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ANALYSIS OF GENETIC DIVERSITY IN GENUS *AVENA*

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Abstract

Wild oats is the worst weed of wheat worldwide but, the oats are also used as food for people and as feed for animals, especially poultry and horses. Straw of oats is used as animal bedding and sometimes as animal feed. To improve yield and quality of oat, presence of sufficient genetic diversity in the germplasm is an important prerequisite. Due to its nutritional importance 10 varieties of oat were used for genetic diversity analysis. This study will provide a new trend in research that oat can be used as cultivated crop varieties and it has sufficient diversity for improvement like other major crops. On an average, 30.3 alleles per genotype were amplified using RAPD primers. Mean genetic distance estimates ranged from 15 to 81%. Size of scorable fragments ranged from approximately 250 to >1000 bp. A high level of genetic dissimilarity (GD= up to 81%) was estimated among the 10 genotypes. Entries were grouped in clusters using cluster analysis. On the basis of dendrogram, most diverse genotypes were identified for future breeding programs.

Key words: Oat, cultivar, genetic diversity, Randomly Amplified Polymorphic DNA, Cluster analysis, dendrogram.

INTRODUCTION

The genus *Avena* includes world's worst weeds Viz *Avena fauta* and *A. sterilis*, infesting wheat and other small grains but, the cultivated oats (*A. sativa*) is used as human food as well as feed for cattle. Oats are unsuitable for making bread on their own, they are often served as a porridge made from crushed or rolled oats and are also baked into cookies, which can have added wheat flour. As oat flour or oatmeal, they are also used in a variety of other baked goods

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and cold cereals, and as an ingredient in muesli and granola. Oats may also be consumed raw, and cookies with raw oats are becoming popular. Oats also have non-food uses. Oat straw is also used in corn dolly making, and it is the favourite filling for home made lace pillows. Oat extract can be used to soothe the skin conditions, e.g. in baths, skin products, etc.

Like any other crop species the first step in oat improvement is full assessment of the local materials, including collection, evaluation and molecular characterization of germplasm lines. Often, local varieties of crops are of excellent quality and flavour, have a good level of resistance to pests and diseases and may be superior to exotic materials (Williams *et al*., 1991). Knowledge about germplasm diversity and genetic relationships among breeding materials could be an valuable aid in crop improvement strategies. A number of methods are currently available for analysis of genetic diversity in germplasm accessions, breeding lines and segregating populations. These methods have relied on pedigree data, morphological data, agronomic performance data, biochemical data, and molecular (DNA-based) data **(**Mohammadi and Prasanna, 2003). Accurate assessment of the levels and patterns of genetic diversity can be valuable in crop breeding for diverse applications including (i) analysis of genetic variability in cultivars (Smith, 1984; Cox *et al*., 1986), (ii) identifying diverse parental combinations to create segregating progenies with maximum genetic variability for further selection (Barrett and Kidwell, 1998), and (iii) introgressing desirable genes from diverse germplasm into the available genetic base (Thompson *et al*., 1998). Significant emphasis is being paid to comprehensive analysis of genetic diversity in numerous crops, including major and minor field crops.

The use of DNA marker technology in varietal improvement has progressed rapidly during the last decade (Hoisington *et al*., 1998). The discovery and use of molecular markers based on DNA differentiation offers great opportunity to understand and identify the diverse genetic material in crop species. Molecular markers detect variation of the DNA sequences among cultivars and therefore directly bypass problems related with environmental effects and thus has many applications of value to crop improvement. Several molecular marker types are available and they each have their advantages and disadvantages. DNA-based markers have shown promise in expediting plant-breeding procedures. It would be useful to identify the genetic diversity prevailing in the local germplasm through the use of DNA based marker systems.

