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Resolving Genetic Relationships Among *Aegilops* L. and *Triticum* L. Species Using Analysis of Chloroplast DNA by Cleaved Amplified Polymorphic Sequence (CAPS)

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Abstract: Studying of genetic relationships among *Triticum* L. and *Aegilops* L. species by using Cleaved Amplified Polymorphic Sequence (CAPS) to investigate the polymorphism in chloroplast DNA (cpDNA) among 20 *Aegilops* L. and 7 *Triticum* L. species and one accession of *Amblyopyrum muticum.* 23 chloroplast regions were amplified then Restriction digests were performed on PCR amplicons instructions using 72 single-cut Endonucleases. The rseults indicate that there are 16 accessions from *Triticum* and *Aegilops* species have positive and unique restriction profiles in 5 loci. Inaddition 11 accessions from *Aegilops* and one accession of *Amblyopyrum muticum* have identical restriction profiles in all loci.

Keywords: *Aegilops*, CAPS, chloroplast DNA, genetic relationships, *Triticum*

INTRODUCTION

By 2020, the world demand for wheat will be 40% greater than it is today (Monneveux *et al*., 2000), To face the demands for increasing global food production, plant breeding is adopting new approaches to develop improved cultivars and increase crop yields (Landjeva *et al*., 2007). In last decades, the narrow genetic basis of modern wheat cultivars is well evident (Alnaddaf *et al*., 2011).

It is therefore necessary to broaden the genetic base of wheat through the introgression of novel materials including wild relatives which contain numerous unique alleles that are absent in modern wheat cultivars should be emphasized. The wild relatives of bread wheat, *T. aestivum* L., are considered as potential sources of useful alleles for bread-wheat improvement (Khalighi *et al*., 2008) because The high polymorphism found in wild gene resources of cultivated crops can be important for agricultural crop improvement/breeding purposes (Cenkci *et al*., 2008).

Some *Aegilops* species participated in wheat evolution and played a major role in wheat domestication thus the genus *Aegilops* represents the largest part of the secondary gene pool of wheat and several species have been used in crop improvement programs (Kilian *et al*., 2011).

Phylogeny and evolution Earlier the theories of evolution were based on morphological and geographical variations between organisms (Sharma *et al*., 2008). But recently Molecular markers are very important and strong implements for assessment of genetic relationships, selection of superior plants and study of similarity 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.. (Baghizadeh and Khosravi, 2011).

Selection of the best marker system depends on aim of research and ploid level of studied being (Dehghan *et al*., 2008; Naghavi *et al*., 2005). The use of micro-satellites (Simple Sequence Repeats, SSRs) and Amplified Fragment Length Polymorphisms (AFLPs) are routine methods for quickly and efficiently estimating relationships between lines and populations of many plant species (Khalighi *et al*., 2008). AFLP is an efficient, reproducible technique which combines the reliability of Restriction Fragment Length Polymorphisms (RFLP) and the power of Polymerase Chain Reaction (PCR) technique (Vos *et al*., 1995). RFLPs have been used in evolutionary studies for assuming the relationship between the hexaploid genome of bread wheat and its ancestors (Jena and Khush, 1990). Random Amplified Polymorphic DNAs (RAPD) and Inter Simple Sequence Repeat (ISSR) markers used in evolutionary studies of wheat and rice, respectively (Gale and Devos, 1998; Ribaut *et al*., 1997). Specific markers like STMS (Sequence Tagged Microsatellite Markers) ALPs (Amplicon Length

