academicJournals

Vol. 12(22), pp. 3425-3429, 29 May, 2013
DOI: 10.5897/AJB2013.12410
ISSN 1684-5315 ©2013 Academic Journals
http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Molecular diversity analysis in selected fodder and dual purpose oat (*Avena sativa* L.) genotypes by using random amplified polymorphic DNA (RAPD)

Yogesh Ruwali, Kalyan Singh, Sundeep Kumar and Lalan Kumar*

Department of Genetics and Plant Breeding, College of Agriculture, G. B. Pant University of Agriculture and Technology, Pantnagar-263145, Uttarakhand, India.

Accepted 5 June, 2013

Genetic variability among 15 oat genotypes comprising fodder and dual purpose oat varieties from different geographical regions was analyzed by random amplified polymorphic DNA (RAPD) marker method in Department of Genetics and Plant Breeding, College of Agriculture, Pant University of Agriculture and Technology (G.B.P.U.A. & T.), Pantnagar. The results show appreciably high genetic diversity among the oat genotypes studied. Fifteen (15) primers selected from 20 RAPD primers could amplify 259 clear and identifiable bands, of which 250 bands were polymorphic, accounting for 96.52% genetic polymorphism. All the oat genotypes studied could be distinctly divided into two major groups with the genetic distance level at 0.46 by cluster analysis based on the Jaccard's coefficient of similarity. The cluster break indicated sufficient genetic variability among the genotypes. Clustering pattern of the varieties appeared such that it can be grouped in the genotypes suitable for the fodder purpose and the dual purpose varieties separately. Several polymorphic bands were also found in different genotypes which helped in molecular diversity analysis of these genotypes. The results found are encouraging and indicate that RAPD technique is an easy, quick and reliable technique used for molecular diversity analysis for preliminary selection.

Key words: Oats, RAPD, genetic diversity, polymorphism.

INTRODUCTION

Oats is an annual winter cereal plant with more than 30 genotypes; and is known for its quality fodder and nutritious grains with high lysine and protein content. Though, this crop is a cereal crop of global importance and used for food, feed and forage (Tinker et al., 2009). Differing from other cereal grains such as wheat and barley, oat is rich in the antioxidants α-tocotrienol, α-tocopherol, and avenanthramides, as well as total dietary fiber including the soluble fiber β-glucan etc. (Oliver et al., 2010). In recent years, with the advent of intensified dairy industry in our country, the oat have attracted the attention of breeders for its improvement due to its nutritious quality fodder for livestock and its grains as animal feed with high net energy gains. Since genetic diversity is the mainstay of any successful breeding improvement; among molecular techniques, random amplified polymorphic DNA (RAPD) is the quickest and the simplest (Williams et al., 1990) and rapid technique to elucidate the extent of genetic variability present in the experimental material, thereby helping in selection of the basic genetic material before starting any crossing programmed. The molecular diversity analysis using RAPD markers, RAPD employs single, 10 mers primer with an arbitrary sequence to generate genome specific

*Corresponding author. E-mail: lalan53singh@gmail.com

Table 1. Details of genotypes with their use and origin.

S/N	Genotype	Use	Origin
1.	OL- 9	Fodder	
2.	OL- 125	Fodder	
3.	OS- 6	Fodder	
4.	HFO- 114	Fodder	
5.	EC- 605830	Fodder	
6.	EC- 605834	Fodder	
7.	EC- 605838	Fodder	
8.	UPO- 94	Fodder	Hissar
9.	UPO- 212	Fodder cum Grain	
10.	UPO- 265	Fodder cum Grain	
11.	UPO- 271	Fodder cum Grain	
12.	UPO- 272	Fodder cum Grain	
13.	UPO- 273	Fodder cum Grain	
14.	UPO- 276	Fodder cum Grain	
15.	UPO- 260	Fodder cum Grain	

fingerprints of multiple amplification products.

Polymorphism found in RAPD profile of genotypes could be useful as genetic markers in establishment of genetic identity of cultivars in several crop species (Choudhury et al., 2007; Samec and Nasinec, 1996; Beebe et al., 2000). RAPD markers are also recommended for assessing the genetic purity of cultivars due to certain advantages than protein and RFLP markers (McDonald, 1995). In the present work, the genotypes were screened with 10 Random Amplified Polymorphic DNA markers of which finally eight markers were used to identify the genetic diversity present among the fodder and dual purpose varieties of oats.

