGTTAGGTTGCATT-3'	5'-ATCCACAGAAATTTGGAAAC-3'	Aroma content	8

Table 2: Estimated parameters of genetic variability for quality traits in rice.

Traits	Mean	Range Mini.	Range Maxi.	GCV (%)	PCV (%)	h2 (Broad Sense) (%)	Genetic Advancement 5%	Gen. Adv as % of Mean 5%
GL	08.65	06.26	11.49	10.85	10.85	99.90	01.93	22.34
GW	02.61	01.80	03.34	13.37	13.76	94.40	00.70	26.76
LBR	03.39	02.30	05.20	19.55	19.80	97.50	01.35	39.76
DGL	06.68	03.07	09.04	12.94	12.95	99.90	01.78	26.65
DGW	02.19	01.22	03.07	16.70	16.86	98.10	00.75	34.07
DLBR	03.16	01.13	05.15	22.52	22.71	98.30	01.45	45.99
CGL	08.58	07.11	11.55	10.05	10.16	97.80	01.76	20.47
GER	01.30	01.04	02.33	12.33	12.42	98.60	00.33	25.22
AC %	14.59	05.62	25.87	31.68	31.71	99.80	09.51	65.21

Table 3:

S. No.	Marker	Major Allele Frquency	Gene Diversity	Heterozygosity	PIC	Number of allele	Polymorphic allele	Unique allele	Allele size (bp)	Polymorphic /Monomorphic
1.	RM276	0.9211	0.1454	0.0526	0.1349	2	2	*	100, 150	Polymorphic
2.	RM256	0.4211	0.6551	0.1579	0.5817	3	3	*	100,120,180	Polymorphic
3.	RM259	0.4474	0.6053	0.8947	0.5212	3	3	*	120,150,350	Polymorphic
4.	RM223	0.5000	0.5000	1.0000	0.3750	2	*	*	160,300	Monomorphic
5.	RM236	0.5263	0.4986	0.9474	0.3743	2	*	*	100,220	Monomorphic
6.	RM234	0.8947	0.1884	0.0000	0.1706	2	2	2	170,190	Polymorphic
7.	RM142	0.8684	0.2285	0.2632	0.2024	2	1	*	190,310	Polymorphic
8.	RM7	0.6316	0.4654	0.0000	0.3571	2	2	*	180,200	Polymorphic
9.	RM201	0.8421	0.2659	0.0000	0.2306	2	2	3	170,190	Polymorphic
10	RM341	1.0000	0.0000	0.0000	0.0000	1	*	*	200	Monomorphic
11	RM42	0.5263	0.4986	0.6316	0.3743	2	2	*	200,280	Polymorphic
12	BADEX7-5	0.4474	0.6385	0.6842	0.5635	3	3	*	150,195,210	Polymorphic
-	Mean	0.6689	0.3908	0.3860	0.3238	2.1667		5		

References

1 Anand D, Prabhu KV, Singh AK. Analysis of molecular diversity and fingerprinting of commercially grown Indian rice hybrids. J Plant Biochem Biotechnol. 2012; 21(2):173-79.

2 Choudhary B, Khan ML, Dayanandan S. Genetic structure and diversity of indegenous rice (*Oryza sativa*) varieties in the Eastern Himalyan region of Northeast India. Springer Plus. 2013: 2(1):228-38.

3 Cuevas RP, Daygon VD, Corpuz HM, Reinke RF, Waters DLE, Fitzgerald MA. Melting the secrets of gelatinization temperature in rice. Func. Plant Biol., 2010; 37:439-447.

4 Fernando HKDH, Kajenthini TJC, Rebeira SP, Bamunuarachchige TC, Wickramasinghe HAM. Validation of Molecular Markers for the Analysis of Genetic Diversity of Amylase Content and Gel Consistency among Representative Rice Varieties in Sri Lanka. Tropical Agricultural Research. 2015; 26(2):317-328.

5 Fitzgerald MA, McCouch SR, Hall RD. Not just a grain of rice: the quest for quality. Trends Plant Sci. 2009; 14(3):133-139.

6 Garris AJ, Tai TH, Coburn J, Kresovich S, McCouch S. Genetic structure and diversity in *Oryza sativa* L. Genetics, 2005; 169:1631-1638.

7 Jha VB, Sharma AK, Singh B. Genetic variability for different quantitative traits in early rice.Annals of Agri Bio Research, 2014; 19(1):25-28.

8 Juliano BO. A simplified assay for milled-rice amylose.J Cereal Sci.1971; 16:334-340.

9 Lang NT, Buu BC. a. Gen waxy (Wx) in grain quality by marker molecular. Agri. publish House. 2004; 9:1170-1172.

10 Lang NT, Buu BC. b. Fine mapping with microsattelite marker to detected aroma in rice. Agri. publish House. 2004; 192-199.

11 Lingaiah N, Venkanna V, Cheralu C, Chandra BS. Variability, heritability and genetic advance for yield and yield attributing traits in mid early genotypes of rice (*Oryza sativa* l.). International J. Advanced Research in Sci., Eng. Tech. 2014; 1(4):179-180.

12 Mahalingam A, Saraswathi R, Ramalingam J. Simple sequence repeat (SSR) markers for assessing genetic diversity among the parental lines of hybrid rice (*Oryza sativa* L.) African Journal of Biotechnology 2013; 12(33):5105-5116.