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Polymorphisms) or (Sequence-Tagged Sites) STS markers have proved to be extremely valuable in the analysis of gene pool variation of crops during the process of cultivar development and classification of germplasm. These markers are extremely sensitive and can detect allelic variability during cultivar development (Sharma *et al*., 2008). Many cytoplasmic and cytological studies (Tsunewaki *et al*., 1976; Teoh and Hutchinson, 1983) and an isozyme analysis (Benito *et al*., 1987) were done to reveal the genome relationships of *Triticum-Aegilops* species. More recent analyses have been focused on molecular markers in nuclei (Dvorak and Zhang, 1992; Sasanuma *et al*., 1996; Wang *et al*., 2000; Huang *et al*., 2002a, b; Sallares and Brown, 2004) or organelles (Tsunewaki and Ogihara, 1983; Terachi and Tsunewaki, 1986; Ogihara and Tsunewaki, 1988; Terachi and Tsunewaki, 1992). Chloroplast DNA (cpDNA) has been used extensively to infer plant phylogenies at different taxonomic levels (Gielly and Taberlet, 1994). Direct sequencing of Polymerase Chain Reaction (PCR) products is now becoming a rapidly expanding area of plant systematic and evolution (Clegg and Zurawski, 1991). Provan *et al*. (2004) recently used the entire chloroplast genome sequences of *Oryza*, *Triticum* and *Zea* to screen the chloroplast genomes of grasses to identify SSRs. Similarly, Takahashi *et al*. (2005) compared *Saccharum* and *Zea* chloroplast genome sequences to search for regions of high variability for use in a phylogeny of *Saccharum*, Daniell *et al*. (2006) surveyed complete chloroplast genome sequences of four *Solanaceae* species and Timme *et al*. (2007) compared complete chloroplast genome sequences of *Helianthus* and *Lactuca* (*Asteraceae*). Lastly, Kress *et al*. (2005) compared the complete chloroplast genomes of *Atropa* and *Nicotiana* to find an appropriate region for DNA barcoding in plants. Ogihara and Tsunewaki (1988) stated cpDNA is highly suitable for classification of cytoplasms and phylogenetic study among divergent plant species. And they classified cpDNAs from 42 lines of *Triticum* and *Aegilops* into 17 types by analysis using 13 restriction endonucleases and they investigated phylogenetic relationships among these chloroplast genome types. These data provide a molecular basis for the genetic diversity of cytoplasms in *Triticum* and *Aegilops*. The goal of this study is to Resolve genetic relationships among *Triticum* L. and *Aegilops* L. species using Cleaved Amplified Polymorphic Sequence (CAPS) to investigate the polymorphism in chloroplast DNA (cpDNA) among 20 *Aegilops* L. and 7 *Triticum* L. species and one accession of *Amblyopyrum muticum.*

MATERIALS AND METHODS

Plant materials: The plant material consisted of 28 accessions (Table 1). Twenty accessions representing (20) *Aegilops* L species: *Ae. crassa*, *Ae. tauschii*, *Ae. umbellulata*, *Ae. triuncialis*, *Ae. Juvenalis*, *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. uniaristata*, *Ae. sharonensis.* (7) accessions representing 7 *Triticum* L. species: *T. monococcum*, *T. urartu*, *T. dicoccoides*, *T. durum*, *T. turgidum*, *T. dicoccon*, *T. aestivum* and one accession of *Amblyopyrum muticum*. 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. Analysis were performed in the Laboratory of Molecular Biology and Biotechnology-Department of Molecular Biology and Biotechnology, Atomic Energy Commission, Damascus, Syria in 2011.

DNA extraction: We extracted chloroplast DNA (cpDNA) from young leaves of a single plant for each accession using the Dorokhov and klocke (1997). Recovered DNA pellets were dried under the laminar flow and then resuspended in 150 μL of doubled distilled and sterilised water. 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 20 μL volume containing 10×PCR buffer (Eurobio), dNTPs (10 mM) (Mix Roche), 10×MgCl2 (50 mM) (Eurobio), forward primer (15 μM) (Invitrogen), reverse primer (15 μM) (Invitrogen), Taq polymerase (5 U/μL) (Eurobio). DNA was added to each PCR at a rate of 20 ng and the total volume was adjusted with dd H2O to 20 μL. For 35 cycles, PCRs were subjected to 94ºC for 30 s for DNA denaturation, 46.6-58.5ºC for 1 min for annealing of primers, 72ºC for 1 min for extension of the target chloroplast region and 72ºC for 5 min for final extension. Two μL of each PCR product was loaded into 1.8% agarose gel that was run at 120 V for 30 min. Twenty

