Int.J.Curr.Microbiol.App.Sci (2018) *7*(4): 548-555

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 7 Number 04 (2018)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2018.704.064

Genetic Diversity Analysis of Rice Landraces of NW Himalayas Using RAPD and ISSR Markers

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Keywords

RAPD, ISSR, Genetic diversity, Rice landraces

Article Info

Accepted:
07 March 2018
Available Online:
10 April 2018

A B S T R A C T

Forty seven landraces of rice collected from different agro-climatic zones of Himachal Pradesh along with three check varieties were used in the present investigation for the diversity studies using molecular markers to know the their genetic relationship. Higher level of polymorphism was detected by both RAPD and ISSR analysis but the ISSR polymorphism percentage was higher than that of RAPD. Using fifteen RAPD and eleven ISSR primers, 81.2 and 86.4 percent of DNA polymorphism could be detected among these genotypes. The average Jaccard's similarity coefficient based on RAPD, ISSR and combined RAPD and ISSR analysis was 0.49, 0.62 and 0.57, respectively. Combined analysis of RAPD and ISSR showed that out of 50 landraces, 44 are grouped in Cluster I. Grouping of majority of the genotypes into one cluster suggested affinity among the genotypes indicating their origin in same geographical area. RAPD showed the landraces 'IC 3131155' and 'Sukara' as most divergent ones, while the land races 'Local Variety' and 'Lalzhini' are most diverse as per the ISSR result. The present study revealed the existence of sufficient amount of genetic variability among the landraces, which could be exploited further in the breeding programme.

Introduction

Rice is highly polymorphic species with wide geographical and eco-genetic differentiation. Many farmers in India still grow local land races under different names and they also bring some varieties from distant places and start cultivating them with local name (Singh *et al.*, 2006). The indigenous rice germplasm of Northwest Himalayas, including Himachal Pradesh is enriched with wide genetic diversity and valuable gene system for yield attributes and adaptability. In Himachal Pradesh red pericarped and purple leaved rice landraces during long course of evolution has been individualized and conserved by the farmers which contain 2-3 times higher iron and zinc content than that of white rice varieties fetching premium prices in the market. These are also used by the local farmers to fight the wild rice problems. These landraces are neither exhaustive nor sufficient to represent fully the genetic diversity of the region and also facing threat from high yielding varieties (HYVs). Hence, their identification, conservation and classification

need documentation for utilization in breeding (Bhuyan *et al.,* 2007). DNA markers are known to be powerful and reliable tools for discerning variation within the plant germplasm. Among developed genetic markers, Random Amplified Polymorphic DNA (RAPD) and Inter Simple Sequence Repeats (ISSR) have been widely used for diversity analysis by several groups (Virk *et al.*, 2000; Qian *et al.*, 2001; Bhuyan *et al.*, 2007; Mathure *et al.*, 2010). The present study RAPD and ISSR markers are used to evaluate the pattern of genetic variability and relatedness among landraces of Himachal Pradesh. The data generated from the study will be useful for of maintenance and differentiation of various landraces which are preserved. It would also be helpful to the plant breeders to select readily the diverse parents which will add new germplasm base for future rice breeding progammes.

