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MOLECULAR CHARACTERIZATION AND GENETIC DIVERGENCE REVEALED BY RAPD IN OATS (*AVENA SATIVA* L.)

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SUMMARY

Genetic divergence among oat genotypes from three different geographical blocks constituting nine oat genotypes viz., KENT, WRIGHT, OS-6, JHO-822, OL-9, JHO-851, OL-88, UPO-94 and UPO-212 was analyzed by Random Amplified Polymorphic DNA method in Department of Genetics & Plant Breeding, College of Agriculture, G. B. P. U. A. & T., Pantnagar. The results showed that the diversity among oat varieties was high at DNA level. Nine primers selected from 15 RAPD primers could amplify 245 clear and identifiable bands, of which 236 bands were polymorphic, accounting for 96.32 per cent genetic polymorphism. All oat genotypes studied could be distinctly divided into two major groups with the genetic distance level at 0.60 by cluster analysis based on the Jaccard's coefficient of similarity. The cluster break was further supported by the pedigree relationships among the genotypes. Several unique identifiable bands were also found in different genotypes which would help in characterization of these genotypes. The results found were encouraging and advocated that RAPD technique could be used for classification, identification and evolution studies.

Key words : Oats, divergence, RAPD, cluster

Oats is an annual or perennial herbaceous plant with more than 30 varieties, possibly possessing considerable complex genetic relationship, while most of these are economic crops which are known for their nutritious value when used as forage. In recent years, there is increasing need of hour for conduction of efficient breeding programmes yielding transgressive genotypes which would add to the further development of this dual purpose crop. Genetic diversity is of prime importance for the survival, adaptation to certain agro-climatic conditions, success and improvement of any crop species. The DNA fingerprinting by using RAPD markers is an easy and rapid technique for the diversity analysis and molecular characterization of genotypes concerned. RAPD markers offer many advantages such as higher frequency of polymorphism, rapidity (Fahima *et al.*, 1999), technical simplicity, requirement of a few nanograms of DNA, no requirement of prior information of the DNA sequence and feasibility of automation (Subudhi and Huang, 1999). In the present work, the genotypes were screened with 15 Random Amplified Polymorphic DNA markers of which finally nine markers were used to characterize the experimental oat genotypes at molecular level and study the genetic divergence between them.

MATERIALS AND METHODS

The plant material used in the present study was collected from different places (Table 1). CTAB procedure was used for isolation of DNA (Doyle and Doyle, 1987). The CTAB-DNA precipitation was washed and purified by treatment with RNAse and quantified by taking the absorbance on Genesys UV spectrophotometer. The PCR amplification was done by screening 15 RAPD primers. 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). Pair-wise similarity and cluster analysis were done on the basis of presence and absence of bands. Computer software (NTSYS) was used to perform the similarity matrix analysis using 'UPGMA' with Jaccard's coefficient of similarity.

RESULTS AND DISCUSSION

In present investigation an attempt has been made to analyze oat genotypes from different geographical blocks which revealed a high level of genetic diversity with the per cent polymorphic bands value at 96.32 per cent. Thus, sufficient variability was

TABLE 1
Plant material used

S. No.	Genotypes	Origin	Pedigree	Year
1.	KENT	Australia	Introduction from USA	1975
2.	WRIGHT	USA	Introduction from USA	-
3.	OS-6	Haryana (India)	HFO 10 x HFO 55	1981
4.	JHO-822	Jhansi (India)	IGO 4268 x Indio-6-5-1	1989
5.	JHO-851	Jhansi (India)	Selection from Japanese germplasm	1998
6.	OL-9	Ludhiana (India)	NP Hybrid x Kent	1987
7.	OL-88	Ludhiana (India)	Derivative of Appler	-
8.	UPO-212	Pantnagar (India)	US 1492 x 1990	1990
9.	UPO-94	Pantnagar (India)	CVRC no. 19E	1981

present among geographically isolated oat cultivars. This could be beneficially exploited in various breeding programmes. Of the 15 RAPD primers screened, nine primers (Table 2) were selected in our analysis for the clear and polymorphic DNA amplification patterns which anmlified 245 bands ranging from 300 to 2400 bp of which 236 bands were polymorphic and five bands were found unique. The unique bands found among different oat genotypes can be used for identification of the respective genotypes. UPO-212 gave unique band at 1800 bp and 1400 with primer 1, JHO-822 gave two unique bands at 1500 and 1700 bp with primer 3 and OS 6 produced unique band at 1350 bp with primer 8 (Fig. 1). These unique PCR amplification products when separated by electrophoresis in agarose gels can be used to derive more complex (Kalendar *et al*., 2006) and specific SCAR/ CAPS marker (Orr and Molnar, 2008).