MATERIALS AND METHODS

Plant material and DNA extractions

Twenty (20) common oat (*Avena sativa* L.) genotypes (Table 1) seed were surface sterilized and germinated. 1 g etiolated seedlings was used for rapid extraction of genomic DNA (Doyle and Doyle, 1987). Cetyl trimethyl ammonium bromide (CTAB) is a cationic detergent which solubilizes membranes and forms a complex with DNA. After cell disruption and incubation with hot CTAB isolation buffer, proteins were extracted by chloroform: isoamyl alcohol (24:1). CTAB-DNA was precipitated with isopropanol. The DNA pellet resulting after centrifugation was washed, dried and redissolved. The contamination of RNA was removed by using RNaseA (Invitrogen). Quality and quantity of DNA was checked by agarose gel electrophoresis and ultraviolet (UV) spectrophotometer (Spectronic Genesys).

Polymerase chain reaction (PCR) amplification and RAPD analysis

Twenty (20) RAPD primers were screened for PCR amplification (Table 3). Amplifications were carried out in volumes of 25 µl reaction containing 1X KCl buffer (Fermentas) containing 0.2 mM dNTPs, 30 ng of each 10 mer forward and reverse primer, 1.5 mM $MgCl_2$, 0.8 U Taq DNA polymerase (Fermentas) and 100 ng of DNA. Thermal cycler reaction were carried out according to the following temperature profile 4 min initial denaturation at 94°C; 37 cycles of 94°C for 1 min, 50°C for 45 s, 72°C for 1 min and final extension of 7 min at 72°C; and final hold 4°C. All amplifications were performed twice and independently to make sure that the results were correct. Electrophoresis was done at 50 V for 4 h in 1 X TBE electrophoresis buffer for RAPD. Gels were documented using Gel Doc system (Bio-Rad).

Data analysis

The genetic similarity was estimated using the unweighted pair group method using arithmetic average (UPGMA) clustering algorithm (NTSYpc, version 2.1) with the data of 15 primer pairs that presented polymorphism.

RESULTS

Screening of primers and genetic diversity analysis

Out of 20 RAPD primer, 15 were selected in our analysis for clear and polymorphic DNA amplification patterns (Table 2). Two DNA ladders of different sizes were used to ascertain the band position and maximum band size. Maximum band size of 1950 bp was obtained; while the average band size range was 500 to 1900 bp (Figure 1). The banding patterns obtained with 15 selected primers, genotypes could be distinguished from each other on the basis of several polymorphic bands of various sizes (Figure 1). Based on the estimated genetic similarity matrix, the highest genetic similarity value (0.793) was noticed between UPO-260 and -276 followed by 0.758 between UPO-260 and -273. All primers involved in the

Table 2. RAPD profile of amplified products.

Total number of primers tested	20
Number of polymorphic primers	15
Total number of monomorphic primers	5
Total number of amplified product	259
Size range of amplified products (in bp)	1950 to 300
Average number of bands per primer	17.26
Total number of unique bands identified	1
Total number of polymorphic bands identified	250
Total number of monomorphic bands identified	9
Percentage of all bands that were polymorphic (PPB)	96.52%

Table 3. Amplified products and polymorphism obtained with RAPD primers.

S/N	Primer sequence	Amplified product	Polymorphic band (s)	Monomorphic band (s)	Percent (%) polymorphism	GC content (%)
1	AGCGCCATTG	23	23	0	100	60.00
2	GTCGCCGTCA	19	18	1	94.73	70.00
3	TGGTCGCTGA	18	17	1	94.44	60.00
4	GTGATCGCAG	10	9	1	90.0	60.00
5	TTTGCCCGGT	9	9	0	100	60.00
6	TCGCCGCAAA	30	29	1	96.67	60.00
7	CAGCGACTGT	26	25	1	96.15	60.00
8	AGCAGGTGGA	3	3	0	100	60.00
9	CTGCGCGACT	21	20	1	95.23	70.00
10	ACGAGGCTGT	19	18	1	94.73	60.00
11	GACTAGCGAC	7	7	0	100	60.00
12	ATAGCGGCGA	8	7	1	87.5	60.00
13	TGCCGCCATA	29	29	0	100	60.00
14	GACCCTCTCA	24	23	1	95.83	60.00
15	TCGAGGACGA	13	13	0	100	60.00