Materials and Methods

In the present study, the material comprised 47 land races of rice collected from different parts of Chamba, Kangra, Kullu, Shimla and Mandi districts of Himachal Pradesh and maintained at Rice and Wheat Research Centre, Malan of CSK Himachal Pradesh Agriculture University, Palampur, India. Besides, there were three checks, RP-2421, HPR-2143 and China-988 (Table 1). Seeds of 50 landraces of rice were grown in glass house under controlled conditions. After 30 days of growth leaves were plucked and frozen in liquid nitrogen for DNA extraction. Genomic DNA of e 50 landraces were isolated following the CTAB method of (Murray and Thompson, 1980). About 1g fresh juvenile leaves were collected from each genotype, cut into small pieces with sterilized scissor and ground to fine powder in liquid nitrogen (-196^{o}C) in a oven baked pestle and mortar. The quality of DNA was determined by electrophoresis on agarose gel. PCR amplification was performed in 20 µl volume consisting of 1.6 µl of dNTP mix (0.2 mM each of dATP, dGTP, dCTP and dTTP), 0.16 µl TaqDNA polymerase, 2 µl DNA template, 1.6 µl of 100 µM primer, 2 µl of 10x PCR buffer, 1.2 µl of $MgCl_2$ (25 mM) and 11.44 µl of sterilized distilled water. Reaction mixture was vortexed and centrifuged briefly. Amplification was carried out in a thermal cycler, programmed for 5 min at 94^{o}C for initial denaturation and 39 cycles consisting of 1 min at 94^{o}C, 1 min at 37^{o}C and 2 min at 72^{o}C with final 7 min extension at 72^{o}C using the fastest ramp times between the temperature transitions.

For ISSR assay, 10pmol of each primer was used keeping other ingredients the same as that of RAPD. For this, the initial denaturation was at 95^{o}C for 4 min, followed by 45 cycles consisting of 30sec at 94^{o}C, 45sec at 52^{o}C and 2min at 72^{o}C with final 5 min extension at 72^{o}C. After amplification, 12 µl of the amplified product from each sample was resolved on agarose gel (1.4% for RAPD and 2% ISSR) in 1x Tris acetate-EDTA (TAE) buffer (242 gTris, 57.1 ml glacial acetic acid, 100 ml EDTA, pH 8.0). Ethidium bromide (0.5 µg ml^{-1}) was added in the buffer as inter calating agent. To estimate the size of amplified DNA fragments, 1 kb DNA ladder was used as marker. The gel was run at 120V for 2 hr. After electrophoresis, the gel was viewed and stored in the Gel Documentation System.

Resuls and Discussion

The 15 RAPD primers produced a total of 154 markers (Table 2). The number of polymorphic markers and percentage of polymorphism was 125 and 81.2, respectively. The number of markers ranged from 4 to 16. Four primers exhibited 100% polymorphism.

Table.1 The details of the genetic stocks

	Landraces	Location/Source
Red pericarp rice	Jhinjan, Kijun, Tiyun, Sukara, IC3131171, IC3131180, SukaraDhan	Chamba
	DesiDhan, Ram Jawain, AchhooBaldhar, Achhoo	Kangra
	Jattoo, Deval, Matali, BhrighuDhan	Kullu
	Chohatoo	Shimla
	IC3131159	Mandi
Purple leaved rice	RLC-3, LalNakanda 41, TotuDhan, KalooDhan, Lalzhini, R-575, Purple Baldhar,TaptaBaldhar, Kaladhan-1, Kaladhan-2, KrishanDhan, China Purple, HPLC-130, HPR-1194, HPR-2089, HPR-2178, Palampur Purple, Nagrota Purple	Kangra
	IC3131183	Chamba
Quality rice	IC3131155	Mandi
	IC3131165, IC3131166	Kullu
	LC99-1B, LC99-4B, LC99-5B, Local variety, RajpurBasmati, Ram Jawain-100, Kalizhini, Chitizhini	Kangra
Checks	RP-2421, HPR-2143	Kangra
	China-988	China

Table.2 Primer codes, sequences, the number of scored band and number of polymorphic bands in RAPD and ISSR

