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Research Article

Genetic Relationships among *Aegilops* L. and *Triticum* L. Species based on the Internal Transcribed Spacer Sequences of nrDNA (ITS)

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Abstract: Studying of genetic relationships among *Triticum* L. and *Aegilops* L. species by direct sequencing of PCR-amplified Internal Transcribed Spacer (ITS) of nuclear ribosomal DNA to investigate the polymorphism in nucleotide sequences among 19 *Aegilops* L. and 7 *Triticum* L. species. ITS sequences were aligned with CLUSTAL W 2.1 multiple sequence aligment program. The phylogenetic relationships among species were reconstructed using Unweighted Pair Group Mean Arithmetic average (UPGMA) and Neighbor-Joining (NJ) methods. ITS region ranged from 600 to 602 bp. The length of ITS1 was 221-222 bp and ITS2 was 215-217 bp. The 5.8S subunit was 163 bp long. The G+C content of the ITS1 region ranged from (60.3 to 63.9) % in all *Triticum* and *Aegilops* species. The G+C content of the 5.8S subunit was entirely identical in all species, 59.5%. The G+C content of the ITS2 region ranged from 59.9 to 63.5%. There were 67 variable sites (11.12%) in the entire ITS region. The phylogenetic relationships among species were reconstructed using Unweighted Pair Group Mean Arithmetic average (UPGMA) were discussed.

Keywords: *Aegilops*, genetic relationships, internal transcribed spacer, ITS, *Triticum*

INTRODUCTION

Ribosomal RNA genes (known as ribosomal DNA or (rDNA) are found as parts of repeat units that are arranged in tandem arrays, located at the chromosomal sites known as Nucleolar Organizing Regions (NORs). Each repeat unit consists of a transcribed region (having genes for 18S, 5.8S and 26S rRNAs and the external transcribed spacers i.e., ETS1 and ETS2) and a Non-Transcribed Spacer (NTS) region. In the transcribed region, Internal Transcribed Spacers (ITS) are found on either side of 5.8S rRNA gene and are described as ITS1 and ITS2 (Sharma *et al*., 2002). These regions can be readily amplified by PCR and sequenced using universal primers (White *et al*., 1990). Universal PCR primers designed from highly conserved regions flanking the ITS and its relatively small size (600-700 bp) enable easy amplification of ITS region due to high copy number (up to-30000 per cell) (Dubouzet and Shinoda, 1999) of rDNA repeats. However, DNA from many plant species may not be amplified by the currently available primers and thus species-specific ITS primers would be desirable (Lin *et al*., 2010). there is now a set of seven ITS universal primers (White *et al*., 1990; Sun *et al*., 1994) that are widely applied for diagnostic (Linder *et al*., 2000) and phylogenetic studies in many angiosperm families (Baldwin *et al*., 1995; Haider, 2011). Because of their different rates of evolution, ITS regions presented high variability and faster rates of evolution (Baldwin *et al*., 1995; Barker *et al*., 1988; Kollipara *et al*., 1997; Downie and Kartz-Downie, 1996) and could be highly applicable in a broad range of organisms including plants, fungi and animals (White *et al*., 1990). The ITS polymorphism might occur at the genus, species or individual levels, making it to be a useful source of molecular characters for phylogenetic studies in many angiosperms (Nwakanma *et al*., 2003). This makes the ITS region an interesting subject for evolutionary/phylogenetic investigations (Baldwin *et al*., 1995; Hershkovitz and Zimmer, 1996; Hershkovitz *et al*., 1999; Nwakanma *et al*., 2003; Gulbitti-Onarici *et al*., 2009) as well as biogeographic investigations (Baldwin, 1993; Suh *et al*., 1993; Hsiao *et al*., 1994; Dubouzet and Shinoda, 1999; Sharma *et al*., 2002). ITS loci has properties that were claimed to be advantageous for purposes of phylogenetic reconstruction: Biparental inheritance-Universality-Simplicity -Intragenomic uniformity-Intergenomic variability-Low functional (González-Chavira *et al*., 2006). In the last decade numerous molecular markers and techniques were used for studies on origin, evolution and relationships in the wheat