Table 1: List of accessions used in the study

1. *Ae.crassa*	8. *Ae. caudata*	15. *Amblyopyrum muticum*	M2. *T. monococcum*
2. *Ae. tauschii*	9. *Ae. biuncialis*	16. *Ae. cylindrica*	U2. *T. urartu*
3. *Ae. umbellulata*	10. *Ae. ovata*	17. *Ae. kotschyi*	Di2. *T. dicoccoides*
4. *Ae. triuncialis*	11. *Ae. neglecta*	18. *Ae. longissima*	D2. *T. durum*
5. *Ae. juvenalis*	12. *Ae. speltoides*	19. *Ae. bicornis*	Tr2. *T. turgidum*
6. *Ae. comosa*	13. *Ae. ventricosa*	20. *Ae. uniaristata*	Dc2. *T. dicoccon*
7. *Ae. peregrina*	14. *Ae. searsii*	21. *Ae. sharonensis*	T5. *T. aestivum*

three chloroplast regions were amplified then Restriction digests were performed on PCR amplicons instructions using 72 single-cut Endonucleases.

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 0.5x buffer and visualised under UV lights.

Data analysis: CAPS data were scored as (1) when the amplification product of a targeted locus was positively restricted and as (2) when positive unique restriction profile and as (0) when negatively restricted.

RESULTS

Amplification of chloroplast loci in *Triticum* L. and *Aegilops* L species: All universal primer pairs used to target the 23 chloroplast loci generated PCR amplicons of the appropriate size when applied to template DNA from all of the species analyzed. Generally, a single, clear band was observed when the PCR products generated were fractionated through agarose gels and visualized by ethidium bromide staining. There was no size variation among species analyzed for 22 out of 23 loci amplified. The exception to this rule was the amplicons of *trnL-F* (Fig. 1), where *Ae. comosa* yielded amplicons of around 460 bp, whereas those generated from all remaining *Triticum* species and *Ae.* crassa were approximately 410 bp.

Cleaved Amplified Polymorphic Sequence (CAPS) analysis: The restriction of amplicons of each locus targeted using (1-5) endonucleases was either complete or absent There was failure to restrict across all 28 species for 12 of the 72 locus-enzyme combinations tested, while 55 of the remaining combinations had a positive and identical restriction profile in all samples. The remaining 5 combinations showed different levels of variation among species analysed:

- ***psbE&psbF*-HpaII** resulted in identical and positive restriction in *Ae. speltoides* and *T.* durum, *T. turgidum*, *T.* dicoccon, *T.* aestivum but failed to restrict in the remaining species (Fig. 2).
- ***orf62*-HindII** resulted in identical and positive restriction in *Ae. triuncialis*, *Ae. caudate*, *Ae. searsii* but failed to restrict in the remaining species.
- ***trnL-F*-HinfI** resulted in positive unique restriction in *Ae. triuncialis*, *Ae. comosa*, *Ae. caudate*, *Ae.* searsii, *Ae. cylindrica* and positive restrictions in the remaining species.
- ***trnK*intron-Hinf I** was unique for *Ae. peregrina* and positive restriction profile.

Fig. 1: Amplification of *trnL-F* in 7 *Triticum* and 2 *Aegilops* species M: 100 bp DNA ladder; M2: *T. momnococcum*; U2: *T. urartu*; Di2: *T. dicoccoides*; D2: *T. durum*; Tr2: *T. turgidum*; Dc2: *T. dicoccon*; T5: *T. aestivum*; 1: *Ae. crassa*; 6: Ae. comosa

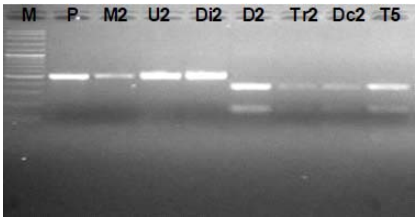

Fig. 2: Restriction of *psbE and F*-HpaII loci in 7*Triticum* species M: 100 bp DNA ladder; P: template amplicon; M2: *T. momnococcum*; U2: *T. urartu*; Di2: *T. dicoccoides*; D2: *T. durum*; Tr2: *T. turgidum*; Dc2: *T. dicoccon*; T5: *T. aestivum*