Primer code	Sequence 5'……3'	Number of scored bands	Number of Polymorphic bands
RAPD			
OPF-05	CCGAATTCCC	10	9
OPF-09	CCAAGCTTCC	10	7
OPF-16	GGAGTACTGG	6	5
OPJ-13	CCACACTACC	10	6
OPJ-20	AAGCGGCCTC	9	4
OPX-13	ACGGGAGCCA	9	8
OPX-20	CCCAGCTAGA	16	11
OPA-10	GTGATCGCAG	10	7
OPA-13	CAGCACCCAC	9	9
OPQ-05	CCGCGTCTTG	10	8
OPQ-06	GAGCGCCTTG	11	9
OPQ-10	TGTGCCCGAA	16	16
OPD-02	GGACCCAACC	11	11
OPD-05	TGAGCGGACA	8	6
OPU-15	ACGGGCCAGT	9	9
Total		**154**	**125**
ISSR			
UBC8 10	$(GA)_8$ T	**12**	**12**
UBC8 14	$(CT)_8$ A	**4**	**2**
UBC8	$(CT)_8$ G	**7**	**7**
UBC8	$(GA)_8$ YT	10	10
UBC8	$(GA)_8$ YC	**2**	**1**
UBC8	$(CT)_8$ RA	**3**	**1**
UBC8	$(CT)_8$ RG	**4**	**3**
UBC8	$(GT)_8$ YC	**4**	**3**
UBC8	$(TG)_8$ RT	**4**	**3**
UBC8	$(TG)_8$ RC	**6**	**6**
UBC8	$(GACA)_4$	**3**	**3**
Total		**59**	**51**

Fig.1 RAPD marker profile of primer OPQ-5, M=1 kb DNA ladder, 1-50: rice genotypes

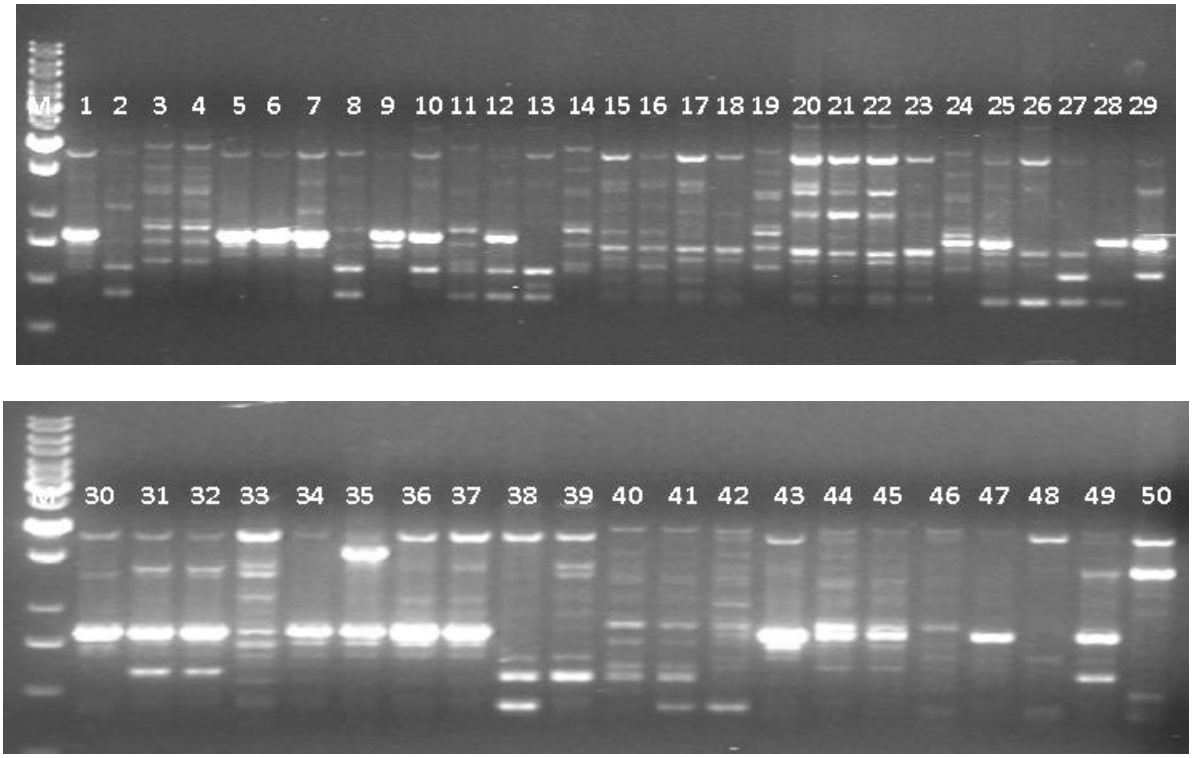


Fig.2 ISSR marker profiles of primer UBC8-68. M=1 kb DNA ladder, 1-50: rice genotypes