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group: chloroplast and nuclear microsatellite markers (Lelley *et al*., 2000; Ishii *et al*., 2001), chromosome-specific low-copy DNA (Liu *et al*., 2003), nuclear genes (Caldwell *et al*., 2004) and ITS has also been used to study the evolution of wheat species in the early 1980s (Peacock *et al*., 1981; Dvorák and Appels, 1982; Wang *et al*., 2000; Rudnóy *et al*., 2005). The evolution of the ITS region is more complicated in hybrid and polyploid species (Baldwin *et al*., 1995; Wendel *et al*., 1995; Waters and Schaal, 1996). Baldwin *et al*. (1995) proposed that ITS sequences would provide direct evidence of reticulate evolution if concerted evolution failed to homogenize the repeat units contributed by different parental species when the hybridization event was recent, or if nrDNA repeats were at different loci in the parental genomes and interlocus gene conversion was inoperative in their hybrid, or if the hybrid was asexual. Since the history of polyploid wheats is relatively short (Mori *et al*., 1995) and the ITS repeats in polyploid wheats are located at different loci (Dubkovsky and Dvorák, 1995; Badaeva *et al*., 1996), it may be possible to identify ITS sequences of different parental origins in polyploid wheats and hence to identify their progenitors. In addition, the *Triticum* complex is a good model system for studying how hybridization and polyploidization could possibly affect the evolution of nrDNA (Zhang *et al*., 2002). Numerous studies have demonstrated the utility of the ITS region (Hsiao *et al*., 1995a; Wang *et al*., 2000; Blattner, 2004; Jakob *et al*., 2010) for resolving relationships among closely related species in *Triticeae* and other plant species (Hsiao *et al*., 1994, 1995a, b; Baldwin *et al*., 1995; Goel *et al*., 2002; Sharma *et al*., 2002; Zhang *et al*., 2002; Alvarez and Wendel, 2003; Bordbar *et al*., 2011). Hsiao *et al*. (1995a) studied the sequence of the ITS region of 30 diploid *Triticeae* species representing 19 genomes. they suggest that the sequence of the ITS is variable enough to differentiate closely related species (Hsiao *et al*., 1994). Nalini *et al*. (2007) found out that the polymorphic ITS fragments were correlated with Quantitative Trait Loci (QTL) such as the spike size and the number of spikelets per spike. And the polymorphic ITS fragments detected there should be further cloned and sequenced in order to screen for interesting agronomic traits with potential use in wheat breeding. Carvalho *et al*. (2009a) In their previous study performed in 48 old Portuguese bread wheat cultivars, they found out nine ITS PCR-RFLP patterns with the enzymes *AluI*, *HpaII* and *TaqI* (three patterns per enzyme). ITS PCR-RFLP markers revealed a lower percentage of polymorphism when compared with other DNA markers such as ISSRs (Carvalho *et al*., 2009b). Carvalho *et al*. (2011) In their present study, 51 durum wheat cultivars showed 40% of ITS variation and this lower level of polymorphism could be due to the absence of the D genome. Low ITS variation was previously reported for *Triticeae* (Zhang *et al*., 2002) and other taxa such as *Cucurbitaceae* (Jobst *et al*., 1998); *Oleaceae* (Jeandroz *et al*., 1997) and *Vigna* (Saini *et al*., 2008). So Carvalho *et al*. (2011) concluded that the knowledge of the genetic relationships and phylogenies among the durum wheat cultivars and their botanical varieties might contribute for the designing of intraspecific crosses between the genotypes studied there, with potential interest for wheat improvement. The sequence data of the ITS region has also been studied earlier to assess genetic diversity in cultivated barley (Petersen and Seberg, 1996). Sharma *et al*. (2002) used Internal Transcribed Spacer (ITS) sequences of ribosomal DNA of wild barley and their comparison with ITS sequences in common wheat to assess genetic diversity at the intraspecific level in barley and wheat.

Variations in length and sequence of the ITS have been used for taxonomic study in trees (Campbell *et al*., 1995; Baldwin, 1992; Lin *et al*., 2010) used ITS region sequence to identify phylogenetic analysis in intraspecies population of the genus *Zanthoxylum*. Sun *et al.* (1994) studied the phylogenetic relationships of the genus *Sorghum* and related genera by sequencing the ITS region. Their results were consistent with those based on chloroplast DNA analysis (Cao, 1997). Moore and Field (2005) were able to identify the roots of different plant species in mixed samples using sequences of the nuclear ITS region. Sequencing of the same region proved useful for DNA barcoding of members of the *Cycadales*. Singh *et al*. (2008) were used Internal Transcribed Spacer (ITS) sequences of nuclear ribosomal DNA to assess genetic diversity and phylogenetic relationships in chickpea (*C. arietinum*) cultivars and its related wild species. Their present comprehensive study has provided novel insights in the phylogeny of genus *Cicer*. Linder *et al*. (2000) developed a method that uses (ITS) region of the nuclear ribosomal DNA (rDNA) repeat to identify most underground plant parts to the level of species. Their technique should allow the below-ground parts of plants in any system to be identified and thereby open new possibilities for the study of below-ground plant communities.