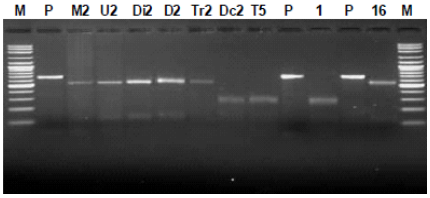

Fig. 3: Restriction of *trnT-L*-TaqI loci in 7 *Triticum* and 2 *Aegilops* species M: 100 bp DNA ladder; P: template amplicon; [M2: *T. momnococcum*; U2: *T. urartu*; Di2: *T. dicoccoides*; D2: *T. durum*; Tr2: *T. turgidum* (unique restriction profiles)]; Dc2: *T .dicoccon*; T5: *T. aestivum*; 1: *Ae. crassa*; [16: *Ae. cylindrica* (unique restriction profile)]

- ***trnT-L*TaqI** was unique for *Ae. tauschii*, *Ae. ventricosa*, *Ae. cylindrica*, *T. monococcum*, *T. urartu*, *T. dicoccoides*, *T. durum*, *T. turgidum* and positive restriction in the remaining species (Fig. 3).

Based on morphological and genetic analysis, the genus *Aegilops* is separated into five sections: *Aegilops*, *Comopyrum*, *Cylindropyrum*, *Sitopsis* and *Vertebrata*, with combinations of the genomes C, D, M, N, S and U (Van Slageren, 1994).

***Aegilops*:** *Ae. biuncialis*, *Ae. Ovata* = *Ae. geniculata*, *Ae. neglecta*, *Ae. peregrine*, *Ae. umbellulata*, *Ae. triuncialis*, *Ae. kotschyi*.

Ae. biuncialis, *Ae. ovata*, *Ae. neglecta*, *Ae. umbellulata*, *Ae. kotschyi*, These species showed identical restriction profiles using restriction enzymes in 23 chloroplast regions. *Ae. triuncialis* differed from another species in the same section in (*orf62*, *trnL-F*). whereas *Ae. peregrine* had uniqe restriction profile in (*trnK* intron) loci.

***Comopyrum*:** *Ae. comosa*, *Ae. uniaristata* two species showed identical restriction profiles using restriction enzymes in 22 chloroplast regions and differed in *(trnL-F*) loci.

***Cylindropyrum*:** *Ae. caudate*, *Ae. cylindrica* two species showed identical restriction profiles using restriction enzymes in 21 chloroplast regions and differed in two loci *(trnT-L , orf62*)

***Sitopsis*:** *Ae. bicornis*, *Ae. longissima*, *Ae. searsii*, *Ae. sharonensis*, *Ae. speltoides*.

Ae. bicornis, *Ae. longissima*, *Ae. sharonensis* these species showed identical restriction profiles using restriction enzymes in 23 chloroplast regions. But, *Ae. searsii* differed on two chloroplast regions (*orf62*, *trnL-F*) compered with another species in the same section. whereas *Ae. speltoides* differed in (*psbE&psbF*) loci.

***Vertebrata*:** *Ae. Juvenalis*, *Ae. tauschii*, *Ae. crassa*, *Ae. ventricosa.*

Ae. crassa, *Ae. Juvenalis* two species showed identical restriction profiles using restriction enzymes in 23 chloroplast regions. whereas *Ae.tauschii* and *Ae. ventricosa* showed identical restriction profiles using restriction enzymes in 23 chloroplast regions and differed on that these two species in the same section in *trnT-L* loci.