RAPD employed to categorize the genotypes belonging to different geographical regions and the relationship among different oat genotypes using the genetic similarity value point revealed that OS-6 and KENT and also OL-9 and WRIGHT with genetic similarity value of 0.851 had highest portion of common genetic region, while JHO-851 and OL-9 had the lowest genetic similarity value (0.468) revealing that they are the most diverse pair of genotype used in the experimental material. Based on the estimated genetic similarity matrix in Table 3, the highest genetic similarity value (0.851) was noticed between two pairs, OS-6 and KENT and also in OL-9 and Wright followed by (0.787) between UPO-212 and KENT. The other primers resulted in complex bands, which can assist to discriminate with each other. Above all, it can be anticipated that RAPD-PCR could be exploited as the basis of molecular techniques for oat genotypes in analysis of genetic diversity and too molecular characterization.

Cluster Analysis

Cluster analysis was carried out based on the RAPD data by NTSYSpc 2.11V software, which showed that all oat genotypes studied, could be clustered into two groups breaking at 0.60 Jaccard's coefficient of similarity (Fig. 2). The major gene cluster consisted of six oat genotypes viz., KENT (Australia), OS-6 (India), UPO-212 (Pantnagar, India), JHO-851 (Jhansi, India), OL-88 (Ludhiana, India) and UPO-94 (Pantnagar, India), while the minor gene cluster comprised three oat genotypes viz., WRIGHT (USA), OL-9 (Ludhiana, India) and JHO-822 (Jhansi, India). Within the major gene

TABLE 2
RAPD primer, amplified products and obtained polymorphism detail

Primer (Genei code)	No. of amplified products	Polymorphic band (s)	Monomorphic band (s)	Per cent polymorphism
1. (4013-079)	23	23	0	100.00
2. (4013-080)	40	38	2	95.00
3. (4013-081)	36	34	2	94.44
4. (4013-082)	8	7	1	87.50
5. (4013-083)	17	16	1	94.11
6. (4013-084)	9	9	0	100.00
7. (4013-085)	59	58	1	98.30
8. (4013-086)	50	48	2	96.00
9. (4013-087)	3	3	0	100.00

