Environment & Ecology 35 (3) : 1708—1713, July—September 2017
Website: environmentandecology.com ISSN 0970-0420

DNA Fingerprinting and Divergence Analysis in New Plant Type of Rice using RAPD Markers

G. K. Koutu, Yogendra Singh, Ekta Hedau, D. K. Mishra

Received 2 September 2016; Accepted 5 October 2016; Published online 28 October 2016

Abstract India is the second most populous nation, stands first in area, second in production, followed and preceded by China on these two aspects. Increasing yield is still the most important objective of rice breeding programs in developing countries because of the growing demand for food resulting from population growth and a reduction in area devoted to rice production. Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur has developed NPT (New Plant Type) lines through tropical japonica × Indica hybridization using wide capability gene sources. At morphological level these line have shown potential for crop improvement programme. In present study genomic fingerprinting and divergence analysis has been done in New Plant of Rice using RAPD markers. For this 90 NPT lines were selected and 15 RAPD primers were used for generating genomic finger prints and assessment of genetic diversity. The present investigation was undertaken to analyze the relatedness and distances among ninety New Plant Type (NPT) of rice using fifteen randomly amplified polymorphic DNA (RAPD) markers. The DNA amplification pattern revealed that a total number of 107 RAPD loci were amplified with an average of 7.13 loci per primer comprising 83 polymorphic loci (77.57%) and 24 monomorphic loci (22.43%). In the clustering pattern using RAPD primers all genotypes were grouped in to two groups having six and eighty four lines. The major cluster was further sub grouped in to four small sub groups having three, four, forty eight and twenty nine lines respectively. Wide divergence was detected among all lines. Further, the selective lines from these clusters may be used as potential donors for future hybridizations/crop improvement programs to develop desired varieties.

Keywords New Plant Type, Rice, Genomic fingerprinting, Divergence analysis, RAPD markers.

Introduction

Rice (*Oryza sativa* L) is the most important human food crop in the world. Rice is known as the grain of life, and is synonymous with food for Asians. More than 90% of the world's rice is grown and consumed in Asia, where 60% of the calories are consumed by 3

G. K. Koutu, Y. Singh*, E. Hedau, D. K. Mishra
Department of Plant Breeding & Genetics,
Jawaharlal Nehru Krishi Vishwa Vidyalaya,
Jabalpur (MP), India
e-mail : yogendrasinghbt@gmail.com
*Correspondence

Table 1. Details of selected lines included for molecular analysis.

Sl. No.	NPT Line No.	Sl. No.	NPT Line No.	Sl. No.	NPT Line No.
1	NPT (F13-14) 4	31	NPT 86	61	NPT (F12-13) 18
2	NPT (F13-14) 20	32	NPT 88	62	NPT (F12-13) 20
3	NPT (F13-14) 22	33	NPT 89	63	NPT (F12-13) 25
4	NPT (F13-14) 30	34	NPT 90	64	NPT (F12-13) 32
5	NPT (F13-14) 32	35	NPT 91	65	NPT (F12-13) 35
6	NPT (F13-14) 35	36	NPT 92	66	NPT (F12-13) 38
7	NPT (F13-14) 38	37	NPT 14-1	67	NPT (F12-13) 41
8	NPT (F13-14) 39	38	NPT 14-2	68	NPT (F12-13) 43
9	NPT (F13-14) 43	39	NPT 14-3	69	NPT (F12-13) 53
10	NPT (F13-14) 45	40	NPT 14-4	70	NPT (F12-13) 54
11	NPT (F13-14) 47	41	NPT 14-6	71	NPT (F12-13) 55
12	NPT (F13-14) 48	42	NPT 14-9	72	NPT (F12-13) 58
13	NPT (F13-14) 67	43	NPT 14-11	73	NPT (F12-13) 59
14	NPT (F13-14) 76	44	NPT 26-1	74	NPT (F12-13) 60
15	NPT (F13-14) 77	45	NPT 87	75	NPT (F12-13) 61
16	NPT (F13-14) 79	46	NPT (F 13-14) 9	76	NPT (F12-13) 62
17	NPT (F13-14) 82	47	NPT (F 13-14) 11	77	NPT (F12-13) 63
18	NPT (F13-14) 84	48	NPT (F 13-14) 24	78	NPT (F13-14) 81
19	NPT (F13-14) 89	49	NPT (F 13-14) 65	79	NPT (F13-14) 95
20	NPT (F13-14) 116	50	NPT (F 13-14) 85	80	NPT (F13-14) 103
21	NPT (F12-13) 8	51	NPT (F 13-14) 92	81	NPT (F13-14) 72
22	NPT (F12-13) 10	52	NPT (F 13-14) 94	82	NPT (F13-14) 1
23	NPT (F12-13) 30	53	NPT (F 13-14) 98	83	NPT (F13-14) 2
24	NPT (F12-13) 34	54	NPT (F 13-14) 106	84	NPT (F13-14) 3
25	NPT (F12-13) 49	55	NPT (F 13-14) 117	85	NPT (F13-14) 10
26	NPT 19	56	NPT (F 13-14) 119	86	NPT (F13-14) 66
27	NPT 22	57	NPT (F 12-13) 1	87	NPT (F13-14) 87
28	NPT 82	58	NPT (F 12-13) 2	88	NPT (F13-14) 12
29	NPT 83	59	NPT (F 12-13) 3	89	NPT (F13-14) 13
30	NPT 84	60	NPT (F 12-13) 13	90	NPT (F13-14) 33

