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Evaluation of Genetic Diversity of Algerian *Aegilops Ventricosa* Tausch. Using Inter-Simple Sequence Repeat (ISSR) Markers

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ABSTRACT

The genetic polymorphism of thirty Aegilops ventricosa Tausch. accessions sampled from northern Algeria was evaluated using inter-simple sequence repeat (ISSR) markers (into 5'). Six reproducible primers were used, and 53 out of 62 amplified distinct bands were found with a high score of polymorphism (87.48%). A significant number of specific bands was identified which were useful for genotyping. The majority of ISSR primers showed important resolving power with an average of 10.86, reflecting high informative markers tested in this investigation. SIMQUAL coefficient was used to calculate the genetic similarity among the 30 accessions. This similarity ranged from 0.5806 to 1. Based on Unweighted Pair-Group Method using Arithmetic Averages (UPGMA) method, a dendrogram was established to assess the intraspecific relationships via the genetic distances between the 30 accessions. The ISSR results showed a high variability in the studied collection. These markers could be efficiently applied to estimate the genetic diversity in the Ae. venticosa or others species in the Aegilpos genus.

Keywords: *Aegilops ventricosa Tausch., Genetic diversity, ISSR markers, Northern Algeria.*

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1. INTRODUCTION

For a long time, the state and conservation of the genetic diversity of genus *Aegilops* have been a constant preoccupation of researchers. *Aegilops* species, related to the genus *Triticum,* (Valkoun et al. 1985) represent a large genetic diversity, and are therefore a potential source for many genetic and agronomic traits such as donors of wheat genomes, disease resistance, abiotic stress tolerance, and quality of storage protein)Shewry et al. 1984; Holubec et al. 1993; Van Slageren, 1994; Zaharieva 1996; Schneider et al. 2008).

Aegilops genus is distributed in West Asia and the Mediterranean basin, and includes diploid, tetraploid, and hexaploid species. In Algeria, the survey conducted by ICARDA between 1989 and 1990, identified three tetraploid species: *Aegilops geniculata* Roth., *Aegilops ventricosa* Tausch and *Aegilops triuncialis* L with three other species of diploid *Aegilops* (*Ae. Umbellulata, Ae. caudata* and *A. Tauschii*) collected from eastern Algeria.

Apart from some tetraploid *Aegilops* such as *Aegilops geniculata* studied by Medouri et al. (2014, 2015) and Bandou et al. (2009) or the Karyological study by Baik et al. (2017), the study of genetic variability of other Algerian species is still insufficient. *Aegilops ventricosa* Tausch is one of the least studied tetraploid species, despite their abundance in northern Algeria and their interesting characteristics as a source of resistance genes to certain diseases and different types of stress. *Aegilops ventricosa* Tausch. (also named *Aegilops fragilis* Parl., *Triticum ventricosum* Ces.) is a tetraploid species, having the genomic formula DDNN (Kimber & Tsunewaki 1988). The genome N comes from diploid species *Aegilops uniaristata* Vis. (2n = 14). The genome D is the one from *Aegilops tauschii* Coss. (2n = 2x = 14). (Lilienfeld, 1951). Ii is generally found in the north and north-west of the Sahara, southern France, the Iberian Peninsula and southern Italy (Pignone et al. 1994).

Several approaches and methods have been used to assess the genetic variability in plant genetic resources. Morphological markers, protein and isozymes analysis can be used with electrophoresis, but can have remarkable limitations related to environmental influences, post-translational modifications and paucity of loci (Afzal et al. 2004). On the contrary, the molecular markers are still considered the most used means for the study of this genetic polymorphism, which have only weak limitations and provide the ability to precisely identify the genotypes and determine the genetic relationships between them (Carvalho et al. 2005). Among these markers, Inter-Simple Sequence Repeat (ISSR) became widely used in different areas of plant research (Karaca & Izbirak 2008). ISSR is useful in genetic diversity studies, genome mapping, gene tagging and phylogeny (Blair et al. 1999). The Polymerase Chain Reaction (PCR) can be used to amplify the fragments flanked by two inversely oriented microsatellite motifs (Zietkiewicz et al. 1994). Under the appropriate amplification conditions, this technic produces several products. The revealed polymorphism is essentially of the presence / absence type (Prevost & Wilkinson, 1999). The changes in these regions

(insertions or deletions), as well as in the binding sites are detected as polymorphic bands (Yang et al. 1996).