In this study we study the genetic relationships among *Triticum* and *Aegilops* species inferred from the sequence data of the ITS region of the nuclear rDNA to broaden the genetic base of wheat and improve its yield, quality and resistance to biotic and abiotic stresses.

MATERIALS AND METHODS

Plant materials: The plant material consisted of 26 accessions (Table 1). 19 accessions representing (19) *Aegilops* L species: *Ae. crassa*, *Ae. tauschii*, *Ae. umbellulata*, *Ae. triuncialis*, *Ae. comosa*, *Ae. peregrine*, *Ae. caudata*, *Ae. biuncialis*, *Ae. ovata*, *Ae. neglecta*, *Ae. speltoides*, *Ae. ventricosa*, *Ae. searsii*, *Ae. cylindrica*, *Ae. kotschyi*, *Ae. longissima*, *Ae. bicornis*, *Ae.*

Table 1: Base compositions of ITSl, 5.8S and ITS2 regions of *Triticum* and *Aegilops* species

Triticum and *Aegilops* species		ITS1	% CG	5.8	% CG	ITS2	% CG	ITS total	% CG
Ae. bicornis	25_R_primer-5R	222	61.7	163	59.5	217	63.1	602	61.4
Ae. sharonensis	26_R_primer-5R	222	61.7	163	59.5	217	63.1	602	61.4
Ae. crassa	27_R_primer-5R	222	61.2	163	59.5	216	62.6	601	61.4
Ae. triuncialis	28_R_primer-5R	222	60.7	163	59.5	217	63.1	602	61.4
Ae. peregrina	29_R_primer-5R	222	60.3	163	59.5	217	62.2	602	60.6
Ae. biuncialis	30_R_primer-5R	222	63.0	163	59.5	217	62.2	602	61.5
Ae. ovata	31_R_primer-5R	222	63.0	163	59.5	217	62.2	602	61.5
Ae. neglecta	32_R_primer-5R	222	63.5	163	59.5	217	62.6	602	61.8
Ae. searsii	33_R_primer-5R	222	61.5	163	59.5	217	63.1	602	61.3
Ae. longissima	34_R_primer-5R	222	62.5	163	59.5	217	63.1	602	61.7
Ae. tauschii	35_R_primer-5R	222	61.2	163	59.5	217	60.3	602	60.3
Ae. umbellulata	36_R_primer-5R	222	61.2	163	59.5	217	63.1	602	61.2
Ae. comosa	37_R_primer-5R	222	62.6	163	59.5	217	61.7	602	61.2
Ae. caudata	38_R_primer-5R	222	60.7	163	59.5	217	63.1	602	61.1
Ae. uniaristata	39_R_Rev-Primer	222	61.5	163	59.5	217	63.1	602	61.3
Ae. speltoides	40_R_Rev-Primer	222	61.5	163	59.5	217	63.5	602	61.5
T. turgidum	41_R_Rev-Primer	221	61.5	163	59.5	217	60.8	601	60.6
T. dicoccon	42_R_Rev-Primer	222	61.2	163	59.5	217	63.1	602	61.2
T. aestivum	43_R_Rev-Primer	222	61.2	163	59.5	217	60.8	602	60.6
Ae. ventricosa	44_R_Rev-Primer	222	62.6	163	59.5	217	59.9	602	60.6
Ae. cylindrica	45_R_Rev-Primer	222	63.9	163	59.5	217	60.3	602	61.2
Ae. kotschyi	46_R_Rev-Primer	222	61.2	163	59.5	217	63.1	602	61.2
T. monococcum	48_R_Rev-Primer	222	61.2	163	59.5	215	61.3	600	60.6
T. urartu	25_Rev_5R-Rev	222	61.2	163	59.5	217	61.2	602	60.6
T. dicoccoides	26_Rev_5R-Rev	222	61.5	163	59.5	217	60.6	602	60.5
T. durum	27_Rev_5R-Rev	222	61.5	163	59.5	217	60.6	602	60.5

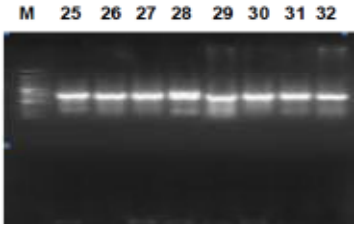

Fig. 1: PCR of ITS in *Aegilops* species
M: 100 bp DNA; 25: *Ae. bicornis*; 26: *Ae. sharonensis*; 27: *Ae. crassa*; 28: *Ae. triuncialis*; 29: *Ae. peregrina*; 30: *Ae. biuncialis*; 31: *Ae. ovata*; 32: *Ae. neglecta*

uniaristata, *Ae. sharonensis*. (7) accessions representing 7 *Triticum* L. species: *T. monococcum*, *T. urartu*, *T. dicoccoides*, *T. durum*, *T. turgidum*, *T. dicoccon*, *T. aestivum* (Table 1). All the accessions were obtained from the Genetic Resources Unit (GRU) at the International Centre for Agricultural Research in the Dry Areas (ICARDA), Aleppo, Syria.