Molecular markers, such as isozymes, restriction fragment length polymorphisms (RFLP) and randomly amplified polymorphic DNA (RAPD) have been used to detect genetic differences in species. Among these molecular markers, RAPD, this was introduced by the use of polymerase chain reaction (PCR) with arbitrary 10-mer primers (Williams, J.G.K. 1990). The use of RAPD has been quite helpful in distinguishing species and identifying genetic variations between and within populations. RAPD markers can be efficient for the study of genetic variability because they make possible the random amplification of many genome regions that can be compared simultaneously. Furthermore, RAPD technique does not require large amounts of DNA nor any previous knowledge about the genome sequences of the species under investigation (Eloisa *et al., 2006*) and can express DNA variations for distinguishing species with less labor and high reliability.

The study of genetic diversity to identify groups with similar genotypes is important for conserving, evaluating and utilizing genetic resources for studying the diversity of pre-breeding and breeding germplasm and for determining the uniqueness and distinctness of the phenotypic and genetic constitution of genotypes with the purpose of protecting a breeder intellectual property rights (Franco *et al.* 2001). The aims of this research were to study genetic diversity in different oat cultivars using RAPD marker.

MATERIALS AND METHODS

Crop Science department, National Agricultural Research Center, Islamabad, Pakistan, very kindly provided seed material used during present study. The seeds were planted in green house of Biotechnology Department, Balochistan University of Information Technology and Management Sciences, Quetta and recommended agricultural practices were carried out. Ten oat varieties cultivated in different regions of the Pakistan were selected for the study of molecular genetic diversity. Ten varieties included in the present study were Palestine, Fatua, Algerian, W.No.11, DN-8, Svon, PD2, Sargodha81, LV65 and S-2000.

Leaf samples were used to isolate total genomic DNA following the protocol described by Weining and Langridge (1991). To remove RNA, DNA was treated with 40 micro-grams RNAse-A at 37°C for 1 hour and samples were stored at 4°C. To use in Polymerase Chain Reaction (PCR) a 1:5 dilution of DNA was made in doubled distilled, deionized and autoclaved water. Ten Randomly Amplified Polymorphic DNA primers (GL-A03, -A04, -A12, -B07, -B19, -C07, -D17, -D18, -

E05 and –E08, purchased from GeneLink, Inc. NY 10532, USA, Table-1) were used. PCR reactions were carried out in 25 μl reaction using protocols of (Devos and Gale, 1992) recommended for wheat. Amplification conditions involved an initial denaturation step of 4 min at 94°C followed by 40 cycles each consisting of a denaturation step of 1 min at 94°C, followed by an annealing step of 1 min at 34°C and an extension step of 2 min at 72°C. The last cycle was followed by 7 minutes extension at 72°C. Depending on the primer sets used, the protocol provided by the primer set supplier was followed. All amplification reactions were performed using the GeneAmp PCR system 2700 (Applied Biosystem). The amplification products were electrophoresed on 2.0% agarose/ TBE gels and visualized by staining with ethidium bromide and viewed under UV light.

For analysis of genetic diversity, every scorable band was considered as single allele / locus and was scored as present (1) or absent (0) (Nei and Li, 1979). The bivariate 1-0 data were used to estimate genetic distances (G.D) following "Unweighted Pair Group of Arithmetic Mean (UPGMA)" procedures described by Nei and Li (1979) and to construct a dendrogram using computer program "PopGene32" version 1.31 http://www.ualberta.ca./fyeh/fyeh).

Table-1. Name, sequence, size, molecular weight and %GC content of 10 RAPD primers used to study genetic diversity in oat cultivars.

