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RESEARCH ARTICLE

ASSESSMENT OF GENETIC VARIATION AMONG DIFFERENT ACCESSIONS OF OAT (*AVENA SATIVA* L.) USING RAPD MARKERS

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ABSTRACT

The present study was carried out to assess the genetic variation in oats (*Avena sativa* L.) at DNA level. The three decamer primers, RAPD primers (GLD-18, GLE-08 and GLA-03) were used to estimate genetic diversity in *Avena sativa* genotypes. The assay revealed a large amount (65%) of polymorphism. The total of 20 bands were scored for three RAPD primers ranging from 4-9 corresponding to an average of 4.2 bands per primer and 13 bands of these were polymorphic. Polymorphic bands for each primer ranged from 0-100%. One primer out of three generated a total of 9 bands of which 9 (100%) were scored as polymorphic. Similarity matrix reveals the maximum similarity between varieties JHO 99-1 (S1) with JHO 822 (S3) and JHO 851 (S4) with JHO 2000-4 (S5) (Similarity Indices 0.880 and 0.903 respectively). While distantly related varieties were JHO 99-2 (S2) and JHO 99-1 (S1) (similarity indices 0.609). The RAPD cluster showed two major clusters namely cluster-I and cluster-II comprising of two varieties each. Cluster-I includes two cultivars namely JHO 99-1 (S1) and JHO 822 (S3). The major cluster-II includes JHO 851 (S4) and JHO 2000-4 (S5). The cultivar JHO 99-2 (S2) occupies a distinct place as revealed in the dendogram constructed. In present investigation RAPD markers revealed high degree of polymorphism (100%) among the five accessions of *Avena* varieties using GLE-08.

INTRODUCTION

Poaceae, formerly called Gramineae, grass family of monocotyledonous flowering plants, a division of the order Poales. They rank among the top five families of flowering plants in terms of the number of species, but they are clearly the most abundant and important family of the Earth's flora. They grow on all continents, in desert to freshwater and marine habitats, and at all but the highest elevations. Plant communities dominated by grasses account for about 24 percent of the Earth's vegetation (Richard W. Pohl, 1978). One of the important genus of the family Poaceae is *Avena* (Oats). Oats (*Avena species*) belongs to family Gramineae / Poaceae. The genus *Avena* L. (Poaceae) belongs to the tribe Aveneae, and contains diploid, tetraploid and hexaploid species, with the basic chromosome number of seven (x=7). All species are self-pollinated annuals that form bivalent at meiosis and have disomic inheritance, with the exception of *A. macrostachya*, which is an out breeding quadrivalent-forming, autotetraploid perennial (Katsiotis *et al.*, 1996). The fundamental phenotypic difference between hulled and hull-less oat gave rise to a formal taxonomical distinction at the subspecies level. Hulled oat is named *A. sativa* subsp. *sativa* and hull-less oat *A. sativa* subsp. *nudisativa* (Rodionova *et al.*, 1994). Molecular investigations showed that *A. sativa* was derived from *A. sterilis* (Zhou *et al*., 1999; Jellen and Beard, 1998). The cultivated oats (A. *sativa)* is used as human food as well as feed for cattle. Oat extract can be used to soothe the skin conditions, e.g. in baths, skin products, etc. (Abbas *et al*., 2008). In the past, this plant was used in India to help opium, morphine and cigarette addicts kick their habits. Green oats are thought to have a sedative effect and are also useful when trying to stop smoking by reducing the craving to smoke. Oat straw liquid extract could also help tobacco users kick the habit by easing withdrawal from nicotine. Wild oats (*Avena species*) are among the most troublesome weed species in grain crops (Muur, J. 1999). These species act as highly competitive weeds with wheat and can cause up to 60% yield loss. The most important reason for their control failure is due to their biodiversity. Biodiversity is most typically seen as genetic polymorphism: the heterogeneity among and within weed species (Khan *et al*., 2010). For many years, the principles of genetics have been applied to crop variety improvement with great success. The utility of DNA-based diagnostic markers is determined to a large extent by the technology that is used to reveal DNA-based polymorphisms. 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, 1990). 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. Over the last few years, polymerase chain reaction technology has led to the development of several nove1 genetic assays based on selective DNA amplification (Krawetz, 1989). 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.

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MATERIALS AND METHODS

The seeds of five accessions (JHO 99-1, JHO 99-2, JHO 822, JHO 851, and JHO 2000-4) were obtained from Indian Grassland and Fodder Research Institute (IGFRI) Jhansi (U.P.) to study the genetic variation based on RAPD markers. RAPD analysis was conducted at Indian Grassland and Fodder Research Institute (IGFRI) Jhansi (U.P.) India, under controlled conditions during 2012.