DNA extraction: Total genomic DNA was isolated from fresh, young leaves as described by Doyle and Doyle (1987). DNA was quantified using Spectrometer and the concentration of all samples was set at 10 ng/µL.

PCR amplification: PCR reactions were carried out in a 25 µL volume containing 10×PCR buffer (Eurobio), dNTPs (10 mM) (Mix Roche), 10×MgC l2 (50 mM) (Eurobio), Taq polymerase (5 U/µL) (Eurobio). ITS region (including ITS 1, 5.8 S and ITS 2) was amplified using the following primer pair (White *et al*., 1990): ITS-4 (5'-TCCTCCGCTTATTGATATGC-3') -ITS-5 (5'GGAAGTAAAAGTCGTAACAAGG-3'), DNA was added to each PCR at a rate of 10 ng and the total volume was adjusted with dd H_2O to 25 µL. For 35 cycles, PCRs were subjected to 95°C for 1min for DNA denaturation, 50°C for 1 min for annealing of primers, 72°C for 1 min for extension of the target region and 72°C for 5 min for final extension. PCR products (1-5 µL) were digested according to manufacturer (Fermentaz). Digested fragments were separated by electrophoresis on 1.8% agarose gel that was run at 100 V for 2 h in TBE 0.5 x buffer and visualised under UV lights (Fig. 1).

Sequence analyses: The PCR products delivered to Leibniz University Hannover-Department of Plant Biotechnology-Hannover-Germany for sequencing, which was conducted in both forward and reverse directions using the amplification primers. ITS sequences were aligned with CLUSTAL W 2.1 multiple sequence aligment program. The boundaries of the ITS region was determined by comparison with the sequence information in (Chatterton *et al*., 1992). The phylogenetic relationships among species were reconstructed using Unweighted Pair Group Mean Arithmetic average (UPGMA) and Neighbor-Joining (NJ) methods.

RESULTS AND DISCUSSION

Earlier studies in the *Poaceae* have focused on morphology, anatomy, taxonomy, physiology, cytology, genetics and crop improvement. They have provided important information, but data based on these

studies are not enough to assess the true relationships between these species. Phylogenetic constructions proposed for the *Triticum* and *Aegilops* species based on these characters are poorly resolved and differ widely in topology. Therefore we used (ITS) regions of the nuclear ribosomal DNA (nrDNA) because it has been shown to be a valuable source of evidence to resolve phylogenetic relationships in many angiosperm groups (Gulbitti-Onarici *et al*., 2009).

The results of this study showed that all *Triticum* L. and *Aegilops* L. species presented a 700-bp PCR product of invariant length (Fig. 1). And The ITS region ranged from 600 to 602 bp. The length of ITS1 was 221-222 bp and ITS2 was 215-217 bp. The 5.8S subunit was 163 bp long (Table 1). The total length of the entire ITS of rDNA among *Triticum* L. and *Aegilops* L. species during the present study were variable and were in agreement with the results of earlier studies. Hsiao *et al*. (1995a) studied the phylogenetic relationships of 30 diploid species of *Triticeae* representing 19 genomes based on the analysis of the ITS region of the 18-26S rRNA gene. The length of the entire ITS region of the *Triticeae* species studied only varied from 596 to 605 nucleotides. In diploid species of the *Triticeae*, the ITS1 region ranges from 216 to 223 bp in length and the ITS2 ranges from 214 to 217 base pairs. Our results were similar to the research of Cao (1997) who used the internal transcribed spacer to determine the phylogenetic relationship of hexaploid wheats among and within the five groups of hexaploid wheat. The ITS 1, 5.8S and ITS2 regions in the five groups of hexaploid wheat contained 222, 163 and 217 bp, respectively. Both spacer regions (ITS1, ITS2) were identical in length to those of Chinese Spring (Chatterton *et al*., 1992), but there was a 1% sequence difference in the spacer regions between Chinese Spring (Chatterton *et al*., 1992) and the common wheat cultivar Columbus (Cao, 1997). Also Wang *et al*. (2000) studied nine diploid species representing six sections of *Aegilops* using direct sequencing of PCR-amplified DNA fragments for ITS region. The ITS region ranged from 601 to 607 bp. The length of ITS1 was 221-226 bp and ITS2 was 215-217 bp. The 5.8S subunit sequence was the most conserved region and was 164 bp long in all the diploid species sequenced. Furthermore, no nucleotide substitution was observed in this subunit. This is agree with our results. In addition Zhang *et al*. (2002) Studied the origin and evolution of tetraploid wheats based on the Internal Transcribed Spacer (ITS) sequences of nuclear ribosomal DNA. The results of their study showed that 2-bp indel was found in ITS1 sequences and 1-bp indel in ITS2 sequences. and The length ranged from 221 to 223 bp in ITS1 and from 216 to 217 bp in ITS2. This is agree in part with our results, there is a 1-bp indel in ITS1 sequences of *T. turgidum* at position 211 and an 3-bp indel in ITS2 sequences of *Ae. crassa* at position (416) and *T. monococcum* at position (510-511). Also Bordbar *et al*. (2011) observed The difference occurred in the length of ITS2, which ranged from 214 to 216 bp. That difference was due to one dinucleotide (AA/TT) deletion at positions 185-186 of ITS2 in some sequences of *Ae. crassa*, *Ae. vavilovii* and *Ae. juvenalis*.