The current work was done in this context. This has been the first study aimed at estimating the genetic variability of the *Aegilops ventricosa* species belonging to 30 accessions collected from different regions of northern Algeria by using ISSR markers.

2. MATERIALS AND METHODS

Collection of plant material

This study was conducted to evaluate the genetic variability in thirty accessions of *Aegilops ventricosa* Tausch., collected from different regions of northern Algeria as shown in Table1.

DNA isolation

Genomic DNA was isolated from fresh leaves samples of 15-day-old-seedlings germinated under totally dark condition at 25°C. 300 mg of healthy young leaves' tissues from 12 individuals was used for DNA extraction using Cetyl Trimethyl Ammonium Bromide (CTAB) method described by Doyle & doyle (1990). DNA concentration and purity were quantified spectrophotometerly, at ratios of 280/260 and 230/260, using Nanodrop. The DNA quality was then checked on 0.8% agarose gel in TBE buffer (1X). The DNA was finally diluted to a final concentration of 50 ng/µl for PCR amplification.

ISSR Amplification

Initially, eleven ISSR primers purchased from Biomatik (Canada) were screened with fives accessions of *Ae. ventricosa* Tausch. Only six primers (Table 2) which presented a higher resolution of bands and polymorphic amplification patterns were selected for ISSR analysis. PCR reactions were carried out in a 20 µl volume mixture containing 50 mg of genomic DNA, 10 µl of premixed 2x HS-Taq Master Mix (Biomatik, Canada), 0.5 µM of ISSR primer and 8 µl of Nuclease-free water. The amplifications were performed in a Techne Prime Thermocycler using the following PCR cycling program: Initial denaturation for 5 min at 94°C, 45 consecutive cycles of 94°C for 30 s, annealing step at 52 °C for 45 s and an extension step at 72 °C for 2 min. After the final cycle, the reaction was held for 5 min at 72 °C for a final extension. 12 µl of ISSR-PCR products were separated on 1.5% agarose gel in 1 x Tris Borate EDTA (TBE) buffer in the presence of 100 bp DNA ladder (Nippon Genetics EUROPE GmbH 100pb DNA ladder) at 100 V constant voltages for 80 min. Gels were stained in Ethidium bromide and photographed under UV by Doc-Print system, VX2, Vilbert Lourmat.

Scoring and data analysis

Using Photo-Capt, Vilber Lourmat, version 1502 Software Program, ISSR data were analyzed, and the molecular weight of each band was calculated. The amplified products were scored for the presence (1) or absence (0) of each of thirty accessions. To ensure reproducibility, the amplifications of DNA with each ISSR primer were repeated thrice. The bands were considered reproducible only after comparing them in the profile of three separate amplifications. The genetic similarity among the 30 accessions was estimated by SIMQUAL coefficient. The genetic relationships between accessions and clustering were developed via the Unweighed Pair Group Method with Arithmetic Average (UPGMA) method based on SAHN (Sequential, Agglomerative, and Hierarchical and Nested clustering) module through Numercial Taxonomy and Multivariate Analysis System, version 2.01b (NTSYS.pc) software (Rohlf 2000). For each primer, a total number of bands (TNB), number of monomorphic bands (NMB), number of polymorphic bands (NPB), percentage of polymorphic bands (PPB), number of specific bands (NSB) were calculated. To evaluate the informativeness of ISSR markers, band informativeness (Ib) and resolving power (RP) of primer were calculated according to Prevost & Wilkinson formula (1999):

$$Ib = 1-[2 \times (0.5 - p)],$$

Where:

p = proportion of the total accessions containing the band;

$$RP = \Sigma Ib.$$

The Average of informativeness of bands (AvIb) represented the measure of closeness of a band to be present in 50% of the genotypes under study.

3. RESULTS

Identification and analysis of ISSR markers

The six selected ISSR primers used to amplify genomic DNA segments from thirty *Ae. ventricosa* accessions included in this study generated 62 different bands in molecular weight ranging from 279 and 2360 bp (Table 3). Out of this number, 53 polymorphic fragments were scored (85.48%). The highest Percentage of polymorphic PCR-amplified fragments was identified in UBC 812, UBC 814 and UBC 849 (100%), while UBC 826 primer gave the lowest value (66.66%). The number of specific bands ranged from 1 to 4. As shown in Table 4, UBC 818, UBC 826 and UBC 880 primers produced one specific band each (993 bp, 1680 bp and 706 bp) presented only in accessions V42, V93 and V53; respectively. In another side, UBC 812 generated four strong specific bands at 1800, 1460 and 1266 pb in V101 and 670 pb found at the accession V34. The electrophoretic profiles of some accessions using ISSR primers UBC880 and specific band (706 bp) have been presented in Figure 1.