DISCUSSION

The results of this study showed that *Aegilops searsii* and *Aegilops caudate* have identical chloroplast type. Sliai and Amer (2011) reported that two species were sisters to each other when they used Nucleotides of 1651 bp from 5.8 S rRNA gene and the intergenic spacers *trnT-trnL* and *trnL-trnF* from the chloroplast DNA. The high similarity in cpDNA observed in the present study for *Ae. sharonensis*, *Ae. longissima* and *Ae. bicornis* (these species which consisted of *Sitopsis* section with *Aegilops searsii* and *Aegilops speltoides*). This is in agreement with Wang *et al*. (1997) which used (PCR-SSCP) To investigate phylogenetic relationships among plasmons in *Triticum* and *Aegilop*. Mendlinger and Zohary (1995) reported that *Aegilops sharonensis* was found to be equally close to *Ae. longissima* and *Ae. bicornis*. And this is consistent with current classifications (Kellogg *et al*., 1996; Sasanuma *et al*., 1996). whereas *Aegilops searsii* was equally distant from *Ae. longissima*, *Ae. bicornis* and *Ae. sharonensis*. And plasmons of *Ae. speltoides* differ greatly from the plasmons of *Ae. bicornis* (S^b), *Ae. sharonensis* (S^l), *Ae. longissima* (S^l) and *Ae. searsii* (S^v) (Wang *et al*., 1997) and this is agree with present study, *Ae.* searsii differed on two chloroplast regions (*orf62, trnL-F*) compered with another species in the same section. whereas *Ae.* speltoides differed in (*psbE&psbF*) loci. Eig (1929) recognizes two subsections based on features of the glumes: subsection *Truncata*, contains *Ae. speltoides* and the rest of the species belong to subsection *Emarginata*. Sliai and Amer (2011) revealed that *Ae. speltoides* does not form a monophyletic clade with other *Sitopsis* species and that *Ae. speltoides* is distant from the other four *Sitopsis* species (Goriunova *et al*., 2008; salina *et al*., 2006). This finding is supported by various molecular data ((Provan *et al*., 2004); chloroplast microsatellites, the entire 5' External Transcribed Spacer (ETS) region of the 18S rRNA gene, (Sallares and Brown, 2004)), cpDNA (Bowman *et al*., 1983; Ogihara and Tsunewaki, 1988; Yamane and Kawahara, 2005; Kawahara *et al*., 2008), isoenzymes (Jaaska, 1978), electrophoresis of seedling proteins (Bahrman *et al*., 1988), rRNA (Dvorak and Zhang, 1990), repetitive DNA sequences (Taberlet *et al*., 1991), variation in the restriction patterns of repeated nucleotide sequences (Dvorak and Zhang, 1992), electrophoresis of leaf proteins (Mendlinger and Zohary, 1995), nRFLP (Sasanuma *et al*., 1996; Ciaffi *et al*., 2000; Giorgi *et al*., 2002) and in situ hybridization (Giorgi *et al*., 2003). Nevertheless, some studies suggest that the five *Sitopsis* species form a single group without clear differentiation PCR-SSCP analyses of organellar DNAs, (Wang *et al*., 1997). Nor did van Slageren (1994) recognize subsections within section *Sitopsis*. So Yen *et al*. (2005) suggested that the S-genome species share a same basic genome but each has a unique variety. Intensive studies have been done on The species of section *sitopsis* because these species considered potential B genome donors to polyploid wheat. There are different views about which species donated the B genome (Sarkar and Stebbins, 1956; Riley *et al*., 1958; Rees and Walters, 1965) proposed that *Ae. speltoides* is the donor, their reasoning being based on external morphology, karyotype, chromosome pairing, geographical distribution and nuclear DNA content. Kimber and Athwal (1972), Gill and Kimber (1974) criticized this proposal using chromosome pairing data and C-banding patterns. Sears (1956) pointed out that *Ae. bicornis* could have been the B genome donor, his suggestion was based on the morphology of a synthetic amphidiploid (S^bS^bAA) and chromosome pairing data. Johnson (1972, 1975) proposed that *T. urartu* was the B genome donor and his opinion was based mainly on seed protein profiles. However, the