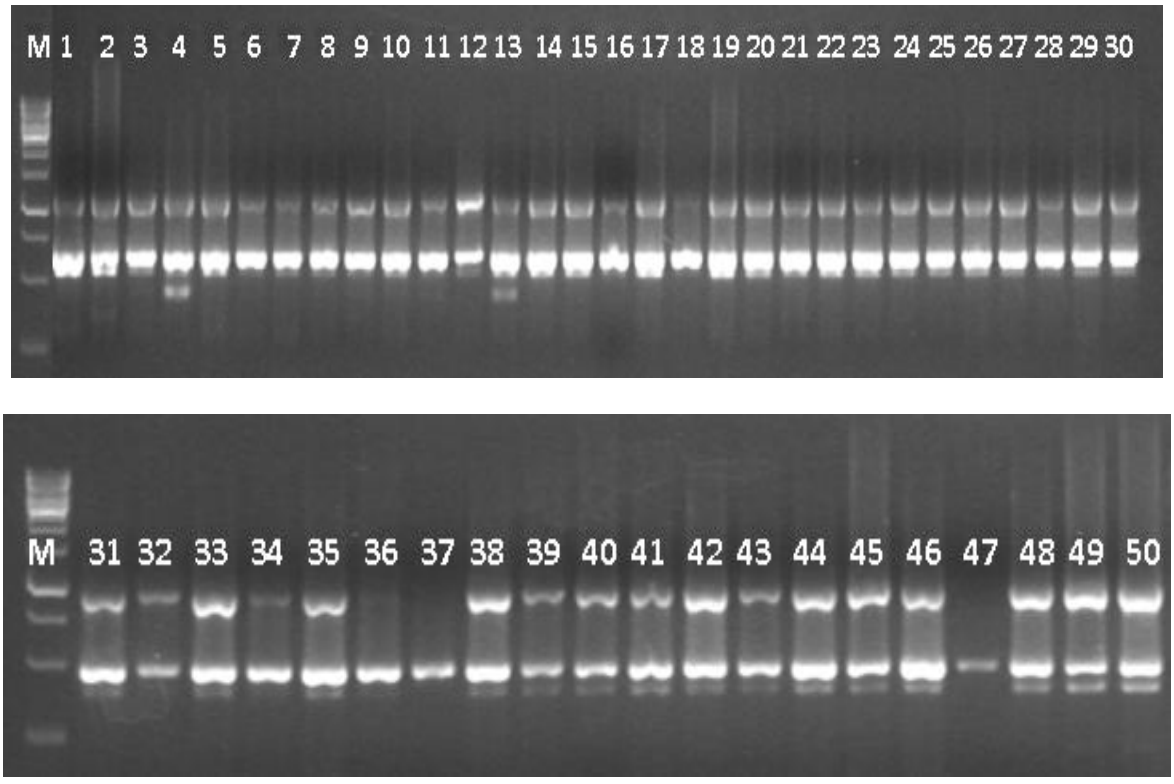


Fig.3 (A) Dendrogram showing relationship among novelty rice landraces with RAPD and ISSR (data)

