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SCoT MARKERS ASSISTED EVALUATION OF GENETIC DIVERSITY IN NEW PLANT TYPE (NPT) LINES OF RICE

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Abstract

Rice being a stable food crop has wide range of diversity and distribution throughout the globe. Recently in hybrid rice breeding program new plant types have been developed through line x tester mating with the use of male sterility system. Genetic diversity and insight amplification among 43 New Plant Type (NTP) lines of rice using 15 start codon targeted polymorphism markers were tested. Out of 25 start codon targeted polymorphism (SCoT) primers, only 15 SCoT primers were amplified and produced 76 alleles with 83.26 per cent polymorphism. Average number of alleles per primer was 5.06, while polymorphism information content ranged from 0.28 to 0.67 with an average of 0.50 per primer. Dandogram cluster falls in two major group having 31 and 12 NPT lines respectively.

Introduction

Rice (*Oryza sativa* L.) belonging to Poaceae and a staple food of India and many parts of the world. It is consumed by one third of the world's population and occupies almost one-fifth of the total land area (Kahani *et al*. 2015, Rao *et al*. 2016). Furthermore, rice is an ideal model plant for the study of cereals genetics and genome organization due to its diploid genetics. It consists of small genome size ~430 Mb, significant level of genetic polymorphism, large amount of well conserved genetically diverse material and compatible wild species. South East Asia, including India is the centre of rice diversity and high level of diversity has been reported both at inter- and intra- specific levels (Singh *et al*. 2016).

Recently, New plant type (NPT) lines of rice for hybrid rice breeding program have been carried out in line x tester mating design involving three cytoplasmic male sterility (CMS) lines and six testers. Using CMS several new plant types of rice have been developed for a specific trait. Repeated crossing and selection of desirable trait bearing plant lead to reduction of existing diversity in the plant genotypes. Thus knowledge of genetic diversity in the germplasm provides an opportunity for plant breeders to develop new and improved cultivars with desirable characteristics, which include both farmer-preferred traits as well as breeders preferred traits. Crop improvement work is not possible due to lack of appropriate evolutionary and genetic diversity knowledge. Assessment of genetic diversity within and between plant populations is routinely performed at morphological and biochemical (allozyme) level in the pregenomic era, and DNA or molecular level in post genomic era (Sharma *et al*. 2012). Molecular markers show a similar mode of inheritance for dominant/recessive or codominant traits. At present, DNA based marker technology is non-destructive, not affected by environmental factors, require less quantity of samples and entail small experimental setup (Ma *et al*. 2011) and equipment's for assessment of morpho-molecular parameters (Kanawappe *et al*. 2011). Molecular markers are the useful approach for the assessment of genetic variation and in the elucidation of genetic relations within

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and among the species. Various molecular markers are used for the genetic diversity analysis in many crop plants such as RFLP, RAPD, SCoT, SSR, SNP, ISSR and AFLP etc (Idrees and Irshad, 2014, Sharma *et al.* 2015).

Now days, a novel molecular marker known as start codon targeted polymorphism (SCoT) targets on short ATG start codon in plant genes has been reported(Collard and Mackill 2009). SCoT marker is gaining popularity for its superiority over other dominant DNA marker system like RAPD and ISSR for higher polymorphism, better marker resolvability and reliable bands which can be used for effective population studies, genetic mapping in different plants and in marker assisted selection programs (Gorji *et al.* 2011). SCoT marker has been successfully employed in genetic diversity analysis and fingerprinting of a number of agricultural and horticultural crop species (Mulpuri *et al*. 2013). The present study was aimed to assess the genetic diversity among and amplification insights of NPT lines of rice developed from Indica and Japonica crosses at Jwaharlal Nehru Krishi Vishwa Vidhalya Jabalpur, India.

Materials and Methods

Seeds of 43 Jawahar New Plant Type (NPT) lines of rice were procured from germplasm bank, Seed Technology Centre, JNKVV, Jabalpur, India. From each lines, total genomic DNA was isolated from young leaf samples (14-15 days old) using CTAB DNA extraction method with minor modifications described by Saghai-Maroof *et al.* (1984)

Total 25 SCoT primers were screened for SCoT analysis and 15 primers were selected for genetic diversity analysis on the basis of sharp and clear banding pattern (Table 1). PCR reaction mixture and protocol was standardized to get good quality amplification. The sequences of SCoT primers with GC content and melting temperature is shown in Table 1.

Table 1. List of SCoT Primers used in the present investigation.

