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Genetic diversity assessment of *Aegilops germplasm* by RAPD molecular markers

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ABSTRACT

Study of the genetic diversity in plant inherited stores in order to classify the germplasm regarding resistance to biotic and biotic stress and also preventing from genetic erosion is one of the basic and fundamental steps in the most breeding programs .Iran is one of the most important centers of genetic diversity due to having different climates and the old civilization. In order to distinguish and evaluate different genetic of *aegilops germplasm*, which were collected from west part of the Iran, 80 ecotypes of *aegilops* by RAPD molecular marker were assessed .DNA extraction was done by modified CTAB method. After DNA extraction stages, complement gene locuses were amplified by 18 RAPD primers. These primers produced 183 bands, that 151 bands (about 82%) were polymorphic. Cluster analysis based on the resulting data was performed using UPGMA method and Dice's similarity coefficient in NTSYS software. The resulting dendogram categorized the accessions into 8 groups in 69% similarity. Principle Component Analysis (PCA) was performed too, 2 and 3 dimensions graphs using 18 primers were drawn.

KEYWORDS

Aegilops;
Genetic diversity;
RAPD molecular marker;
NTSYS software.

INTRODUCTION

Aegilops is a genus of plants generally known as goatgrasses and belonging to the grass family, Poaceae. There are about 23 species and numerous sub species in the genus. Various members of the genus are classed as agricultural weeds. Growing through the winter, they resemble winter wheat. They are able to hybridize with various types of wheat and are sometimes classified as members of the wheat genus, *Triticum*. *Aegilops* is a winter annual grass, vegetatively similar to wheat in the seed stage. The leaves are alternately arranged with auricles at their base and occasional hairs extending along the margins. The flower spike is cylindrical and distinct from wheat. Two to four flowers are arranged in each of the spikelets which form the elongate cylindrical spike. It is jointed in appearance and each joint contains one to three seeds. The glumes on the top spikelet have long awns. The seed of jointed goatgrass ripens before winter wheat and shatters easily. The genus *Aegilops* L. contains approximately 23 species of diploid and polyploid annual grasses, which originated

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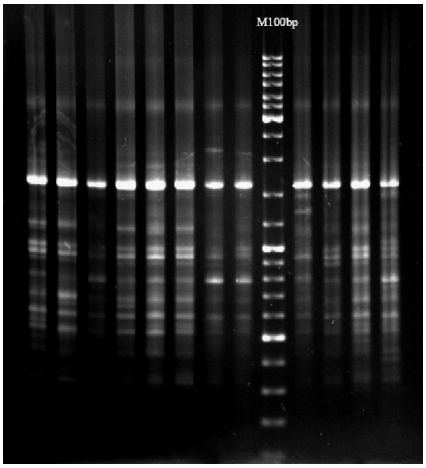

Figure 1 : sample of electrophoresis in 1% agarose gel

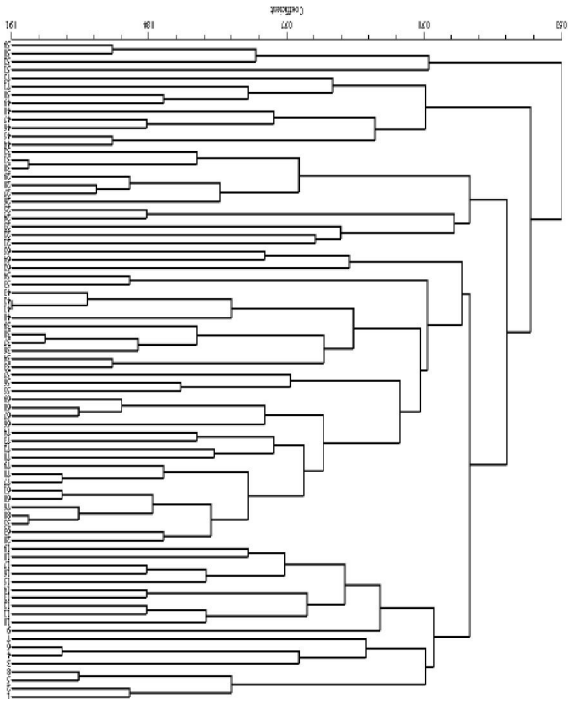

Figure 2 : Achieved dendrogram by UPGMA method and Dice's similarity coefficient for 80 ecotypes using 18 primers

around the Mediterranean region and in Western and Central Asia[12]. One of the basic legs of plant breeding is access and information of amount of diversity in genetic sets and different stages of breeding projects. Estimating of genetic composition of field crops and or genetic sets and also appointment of relationship between them has been usual since very olden times. From a historical point of view, this estimating has accomplished based on sexual organs biology, ecogeographical data, biology, pigments, pedigrees and assessment of agricultural characteristics. Although these markers and