billion Asians [1]. According to Food and Agriculture Organization (FAO) of United Nations (UN) 80% of the world rice production comes from seven countries only. India is the second most populous nation, stands first in area, second in production, followed and preceded by China on these two aspects. The other major rice growing countries are Indonesia, Vietnam, Bangladesh, Thailand, Myanmar and Philippines. The green revolution of the 1970s resulted in remarkable increase in rice production. However since then the rate of production in most rice growing countries has slowed down and now has reached a plateau. Contributing factors include a higher population growth rate and the conversion of some highly productive rice lands for industrial and residential purpose. These developments have pushed rice cultivation to less productive land including saline and drought prone areas. Increasing yield is still the most important objective of rice breeding programs in developing countries because of the growing demand for food resulting from population growth and a reduction in area devoted to rice production. The International Rice Research Institute (IRRI) began developing the new plant type (NPT) rice through ideotype breeding approaches in 1989 [2]. The goal was to develop an NPT with a yield potential 20–25% higher than that of existing semi dwarf rice varieties under a tropical environment during the dry season. The NPT was designed based on the results of stimulation modeling and the new traits were mostly morphological since these are easier to select than physiological traits in breeding programs. The proposed NPT has a low tillering capacity (3 to 4 tillers when direct-seeded), few un-productive tillers, 200 to 250 grains per panicle, a plant height of 90 to 100 cm, thick and sturdy stems, leaves that are thick, dark green, and erect, a vigorus

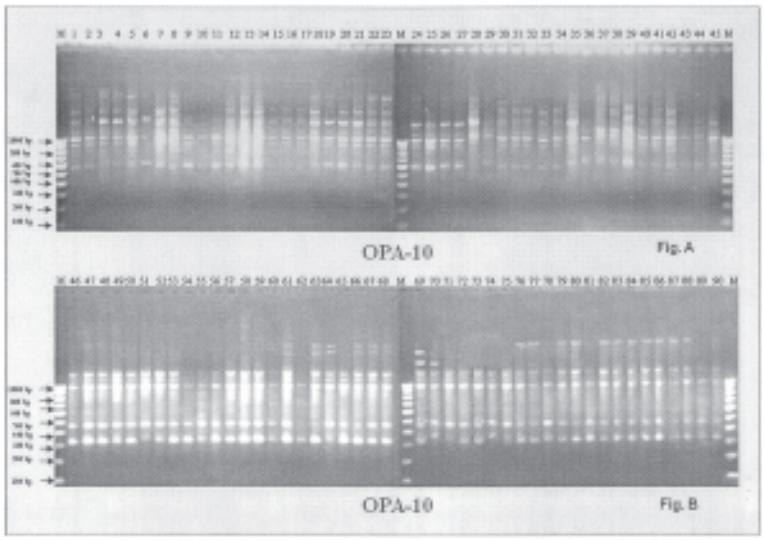

Fig. 1. Amplification profile of the DNA of 90 rice NPT lines using the primer-OPA 10.

root system, 100 to 130 days' growth duration, and increased harvest index [3].

Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur has developed New Plant Type (NPT) lines through tropical japonica × Indica hybridization using wide capability gene sources. These lines combines strong clum, short stature, dark green erect leaves, longer panicles, high grain numbers with improved grain quality which are at various stages of testing and are capable of high yield. In the last two decades, a rapid progress has been made towards the development and application of molecular marker technology in plant genome analysis. Molecular markers are considered best for the analysis of genetic diversity and cultivar identification since they are indifferent to development stage or environment. Molecular markers are also called DNA markers. It is a DNA sequence that is readily detected and whose inheritance can be easily monitored. The use of molecular markers is based on naturally occurring DNA polymorphism, which forms the basis for designing strategies to exploit applied purpose. Random Amplified Polymorphic DNA (RAPD) marker system was developed by Welsh and McClelland in [4]. Random Amplified Polymorphic DNA (RAPD) is performed in conditions resembling those of PCR using genomic DNA from the target and a single short oligonucleotide (generally 10 mer). The DNA amplification product is generated from a region that is flanked by a part of 10 b.p priming site in the appropriate orientation. A particular fragment generated for one individual but not for other represents DNA polymorphism and can be used as a genetic marker. The present study was done having objective (i) Generation of DNA fingerprints (molecular profiling) and (ii) Divergence analysis at molecular level. To achieve these objective 90 NPT lines of rice were selected and 15 RAPD primers were used for generating DNA finger prints and assessment of genetic diversity. These DNA finger prints will help in estimation of genetic divergence and selection of appropriate donor for further rice improvement.

(The financial assistance for this study in form of Adhoc project entitled Molecular characterization of New Plant Types (NPTs) of Rice Developed by JNKVV, Jabalpur (MP). is provided by Madhya Pradesh Council of Science and Technology, Bhopal is dully acknowledged).

Table 2. RAPD primers used in study (with sequence).

Sl. No.	Primer code	Primer sequence (5´–3´)	GC content (%)
1	OPA01	CAGGCCCTTC	70 %
2	OPA02	TGCCGAGCTG	70 %
3	OPA03	AGTCAGCCAC	60 %
4	OPA04	AATCGGGCTG	60 %
5	OPA05	AGGGGTCTTG	60 %
6	OPA07	GAAACGGGTG	60 %
7	OPA10	GTGATCGCAG	60 %
8	OPA12	TCGGCGATAG	60 %
9	OPA13	CAGCACCCAC	70 %
10	OPB01	GTTTCGCTCC	60 %
11	OPB03	CATCCCCCTG	70 %
12	OPB04	GGACTGGAGT	60 %
13	OPB06	TGCTCTGCCC	70 %
14	OPB07	GGTGACGCAG	70 %
15	OPB09	TGGGGGACTC	70 %

Table 3. Number of RAPD loci and percentage of polymorphism in NPT lines genomic DNA.

Sl. No.	Primer code	Range of loci scored (bp)	Total loci	Mono-morphic loci	Polymorphism No. of loci	%
1	OPA01	350-2000	06	03	03	50
2	OPA02	450-2000	08	02	06	75.0
3	OPA03	200-1800	08	02	06	75.0
4	OPA04	500-1500	08	03	05	62.5
5	OPA05	280-1750	08	01	07	87.5
6	OPA07	300-1600	09	01	08	88.9
7	OPA10	290-2000	08	01	07	87.5
8	OPA12	280-2150	09	02	07	77.8
9	OPA13	260-1700	06	01	05	83.3
10	OPB01	340-1650	08	01	07	87.5
11	OPB03	260-1100	05	01	04	80.0
12	OPB04	150-1000	06	01	05	83.3
13	OPB06	600-2500	07	02	05	71.4
14	OPB07	400-2200	06	01	05	83.3
15	OPB09	350-1550	05	02	03	60.0

Materials and Methods

Plant materials

For the molecular analysis, Ninety promising New Plant Type (NPT) lines were selected on the basis of morphological, quantitative and qualitative traits from Seed Breeding Farm, JNKVV, Jabalpur (MP) (Table 1).

Random amplified polymorthic DNA (RAPD) primers

Twenty five RAPD primers previously reported in rice were got synthesized from Bangalore Genei Pvt. Ltd, Bangalore. The details of operon code, sequence of the primers and GC content are given in Table 2.

DNA isolation

The isolation of genomic DNA from leaf sample was undertaken following the method describe by Mukherjee [5] with slight modifications. The PCR reaction was performed in a volume of 20 µl reaction set up having 30 ng of template DNA, 800 µM of dNTPs mix, 1.0 U of Taq DNA polymerase, Ix Reaction buffer, 0.3 µM of Primer and rest of deionized water. The PCR amplification was achieved in M.J Research Thermo cycler (PTC 200). The PCR conditions were initially 5 min denaturing step at 94°C, followed by 41 cycles having denaturing at 94°C for 1 min, annealing at 52°C for 1 min, polymerization at 72°C for 2 min and incubation at 72°C for 5 min. A set of 10–25 mer nucleotides RAPD primers was used for PCR amplification.