Sl. No.	Primer	Forward Sequence (5'-3')	GC content	T_m
1	SCoT2	CAACAATGGCTACAACCC	55'6	53.6
2	SCoT6	CAACAATGGCTACCACGC	55.6	54.4
3	SCoT9	CAACAATGGCTACCAGCA	50.0	52.9
4	SCoT13	ACGACATGGCGACCATCG	61.1	58.0
5	SCoT14	ACGACATGGCGACCACGC	66.7	61.3
6	SCoT18	ACCATGGCTACCACCGCC	66.7	60.7
7	SCoT19	ACCATGGCTACCACCGGC	66.7	60.7
8	SCoT26	ACCATGGCTACCACCGTC	61.1	57.3
9	SCoT28	CCATGGCTACCACCGCCA	66.7	60.7
10	SCoT30	CCATGGCTACCACCGGCG	72.2	61.8
11	SCoT31	CCATGGCTACCACCGCCT	66.7	60.4
12	SCoT32	CCATGGCTACCACCGCAC	66.7	59.1
13	SCoT33	CCATGGCTACCACCGCAG	66.7	58.3
14	SCoT35	CATGGCTACCACCGGCCC	72.2	61.7
15	SCoT36	GCAACAATGGCTACCACC	55.6	54.2

The presence or absence of each of the observed bands for each of 30 primers was scored 1 or 0, respectively and then a matrix of 0 and 1 digits in excel software was provided. Genetic diversity amongst NPT lines of rice was evaluated by the Jaccard's similarity coefficient. The similarity matrix was subjected to cluster analysis of UPGMA method, and a dendrogram was generated using NTSYS software (version 2.02e). Principle component analysis (PCA) was also done. Polymorphic information content (PIC) of each of the analyzed SCoT primers were measured using the formula PIC = 1-Σpi^2 (Powell *et al.* 1996), where p_i is the frequency of the i^{th} allele. Resolving power (Rp) is the ability of a primer to detect level of variation between individuals and it is calculated according to methods described by Prevost and Wilkinson (1999).

$Rp = \Sigma Ib$,

Where Ib (band In formativeness) takes the values of: 1–[2|0.5–*p*|], where *p* is the proportion of individuals containing the band.

Marker index (MI) was computed as PIC × EMR

Where EMR (Effective multiplex ratio) was defined as EMR= nβ: n= total number of bands, β= total number of polymorphic bands.

Results and Discussion

Rice crop has emerged as a vast diverse crop plant which is grown throughout the world. It has a large number of varieties/germplasm in the globe and presently it is given as a high impetus for increasing the production and productivity in the climate changing scenario. In the present study genetic diversity was assessed using molecular markers and found significant amount of diversity in the studied NPT lines of rice. Genetic diversity is necessary for any crop improvement program done by accessing morphological and molecular data. The use of advanced molecular technologies is a potential approach to understand their diversity. Genetic diversity analysis using molecular markers are non-destructive, not affected by environmental factors, requireless amount of samples, entail small size of experimental setup and equipment for measuring physiological parameters (Sharma *et al.* 2012). DNA fingerprinting is routinely employed by researchers to study the extent of genetic variation in the germplasm or cultivars and further group them into specific categories (Panwar *et al*. 2010). In the present study, 15 SCoT markers were used to characterize and evaluate the genetic diversity of 43 rice NPT lines. A total 76 alleles were produced with amplicon size varied from ~250bp-2500bp. Sixty four alleles were polymorphic with 83.26 per cent polymorphism. Average number of amplicon per primer was 5.06, with an average of 4.26 polymorphic bands per primer. The estimates of polymorphism information content ranged from 0.28 to 0.67 with an average of 0.50 per primer. Marker index (3.36 to 18.72) and effective multiplex ratio (6 to 36) with an average of 11.28 and 22.4 per primer was found respectively. Value of resolving power ranged from 2.78 to 8.42 with an average of 6.24 per primer Table 2. Earlier, Shahlaei *et al*. (2014), Baghizadeh and Dehghan (2018) were also carried a study in rice germplasm and observed similar results. The DNA profile data derived from SCoT primers were subjected to calculate the genetic similarity and the matrix index. The similarity matrix used to determine level of relatedness among the NPT rice line studied. Pair-wise estimates of similarity index ranged from 0.52 to 1.00. Khan *et al.* (2017) also found significant level of similarity index in Mentha genotypes using SCoT markers.