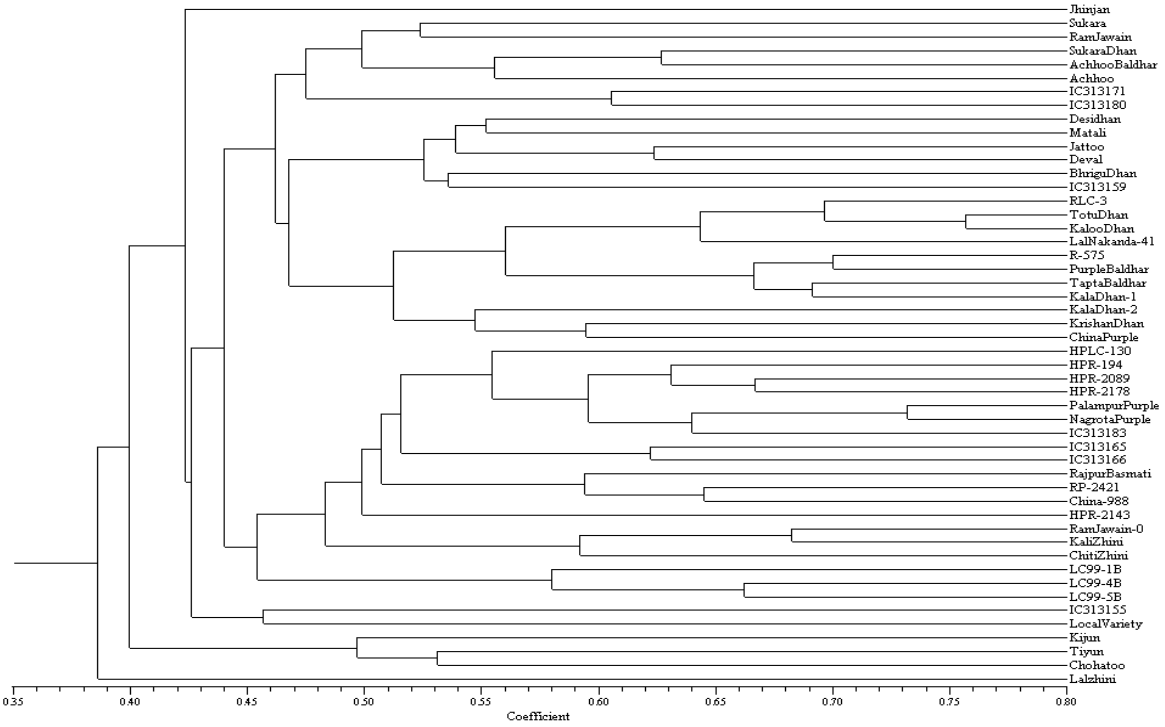
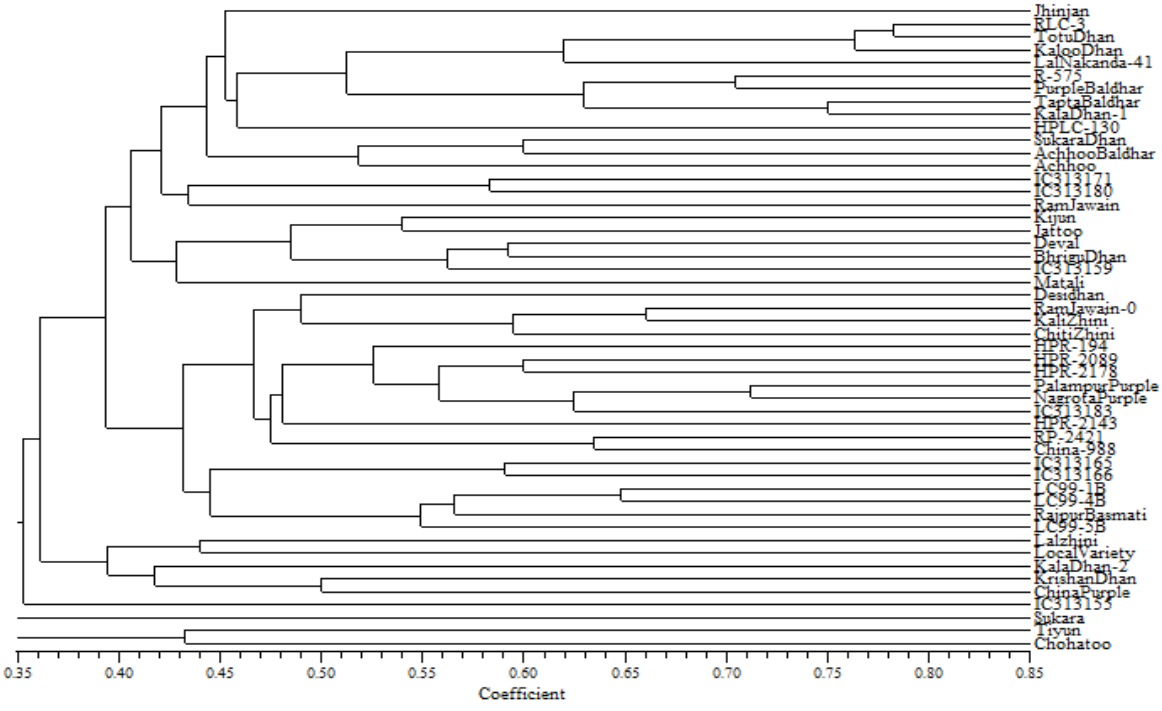