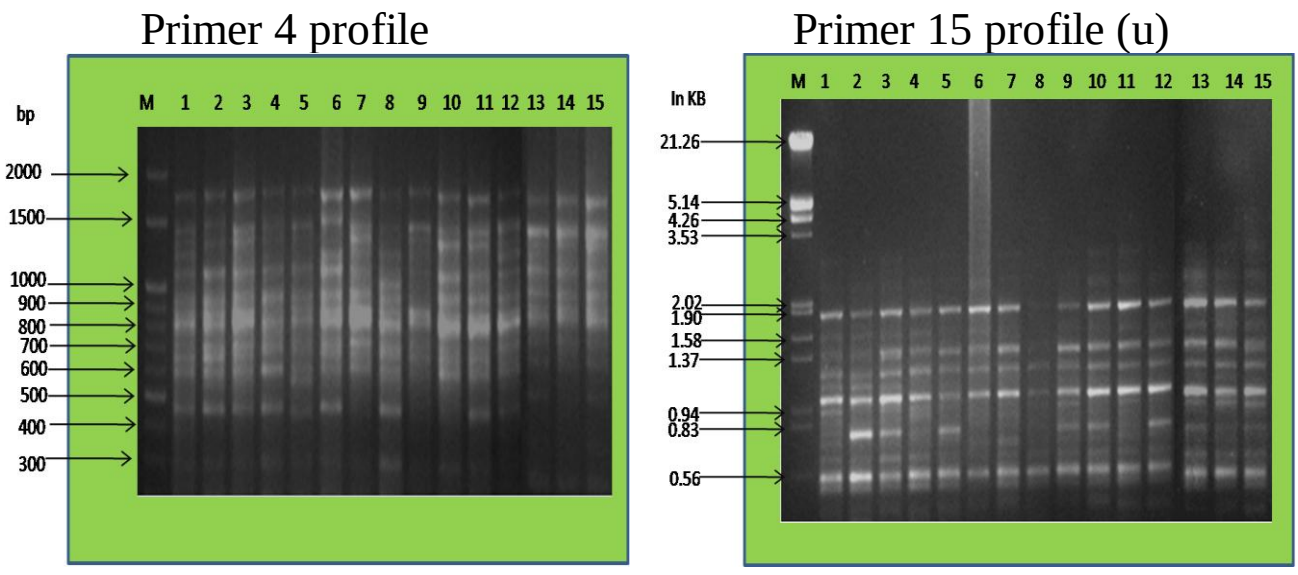

Figure 1. RAPD profile of oat genotypes generated by the different primers on agarose gel.