13 Moller EM, Bahnweg G, Sandermann H, Geiger HH. A simple and efficient protocol for isolation of high molecular weight DNA from filamentous fungi, fruit bodies and infected plant tissues. Nucleic Acids Res. 1992; 20(22):6115-6116.

14 Nachimuthu VV, Muthurajan R, Duraialaguraja S, Sivakami R, Pandian BA, Ponniah G. Analysis of population structure and genetic diversity in rice germplasm using SSR Markers: An initiative towards association mapping of agronomic traits in *Oryza sativa*. Rice. 2015; 8:30-54.

15 Nagaraju J, Kathirve lM, Kumar RR, Siddiq EA, Hasnain SE. Genetic analysis of traditional and evolved Basmati and non-Basmati rice varieties by using fluorescence-based ISSR-PCR and SSR markers. Proc. Natl. Acad. Sci. USA. 2002; 99:5836-5841.

16 Pacada IG, Coloma LP, Luciano VP, Rigor AT. Morphological evaluation and diversity analysis of restorer and maintainer line using SSR markers. Philippines. Journal of Crop Science. 36(Supplement no.1). 2011, 31-32.

17 Pachauri V, Taneja N, Vikram P, Singh NK, Singh S. Molecular and morphological characterisation of Indian

farmers rice varieties (*Oryza sativa* L.). AJCS. 2013; 7(7):923-32.
18 Rajendran N, Mukherjee L, Reddy KK, Shashidhar HE. DNA fingerprinting and estimation of genetic diversity among hybrid rice parental lines (*Oryza sativa* L.) using simple sequence repeats (SSR) markers. Journal of Plant Breeding and Crop Sciences. 2012; 4(11):169-174.
19 Sabouri H. QTL detection of rice grain quality traits by microsatellite markers using anindicarice (*Oryza sativa* L.) combination. Journal of Genetics, 2009; 88:1.
20 Selvaraj CI, Nagarajan P, Thiyagarajan K, Bharathi M, Rabindran R. Genetic parameters of variability, correlation and path coefficient studies for grain yield and other yield attributes among rice blast disease resistant genotypes of rice (*Oryza sativa* L.). African Journal of Biotechnology, 2011; 10(17):3322-3334.
21 Shah G, Sasidharan N, Chakraborty S, Trivedi R, Ravikiran R, Davla D. Genetic diversity and molecular analysis for fertility restorer genes in Rice (*Oryza sativa* L.) for wild abortive (WA) cytoplasm using microsatellite markers. Journal of Agricultural Technology 2001; 8(1):261-271.
22 Shah SM, Naveed SA, Muhammad A. Genetic diversity in basmati and non-basmati rice varieties based on microsatellite markers. Pakistan Journal of Botany, 2013; 45:423-431.
23 Shrivastava A, Mishra DK, Koutu GK, Singh SK, Prakash V, Sohgaura N. Assessment of genetic diversity of restorer and maintainer lines of rice using SSR markers. The Ecoscan. 2015; 9(1&2):265-269.
24 Singh B, Mishra MK, Naik RK. Genetic diversity among some traditional aromatic rice (*Oryza sativa* L) varieties of orissa. Indian J. Agric. Res. 201; 44:141-145.
25 Singh D, Kumar A, Sirohi A, Kumar P, Singh J, Kumar V *et al*. Improvement of Basmati rice (*Oryza sativa* L.) using traditional breeding technology supplemented with molecular markers. African Journal of Biotechnology. 2011; 10(4):499-506.
26 Singh N, Choudhury DR, Tiwari G, Singh AK, Kumar S, Srinivasan K *et al*. Genetic diversity trend in Indian rice varieties: an analysis using SSR markers. BMC Genetics. 2016; 17:127, 1-13.
27 Singh RK, Sharma RK, Singh AK, Singh VP, Singh NK, Tiwari SP *et al*. Suitability of mapped sequence tagged microsatellite site markers for establishing distinctness, uniformity and stability in aromatic rice. Euphytica. 2004; 135:135-143.
28 Singh RK, Singh US, Khush GS. Breeding aromatic rice for high yield and improved grain quality, In: Aromatic Rices. Oxford & IBH Publishing Co. Pvt Ltd, New Delhi. 2000a, 71-105.
29 Singh RK, Singh US, Khush GS. Genetics and Biotechnology of quality traits, In: Aromatic Rices. Oxford & IBH Publishing Co. Pvt Ltd, New Delhi. 2000b.
30 Singh SK, Sharma S, Koutu GK, Mishra DK, Singh P, Prakash V, Kumar V, Pal N. Genetic Diversity in NPT Lines Derived from *indica*x *japonica* Sub-species Crosses of Rice (*Oryza sativa* L.) Using SSR Markers. Scholarly Journal of Agricultural Science. 2014; 4(3):121-132.
31 Verma H, Patha kK, Rathi S, Sarma RN. Association analysis for grain quality traits in rice.Indian J. Genet. 2015; 75(4):506-509.
32 Wong SC, Yiu PH, Bong STU, Lee HH, Neoh PNP, Rajan A. Analysis of Sarawak Bario rice diversity using microsatellite markers. American J. Agri. Bio. Sci. 2009; 4(4):298-304.
33 XiLan L, Chuan Hua C, Guang Lin L, Yuan Meng C. Genetic diversity analysis of high quality conventional rice varieties grown in Guangxi and Guangdong using SSR markers. Guangxi Agricultural Sciences. 2010; 41(4):303-306.