Fig.3 (B) Dendrogram generated using UPGMA analysis, showing relationship among 50 landraces of rice from Himachal Pradesh using RAPD and ISSR data (A) RAPD (B) ISSR



Out of these four, OPQ-10 generated maximum number of polymorphic markers. The highest polymorphism percentage by RAPD was shown by quality rice landraces (94.8%), followed by red pericarp (92.1%) and purple leaved rice landraces (86.7%).

Based on RAPD markers, maximum similarity (0.78) was observed between 'RLC-3' and 'TotuDhan' and minimum similarity (0.20) was observed between 'Chohatoo' and 'IC3131165'. The dendrogram generated by the RAPD markers showed three clusters with a similarity coefficient of 30.5%. The largest cluster I comprised of 41 genotypes and cluster II consisted of purple leaved rice and one quality rice *i.e.* Local Variety. Cluster III consisted of two genotypes of red rice (Tiyun and Chohatoo). Two genotypes (IC3131155 and Sukara) did not fall in the defined clusters. Cluster I had two sub-clusters I_a and I_b and sub-cluster I_a had two further sub-groups I_{a-1} and I_{a-2} and out of these two I_{a-1} had all purple leaved rice, with similarity coefficient of 46%. Besides one red pericarp rice, Jhinjan was also associated with this group at a similarity coefficient of 45.0%. Sub-group I_{a-2} had maximum genotypes of red pericarp rice landraces. I_b had nine genotypes of quality rice, six genotypes of purple leaved rice, three checks and one genotype of red rice.

Eleven primers were selected to detect polymorphism based on their reliability (Table 2). These primers amplified a total of 59 ISSR markers among the 50 novelty rice landraces, of which 51 (86.4%) were polymorphic with an average of 4.6 per primer (Fig. 2). The ISSR polymorphism percentage was higher than that of RAPD, in accordance with the Qian *et al.,* 2000. The number of markers produced by different primers exhibited polymorphism. UBC8-10 generated maximum number of polymorphic markers while UBC8-41 and UBC8-43 generated least polymorphism. Red pericarp and quality rice showed the highest polymorphism (86.7%), followed by purple leaved rice (Fig. 1). Maximum similarity (0.78) was observed between 'China Purple' and 'HPLC-130' and minimum similarity (0.30) between 'Kijun' and 'Chitizini'. The dendrogram generated by the ISSR markers showed 6 clusters with an overall similarity coefficient of 56.5%. Cluster I (Jhinjan and Achhoo) and cluster II (Kijun and BhriguDhan) constituted of red pericarp landraces with similarity coefficient of 58.0%. Cluster III was largest and further divided into two sub-clusters III_a and III_b and III_a included twelve genotypes of red pericarp ones, one purple leaved rice and two genotypes of quality rices (IC 3131155 and IC 3131166). Sub-cluster III_b constituted of 16 of 19 of purple leaved rice and one genotype of quality rice landraces (IC 3131166) at similarity level of 59.5%. Cluster IV had four genotypes of quality rice landraces and one genotype of red pericarped ones *i.e.* IC 3131159 at similarity coefficient of 58.0%. Cluster V included all the three checks while Cluster VI constituted three genotypes of quality rice landraces (LC99-1B, LC99-4B and LC99-5B) at similarity level of 58.5%. Checks of RAPD analysis were IC 3131183, Local variety and Lalzhini which were independent and did not fall in any cluster (Fig. 3B).