In UPGMA cluster analysis, maximum number of NPT lines (31) was represented in Cluster I whereas 12NPT lines were placed in cluster II. Cluster I was divided into two sub-clusters 'A_1' and 'A_2' at 60% similarity coefficient. Cluster'A_1' represented 10 NPT lines and 'A_2' had 2 NPT lines namely NPT-81-04 and NPT-81-35-01. Sub-cluster 'A_1' was further divided into 'A_3' and

'A_4' at 70% similarity coefficient. Sub-sub-cluster 'A_3' included the NPT lines namely NPT-10-123, NPT-83, NPT-14-11, NPT-19-01, NPT-65, NPT-87 and NPT-14-12 while the sub-cluster 'A_4' were represented by three NPT lines namely NPT-10-113, NPT-19-02 and NPT-14-03, respectively. Cluster II was divided into Sub-cluster 'B_1' and 'B_2' at 645 similarity coefficient. Cluster 'B_1' represented two NPT lines namely NPT-81-11 and NPT-81-01. Cluster 'B_2' further divided into sub-cluster 'B_3' and 'B_4' at 66% similarity coefficient. Sub-cluster'B_3' was represented by a single genotype NPT-14-05 whereas sub-cluster 'B_4' had 28 NPT lines (Fig. 1). Similar to the present clustering patterns earlier work have also been reported by Pakseresht *et al*. (2013) and Khan *et al.* (2017).

Table 2. Banding patterns of NPT lines of rice with 15 SCoT primers.

Sl. No	PR	TB	BZ	MB	PB	PP	PIC	EMR	MI	R_P
1	SCoT-2	5	250-1500	1	4	80	0.51	20	10.2	5.68
2	SCoT-6	5	230-1600	1	4	80	0.59	20	11.8	5.22
3	SCoT-9	3	280-2000	1	2	66.66	0.64	6	3.84	2.78
4	SCoT-13	6	270-2400	1	5	83.33	0.58	30	17.4	6.98
5	SCoT-14	6	260-2200	0	6	100	0.52	36	18.72	7.00
6	SCoT-18	6	250-2400	1	5	83.33	0.38	30	11.4	8.42
7	SCoT-19	6	250-2400	1	5	83.33	0.43	30	12.9	7.38
8	SCoT-26	5	260-2500	1	4	80	0.67	20	13.4	4.92
9	SCoT-28	5	250-2000	0	5	100	0.59	25	14.7	5.36
10	SCoT-30	5	280-2500	2	3	60	0.42	15	6.30	7.00
11	SCoT-31	5	250-2400	0	5	100	0.54	25	13.5	5.80
12	SCoT-32	6	270-2200	1	5	83.33	0.53	30	15.9	7.58
13	SCoT-33	4	260-1400	1	3	75	0.28	12	3.36	5.56
14	SCoT-35	5	250-1800	0	5	100	0.39	25	9.75	8.30
15	SCoT-36	4	280-1400	1	3	75	0.51	12	6.12	5.74
TL	-	76	-	12	64	-	-	-	-	-
MN	-	5.06	-	0.80	4.2	83.26 %	0.50	22.4	11.28	6.24

Where, TL= total, MN= mean, PR= primer, TB= total bands, MB= monomorphic bands, PB= polymorphic bands, PP= percent polymorphism, PIC= polymorphism information content, EMR= effective multiplex ratio, MI= marker index, R_p= resolving power, BZ= band size in bp.

Two and three dimensional scaling of PCA analysis placed all the 43 NPT lines of rice into three groups. First contained12 NPT lines such asNPT-14-11, NPT-19-01, NPT-10-123, NPT-83, NPT-87, NPT-10-113, NPT-81-04, NPT-81-35-01, NPT-14-12, NPT-14-01, NPT-14-03, NPT-19-02 and these NPT lines were placed closely due to more similarity among them. Second group contained 29 NPT lines *viz.*, NPT-14-05, JNPT-832, NPT-14-10, NPT-10-17, NPT($_S$)-10-01, NPT-14-09, NPT-81-16, NPT-201-18, NPT-201-26-01, JNPT-831, JNPT-828, JNPT-821, JNPT-827, JNPT-822, NPT-14-07, NPT-14-06, NPT-14-04, NPT-10-116, NPT-14-02, NPT-14-01, NPT-81-26-01, NPT-81-29, NPT-81-65, NPT-81-10, NPT-10-24, NPT-10-65, NPT-86, NPT-85 and NPT-82 were placed closely with more similarity and group third group having two NPT lines *i.e*.NPT-81-01and NPT-81-11 (Fig. 2).

