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AGRONOMIC AND POLYMORPHISM STUDY OF WILD OAT (*AVENA FATUA* L.) BIOTYPES AT DNA LEVEL

IMTIAZ KHAN[1*], GUL HASSAN[1], MUHAMMAD ISHFAQ KHAN[1], NAQIB ULLAH KHAN[2] AND KHAN BAHADER MARWAT[1]

[1]Department of Weed Science, NWFP Agricultural University Peshawar 25130, Pakistan
[2]Department of Plant Breeding and Genetics, NWFP Agricultural University Peshawar
[*]Corresponding author E-mail: imtiazagri@yahoo.com

Abstract

Laboratory studies were conducted to compare the biotypes of wild oats collected across North West Frontier Province (NWFP) and Federal capital areas of Pakistan. The wild oats biotypes included were named by their area from where collected i.e., Mardan, Malakand, Karak, D.I.Khan, NARC-Islamabad, Charsada, Peshawar, Swat and Kohat. The data were recorded for plant height (cm), leaf lamina width (cm), leaf lamina length (cm), number of leaves plant^{-1} and polymorphism at DNA level. Different biotypes appeared to differ in their competitive ability, as due to variation in agronomic parameters. Range of amplified fragments was from < 250 to > 1000 bp in size. The degree of genetic polymorphism ranged from 0 to 100%, indicating that the studied germplasm of wild oat is genetically very diverse and possesses a higher degree of polymorphism. Consequently their competitive ability will also be varying. Thus, in the areas of the more competitive wild oats biotypes more attention will be needed on the control of wild oats as compared to the less competitive genotypes. Alternately, if using wild oat as a food or feed crop, then based on genetic distance estimates and dendrograms analysis, the most diverse biotypes could be used in future breeding programs aimed at improving genetic variability of wild oat germplasm.

Introduction

Wild oats (*Avena* spp.*)* is one of the most troublesome weed species in grain fields (Muur, 1999). Wild oats is highly competitive weed with wheat and can cause up to 60% yield loss. Due to resemblance with wheat at seedling stage, wild oats is difficult to control manually; therefore, herbicide application becomes inevitable. The most important cause of weed control failure is due to weed biodiversity. Biodiversity is most typically seen as genetic polymorphism: the heterogeneity among and within weed species (Khan *et al*., 2007).

Genetic diversity is of prime importance for the survival, adaptation to certain agro-climatic conditions, success and improvement of any crop species. If there is not enough genetic diversity in the germplasm, it is practically possible to increase the yield and other desirable characters of the crop, because selection for the improved genotypes depends on the availability of genetic variability within the breeding material. There is a dire need to characterize the indigenous germplasm of wild oat using available methods. Genetic diversity is, therefore, a key component for conservation efforts associated with population management (Andayani *et al*., 2001).

Salem *et al*., (2007 summarize numerous studies on the use of the random amplified polymorphic DNA (RAPD) technique on rice, corn, wheat, sorghum, barley, rye and oats to examine its feasibility and validity for assessment of genetic variation, population genetics, mapping, linkage and marker assisted selection, phylogenetic analysis, and the detection of somaclonal variation. Molecular markers have entered the scene of genetic

improvement in different fields of agricultural research. The simplicity of the RAPD technique made it ideal for genetic mapping, plant and animal breeding programs, and DNA fingerprinting, with particular utility in the field of population genetics. But no such work has been undertaken in Pakistan previously. Hence, the research was conducted with these objectives:

1. To evaluate the biotypes for physiological and agronomic traits.
2. To caution the weed managers regarding the competitive genotypes.
3. To evaluate the genetic diversity in wild oat biotypes using molecular markers.

Materials and Methods

1. Whole plant studies: Ten biotypes of wild oats (*Avena fatua* L.) were used in present study. The seeds were collected from different districts of NWFP (D.I. Khan, Karak, Peshawar, Charsadda, Mardan, Malakand, Swat and Kohat and Federal Capital areas (NARC, Islamabad), Pakistan. The studies were conducted at Weed Science Research Laboratory, Department of Weed Science, NWFP Agricultural University Peshawar, Pakistan during 2004-2005 (Table 1a). Ten seeds of each biotype were placed in plastic pots having 15 cm dia. and 12 cm depth. From the start of the experiment, the required quantity of water was applied to each pot.

1.1. Plant height (cm): For recording wild oats plant height (cm), plant height of all the plants in each biotype was measured from the ground surface in the pot to the growing point at physiological maturity and then the average was taken.