TABLE 1 : collected aegilops ecotypes

Region of collection	No of Ecotype
zanjan province	1-2-3-4-5-6-7-8-9-10
kordestan province	11-12-13-14-15-16-17
kermanshah province	18-19-20-21-22-23-24
ilam province	25-26-27-28-29-30-31-32
lorestan province	33-34-35-36-37-38-39-40-41-42-43
hamedan province	44-45-46-47-48-49
Chaharmahal & bakhtiari province	50-51-52-53-54-55-56-57
Khozestan province	58-59-60-61-62- 63-64-65-66-67-68-69
markazi province	70-71-72-73-74-75
Khohkiloei & boerahmad province	76-77-78-79-80

TABLE 2 : Applied random 10 nucleotide primers

Primer specified code	Primer sequence	Primer specified code	Primer sequence
53(sina gene)	CTCCCTGAGC	A(sina gene)	GGTCTCCTAG
54(sina gene)	GTCCCAGAGC	B(sina gene)	CGGAGAGCGA
62(sina gene)	TTCCCCGTCG	C(sina gene)	CCGGCATAGA
63(sina gene)	TTCCCCGCCC	D(sina gene)	TGGGCTCGCT
66(sina gene)	GAGGGCGTGA	E(sina gene)	ACTTGTGCGG
69(sina gene)	GAGGGCAAGA	F(sina gene)	CCCACTGACG
70(sina gene)	GGGCACGCGA	G(sina gene)	CTGAGGAGTG
71(sina gene)	GAGGGCGAGG	H(sina gene)	GGTCAACCCT
		I(sina gene)	GCGGGAGACC
		J(sina gene)	CCTCACCTGT

TABLE 3 : Parameters of amplification

1	Initial denaturation programme	2 min at 94°C
		1 min at 92°C
2	40 cycles programm	1 min at 35°C
		1 min at 72°C
3	End programme of completion of DNA extension	5 min at 72°C

similar ways of genetic diversity estimating are usually very useful, but as for wide development in connection to molecular biology, more possibilities is created that can be undertaker for superior needs in connection to development of markers for assessment and estimating of genetic diversity of animal and plant masses. Between different aspects of usage of DNA based markers, DNA based fingerprinting has been the most interesting. Attractiveness of DNA based fingerprinting is because of its differentiation ability, pragmatism and abundance. Molecular markers are very important and strong implements for assessment of genetic relationships, selection of superior plants and study of similar-

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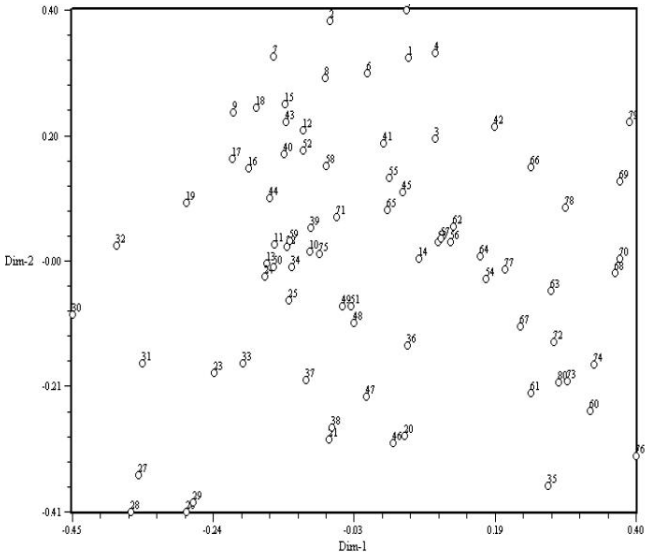

Figure 3 : Two dimensions graph related to Principle component analysis for 80 ecotypes using 18 primers