Based on the results of this study The G+C content of the ITS1 region ranged from (60.3 to 63.9) % in all *Triticum* and *Aegilops* species. The G+C content of the 5.8S subunit was entirely identical in all species, 59.5%. The G+C content of the ITS2 region ranged from 59.9 to 63.5% (Table 1). But this G+C content were differ according to Wang *et al*. (2000) mentioned that The GC content of the *Aegilops* species was fairly equivalent, 61.1-62.9%. so The G+C contents of the sequences in the study of Zhang *et al*. (2002) were varied from 59 to 62% for ITS1 and from 60 to 65% for ITS2. In addition, Nalini *et al*. (2007) reported in identification and characterization of some ITS variants from hexaploid wheat (*Triticum aestivum* L.) The length of the three ITS variants were found to be same but showed variation in their %G+C. Generally %G+C was slightly more in ITS1 (range: 59.00-60.36) than in ITS2 (range: 60.82-61.75). on the other hand Asay *et al*. (1992) observed that mountain rye and wheat (*Triticum aestivum*) have complete homology in the 5.8 s gene but have different nucleotides at 13 and 30 locations in the ITS1 and ITS2 regions, respectively. nevertheless, the AT 39% and GC 61% contents are the same as those in wheat. that is in contrast with 25% AT and 75% GC in rice. The ITS region (601 bp) in mountain rye is 1bp shorter than that in bread wheat (602) and 4 bp longer than that in barley (*Hordeum vulgare*).

The available studies have indicated that may be there is a relationship between the comparatively high GC content and adaptation to the environment. however it is evident that if ITSs are subject to select pressure, temperature is only one factor (Torres *et al*., 1990). This hypothesis certainly is preliminary and needs to be further tested.

Our present study showed that There were 67 variable sites (11.12%) in the entire ITS region. The ITS1 region had 28 variable sites and the ITS2 region had 39 variable sites. In the 5.8 s gene there were no variable sites. And that 16 of 62 variable sites were in *T. dicoccon*, *Ae. kotschyi*, *T. durum* and one in *Ae. biuncialis.* whereas, Sharma *et al*. (2002) indicated that The sequence data showed that the total variable sites in wild barley (7.4%) were comparable with the total variable sites in wheat (6.90%). Also Cao (1997) reported that there were 13 variable sites (2%) in the ITS1 and ITS2 regions among the five groups of hexaploid wheat.

In the current study, among the substitutions, transitions were more frequent than transversions, the transitions in ITS1 were 17 while in ITS2 were 22. the transversions were 10 in both ITS1, ITS2 and among the indels, deletions were one in ITS1 and 7 in ITS2 (Table 2).

Table 2: The gray bases indicate transition, transversion, deletion

30_R_primer-5R	TCTATTTAAT
27_Rev_5R-Rev	**G**CTATTTAAT
41-R_Rev-Primer	-CTAT**AA**AAT