1.2. Lamina width (cm): For recording wild oat lamina width (cm) of all the leaves of a plant in pot with the help of a scale, then average was taken and recorded.

1.3. Lamina length (cm): For recording wild oat lamina length (cm), all the leaves of plants in a pot were measured with the help of a scale, then average was computed and recorded.

1.4. Number of leaves plant^{-1}: For recording number of leaves plant^{-1}, # of leaves in each biotype were counted in all plants in a pot and then average was taken for each biotype.

2. Molecular level studies:

2.1. Raising of plant material: The seeds of the above referred biotypes in the whole plant experiment were sown in plastic pots under the protocol narrated in the whole plant experiment. The plants were raised under the greenhouse environment, at the Institute of Biotechnology and Genetic Engineering, NWFP Agricultural University, Peshawar. The plant material was collected by harvesting leaves from each treatment at 4-leaf stage and subjecting to further analyses.

2.2. Primers used: Details of RAPD primers are provided in Table 1b. Laboratory and DNA level studies were conducted in the Institute of Biotechnology and Genetic Engineering (IBGE), NWFP Agricultural University, Peshawar.

Table 1a. Different biotypes of wild oat collected from various District of NWFP.

S. No.	Name of biotype	Biotype code	Collection site
1.	D.I. Khan White	DW	Thatha Balochan, D.I. Khan
2.	D.I. Khan Black	DB	Rakh Shah Kot, D.I. Khan
3.	Karak White	KW	Lakki Ghundakki, Karak
4.	Karak Black	KB	Babul Khel, Karak
5.	Peshawar	P	Malkandher Farm, Peshawar
6.	Charsadda	C	Mvliano Kala, Charsadda
7.	Mardan	M	Lundkhawar, Mardan
8.	Malakand	MK	Dargai, Malakand
9.	Swat	S	Batkhela, Swat
10.	NARC, Islamabad	ISL	Islamabad

Table 1b. Detail of RAPD primers.

S. No.	Name	Sequence	Size	Mol. Wt.	% 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.	GLC-07	GTCCCGACGA	10	3012.99	70
5.	GLB-19	ACCCCCGAAG	10	2981.97	70

2. 3. DNA extraction protocol:

a. Collect 10 cm long fresh leaf.
b. Put in Liquid Nitrogen.
c. Crush leaf material to fine powder.
d. Add 500 µl DNA extraction buffer and mix well.
e. Add 500 µl of Phenol-Chlorofom-isoamyalcohol (25:24:1) and mix until homogenous mixture is obtained.
f. Centrifuge at 5000 rpm for 5 minutes.
g. Take supernatant in a fresh tube.
h. To precipitate DNA, add 50 µl 3M Sodium acetate (*pH* 4.8) and 500 µl isopropanol and mix gently.
i. To make pellet, centrifuge 5000 rpm for 5 minutes.
j. Discard supernatant, dried at for 1 hour at 37^0C and add 40 µl TE buffer to the pellet.

For genetic diversity, 5 Randomly Amplified Polymorphic DNA (RAPD) primers viz., GLA-03, GLA-04, GLA-12, GLC-07 and GLB-19 (Table 1b) were used in the studies. For statistical analysis, each band was considered as single locus / allele. Only scorable bands were included in the analysis. Bands were scored as present (1) / absent (0), and a bi-variate (1-0) data matrix was generated. Genetic distances were calculated using Unweighted Pair Group of Arithmetic Means (UPGMA) procedure as suggested by Nei & Li (1979).