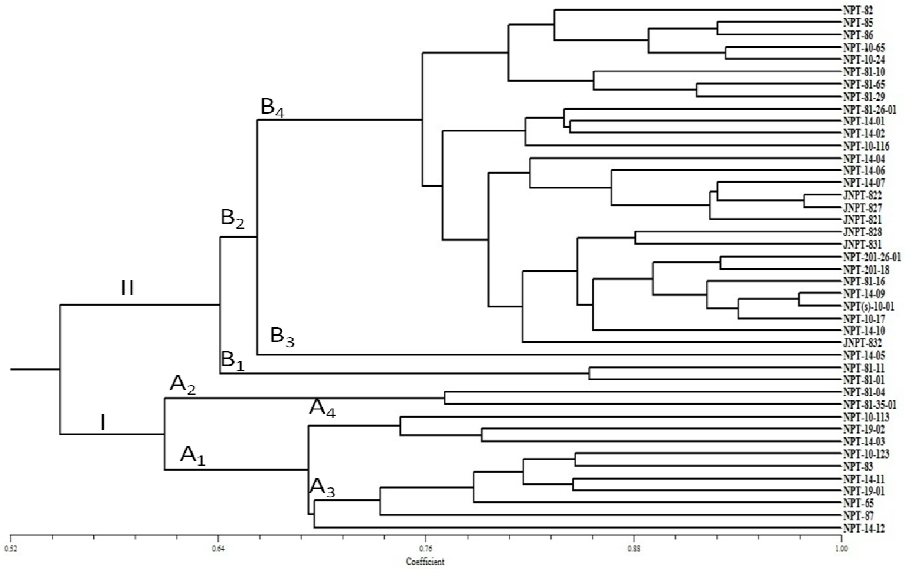

Fig. 1. Dendrogram generated using UPGMA clustering method among 43 NPT line of rice using SCoT markers.

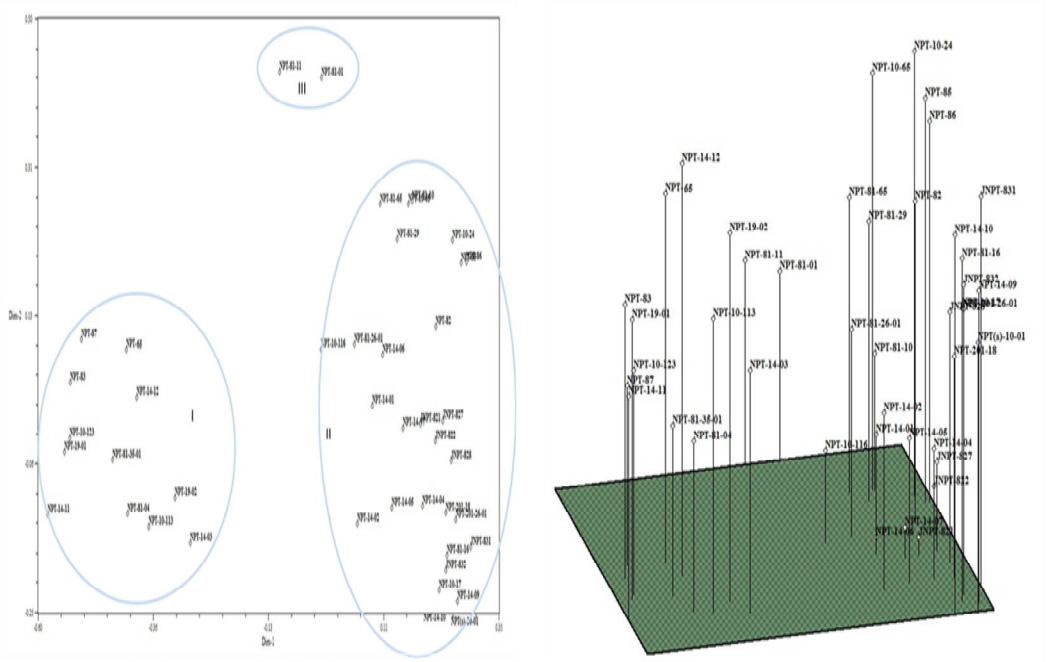

Fig. 2(a). Two Dimensional Principal Component Analysis (b) Three Dimensional Principal Component Analysis based on Euclidean Cluster Analysis in NPT lines of rice using SCoT markers.