The six ISSR primers showed a high variation in the band informativeness (Ib) and resolving power (Rp). Average of Ib ranged from 0.379 (UBC 814) to 0.691 (UBC 880), while resolving power ranged from 6.066 (UBC 814) to 20.666 (UBC 826) (Table 2). The primer UBC 826 with (AC) motif, showed the highest values of these last parameters.

Genetic diversity and clustering

The SIMQUAL similarity coefficients calculated for all the thirty accessions of *Ae. ventricosa* varied from 0.5806 to a maximum of 1. The SIMQUAL similarity coefficients went from 45 to 65 % showing that accessions seemed to be more or less similar to each other. The results of UPGMA cluster analysis using ISSR markers clearly revealed two main clusters (Figure 2). The cluster I included the majority of accessions studied (24 accessions), whereas the cluster II contained only six accessions (V42, V47, V51, V95, V65 and V106). The first cluster was divided into two sub clusters (I-1 and I-2) which comprised of 11 and 13 accessions; respectively. Within this

cluster, all accessions showed a genetic distance to each other except for V10 and V18, which appeared identical with 100% of similarity. Also, it was clear from the sub cluster I-2 that the accession V101 was more genetically dissimilar from the rest of the accessions. The second cluster was less polymorphic than the first, where among the six accessions, three accessions (V47, V51 and V95) had a homology of 100%. The accessions V42 and V101 were grouped in two different and farthest clades, and found to be the most diverse with 58.06% of similarity.

4. DISCUSSION

By examining the results of this study, in order to estimate the level of genetic diversity of *Ae. ventricosa* accessions collected from different Algerian areas and despite the limited number of ISSR markers used, it could be said that a great genetic diversity existed within the collection. Looking at the output of the ISSR primers, it was found that they well expressed a large variance between the genomic DNA sequences of the *Ae. ventricosa* accessions. The average of percentage of polymorphic bands was estimated at big value of 87.89%. This was contrary to the expectations, especially if the example of UBC 812 with the (GA) motif, which gave 100% of polymorphism with four discriminant bands reflecting the abundance of this marker across the *Ae. ventricosa* genome was considered. This was contrary to what was customary in terms of the weakness of this sequence in presenting the low level of variability in plants (Akkaya et al. 1992). Konstantinos & Bebeli (2010), reported that the same GA primers group as: UBC 810= (GA)8T, UBC 811= (GA)8C and UBC 842= (GA)8YG which presented a highly polymorphism (100%) among 44 accessions. representing seven diploid and tetraploid *Aegilops* species. The results of this examination did not agree with those of Galaev et al. (2004) who conducted a study to detect the introgression of Genome Elements of *Aegilops cylindrica* Host. into the *Triticum aestivum* L. genome by ISSR and SSR methods, where the mean polymorphism was within 54% using (GA)9C primer. Compared to the results of this study (8.83), the average of number of polymorphic bands recorded by Haider et al. (2010), using 20 selected primers to compare the efficiency of ISSRs and RAPDs in resolving genetic relationships among 20 *Aegilops* species on the one hand, and Triticum on the other hand, was slightly higher than those which had the value of 9.55. This divergence can be explained by the number of accessions and the different structure of ISSR primers used in each study. The lowest polymorphism values were scored with UBC826 primer (AC)8C, which was contradictory to what has been reported by Carvalho et al. (2009) who found a 100% rate of polymorphism that used the same primer to evaluate the genetic variability among 99 varieties of Old Portuguese wheat by ISSR assays. This result could be explained by the difference of the genomic distribution and frequency of microsatellites between wheat and *Ae. ventricosa* (Morgante & Olivierin, 1993).

The twelve specific bands registered (Table 3) could be considered as an exclusive inter simple sequence repeats markers, and can be used for screening and rapid characterization of *Ae. ventricosa* genotypes. The average of resolving power (Rp) recorded at 10.86, was another parameter used to estimate the effectiveness and ability of primers to distinguish between genotypes (Prevost & Wilkinson 1999). Based on this information, the UBC 826 primer had the highest value of resolving power. In this sense, the most informative ISSR primer can be considered for distinguishing the *Ae. ventricosa* accessions studied. In this study, RP was not significantly related to the other indices (PPB, NSB).