nuclear genome of *T. urartu* was later shown to be nonhomologous to the B and identical to the A genome (Chapman *et al*., 1976; Dvorak, 1976). Also Tsunewaki and Ogihara (1983) stated *T. urartu* cannot be the B genome donor to Emmer and common wheats, as was proposed by Johnson (1972, 1975) because its chloroplast DNA restriction fragment pattern differs from those of Emmer and common wheats by at least eight fragments. Feldman (1978) created the new species, *Ae. searsii*, formerly considered to be a variant of *Ae. longissima* and proposed that it is the B genome donor based on karyotype, geographical distribution and chromosome pairing data. Recently, Kushnir and Halloran (1981) suggested that *Ae. sharonensis* is the B genome donor based mainly on morphological and physiological characteristics of a synthetic amphidiploid (S^1S^1AA) and chromosome pairing data. Thus, almost all species of the *sitopsis* section have been nominated as B genome donor. Recent studies showed that *Ae. speltoides was the main contributor of the B genome of polyploid wheats* (Huang *et al*., 2002b). On the basis of chondriome divergence *Ae. speltoides* seems to be the cytoplasm donor (female parent) of the tetraploid wheats (Wang *et al*., 2000; Rudnoy *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, 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*. Wang *et al*. (1997) stated cpDNAs of emmer and common wheats most resemble cpDNAs of *Ae. speltoides* and greatly differ from cpDNAs of *T. monococcum*, *Ae. bicornis*, *Ae. sharonensis* and *Ae. searsii*. Also the current study showed identical restriction profiles between *Ae. speltoides* and (*T. durum*, *T. turgidum*, *T. dicoccon*, *T. aestivum*) in all loci except one loci we observed positive restriction profiles for (*Ae. speltoides*, *T. dicoccon*, *T. aestivum*) in (*trnT-L*-TaqI) and unique restriction profiles for (*T. durum*, *T. turgidum*) in the same loci.

T. urartu, *T. monococcum* belong to the *E*inkorn wheat group (Mizumoto *et al*., 2002). These two species proposed as the A genome doner to polyploid wheats (Dvorak *et al*., 1993; Takumi *et al*., 1993). our results supported these proposed, *T. monococcum* and *T. urartu* have a similar restriction profiles with *T. dicoccoides*, *T. durum*, *T. turgidum*, *T.* dicoccon, *T.* aestivum except two loci (*psbE&psbF*, *trnT-L*). *T. monococcum* was suggested as the A genome doner (sax, 1922; Gill and Kimber, 1974; Gill and chen, 1987) and it is one of the most ancient crops domesticated in the Middle East (Harlan, 1980). Later, it was revealed that the A genome of *T. urartu* is closer to polyploid wheats than *T. monococcum* genome and therefore *T. urartu* was proposed as the A genome doner (Zhang *et al*., 2006). Ehtemam *et al*. (2010) revealed That SSR analysis showed a close relationship between the diploid *Triticum* species. Huang *et al.* (2002b) stated that the information on *Acc-1* and *Pgk-1* gene families allowed to revisit the question of phylogenetic relationships among wheat and its relatives, Their results agree with some well established facts: the close relatedness of *T. monococcum* and *T. urartu*. Based on the calculated genetic similarities, The A genomes occurring among the polyploids appeared to be more similar to that of the diploid species *T. monococcum* than the other diploids. This observation is partly in accordance with (Johnson, 1975). Tsunewaki and Ebona (1999) pointed out that the A genome donor to *T. aestivum*, *T. turgidum* and *T. durum*, is *T. monococcum* and to *T. dicoccoides* and *T. dicoccum* is *T. urartu*. Tsunewaki and Ogihara (1983) found no differences between *T. monococcum* and *T. urartu* in their chloroplast DNA. The present results support this conclusion. Inaddition 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*. Also our present study showed high similarity in cpDNA between *Einkorn* and *Aegilops* in all chloroplast loci except five loci (*psbE&F*-HpaII, *orf62*-HindII, *trnL-F*-HinfI, *trnK intron*-HinfI, *trnT-L*-TaqI). In (*trnT-L*-TaqI) *T. monococcum* and *T. urartu* have a unique restriction profiles with *T. dicoccoides*, *T. durum*, *T. turgidum*, *Ae. tauschii*, *Ae. ventricosa*, *Ae. cylindrica.*

Based on the results of this study *Ae. umbellulata*, *Ae. ovate*, *Ae. biuncialis*, *Ae. uniaristata*, *Amblyopyrum muticum* (*Ae. mutica*) have identical restriction profiles. Terachi *et al.* (1984) pointed that *Ae. umbellulata* which chloroplast genome is closest to that of *Ae. ovata* among all of the species tested, showed seven ctDNA restriction fragment changes from that of *Ae. Ovate* This fact may suggests a very ancient origin of this specie, compared to those of other tetraploid species of the same Polyeides section. Inaddition they found that Chloroplast genome of *Ae. biuncialis* is identical with that of *Ae. umbellulata* concluded that *Ae. umbellulata* is the cytoplasm donor to *Ae. biuncialis*. Al-ahmar (2010) reported that the two species *Ae. biuncialis* and *Ae. ovata* were the closest genetically. More over according to Wang *et al*. (1997)