$$GDxy = 1 - Nxy \; / \; Nx + Ny - Nxy$$

Results and Discussion

1. Whole plant studies: The data analysis of different morphological characters showed significant differences in mean values for all the traits except leaf lamina width (Table 2). The maximum plant height was recorded in wild oats biotype of Malakand (21.22 cm) followed by Mardan (16.38 cm) and D.I. Khan (15.58 cm). While wild oats biotypes of NARC-Islamabad and Swat fail to germinate. The lowest (7.70 cm) plant height was recorded in biotypes of Peshawar. Data for mean values of different wild oat biotypes for leaf lamina width showed that maximum lamina width was recorded in wild oat biotype of Karak (0.530 cm) which was found statistically at par with other biotypes and ranged from 0.390 to 0.496 cm. (Table 2). The lowest leaf Lamina width was recorded in Mardan and D.I.Khan biotypes (0.390 cm). Data regarding the effect of different wild oat biotypes on leaf lamina length (cm) revealed that maximum leaf lamina length was noticed in Peshawar wild oats biotype (13.20 cm) and Charsadda (12.67 cm) followed by Malakand (11.17). The lowest leaf lamina length was recorded in D.I. Khan (9.160 cm) oats biotype. In case of number of leaves plant^{-1} (Table 2), data manifested that maximum number of leaves plant^{-1} were recorded in Kohat (5.40) and Karak (5.10) wild oat biotypes. The lowest number of leaves plant^{-1} was recorded in D.I.Khan wild oats biotype (3.70). The number of leaves plant^{-1} of all wild oats biotypes statistically comparable to each other except NARC-Islamabad and Swat, which fail to germinate. The canopy development has an enormous role in determining the competitive ability of different species and biotypes. Thus, although the trait under reference was statistically having no differences in mean values, yet a spread in data indicates a differential competitive status of various biotypes. Our finding are great analogy with the result of Hassan & Khan (2007) and Khan et al (2008) who reported that different wild oats biotypes contemplated exhibit that they differ in their competitive ability as their performance varied in the morphological and physiological and agronomic parameters. Tessema & Tanner (1997) reported that plant height was most closely associated with weed competitive ability of different wild oats biotypes.

Molecular level polymorphism studies: For genetic diversity, five Randomly Amplified Polymorphic DNA (RAPD) primers viz., GLA-03, GLA-04, GLA-12, GLC-07 and GLB-19 were used in the studies. The banding pattern obtained by using RAPD primer Genelink-A03 is presented in Fig. 1. All the biotypes of wild oat showed various levels of genetic polymorphism for the loci detected using prime GL-A03. A total of 21 alleles (bands) were amplified in 10 biotypes giving an average of 2.1 alleles per biotype. The size of amplified fragments, estimated by using a 1kb DNA ladder, ranged from > 250 to >1000 bp. Genetic distances (GD) among the 10 biotypes are presented in Table 3, and range from 0% to 75%. Maximum genetic distance (75%) was estimated for 4 comparisons viz D.I. Khan (Black)-D.I.Khan (white), D.I.Khan (black)-Peshawar, D.I.Khan (black)-NARC (Islamabad) and Peshawar-Swat, while 4 comparisons showed homozygosity for the loci amplified by prime GL-A03. Analogous results are also reported by Chauvel & Gasquez (1993). Their results indicate that *Alopecurus myosuroides* is an allogamous self-incompatible plant with a high level of genetic polymorphism (60% of loci polymorphic, average heterozygosity = 0.21) and with a very low genetic differentiation among populations (Nei's distances less than 0.06) from wide geographical origins.

Table 2. Comparison of different wild oat biotypes.

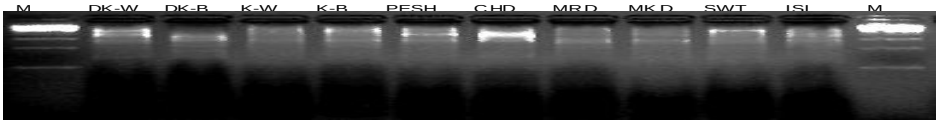
Biotypes	Plant height (cm)	Leaf lamina width (cm)	Leaf lamina length (cm)	No. of leaves plant^{-1}
Mardan	16.38b	0.390	9.206b	4.400a
Malakand	21.22a	0.496	11.17ab	4.200a
Karak	15.24bc	0.530	9.780ab	5.100a
D.I.Khan	15.58cd	0.390	9.160b	3.700a
Charsada	11.38d	0.456	12.67ab	4.866a
Peshawar	7.700e	0.416	13.20a	4.732a
Swat	0.00f	0.00	0.00c	0.00b
Kohat	14.82bcd	0.430	10.17ab	5.400a
NARC, Islamabad	0.00f	0.00	0.00c	0.00b
LSD $_{(0.05)}$	3.644	NS	3.644	3.644

Table 3. Genetic distance (in percentage) among 10 biotypes of wild oat using RAPD Prime GL.-A03.