Electrophoresis

Horizontal submerged gel electrophoresis unit was used for fractionating RAPD markers on Agarose gel (1.8%), prepared by dissolving appropriate amount of Agarose in 1x TAE/TBE buffer as per Sambrook et al. [6] and adding ethidium bromide stain (1.5 µg/ml). For each well, DNA sample and DNA loading dye (6x) were mixed in ratio of 5:1, v/v and loaded with a micropipette. Electrophoresis was done at 80 V for 4 h in 1× TAE electrophoresis buffer. The gel image was viewed and stored in gel documentation (Syngen) system.

Data analysis

Data analysis of RAPD primer based fingerprinting was done using Gel Compar-II, version 3.5 (Applied Maths. U.S.A). Initially all lanes were marked (red) then reference lane (100 b.p ladder) were selected and different bands of 100 b.p ladder (100 b.p to 1000 b.p) were matched (normalized) in all three reference lane.

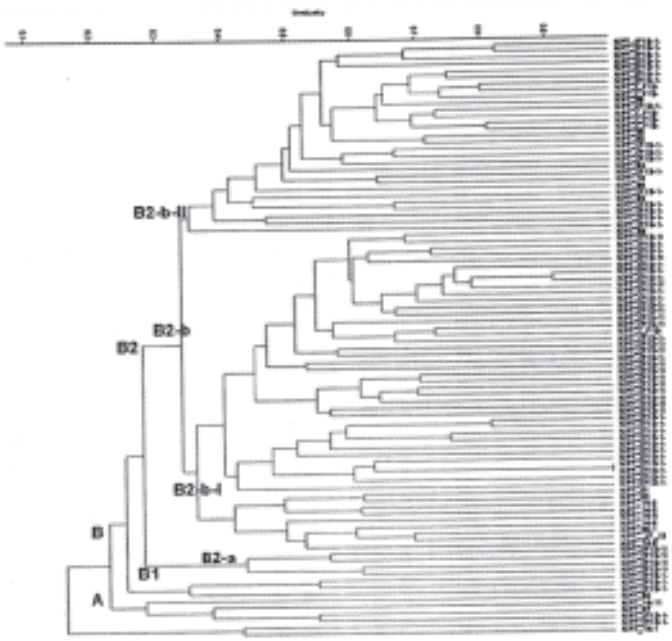

Fig. 2. Dendrogram of rice NPT lines based on RAPD markers.

Then only distinctly visible bands were marked. After this all lanes were integrated and finally combined dendrogram based on all 15 RAPD markers was generated using Jaccard coefficient of similarity and UPGMA cluster analysis.

Results and Discussion

In the present investigation, RAPD markers were employed to assess the genetic diversity among ninety NPT lines of rice. The experiment for analyzing genetic relationship using 15 random primers revealed that all primers showed polymorphism with reproducible and informative profiles (Fig. 1). The DNA amplification pattern revealed that a total number of 107 RAPD loci were amplified with an average of 7.13 loci per primer comprising 83 polymorphic loci (77.57%) and 24 monomorphic loci (22.43%) (Table 3). The range of polymorphism was 50% (OPA-01) to 88.90% (OPA 07). In the clustering pattern using RAPD primers all genotypes were grouped in to two groups having six and eighty four lines respectively at 25% similarity. Again at 30% similarity. The major cluster B was further sub grouped in to two sub groups (B1 and B2) having three and eighty one lines respectively. The B2 sub group was further classified in to B 2-a and B 2-b sub-sub groups consisting of four and seventy seven lines respectively. The B2 -b sub-sub was again classified in to two clusters (B2-b-I and B2-b-II) having forty eight and twenty nine NPT lines respectively at 40% similarity (Fig. 2). Wide divergence was detected among all lines. The similar results have been reported earlier [7, 8,9]. Further, the selective lines from these clusters may be used as potential donors for future hybridizations/crop improvement programs to develop desired varieties.