Isolation of Genomic DNA

Genomic DNA was isolated from seeds using CTAB based method, analyzed and quantified by standard methods.

Polymerase chain Reaction

The three decamer primers**,** GLD-18 (GAGAGCCAAC), GLE-08 (TCACCACGCT), and GLA-03 (AGTCAGCCAC) used for RAPD analysis in *Avena sativa* were selected on the basis of prior knowledge. Polymerase chain reaction (PCR) is a technique used to selectively amplify *in vitro* a specific segment of the total genomic DNA a billon fold. The most requirement of PCR is the availability of a pair of short (typically 20 - 25 nt) oligonucleotide called primers, having sequence complementary to either end of the target DNA segment (called template DNA) to be synthesized in large amount. In a sterile 0.2 ml thin wall PCR tube, following components of the reaction were mixed in the order as Water 18.5 µl, Buffer $Mgcl_2$ 2.5 µl, dNTP mix 1 µl, Taq DNA Polymerase 1 µl (1unit), and Primer 1 µl 6. DNA (50ng) 1 µl, Total 25 µl. A positive control was prepared by adding the vector DNA carrying the gene construct to the reaction mixture instead of the target genomic DNA (presently DNA of the transgenic plant). Negative control tubes were also prepared by adding DNA from the transformed control plant to one tube and no DNA to another tube containing all other PCR components. As the number of PCR cycle increases the amount of the target DNA synthesized increases exponentially. (Table 2.)The PCR involves three basic steps which constitute single cycle. Thermo cycling of DNA was carried out for 39 cycles.1.5% agarose gel was prepared in 0.5 x TE buffer. 2.5 µl gel loading dye was added to each tube containing the amplified DNA. Samples were loaded and electrophoresed at 50 v for 3 hours. The gel was stained with ethidium bromide solution (0.5 µg/ml) then after the gel was visualized under U.V light, band position of amplified DNA were compared with that of the positive control. Then the gel was photographed.

Table 1. Sequence information of primer used with molecular weights.

S.NO	PRIMER	SEQUENCE	SIZE	MOLECULAR WEIGHT
1	GLD-18	GAGAGCCAAC	10bp	3046
2	GLE-08	TCACCACGCT	10bp	2987.98
3	GLA-03	AGTCAGCCAC	10bp	2996.98

Table 2. PCR Thermal Profile

Step	Temperature	Duration
Hot Start	94^0C	5 min
Denaturation	94^0C	1 min
Primer annealing	35^0C	2 min
Extension	72^0C	2 min
Final extension	72^0C	10 min

Data Analysis

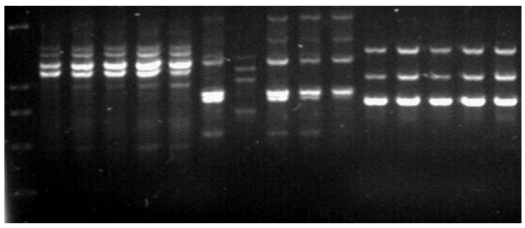
The amplification products were scored for each accesssion because of presence and absence of band .i,e use of binary code 1 and 0 for the presence or absence of band, respectively. Molecular size (bp) of amplified DNA fragment was determined by 100- 300bp ladder. DNA fragment analyses were performed using the SPSS 12.0 computer software.

RESULTS AND DISCUSSION

Random Amplified Polymorphic DNAs (RAPDs) analyze widely used or detecting genetic polymorphism between genotypes at molecular level in many crop species. During the present study three RAPD primers (GLD-18, GLE-08 and GLA-03) were used to estimate genetic diversity in *Avena sativa* genotypes. The assay revealed a large amount (65%) of polymorphism. The bands obtained either polymorphic or monomorphic. The total of 20 bands was scored for three RAPD primers ranging from 4-9 corresponding to an average of 4.2 bands per primer and 13 bands of these were polymorphic. Polymorphic bands for each primer ranged from 0-100%. Primer GLE-08 generated a total of 9 bands of which 9 (100%) were scored as polymorphic. GLD-18 primer generated a total of 7 bands of which 4 **(**57.5%) were scored as polymorphic. GLA-03 primer generated 4 bands of which no one was scored as polymorphic. Therefore the total polymorphism observed using three bands was 65% **(**Table 3**)**. For individual RAPD primers, higher level of genetic polymorphism among the *Avena sativa* varieties was found in case of GLE-08 primer, where higher levels of genetic variability were observed among different comparison indicating its power for identification of individual genotypes. Further the similarity index revealed the maximum similarity between varieties JHO 99-1 (S1) with JHO 822 (S3) and JHO 851 (S4) with JHO 2000-4 (S5) (Similarity Indices 0.880 and 0.903 respectively). While distantly related varieties were JHO 99-2 (S2) and JHO 99-1 (S1) (similarity indices 0.609). (Table 4.)