The rice landraces constitute a rich source of biodiversity and their conservation and utilization requires that their genetic structure is well characterized and understood. Higher level of polymorphism was detected by both RAPD and ISSR analysis as compared to Mathure *et al.,* (2010). Jaccard's similarity coefficient ranged from 0.36 to 0.78. The dendrogram obtained after pooling the data of two types of markers showed three clusters at similarity coefficient of 42.5%. Cluster I is divided into two sub clusters I_a and I_b. and out

of these sub-cluster I_a is further subdivided into three sub-groups I_{a-1}, I_{a-2} and I_{a-3} at similarity level of 46.8%. Sub-group I_{a-1} and I_{a-2} included seven and six genotypes, respectively of red pericarp landraces with genetic similarity of 47.5% and 52.5% while subgroup I_{a-3} contained eleven collections of purple leaved ones with similarity coefficient of 51.2%. Sub-cluster I_b consisted of seven collections of purple leaved landraces, nine genotypes of quality landraces and three checks. The three checks were grouped together with the similarity level of 50.0%. Cluster II consisted of only two genotypes of quality rice while Cluster III comprised of three collections of red pericarp rice *viz.*, Kijun, Tiyun and Chohatoo. The landraces Lalzhini and Jhinjan did not fall in any group (Fig. 3).

The high similarity between 'RLC-3' and 'Totu Dhan' was obtained in RAPD whereas, in ISSR it was maximum in 'China Purple' and 'HPLC-130'. On the other hand minimum similarity was between 'Chohatoo' and 'IC3131165', and 'Kijun' and 'Chitizini' for RAPD and ISSR, respectively. The lower similarity coefficient among genotype reflects their wider diversity and these cultivars can be crossed to widen the genetic base and exploit heterosis. The result of present study showed that both the markers worked effectively. RAPD showed the landraces 'IC3131155' and 'Sukara' as most divergent ones, while the land races 'Local Variety' and 'Lalzhini' are most diverse as per the ISSR result. This revealed the existence of sufficient amount of genetic variability among the landraces, which could be exploited further. The result of present study showed that both the markers worked effectively. Same results were reported by Lalhruaitluanga and Prasad 2009. Inconsistency between RAPD and ISSR analysis may be because reliability and reproducibility of RAPD in question (Wu *et al.,* 2004) and it needs high level of standardization. The primers which proved very informative can be converted to sequence tagged sites (STS) and sequence characterized amplified regions (SCAR) for amplification of specific alleles which could be further utilized in rice genome analysis. The information gained from clustering behavior of landraces can be useful to design strategies for their management in gene banks.

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How to cite this article:

Ritu Sood, Sanchit Thakur, Neelam Bhardwaj and Kaushik, R.P. 2018. Genetic Diversity Analysis of Rice Landraces of NW Himalayas Using RAPD and ISSR Markers. *Int.J.Curr.Microbiol.App.Sci.* 7(04): 548-555.
doi: https://doi.org/10.20546/ijcmas.2018.704.064