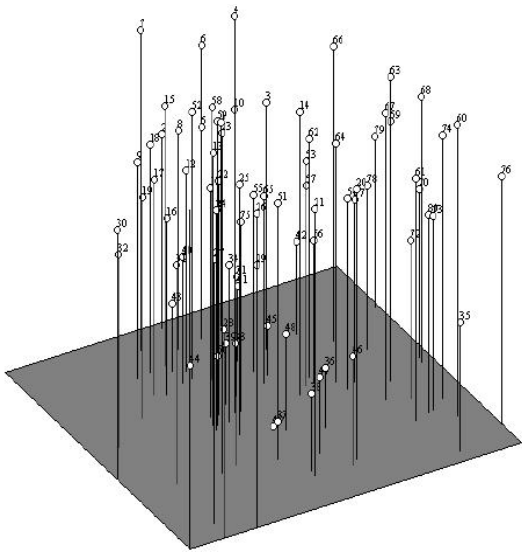

Figure 4 : three dimensions graph related to Principle component analysis for 80 ecotypes using 18 primers

ity or dissimilarity between different samples. Many of DNA markers have been introduced yet. These markers differ from each other for polymorphism degree, dominance or co dominance, distribution in chromosome surface, repeatability, dependence or independence to DNA sequencing and etc.

Selection of the best marker system depends on aim of research and ploid level of studied being[2,5]. Based on morphological and genetic analysis, the genusis separated into five categories: *Aegilops*, *Comopylum*, *Cylindropyrum*, *Sitopsis* and *Vertebrata*, with combinations of the genomes C, D, M, N, S and U[12]. Many cytoplasmic and cytological studies[13,14] and an isozyme analysis[1] were done to reveal the genome relationships of *Triticum-Aegilops* species. More recent analyses have been focused on molecular markers in nuclei[3,4,10,11,17], or organelles[6,14-16].

MATERIAL AND METHODS

Plant material

80 ecotypes of *Aegilops* were collected from west part of the Iran, (TABLE 1).

DNA extraction

The modified CTAB method used for this purpose. In this method, high salt concentration and polyvinylpyrrolidone (PVP) in the extraction buffer used in order to prevent the solubilization of polysaccharides and polyphenols in the DNA extract, respectively. Mild temperature conditions during extraction and precipitation retained. To better lyses of tissue and more obtain of DNA, the sample in extraction buffer incubated in water bath at 55°C for overnight. For completely proteins removal, was repeat chloroform-isoamylalcohol step 4-5 time. DNA precipitation was did using isopropanol at 25°C for 1 hour, then the pellet was washed using wash buffer to remove contaminants. Finally, the pellet dissolved in TE buffer. DNA quantity and quality were estimated both using an UV spectrophotometer by measuring absorbencies at A260 and A280 and 0.8% agarose gel electrophoresis by comparing band intensity with λ DNA of known concentrations. The results showed that the extracted DNA had desirable concentration and quality[2].

PCR amplification

To perform PCR (Polymerase Chain Reaction), the directions of Wantorp et al.[9], with some changes was used.PCR mix contained: 1 µL template DNA in 50 ng/µL concentration; 0.3 µL Taq DNA polymerase in 5 unit/µL C.; 2.5 µL reaction buffer in 10×C. [500 mM $Mgcl_2$ and Tris-HCl (PH=8.4)]; 2 µL $Mgcl_2$ in 50 mM C.; 2.5 µL dNTP in 2.5 mM C.; 2 µL primer in 2 µM C.; 14.7 µL ddH_2O. Final volume of reaction solution was 25 µL. PCR reactions were performed by 18 random primers having 10 nucleotide (TABLE 2) in eppendorf thermal cycler apparatus. PCR amplifica-

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tion was performed by parameters given in TABLE 3.

The PCR products were separated by electrophoresis in 1% agarose gel and subsequently stained with ethidium bromide[7,9,18]. 100 bp size marker was used to score achieved bands.

RESULTS AND DISCUSSION

Applied 18 primers produced183 evident bands. These bands were graded in the form of o&1 matrix in Excel software , that were in the order of presence of band:1, and the absence of band: 0.151 bands (about 82%) of total bands were polymorphic (Figure 1). Analysis of resulting data (o&1 matrix) was performed using UPGMA method and Dice's similarity coefficient in NTSYS software. The resulting dendrogram categorized the accessions into 8 groups in 69% similarity (Figure 1 and 2)[8].

Principle coordinates analysis (PCA)

PCA was performed too 2 and 3dimensions graphs using 18 primers were drawn (Figure 3 & 4). Achieved classification results of cluster analysis didn't agree with results of principle component analysis sufficiently[8].

Since values of first three components as follows: $h_1 = 22.14\%$, $h_2 = 8.7\%$, $h_3 = 6.24\%$ and these three components explain 37.08% of diversity of evaluated ecotypes totally, it seems that selection of primers has been suitable and classification based on cluster analysis is better than 2 and 3 dimensions graphs.

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