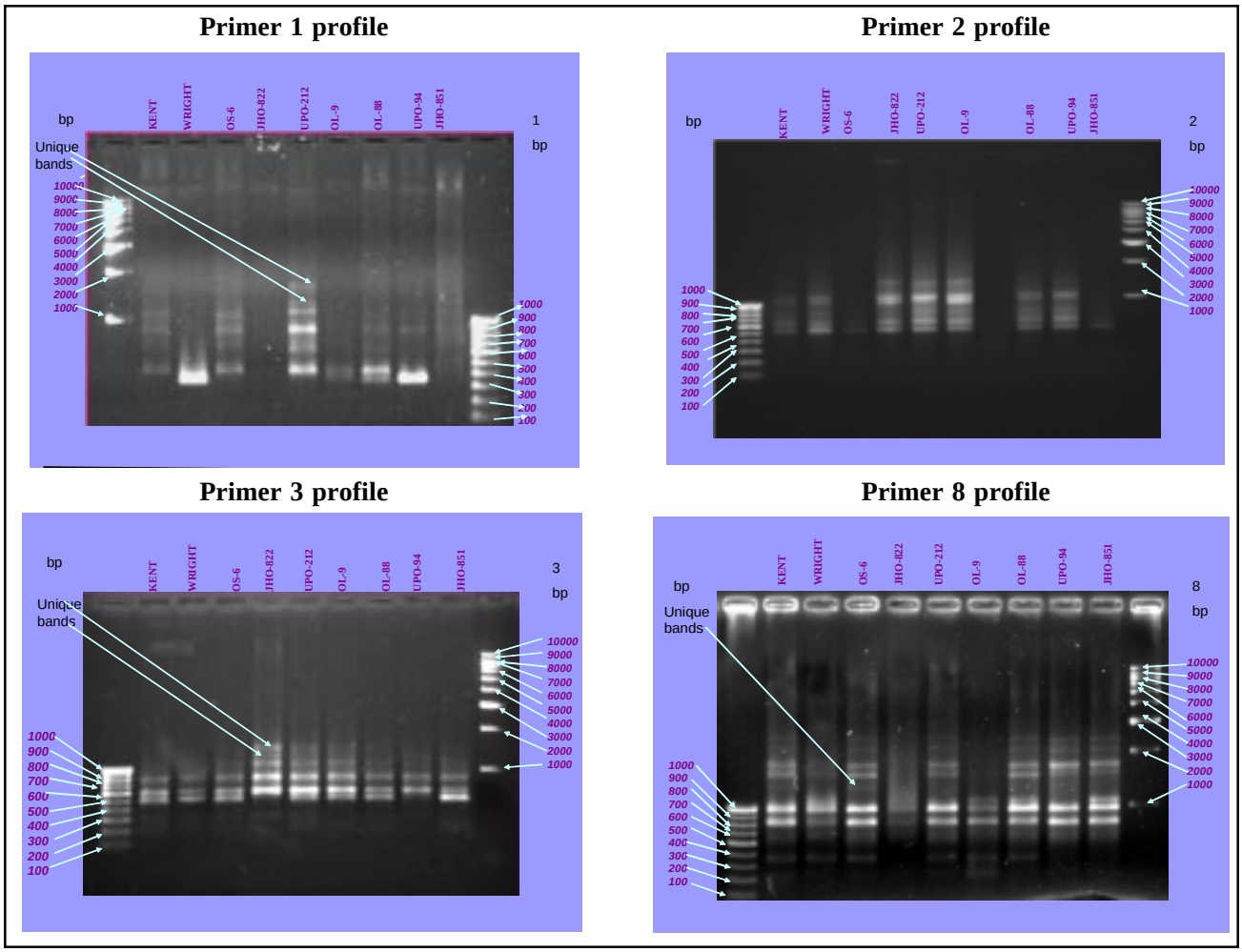

Fig. 1. RAPD profile of oat genotypes generated by the different primers on agarose gel.

TABLE 3
Similarity coefficient between genotypes

	KENT	WRIGHT	OS-6	JHO-822	UPO-212	OL-9	OL-88	UPO-94	JHO-851
KENT	1.000								
WRIGHT	0.659	1.000							
OS-6	0.851	0.553	1.000						
JHO-822	0.638	0.723	0.574	1.000					
UPO-212	0.787	0.574	0.723	0.638	1.000				
OL-9	0.595	0.851	0.531	0.702	0.595	1.000			
OL-88	0.765	0.765	0.659	0.531	0.638	0.744	1.000		
UPO-94	0.744	0.659	0.595	0.638	0.574	0.553	0.765	1.000	
JHO-851	0.702	0.531	0.723	0.510	0.574	0.468	0.595	0.617	1.000

cluster KENT and OS-6 were not further separated indicating the high level of genetic similarity between the two (> 85%), similar case was there with the minor cluster among WRIGHT and OL-9 indicating ancestral relationship between them.

The secondary gene cluster was formed within the major gene cluster at 0.648 Jaccard's coefficient of similarity. The secondary gene cluster divided the major gene cluster into two sub-groups. One of it consisted of four oat genotypes viz., KENT, OS-6, UPO-212 and JHO-851 and the latter consisted of two genotypes OL-88 and UPO-94. KENT and UPO-212 were further close

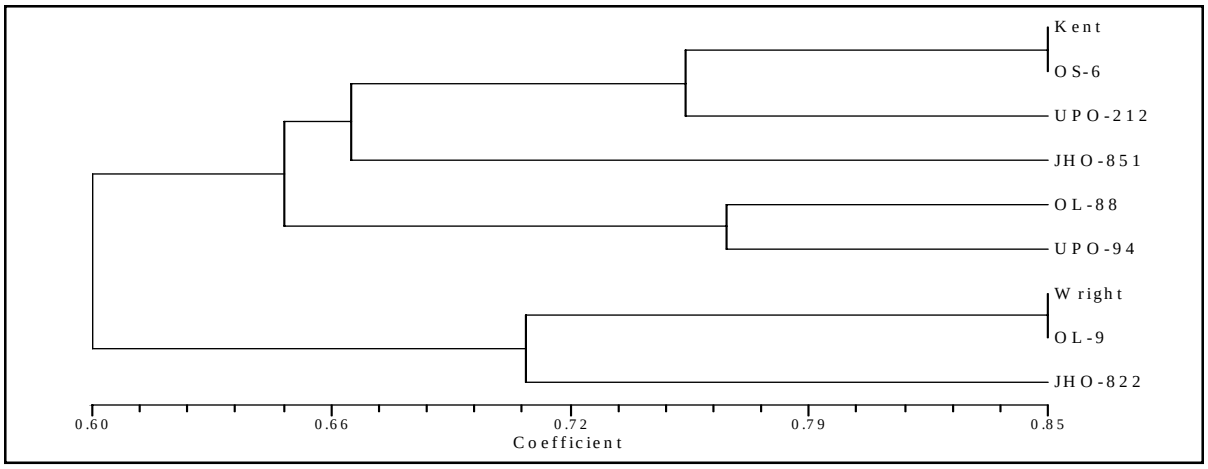

Fig. 2. Dendrogram of genetic distance constructed from RAPD markers.

enough which can be explained by the ancestral relationship as KENT was used as male parent in UPO-212 deriving cross.

To the best of our knowledge, this is one of the few reports analyzing inter-continental genetic diversity in oat genotypes detected by molecular marker. RFLPs, PCR-based SSRs (Pal, 2002), SCAR and CAPS markers in oat genomic region have been developed for various important quality traits and RAPD provided the base line for such results as it could be turned to CAPS easily; these regions may be used in classification of other crops (Orr and Molnar, 2008). RAPD and SSR analysis of wild oats showed high genetic diversity between these genotypes (Zahid *et al*., 2008). The diversity level between the different oats species revealed by RAPD had great significance in oat species conservation and breeding (Loskutov and Perchuk, 2000) and (Wright *et al.,* 2003). In this study, only nine oat genotypes were analyzed, further investigations will include the analysis of more genotypes to allow the resolution of varieties status more detailed 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 oats and also characterization of genotypes.

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