Although the yield potential of the NPT rice is limited by poor grain filing and susceptibility to insect pest and diseases [10]. Still they are valuable genetic materials used in rice breeding programs worldwide. NPT lines of JNKVV, Jabalpur have potential to develop drought tolerance variety for rain fed eco-

system. At JNKVV Jabalpur Extra early Hybrid Rice JRH-% and JRH-8 have already been developed using NPT lines. The concept of genetic distance has been of vital utility [11]. Genetic diversity is the key determinant of germplasm utilization in crop improvement. Population with high level of genetic variation is the valuable resource for broadening the genetic base in any breeding program. Molecular markers help us to understand the level of genetic diversity that exists among traditional races, varieties and exotic accessions which can be exploited in rice breeding programs. Among molecular marker systems used to identify and assess the genetic diversity and phylogenetic relationships in plant genetic resources, random amplified polymorphic DNA (RAPD) technique is the fastest and simplest. According to Manjarrez-Sandoral et al. [12] the accurate estimation of genetic diversity among germplasm sources may increase the efficiency of plant breeding / crop improvement. Similarly, evaluation of genetic diversity among can provide predictive estimate of genetic variation among segregating progeny for pure line cultivar development [13]. It can also help in predicting the degree of heterosis or combining ability in the progeny of some parental combinations [14]. Molecular approaches are more reliable for assessment of genetic divergence in rice [15] and these are being used by various workers time to time [16, 17].

References

1. Khush GS (1997) Origin, dispersal, cultivation and variation of rice. Pl Mol Biol 35 : 25—34.
2. Khush GS (1995) Breaking the yield frontier of rice. Geo J 35 : 329—332.
3. Peng S, Khush GS, Cassman KG (1994) Evaluation of a new plant ideotype for increased yield potential. In: Cassman KG, editor. Breaking the yield barrier. Proc Workshop on Rice Yield Potential in Favorable Environments. Los Baños (Philippines): Int Rice Res Inst, pp 5—20.
4. Welsh J, Mc Clelland M (1991) Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acid Res 18 : 7213—7218.
5. Mukherjee PK (1999) Rapid extraction of plant genomic DNA suitable for restriction analysis and PCR amplification. Asian Pacific J Mol Biol Biotechnol 7 : 95—96.
6. Sambrook J, Fritsch EF, Maniatis T (1981) Molecular cloning: A laboratory manual. 2nd edn. Vol 3. Cold Spring Harbor Lab, Cold Spring Harbor, New York, USA.
7. Nachimuthu VV, Muthurajan R, Duraialaguraja S, Sivakami R, Balaji Pandian AB, Ponniah G, Gunasekaran K, Swaminathan M, Suji KK, Sabariappan R (2015) 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: 8:30. DOI 10.1186/s12284-015-0062-5.
8. Kanawapee N, Sanitchon J, Srihaban P, Theerakulpisut P (2011) Genetic diversity analysis of rice cultivars (*Oryza sativa* L.) differing in salinity tolerance based on RAPD and SSR markers. Electronic J Biotech DOI: 10.2225/vol14-issue6-fulltest-4.
9. Rabbani MA, Pervaiz ZH, Masood MS (2008) Genetic diversity analysis of traditional and improved cultivars of Pakistani rice (*Oryza sativa* L) using RAPD markers. Elect J Biotech vol. 11, no. 3 (In press).
10. Khush GS, Peng S (1996) Breaking the yield frontier of rice. Increasing yield potential in wheat: Breaking the barners. Reynolds MP, Rajaram S, McNad A (eds). Proc Workshop. 26—28 Mar, 1996. Int Maize and Wheat Improv Cent, Ciudad Obregon, Sonora, Mexico, pp 36—51.
11. Arunachalam V (1981) Genetic distance in plant breeding. Ind J Genet 41 : 226—236.
12. Manjarrez-Sandoral P, Carter TE, Webb DM, Burton JW (1997) RFLP genetic similarity estimates and coefficient of parentage as genetic variance predictors for soybean yield. Crop Sci 37 : 698—703.
13. Barbosa-Nato JF, Sorrels ME, Ciser G (1996) Prediction of heterosis in wheat using coefficient of parentage and RFLP based estimates of genetic relationship. Genome 39 : 1142—1149.
14. Moghaddam ME, Trethowan RM, William HM, Reizai A, Arzani A, Mirlohi AF (2005) Assessment of genetic diversity in bread wheat genotypes for tolerance to drought. Euphytica 141 : 147—156.
15. Yogendra Singh (2011) Molecular approaches to assess genetic divergence in rice. GERF Bull Biosci 2 : 41—48.
16. Singh Yogendra, Pani DR, Khokhar D, Singh US (2013) Agro-morphological characterization and molecular diversity analysis of aromatic rice germplasm using RAPD markers. Oryza 50 : 26—34.
17. Saker MM, Youssef SS, Abdallah NA, Bashandy HS, El-Sharkawy AM (2005) Genetic analysis of some Egyptian rice genotypes using RAPD, SSR and AFLP. Afr JBiotech 4 : 882—890.