S. No.	Oligo Name	Sequence	Size	Molecular weight	%GC
1	GLA-03	AGTCAGCCAC	10	2996.98	60
2	GLA-04	AATCGGGCTG	10	3068.02	60
3	GLA-12	TCGGCGATAG	10	3068.02	60
4	GLB-07	GGTGACGCAG	10	3093.03	70
5	GLB-19	ACCCCCGAAG	10	2981.97	70
6	GLC-07	GTCCCGACGA	10	3012.99	70
7	GLD-17	TTTCCCACGG	10	2978.98	60
8	GLD-18	GAGAGCCAAC	10	3046	60
9	GLE-05	TCAGGGAGGT	10	3108.04	60
10	GLE-08	TCACCACGGT	10	2987.98	60

RESULTS AND DISCUSSION

An example of PCR amplification profile of ten oat genotypes using RAPD primer GL-D18 is presented in Fig.-1. Molecular sizes of

amplified fragments ranged from approximately 250 to more than 1000 bp. In an earlier report Chen et al., (2000) observed 900 -1600 bp fragment size amplified using RAPD primers in different sub-species of different crops. During present study a total of 303 DNA fragments were amplified in 10 oat genotypes using ten RAPD primers, giving an average of 30.3 alleles per genotype. The results of genetic dissimilarity analyses showed that extensive genetic diversity (average G.D. ranging from 15 - 81%) existed in 10 genotypes used during present study (Table-2). Most of the comparisons showed moderate estimates of genetic distance using "UPGMA" method. The detection of moderate level of genetic diversity during present study was in agreement with the previous reports where RAPD and SSR markers detected high level of genetic polymorphsim (Welsh and McClelland 1990; Dos Santos *et al*., 1994) in different crop species.

The bivariate (1-0) data and dissimilarity coefficient matrices of ten oat varieties based on the data of 10 RAPD primers (using UPGMA method (Nei and Li, 1979) were used to construct dendrogram using computer program "popgene32" (Fig.-2). For the dendrogram constructed from data using RAPDs, the genotypes were grouped in five main groups (A, B, C, D and E) (Fig.-2). Groups C and E were smallest comprised of 1 genotype only. Groups A and D were the largest consisted of 3 genotypes and group B comprised of 2 genotypes. Based on the dendrogram analyses, variety Palestine and LV65 was most distantly related from one another. In genetic diversity analyses the comparisons among the 2 genotypes (Palestine and LV65) showed high estimate of genetic distance (GD = 65 %). This finding was further strengthened by average genetic diversity analyses (Table-2) where the two genotypes showed higher levels of genetic dissimilarity with rest of the genotypes used during present studies. The polymorphism in the 10 oat varieties were analyzed in the form of dendrogram and total genetic distance analysis. Those varieties which have high genetic distance and away from each other on the dendrogram are more genetic diverse, and these polymorphism in the form of bands presence and absence were used as the basis for genetic variation within a species.

Present findings further strengthened previous reports (Hallden *et al.,* 1994; Chen *et al.,* 2000) that the RAPD and SSR markers can be used effectively to estimate genetic distances among genotypes/lines/cross combinations. This genetic variation data also support cultivation of oat as a crop because like other nutritious crop it has a lot of desirable characteristics which through proper breeding program can be used for the welfare of human beings. However, it is suggested that more molecular data is required to have better

understanding of the presence of genetic variability in oat germplasm and consequently more efficient utilization of existing variability for improvement of oat crop in Pakistan.

Table-2. Average estimates of genetic distances among 10 oat varieties using 10 RAPD primers.

	Palestine	Fatua	Algerian	W.no.11	DN-8	Svon	PD2	Sargodha81	LV65
Palestine									
Fatua	0.48								
Algerian	0.45	0.47							
W.no.11	0.41	0.25	0.49						
DN-8	0.56	0.60	0.52	0.64					
Svon	0.52	0.49	0.47	0.38	0.64				
PD2	0.61	0.60	0.72	0.57	0.67	0.72			
Sargodha81	0.62	0.53	0.54	0.61	0.40	0.64	0.63		
LV65	0.65	0.65	0.70	0.43	0.81	0.61	0.55	0.81	
S-2000	0.37	0.32	0.51	0.15	0.60	0.30	0.54	0.54	0.36