The high SIMQUAL coefficient of similarity values and UPGMA phenogram suggested that 30 accessions of *Ae. ventricosa* were very various despite the limited number of accessions analyzed and the restrict collection area. Through this, it could be suggested that the adaptation to the environmental conditions changed with altitude, latitude, longitude, rainfall, soil type, temperature, and this might be responsible for this genetic diversity (Fahima et al. 1999; Nevo et al. 1986; Erskine et al. 1989).

5. CONCLUSION

Inter Simple Sequence Repeat (ISSR) markers performed in the present work provided enough reproducible fingerprinting profiles and variability to assess the genetic diversity of Algerian *Aegilops ventricosa* populations. UPGMA analysis clearly separated the selected genotypes into distinct groups. In addition to this technique, other morphological, biochemical and molecular tools can be used to further studies of the intra and inter-specific genetic diversity in *Aegilops* species.

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Table 1. Code and origin of collected *Ae. ventricosa* accessions

N°	Accession code	Province	N°	Accession code	Province
1	V88	Lambridi,Batna	16	V65	Tadjenanet, Mila
2	V18	El Madher, Batna	17	V106	Oued Athmania, Mila
3	V95	Oued Chaaba, Batna	18	V40	Guettar El Aich, Constantine
4	V93	Oued Chaaba, Batna	19	V10	Boughni, Tizi Ouzou
5	V14	Mechta Azem, Batna	20	V09	Draa El Mizane, Tizi Ouzou
6	V11	Hamla, Batna	21	V104	Souk El Had, Chelef
7	V25	Hamla, Batna	22	V34	El Hachimia, Bouira
8	V51	Remila, Khanchla	23	V69	Djandel, Ain Defla
9	V52	Remila, Khanchla	24	V101	Bouhaloufa, Ain Defla
10	V33	Remila, Khanchla	25	V47	Medéa
11	V02	Oum El Boughi	26	V62	Medéa
12	V55	Oum El Boughi	27	V07	Tiaret
13	V79	Hanchir Toumghani, Oum El Bouaghi	28	V03	Mellakou, Tiaret
14	V53	Oued Zenati, Guelma	29	V67	Saïda
15	V12	Tamlouka, Guelma	30	V42	El Mansoura, Tlemcen

Table 2. Sequence and GC (%) of ISSR primers used

N°	Primer code	Sequence 5'-3'	GC content (%)
1	UBC 812	GAGAGAGAGAGAGAGAA	47
2	UBC 814	CTCTCTCTCTCTCTCTA	47
3	UBC 818	CACACACACACACACAG	52
4	UBC 826	ACACACACACACACACC	52
5	UBC 849	GTGTGTGTGTGTGTGTYA	47
6	UBC 880	GGAGAGGAGAGGAGA	60

Y = (C, T)

Table 3. Parameters of genetic variation exhibited by ISSR markers

Primers	TNB	NPB	NMB	PPB	NSB	AvIb	RP	Bands size (bp)
UBC 812	9	9	0	100	4	0.381	6.866	577-1800
UBC 814	8	8	0	100	3	0.379	6.066	430-2360
UBC 818	14	12	2	85.714	1	0.507	14.2	360-1540
UBC 826	15	10	5	66.667	1	0.688	20.666	279-1755
UBC 849	8	8	0	100	3	0.395	6.333	433-1173
UBC 880	8	6	2	75	1	0.691	11.066	426-1500
Total	62	53	9	/	14	/	/	/
Average	10.33	8.83	1.5	87.89	2.33	0.506	10.866	279-2360

TNB: Total number of bands, NPB: Number of polymorphic bands, NMB: Number of monomorphic bands, PPB: Percentage of polymorphic bands, NSB: Number of specific bands AvIB: Average of informativeness bands, RP: Resolving power

Table 4. Specific bands identified for each ISSR primers

Primers	Specific bands (size bp)	Accessions
UBC 812	1800	V101
	1460	V101
	1266	V101
	670	V34
UBC 814	2360	V09
	1840	V09
	858	V101
UBC 818	993	V42
UBC 826	1680	V93
UBC 849	980	V93
	786	V42
UBC 880	706	V53