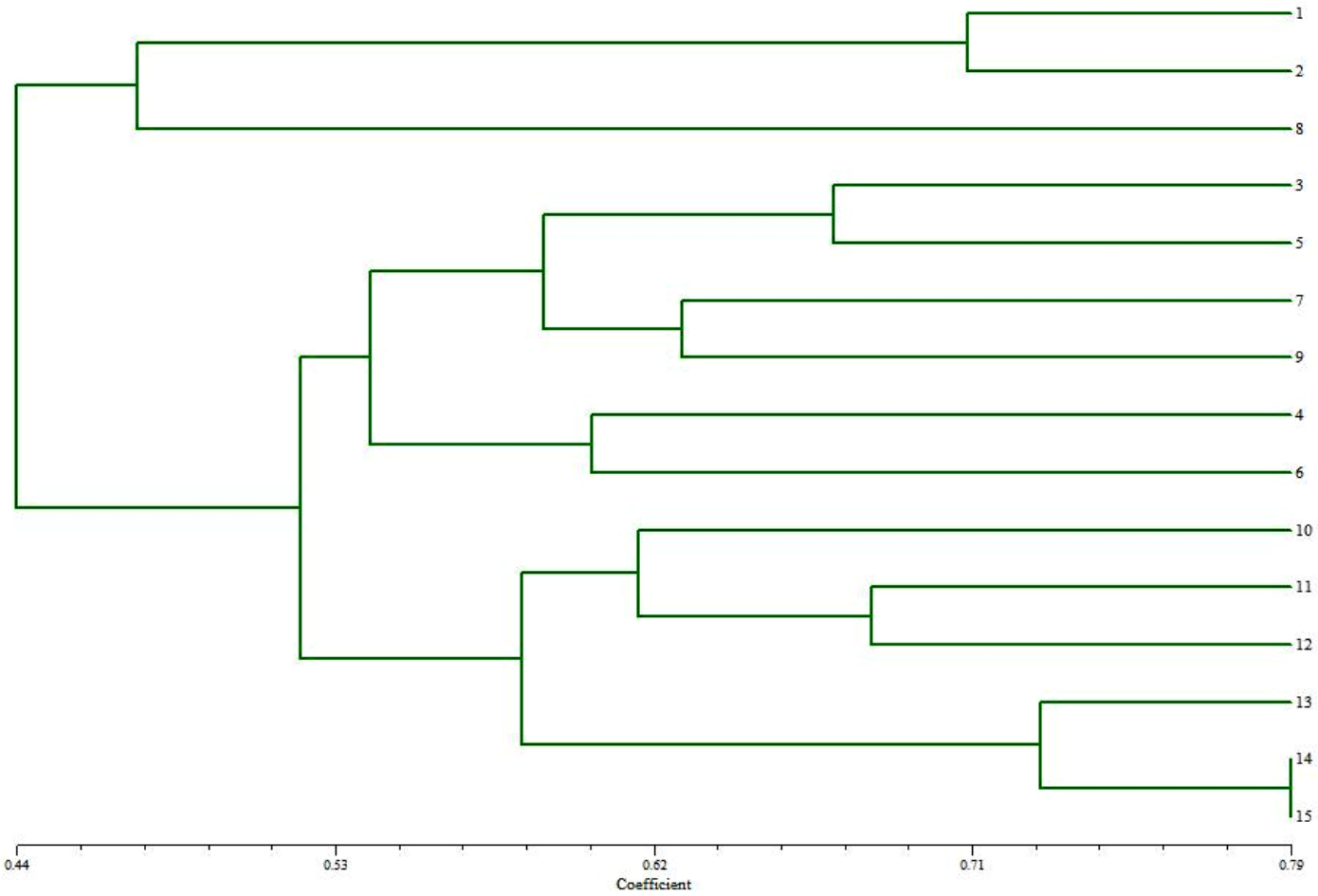

Figure 2. Dendogram of genetic distance constructed from RAPD markers.

analysis resulted in complex bands, which can assist to discriminate with each other. Clustering pattern of the varieties appeared such that it separately grouped the fodder purpose and the dual purpose varieties, with the only exception of UPO-212 which is one of the best dual purpose oat variety. From the study, it can be anticipated that RAPD-PCR could be exploited as the basis of molecular techniques for oats genotypes in analysis of molecular diversity.

Cluster analysis

The dual as well as fodder purpose oat genotypes included in this study were divided into two major groups after cluster analysis based on the RAPD data analyzed by NTSYpc, version 2.11V software at 0.46 jaccard's coefficient of similarity (Figure 2). At this cut-point, three genotypes were grouped into a minor cluster while 12 genotypes comprising mostly of dual purpose varieties were in a major cluster. The major cluster was further broken into two sub clusters with evenly distributed genotypes. Within a sub-cluster in major cluster, the genotypes UPO-260 and -276 were not further separated thus indicating soaring degree of genetic association among them (> 79%). Most of the fodder and dual purpose varieties evaluated in this study are elite varieties developed by various oat breeders and being evaluated so that a diverse combination of parents could be formed for an efficient hybridization programme in oat, which may result in desirable transgressive segregantes.