Ae. biuncialis arose from *Ae. umbellulata* as mother and *Ae. umbellulata* is closely related to the plasmons of *Ae. uniaristata* and they stated that *Ae. umbellulata* is closely related to the plasmons of *Ae. mutica*. inaddition, van Slageren (1994) clamed that *Ae. mutica* should belong to genus *Amblyopyrum* instead of *Aegilops*. whereas Yamane and Kawahara (2005) conclude that *Ae. mutica* should be included in *Aegilops*, based on all available molecular data.

In (Yamane and Kawahara, 2005) study *Ae. comosa* and *Ae. uniaristata* were clustered as sister species in one clade in the trees based on SSRs and cpDNA. In our results *Ae. comosa* and *Ae. uniaristata* have similar restriction profiles except one loci (*trnL-F*-Hinf I) *Ae. comosa* has unique restriction profile in this loci compered with *Ae. uniaristata.*

Using genome analysis and anatomical studies, Lang and Jochemsen (1992a, b) and Kharazian (2007) proved that *Ae. triuncialis* was related to *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*. Sasanuma *et al*. (1996) mentioned *Aegilops caudata* and *Aegilops umbellulata* formed one cluster in the dendrograms. Al-ahmar (2010) showed the two species *Ae. triuncialis* and *Ae. umbellulata* as sister species in The tree diagram. Konstantinos and Konstantinos (2010) observed *Ae. triuncialis* (genome UC) grouped in the same subgroup with *Ae. caudata* (C), which is its progenitor male parent. These evidences have been observed in this study the high similarity in cpDNA obsereved between *Ae. triuncialis* and *Ae. caudata* and differed from *Ae. umbellulata* with two loci *orf62*, *trnL-F*. Inaddition *Ae. umbellulata* and *Ae. comosa* have a positive restriction profile in *trnL-F* loci. But, *Ae. comosa* has distinguished restriction profile in *trnL-F*-HinfI. Wang *et al*. (1997) likewise observed the plasmon of *Ae. umbellulata* is closely related to the plasmons of *Ae. comosa*.

In section *Aegilops* which consists of *Ae. biuncialis*, *Ae. Ovata* = *Ae. geniculata*, *Ae. neglecta*, *Ae. peregrine*, *Ae. umbellulata*, *Ae.triuncialis*, *Ae. kotschyi*. We found These species have identical restriction profiles except two species which we can distinguish its by three loci [*Ae. peregrine* (*trnK* intron), *Ae. triuncialis* (*orf62*, *trnL-F*)]. Al-ahmar (2010) also reported *Ae. kotschyi* and *Ae. peregrine* were the closest genetically. where as Wang *et al*. (1997) mentioned that *Ae. searsii* was mostly similar to *Ae. kotschyi in* cpDNA and *Ae. kotschyi* arose from *Ae. searsii* as mother. This is agree in part with our results *Ae. searsii differed in two loci orf62, trnL-F compored with Ae. kotschyi.* Konstantinos and Konstantinos (2010) noticed that *Ae. kotschyi*-SU, *Ae. peregrina*-SU) grouped closer to the male parent (*Ae. umbellulata*-U) than the female parent (species from *Sitopsis* section possibly *Ae. searsii*-S). This is agree with present study *Ae. kotschyi*, *Ae. umbellulata* have identical restriction profiles. And *Ae. bicornis* and *Ae. kotschyi* have identical chloroplast type based on restriction analysis of cpDNA, this is also in agreement with (Terachi and Tsunewaki, 1986). Queen *et al*. (2004) research indicated that The tree derived from the retrotransposon SSAP data presented (*Ae. biuncialis*, *Ae. neglecta, Ae. triuncialis*) group together.