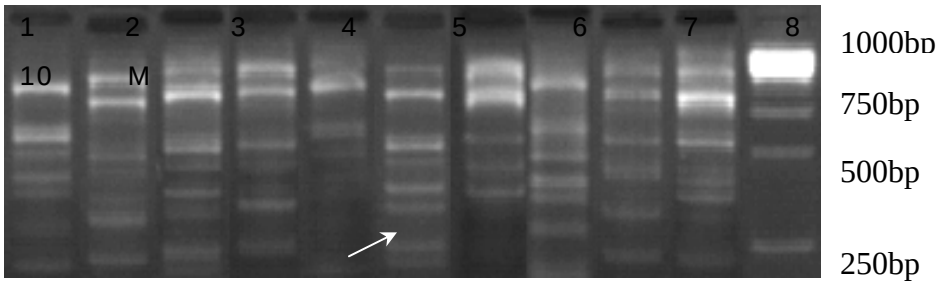

Fig.-1A. PCR amplification profile of 10 oat varieties using RAPD primer D18.
1= Palestine, 2= Fatua, 3= Algerian, 4= W.No.11, 5= DN-8, 6= Svon, 7= PD2, 8= Sargodha81, 9= LV65, 10= S-2000
M = Molecular size marker (1 Kb ladder). Molecular sizes (in bp) are given on right.

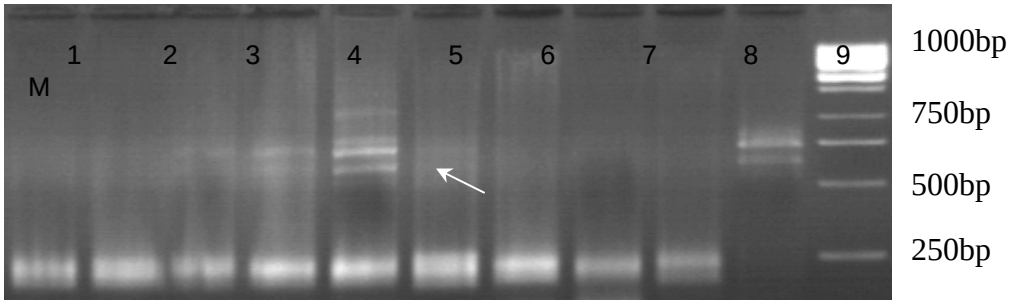

Fig.-1B. PCR amplification profile of 10 oat varieties using RAPD primer E08.
1= Palestine, 2= Fatua, 3= Algerian, 4= W.no.11, 5= DN-8, 6= Svon, 7= PD2, 8= Sargodha81, 9= LV65, 10= S-2000
M = Molecular size marker (1 Kb ladder). Molecular sizes (in bp) are given on right.

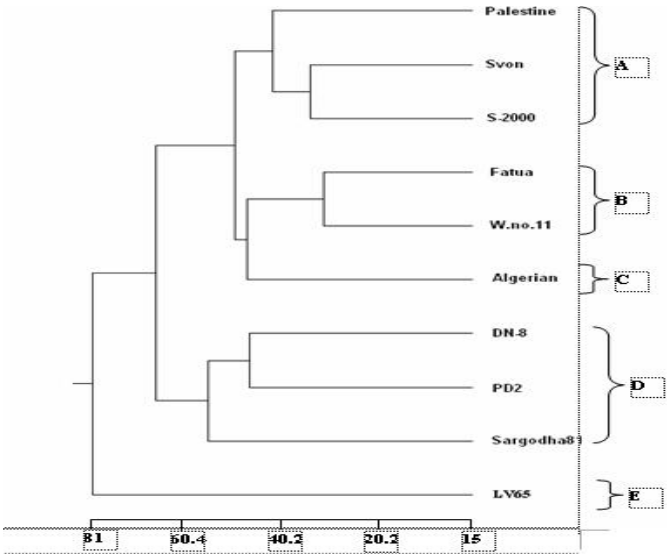

Fig.-2. Dendrogram constructed for 10 oat varieties using 10 RAPD primers.

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