DISCUSSION

To the best of our knowledge, this is one of the few reports analyzing inter-continental genetic diversity in fodder and grain oats genotypes detected by molecular marker. Restriction fragment length polymorphisms (RFLPs), PCR-based single sequence repeats (SSRs) (Pal, 2002), sequence characterized amplified region (SCAR) and cleaved amplified polymorphic sequence (CAPS) markers in oat genomic region have been developed for various important quality traits; these region may

may be used in classification of other crops (Molnar and Orr, 2008). RAPD and SSR analysis of wild oats has shown high genetic diversity between these genotypes (Zahid et al., 2008). The diversity level between different oats genotypes revealed by RAPD has great signify-cances in oats species conservation and breeding (Loskutov and Perchuk, 2000; Wright, 2003). In the present investigation, an attempt has been made to analyze oats genotypes with differential uses as grain and fodder, which revealed a high level of genetic diversity with the 'percent polymorphic bands' value at 96.52%. Thus, sufficient variability is present among fodder and dual purpose oat cultivars' which could be beneficially exploited in various breeding programmes. RAPD was employed to categorize oat genotypes with different cultivation purposes, the relationship among different oat genotypes using the jaccard similarity value, point to the fact that UPO-260 and -276 with genetic similarity value of 0.793 have highest portion of common genetic region while UPO- 94 and - 276 had the lowest genetic similarity value (0.257) revealing that they are the most diverse pair of genotype used in the experimental material.

In this study, 15 cultivated oat varieties were analyzed; further investigations will include the analysis of more varieties to allow the resolution of varieties in great detail and to establish a reliable, quick and convenient authentication system for oat varieties. In a word, with the advantages of high polymorphism and convenience, RAPD could offer a quick and reliable alternation in analyzing the genetic relationship and dissimilarity among oat varieties and also the characterization of genotypes.

ACKNOWLEDGEMENT

This project was supported by project AICRP on forage crops. The authors thank the Director Experiment Station and HOD, Genetics and Plant Breeding; GBPUA&T, Pantnagar for providing the required research facilities.

REFERENCES

Beebe S, Skroch PW, Tohme J, Duque MC, Pedraza F, Nienhuis J (2000).Structure of genetic diversity among common bean landraces of Middle American origin based on correspondence analysis of RAPD. Crop Sci. 40:264-273.

Choudhury PR, Tanveer H, Dixit GP (2007) Identification and detection of genetic relatedness among important varieties of pea (Pisum sativum L.) grown in India. Genetica 130(2):183-191

Doyle JJ, Doyle JL (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue, Phytochem. Bull. 19:11-15.

Loskutov IG, Perchuk IN (2000). Evaluation of interspecific diversity in *Avena* genus by RAPD analysis. Oat Newsletter 46. (http://wheat.pw.usda.gov/ggpages/oatnewsletter/v46/).

McDonald MB (1995). Genetic purity: from protein electrophoresis to RAPDs. Proceedings of the Fifth Annual Corn and Sorghum Industry Research Conference. pp. 256-271.

Molnar SJ, Orr W (2008). SCAR and CAPS Markers Linked to Quality Traits (Oil, β-glucan, and Protein Content) in Oat. Genome 51:421-425.

Oliver RE, Obert DE, Hu G, Bonman J M, Jackson EW (2010). Development of oat-based markers from barley and wheat microsatellites. Genome 6:458-471.

Pal S (2002). Oat-derived SSRs and RFLPs. Crop Science 42:912-918.

Samec P, Nainec V (1996).The use of RAPD techniques for the identification and classification of pisum sativum L. genotype. Euphytica 89:229-234.

Tinker NA, Kilian A, Wight C P, Uszynska HK, Wenzl P, Rines HW, Bjørnstad A (2009). New DArT markers for oat provide enhanced map coverage and global germplasm characterization. BMC Genetics 10:39.

Williams JGK, Ubelik AR, Livak KJ, Rafalsk JA, Tingey SV (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucl. Acid Res. 18(22):6531-6535.

Wight CP, Tinker NA, Kianian SF, Sorrells ME, O'Donoughue LS et al. (2003). A molecular marker map in 'Kanota' x 'Ogle' hexaploid oat (*Avena* spp.) enhanced by additional markers and a robust framework. Genome 46(1):28-47.

Zahid H, Zahoor SA, Khan I, Hassan G, Marwat KB, Ali S, Khan MI(2008). RAPD and SSR analysis of wild oats (*Avena* species) from North West frontier Province of Pakistan. Afr. J. Plant Sci. 2 (11):133-139.