Biotypes	D (W)	D (B)	K (W)	K (B)	P	C	M	MK	S	ISL
DW	-									
DB	75	-								
KW	66	50	-							
KB	33	66	50	-						
P	00	75	66	33	-					
C	33	66	50	66	33	-				
M	66	50	00	50	66	50	-			
MK	33	66	50	66	33	00	50	-		
S	33	66	50	00	75	66	50	66	-	
ISL	00	75	66	33	00	33	66	33	33	-

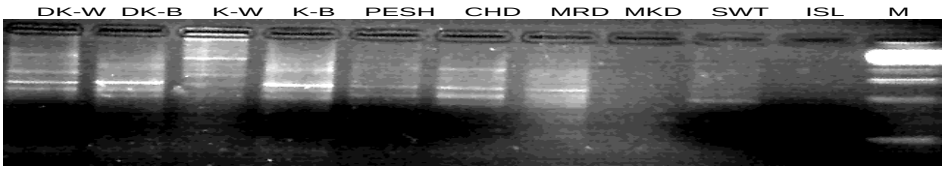
The PCR amplification profile using Genelink-A04 primer (Fig. 2), showed various levels of genetic polymorphism for the loci amplified. Total genomic DNA of 2 biotypes (Malakand and Islamabad) was not amplified by using GL-A04 and so the results are not included in analysis. A total of 32 alleles were observed in 8 biotypes giving an average of 4 alleles per biotypes. The size of amplified fragments ranged from < 500 to >1000 bp. Genetic distances among biotypes (in percentage) are presented in Table 4. The range of genetic distance estimated was 28 to 100%. Maximum genetic distance (100%) was observed for 3 comparisons, D.I.Khan (white)-Swat, Karak (black)-Swat and Mardan-Swat, while one comparison D.I.Khan (black)- Karak (white) showed 72 % homozygosity for the amplified loci. Our results are some what similar with Runzhi *et al*., 2007 who reported that the wild oats populations in China are genetically diverse at a level similar to North America, and the genetic diversity of wild oat in the broad spatial scale is not substantially changed by environment, agronomic practices, or herbicide usage.

The PCR amplification profile of 10 biotypes using Genelink-A12 primer yielded a total of 24 alleles (Fig. 3.), giving an average of 2.4 alleles per biotype. The size of amplified fragments varied from 500 to 1000 bp. Genetic distances estimated for all the possible combinations ranged from 0 to 100% (Table 5). Four pairs of biotypes {Dera Ismail Khan (black)- Peshawar, Dera Ismail Khan (Black)- Swat, Karak (black)-Peshawar and Karak (black)-Swat} showed maximum genetic distance (100%), while three comparisons {Dera Ismail Khan (black)- Karak (black), Charsadda-Islamabad and Mardan-Malakand} revealed no difference (GD =0%).



A total of 21 alleles were amplified in 10 biotypes giving an average of 2.1 alleles per biotype.The size of amplified fragments ranged from >250bp to >1000bp.

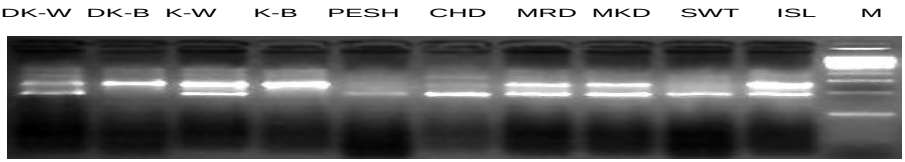
Fig. 1. PCR Amplification profiles of 10 wild oat biotypes using RAPD primer GL-A03.



A total of 32 alleles were amplified in 8 biotypes giving an average of 4 alleles per biotype.

The size of amplified fragments ranged from < 500bp to >1000bp.

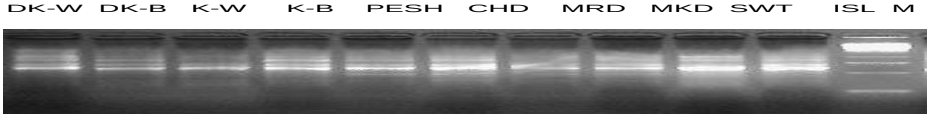
Fig. 2. PCR Amplification profiles of 10 wild oat biotypes using RAPD primer GL-A04.



A total of 24 alleles were yielded giving an average of 2.4 alleles per biotyp

The size of amplified fragments varied from 500bp to 1000bp.

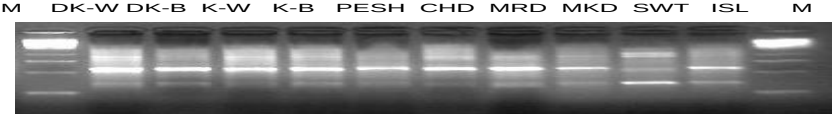
Fig. 3. PCR Amplification profiles of 10 wild oat biotypes using RAPD primer GL-A12.



A total of 38 alleles were amplified in 10 biotypes giving an average of 3.8 alleles per biotype.