TCGTGACCCT GACCAAAACA GACCGCGCAC GCGTCATCCA ATCCGTCGGC
GACGGCATCG TCCGTCGCTC GGCCAATGCC TCGACCACCT CCCCTCCTCG
GAGCGGGTGG GGGCTCGGGG TAAAAGAACC CACGGCGCCA AAGGCGTCAA
GGAACACTGT GCCTAACCCG GGGGCATGGC TAGCTTGCTA GCCGTCCCTC
GTGTTGCAAA GCTATTTAAT CCACACGACT CTCGGCAACG GATATCTCGG
CTCTCGCATC GATGAAGAAC GTAGCGAAAT GCGATACCTG GTGTGAATTG
CAGAATCCCG CGAACCATCG AGTCTTTGAA CGCAAGTTGC GCCCGAGGCC
ACTCGGCCGA GGGCACGCCT GCCTGGGCGT CACGCCAAAA CACGCTCCCA
ACCACCCTCA TCGGGAATCG GGATGCGGCA TCTGGTCCCT CGTCTCGCAA
GGGGCGGTGG ACCGAAGATC GGGCTGCCGG TGTACCGCGC CGGACACAGC
GCATGGTGGG CGTCCTCGCT TTATCAACGC AGTGCATCCG ACGCGCAGCC
GGCATTATGG CCTCAGAATG ACCCAGCAAA CGAAGCGCAT GTCGCTTCGA
CC

Fig. 2: The ITS sequence of *Ae. bicornis* the highly conserved area within the ITS1 is indicated in bold

However, the frequency of such sites in the present study was higher than those reported earlier in the ITS sequences of different species of *Triticeae*. For instance, in a study involving ten genotypes of cultivated barley*, H. vulgare*, only 0.84% substitution sites were available while the indels were completely absent (Petersen and Seberg, 1996). In another study involving different species of *Triticeae* including wild and cultivated barley, no substitutions or indels were detected in a study of two accessions of each species procured from widely separated geographical regions (Hsiao *et al*., 1995a).

A characteristic conserved sequence GGCRY- (4 to 7n) GYGYCAAGGAA (where Y = C or T, R = G or A), was also available in the ITS1 of both *Triticum* L. and *Aegilops* L. species (Fig. 2). In previous studies on many flowering plants this characterstic sequence has been reported in the middle of ITS1 and this sequence is presumed as a recognition site for processing of a primary transcript into the structural rRNA (Liu and Schardl, 1994). So we can notice this conserved sequence in the middle of ITS1 at position (134-150) (GGCGCCGAAGGCGTCAAGGAA) and we observe 2 transversions (G→A) at position 140 in two *Aegilops* species *Ae. bicornis*, *Ae. searsii*.

The phylogenetic tree of 26 *Triticum* and *Aegilops* species generated using the Unweighted Pair Group Mean Arithmetic Average (UPGMA) and Neighbor-Joining (NJ) methods was shown in (Fig. 3). two trees are essentially identical. There were mainly three clades in this tree. The first clade included *T. turgidum*, *T. aestivum*, *T. dicoccon*, *T. durum*. The second clade consisted of *Ae. speltoides*, *Ae. triuncialis*, *Ae. caudata*, *Ae. ovata*, *Ae. biuncialis*, *Ae. umbellulata*, *Ae. peregrina*, *Ae. bicornis*, *Ae. sharonensis*, *Ae. comosa*, *Ae. neglecta*, *Ae. tauschii*, *Ae. cylindrica*, *Ae. ventricosa*. The third clade contained of *T. dicoccoides*, *T. monococcum*, *Ae. uniaristata*, *Ae. searsii*, *Ae. longissima*, *Ae. crassa*, *Ae. kotschyi*, *T. urartu* (Fig. 3).

The wheats (*Triticum* spp.) form a polyploid series with diploid (2 n = 2 x = 14), tetraploid (2 n = 4 x = 28) and hexaploid (2 n = 6 x = 42) forms. The diploid wheats comprise a single genomic group with the genome formula AA (*T. monococcum*, *T. urartu*). The tetraploid emmer wheats are divided into two groups, those with the genome formula AABB (*T. turgidum*) and those with the genome formula AAGG (*T. timopheevi*). On evidence it appears that the wild allotetraploid emmer wheat *T. turgidum* ssp. *dicoccoides* (AABB) arose by amphyploidy between the wild diploid wheat *T. urartu* (AA) and a diploid member of the *Aegilops* genus (BB) (Rudnóy *et al*., 2002). The origin of B genome is still a matter of debate. Polyphyletic origin or divergent evolution of B genome from the donor species are hypothesized. On the basis of chondriome divergence *Ae. speltoides* seems to be the cytoplasm donor (female parent) of the tetraploid wheats (Wang *et al*., 2000). A descendant of *T. turgidum* ssp. *dicocoides*, the *T. turgidum* ssp. *dicoccon* was probably the ancient tetraploid from which hexaploid wheats (AABBDD) may have evolved by hybridisation between the AABB tetraploid as cytoplasm donor and the D genome diploid *Ae. taushii*. (Huang *et al*., 2002). Our results in the first clade reflect these facts (Fig. 3). the current study showed that *Ae. speltoides* was separated from remaining four *Sitopsis* species.