In the present study, SCoT markers showed a moderate level of genetic diversity in the NPT lines of rice. Each polymorphic SCoT marker detected 4-6 alleles with an average 5.06 alleles per locus. The SCoT markers are very effective in detecting the polymorphism in rice lines and could

be used for genetic diversity analysis. The results showed the presence of moderate genetic diversity in the newly developed JNPT lines and would help the rice breeders in selection of suitable parents for breeding purpose and genetic mapping studies.

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References

Baghizadeh A and Dehghan E 2018. Efficacy of SCoT and ISSR markers in assessment of genetic diversity in some Iranian pistachio cultivars. Pistachio Health J. **1**: 37-43.

Collard BCY and Mackill DJ 2009. SCoT polymorphism: a simple, novel DNA marker technique for generating gene targeted markers in plants. Plant Mol. Biol. Rep. **27**: 86-93.

Gorji AM, Poczai P, Polgar Z and Taller J 2011. Efficiency of arbitrarily amplified dominant markers (SCOT, ISSR and RAPD) for diagnostic fingerprinting in tetraploid potato. J. Potato Asso. America. **88:** 226-237.

Idrees M and Irshads M 2014. Molecular markers in plants for analysis of genetic diversity. European Acad. Res. **2**: 2286-4822.

Kahani F and Hittalmani S 2015. Genetic analysis and traits association in F_2 intervarietal populations in rice under aerobic condition. J. Rice Res. **3**(4): 152-158.

Kanawappe N, Sanitchon J, Srihaban P and 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. **14**: 2-2.

Khan N, Singh S and Dhawan SS 2017. Development of species specific SCoT markers and analysis of genetic diversity among Mentha genotypes. In. J. Innov. Sc. Engineer. Tech. **4**: 2348-7968.

Ma H, Yin Y, Guo ZF, Cheng LJ, Zhang L, Zhong M and Shao GJ 2011. Establishment of DNA fingerprinting of Liaojing series of japonica rice. Middle-East J. Sci. Res. **8**(2): 384-392.

Mulpuri S, Muddanuru T and Francis G 2013. Start Codon Targeted (SCoT) polymorphism in toxic and non-toxic accessions of *Jatropha curcas* L. and development of a co-dominant SCAR marker. Plant Sci. **207**: 117-127.

Pakseresht F, Talebi R and Karami E 2013. Comparative assessment of ISSR, DAMD and SCoT markers for evaluation of genetic diversity and conservation of landrace chickpea (*Cicer arietinum* L.) genotypes. J. Physiol. Mol. Biol. **19**: 563-74.

Panwar P, Nath M, Yadav VK and Kumar A 2010. Comparative evaluation of genetic diversity using RAPD, SSR and cytochrome P450 gene based markers with respect to calcium content in finger millet (*Eleusine coracana* L). J. Genetics. **89**:121-33.

Powell W, Gordon CM and Provan J 1996. Polymorphism revealed by simple sequence repeats. Trends in Plant Sci. **7:** 215-222.

Prevost A and Wilkinson MJ 1999. A new system of comprising PCR primers applied to ISSR fingerprinting of potato accessions. Theore. App. Genetics. **98**: 107-112.

Rao M, Grithlahre S, Bisen P, Singh NK, Dar MH, Singh US, Singh PK 2016. Genetics of marker assisted backcross progenies of the cross HUR-105 X Swarna SUB1. In. J. Agri. Environ. Biotech. **9**: 499-505.

Saghai-Maroof MA, Soliman KM, Jorgensen RA, Allard RW1984. Ribosomal DNA spacer-length polymorphisms in barley mendelian inheritance, chromosomal location and population dynamics. Proc.Natl. Acad. Sci. USA. **81**: 8014-8018.

Shahlaei A, Torabi S and Khosroshahli M 2014. Efficacy of SCoT and ISSR markers in assessment of tomato genetic diversity. In. J. Biosc. **5**: 14-22.

Sharma R, Joshi A, Maloo SR and Rajamani G 2012. Assessment of genetic finger printing using molecular marker in plants: A Review- Scien. Res. Impact. **1**: 28-35.

Sharma RS, Maloo SR, Choudhary S and Jain D 2015. Microsatellite markers: an important DNA fingerprinting tool for characterization of crop plants. J. Plant Sci. Res. **31**(4): 1-9.

Singh N, Choudhury DR, Tiwari G, *et al*. 2016. Genetic diversity trend in Indian rice varieties, an analysis using SSR markers. J. BioMed. Central (BMC) Genetics. **17:** 127-139.

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