In section *vertebrata* (*Ae. Juvenalis, Ae. tauschii, Ae. crassa, Ae. ventricosa*) restriction analysis of the chloroplast genome in this study was similar among *Ae. tauschii* and *Ae. ventricosa* and between *Ae. crassa* and *Ae. Juvenalis*. Wang *et al*. (1997) stated That *Ae. crassa* is the maternal parent of *Ae. juvenalis*. Terachi *et al*. (1984) observed a very close resemblance between ctDNAs of *Ae. crassa* and four other species, *T. monococcum* (plasma type A), *Ae. bicornis* (Sb), *Ae. sharonensis* (S') and *Ae. kotschyi* (S''). the same thing we observed in the current study *Ae. crassa, Ae. bicornis*, *Ae. sharonensis*, *Ae. kotschyi* have identical cpDNA, but *T. monococcum* in *Trn T-L* loci has unique restriction profile compared with *Ae. crassa*. The available studies have indicated that *Ae. ventricosa* has a chloroplast genome identical with that of (*Ae. squarrosa* = *Ae. tauschii)* and *Ae. ventricosa* received its cytoplasm from *Ae. squarrosa* (Terachi *et al*., 1984; Tsunewaki, 1980). Wang *et al*. (1997) found *Ae. squarrosa* cpDNA was identical or similar to those of *Ae. ventricosa* and *Ae. cylindrica*. Inaddation, they 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* (van Slageren, 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. Also, The cluster analysis indicated that *Ae. triuncialis* and *Ae. taushii* were grouped together. in our study *Ae. triuncialis* has negative restriction profile in *orf62*-HindII and unique restriction profile in *TrnL-F*-Hinf I compared with *Ae. tauschii. Ae. tauschii has* unique restriction profile in *TrnT-L*-Taq I compared with *Ae. triuncialis.* Naghavi *et al.* (2008) suggested that *Ae. cylindrica* is a relatively new tetraploid species. Some results suggested that D^t genome of bread wheat and D^c genome of *Ae. cylindrica* inherited from different biotype of *Ae. tauschii* (Caldwell *et al*., 2004). Huang *et al.* (2002b) mentioned that Their results agree with some well established facts that *Ae. taushii* being the donor of the D genome to the *T. aestivum* (AABBDD) genome. Our results showed

Table 2: special accessions according to the results of CAPS

Accessions	Unique restriction	Positive restriction	Negative restriction
Ae. cylindrica	*trnT-L*TaqI		
	trnL-F-HinfI		
Ae. triuncialis	*trnL-F*-HinfI		*orf62*-HindII
Ae. caudate	*trnL-F*-HinfI		*orf62*-HindII
Ae. searsii	*trnL-F*-HinfI		*orf62*-HindII
T. durum	*trnT-L*TaqI	*psbE&psbF*-HpaII	
T. turgidum	*trnT-L*TaqI	*psbE&psbF*-HpaII	
Ae. tauschii	*trnT-L*TaqI		
Ae. ventricosa	*trnT-L*TaqI		
T. monococcum	*trnT-L*TaqI		
T. urartu	*trnT-L*TaqI		
T. dicoccoides	*trnT-L*TaqI		
Ae. peregrina	*trnK*intron-HinfI		
Ae. comosa	*trnL-F*-HinfI		
Ae. speltoides		*psbE&psbF*-HpaII	
T. dicoccon		*psbE&psbF*-HpaII	
T. aestivum		*psbE&psbF*-HpaII	

similar restriction analysis between *Ae. tauschii*, *Ae. ventricosa*, *Ae. cylindrica* in all loci except one loci *(trnL-F*). *Ae. tauschii* and *Ae. ventricosa* have positive restriction profile, but *Ae. cylindrica* has unique restriction profile compared with *Ae. tauschii* and *Ae. ventricosa*. Yen *et al*. (2005) indicated that the Results of molecular analyses have shown that genomes S, B, D and A are much more closely related to each other than to other genomes (Dvorak and Zhang, 1990).

The study of genetic relationships among *Triticum* and *Aegilops* species can contribute to broaden the genetic base of wheat and improve yield, quality and resistance to biotic and abiotic stresses of wheat.

CONCLUSION

From the results of this study it is concluded that we can distinguish 16 accessions from *Aegilops* and *Triticum* species in 5 loci (Table 2).

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