Zhang *et al*. (2002) reported that Phylogenetic analysis demonstrated that *Aegilops speltoides* was distinct from other species in *Aegilops* sect. *Sitopsis*. Also Sliai and Amer (2011) revealed that *Ae. speltoides* does not form a monophyletic clade with other *Sitopsis* species (Goriunova *et al*., 2008; Salina *et al*., 2006). Recent studies showed that *Ae. speltoides* was the main contributor of the B genome of polyploid wheats (Huang *et al*., 2002). Inaddition the sequence of one chloroplast gene (*rbcL*, for the Rubisco large subunit) from seven *Triticum* and *Aegilops* species indicated that *Ae. speltoides* is the donor of both the plasmon and B genome of common wheat (Terachi *et al*., 1988; Wang *et al*., 1997; Gupta *et al*., 2008; Al-ahmar *et al*., 2010). And the tree reconstructed based on data of ten EST-SSRs mapped on the B genome showed that *Ae. speltoides* had the closest relationship with *T. aestivum* and *T. durum* (Zhang *et al*., 2006). Yen *et al*. (2005) observed the cytoplasm of *T. turgidum* L. is very similar to that in some races of *Ae. speltoides*.

In our ITS analysis, a close relationship of the sequences from *Ae. tauschii* (*Ae. squarrosa*) and *Ae. cylindrica* was found. Wang *et al*. (1997) reported that *Ae. squarrosa* is the maternal parent of three tetraploids, *Ae. cylindrica*, *Ae. crassa* and *Ae. ventricosa* (Wan *et al*., 2002). Also according to Queen *et al*. (2004) The three D genome-containing *Aegilops* species, comprising the two members of Section *Vertebrata* (*Ae. ventricosa*, *Ae. tauschii*; Van Slageren

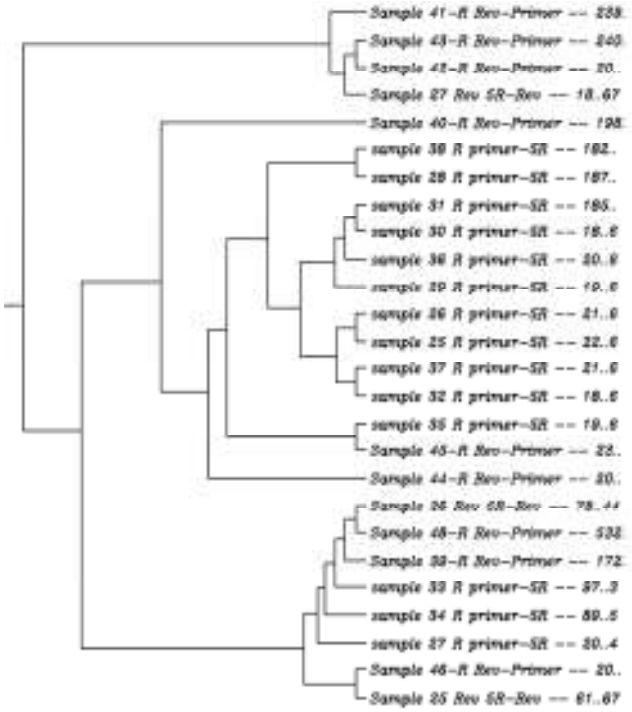

Fig. 3: Phenogram of UPGMA cluster analysis in *Triticum* L. and *Aegilops* L. species

1994) and *Ae. cylindrica* form a clade. More over Kharazian (2008) said *Ae. taushii* and *Ae. cylindrica* have similar genome which it grouped together in the application of Rf data (the migration distance of the band/distance of solvent front) (Jaaska, 1981, 1993) but in the MW (the molecular weight of prolamin bands) are separated. Wang *et al*. (2000) provide an approach to understand the genome evolution of allopolyploid species in genus *Aegilops* through studied the Evolution of parental ITS regions of nuclear rDNA in allopolyploid *Aegilops* (*Poaceae*) species and assumed that they might be largely homogenized by concerted evolution toward one of their other ancestors during the process of hybridization and polyploidization. These evidences have been observed in this study. Based in this conclusion we observed a close relationships between (*Ae. tauschii* and *Ae. cylindrica*), (*Ae. triuncialis* and *Ae. caudata*), (*Ae. comosa* and *Ae. neglecta*), (*Ae. biuncialis*, *Ae. ovata*, *Ae. peregrine* and *Ae. umbellulata*). Wang *et al*. (1997) indicated that *Ae. triuncialis* arose from *Ae. umbellulata* as mother and their results supports the dimaternal origin of *Ae. triuncialis* from reciprocal crosses between *Ae. umbellulata* and *Ae. caudata*. Konstantinos and Bebeli (2010) observed *Ae. triuncialis* (genome UC) grouped in the same subgroup with *Ae. caudata* (C), which is its progenitor male parent. on the other hand they noticed that, *Ae. peregrina*-SU grouped closer to the male parent (*Ae. umbellulata*-U) than the female parent (species from Sitopsis section possibly *Ae. searsii*-S). In addition our ITS sequence-based phylogeny showed *Ae. ovata*, *Ae. triuncialis*, *Ae. peregrina*, *Ae. biuncialis* formed a clade with their common ancestor, *Ae. umbellulata* (UU) (Fig. 3). Wang *et al*. (2000) pointed out that *Ae. umbellulata* (UU) and *Ae. ovata* (UUMM) have the closest relationship among the polyploids with U genome. their results were in agreement with the similarities of ecological and morphological characters of these two species. Wang *et al*. (1997) reported that *Ae. biuncialis* arose from *Ae. umbellulata* as mother.