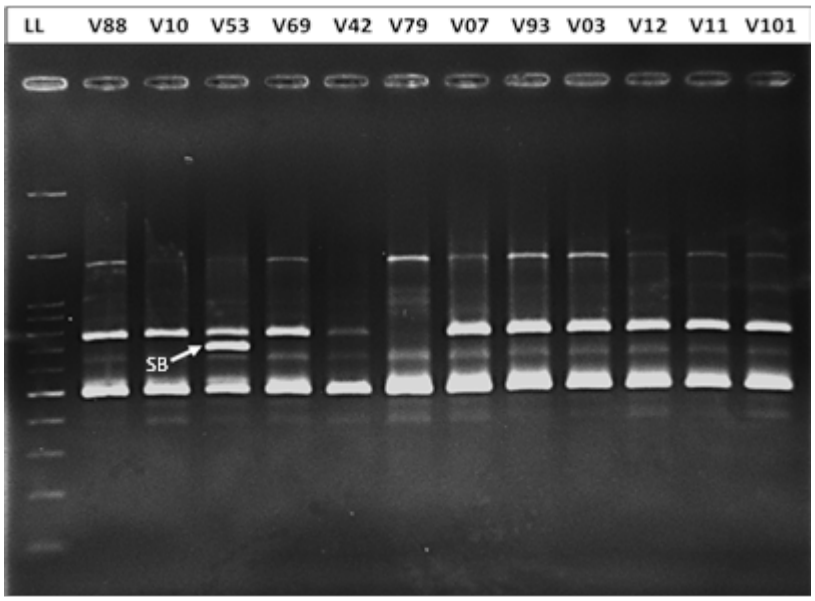

Figure 1. Amplified products with primer UBC 880, visualized on 1.5% agarose gel of some *Ae. ventricosa* accessions. LL: Low ladder; SB: Specific band (706 bp).

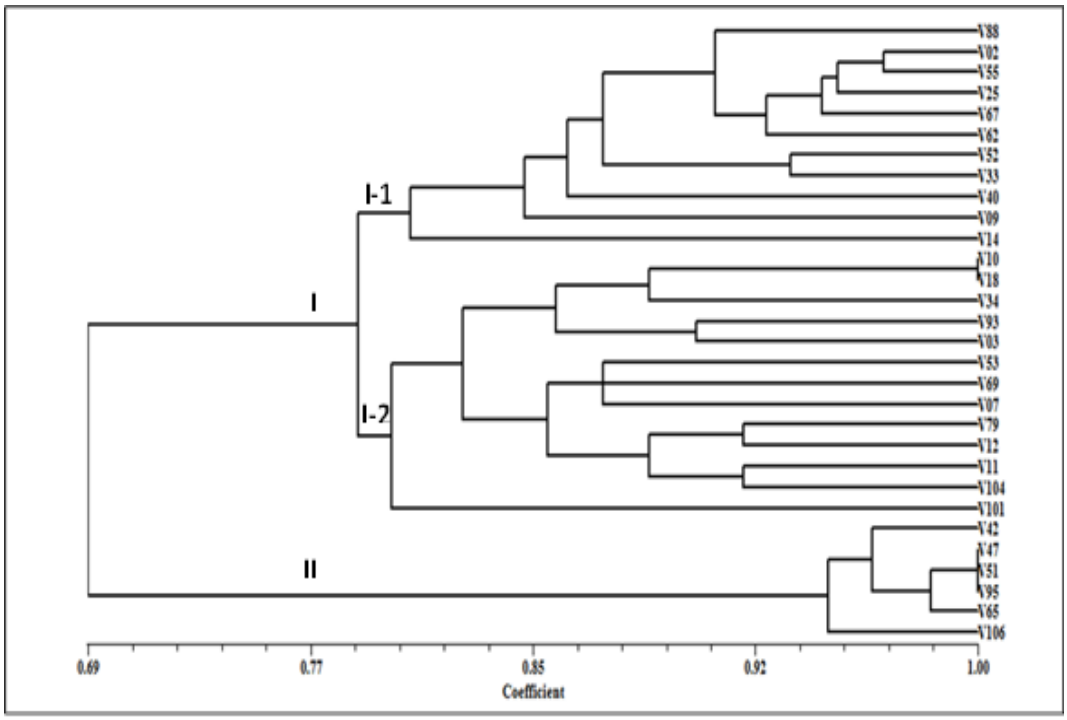

Figure 2. Dendrogram generated using UPGMA cluster analysis based on ISSR markers data.