Table 4. Dice's similarity matrix

	S1	S2	S3	S4	S5
S1	1	0.609	0.880	0.815	0.692
S2		1	0.615	0.643	0.667
S3			1	0.867	0.828
S4				1	0.903
S5					1



M GLD 18 GLE 08 GLA 03

Fig. 1. RAPD profile of five Accessions of*Avena sativa* generated using primers: GLD 18, GLE 08 and GLA 03

Table 3. Total number of Amplified bands and number of polymorphic bands Generated by PCR, Using three randomly selected primers

Name of primers	Nucleotide sequence(5-3`)	Polymorphic bands	Monomorphic bands	%G+C Content	Total bands	Polymorphism
GLD-18	GAGAGCCAAC	4	3	60%	7	57.14%
GLE-08	TCACCACGCT	9	0	60%	9	100%
GLA-03	AGTCAGCCAC	0	4	60%	4	0%
	TOTAL	13	7		20	65%

The RAPD cluster showed two major clusters namely cluster-I and cluster-II comprising of two and two varieties each. Cluster-I includes two cultivars namely JHO 99-1 (S1) and JHO 822 (S3). The major cluster-II includes JHO 851 (S4) and JHO 2000-4 (S5). The cultivar JHO 99-2 (S2) occupies a distinct place as revealed in the dendogram. In present investigation RAPD markers revealed high degree of Polymorphism (100%) among the five accessions of Avena varieties using GLE-08. It is recommended that genetically distant lines observed among the five *Avena sativa* genotype should be used in future breeding programmes for improving yield and quality characteristics of *Avena sativa*. Further it was observed that PCR based assays like RAPD can be used effectively to estimate genetic variability in *Avena sativa* and considering easy handling of the technique, they are specially suitable for breeding programme for

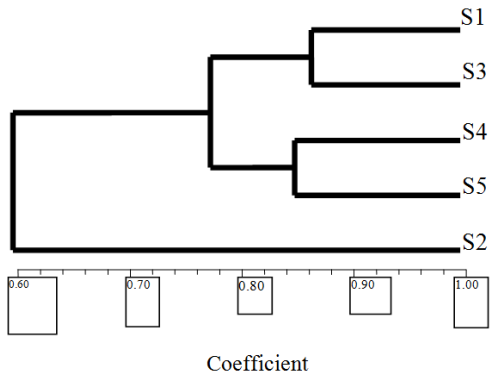


Fig. 2. Dendrogram based on UPGMA showing genetic relationship among five accessions of *Avena sativa* based on Dice's similarity coefficient estimated for RAPD data

where large number of lines/accessions have to be analyzed. The findings are in correspondence with that of other workers. Abbas *et al*., (2008) while investigating genetic diversity in 10 varieties of *Avena sativa* observed considerable amount of genetic variation in case of genetic characters. Prasad *et al.*, (2003) observed same genetic variability in different varieties of *Avena sativa.* Iannucci *et al*., (2011) also observed high level of genetic variability in *Avena sativa.*

Conclusion

The response in the grouping of the accessions based RAPD analysis appears to be under Genetic control leading to the expression of the new genes due to the changing environmental conditions that leads to the adaptation of the plant species. It may be also noticed to varying geographical locations which lead to the expression of the different proteins and hence new genes. 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. It may be concluded that genetic variations exist among the studied cultivars of *Avena sativa* analyzed. Furthermore the results concluded that data can be elaborated to complete profiling of genetic diversity of *Avena sativa* and select more promising lines for the improvement of the crop. Such information may be useful for selecting the diverse parents and monitoring the genetic diversity periodically in breeder's collection of *Avena sativa*. On the basis of present investigation it is concluded that RAPD markers revealed considerable amount of genetic diversity in *Avena sativa.* It is suggested that biochemical and molecular markers, RAPD is the best option to expose inter and intra cultivars variation and is needed to be extend to more germplasm and primers for further study along with morphological traits analysis.

List of Abbreviations

CTAB: Cetyltrimethylammonium Bromide
RAPD: Randomly Amplified Polymorphic DNA
AFLP: Amplified Fragment Length Polymorphism
SSR: Simple Sequence Repeats
UPGMA: Unweighted Paired Group Method Using Arithmetic Averages

Author's Contributions

The work is original and it has been carried out by THR for M.Phil. degree under supervision of RDK at Bundelkhand University, Jhansi (U.P.), India, and at Indian Grassland of Fodder Research Institute (IGFRI), Jhansi (U.P.) India.

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