In the second clade there was two sister group (*Ae. comosa*, *Ae. neglecta*) and (*Ae. sharonensis*, *Ae. bicornis*) (Fig. 3). Mendlinger and Zohary (1995) reported that *Ae. sharonensis* was found to be equally close to *Ae. bicornis*. Wang *et al*. (1997) likewise observed the plasmon of *Ae. umbellulata* is closely related to the plasmons of *Ae. comosa*. and *Ae. neglecta* origin from reciprocal crosses between *Ae. umbellulata* and *Ae. comosa*.

T. urartu, *T. monococcum* belong to the *Einkorn* wheat group (Mizumoto *et al*., 2002). These two species proposed as the A genome doner to polyploid wheats (Dvorák *et al*., 1993; Takumi *et al*.,1993). Our results revealed that there were Two different types of ITS sequences were found in *Triticum* tetraploid species. One type (*T. dicoccoides*) formed a group with *T. monococcum*. and the other type (*T. turgidum*) consisted of a group with other *Triticum* species. This finding is supported by Zhang *et al*. (2002) that mentioned the A genome of *T. dicoccoides* originated from *T. monococcum* based on the ITS 2 sequences of nuclear ribosomal DNA. The similarity between *T. dicoccoides* and *T. monococcum* could be the result of a recent introgression event (Fig. 3).

Tsunewaki and Ogihara (1983) noted that S^b and S' plasma types found in *sitopsis* section showed much closer relation to A plasma type of *Einkorn* wheat than to other plasma types (B, G and S) of the same section. More over according to Wang *et al*. (1997) based on two trees (illustrates the phylogenetic trees constructed by Unweighted Pair-Group Method Using Arithmetic Averages (UPGMA) and Neighbor-Joining (NJ) methods) showed that *Einkorn* is closer to *Aegilops* than to *Triticum*. A similar result was reported by Cenkci *et al*. (2008) using Random Amplification of Polymorphic DNA (RAPD) analysis to estimate the phylogenetic relationships among wild species of *Triticum* and *Aegilops* and cultivars of *Triticum aestivum* and *Triticum turgidum*. And this is agree with present study *T. urartu*, *T. monococcum* had a closer relationships with *Ae. uniaristata*, *Ae. searsii*, *Ae. longissima*, *Ae.crassa*, *Ae. kotschyi.* Terachi *et al*. (1984) observed a very close resemblance between *Ae. crassa* and *T. monococcum*. Whereas, Wang *et al*. (1997) mentioned that *Ae. searsii* was mostly similar to *Ae. kotschyi* and *Ae. kotschyi* arose from *Ae. searsii* as mother. The present results support this conclusion.

CONCLUSION

- ITS 2 was more variable than ITS 1.
- *T. dicoccon, Ae. kotschyi*, *T. durum* were more variable than other species.
- *Einkorn* is closer to *Aegilops* than to *Triticum*.
- *Ae. ovata*, *Ae. triuncialis*, *Ae. peregrina*, *Ae. biuncialis* formed a clade with their common ancestor, *Ae. umbellulata* (UU).
- The similarity between *T. dicoccoides* and *T. monococcum* could be the result of a recent introgression event.
- Conserved area was identical in the ITS 1 of both *Triticum* and *Aegilops* species except two *Aegilops* species *Ae. bicornis*, *Ae. searsii*.
- Transitions were more frequent than transversions and the deletions were one in ITS 1 and 3 in ITS 2.
- The frequency of variable sites in the present study was higher than those reported earlier in the ITS sequences of different species of *Triticeae*.
- We can identify ITS sequences of different parental origins in *Triticum* and *Aegilops* species and hence to identify their progenitors.
- ITS uesd for resolving relationships among closely related species in *Triticeae* and other plant species.

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