The size of amplified fragments ranged from 500bp to 1000bp.

Fig. 4. PCR Amplification profiles of 10 wild oat biotypes using RAPD primer GL-B07.



A total of 37 alleles were yielded giving an average of 3.7 alleles per biotype.

The size of amplified fragments varied from > 250bp to 1000bp.

Fig. 5. PCR Amplification profiles of 10 wild oat biotypes using RAPD prime GL-B19.

Table 4. Genetic distance (in percentage) among 10 biotypes of wild oat using RAPD Primer GL-A04.

Biotypes	D (W)	D (B)	K (W)	K (B)	P	C	M	M	S	ISL
DW	-									
DB	57	-								
KW	50	28	-							
KB	71	50	63	-						
P	86	66	75	60	-					
C	75	57	50	50	40	-				
M	83	83	88	50	33	60	-			
MK	N.A	N.A	N.A	N.A	N.A	N.A	N.A	-		
S	100	80	86	100	66	80	100	N.A	-	
ISL	NA	N.A	N.A	N.A	N.A	N.A	N.A	N.A	NA	-

Table 5. Genetic distance (in percentage) among 10 biotypes of wild oat using RAPD Prime GL-A12.

Biotypes	D (W)	D (B)	K (W)	K (B)	P	C	M	MK	S	ISL
DW	-									
DB	50	-								
KW	25	33	-							
KB	50	00	33	-						
P	75	100	66	100	-					
C	25	75	50	75	66	-				
M	50	66	33	66	50	33	-			
MK	50	66	33	66	50	33	00	-		
S	50	100	75	100	50	33	66	66	-	
ISL	25	75	50	75	66	00	33	33	33	-

Table 6. Genetic distance (in percentage) among 10 biotypes of wild oat using RAPD Prime GL-B07.

Biotype	D (W)	D (B)	K (W)	K (B)	P	C	M	MK	S	ISL
DW	-									
DB	00	-								
KW	25	25	-							
KB	00	00	25	-						
P	40	40	60	40	-					
C	20	20	40	20	20	-				
M	60	60	50	60	25	40	-			
MK	40	40	60	40	00	20	25	-		
S	00	00	25	00	40	20	60	40	-	
ISL	00	00	50	25	25	40	50	25	25	-

Table 7. Genetic distance (in percentage) among 10 biotypes of wild oat using RAPD Prime GL-B19.

Biotypes	D (W)	D (B)	K (W)	K (B)	P	C	M	MK	S	ISL
DW	-									
DB	20	-								
KW	20	00	-							
KB	33	50	50	-						
P	60	50	50	60	-					
C	20	40	40	20	50	-				
M	40	25	25	40	33	25	-			
MK	40	25	25	40	33	25	00	-		
S	50	40	40	20	50	40	25	25	-	
ISL	40	25	25	40	33	25	00	00	25	-

The PCR amplification profile of 10 biotypes of wild oat using GL-B 07 primer showed various levels of genetic polymorphism (Fig. 4). A total of 38 alleles (bands) were amplified in 10 biotypes giving an average of 3.8 alleles per biotypes. The size of amplified fragments ranged from 500 to 1000 bp. The range of genetic distances observed using RAPD GL-B 07 was 0 to 60% (Table 6). Six comparisons showed 60% genetic dissimilarity while 9 comparisons showed no genetic difference using RAPD prime GL-B07.

The banding pattern obtained by using RAPD primer Genelink-B19 is presented in Fig. 5. A total of 37 alleles were amplified in 10 biotypes, giving an average of 3.7 alleles per biotypes. The size of amplified fragments ranged from > 250 to 1000 bp. Genetic distances (GD) among the 10 biotypes ranged from 0% to 60% (Table 7). Maximum genetic distance (60%) was estimated for 2 comparisons (Dera Ismail Khan white-Peshawar and Karak black- Peshawar), while 4 comparisons {Dera Ismail Khan (black)-Karak (white), Mardan-Malakand, Mardan-Islamabad and Malakand-Islamabad} showed homozygosity for the loci amplified using GL-B07.

Conclusions and recommendations: Based on variability in different parameters, it could be assumed that tolerance to herbicides could also vary; consequently different herbicidal doses will be required to kill the infesting biotype in the area. The range of amplified fragments was from < 250 to > 1000 bp in size. The degree of genetic polymorphism ranged from 0 to 100%, indicating that the wild oat germplasm is genetically very diverse and possesses a high degree of polymorphism.

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