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The Genetic Relationships Among *Aegilops* L. and *Triticum* L. Species

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Abstract: Studying of genetic relationships among *Triticum* L. and *Aegilops* L. species is very important for broadening the cultivated wheat genepool, for monitoring genetic erosion and for breeding reasons, because the genus *Aegilops* represents the largest part of the secondary gene pool of wheat, includes the wild relatives of cultivated wheat which contain numerous unique alleles that are absent in modern wheat cultivars and it can contribute to broaden the genetic base of wheat and improve yield, quality and resistance to biotic and abiotic stresses of wheat. Molecular markers have proved valuable in crop breeding. Selection of the best marker system depends on aim of research and ploid level of studied being.

Keywords: *Aegilops*, DNA marker, genetic relationships, *Triticum*

INTRODUCTION

Wheat is the staple food for 35% of the world's population and is becoming increasingly important in the developing world (CIMMYT, 2003). To meet the demand for developing high yielding and stress resistant wheat cultivars, it is desirable to increase the genetic base of this crop (Khalighi *et al*., 2008). There has been a growing concern about the remaining variability in the bread wheat gene pool which is grossly insufficient to address current and future breeding objectives (Rejesus *et al*., 1996). Study of the genetic diversity in plant inherited stores in order to classify the germplasm regarding resistance to biotic and abiotic stress and also preventing from genetic erosion is one of the basic and fundamental steps in the most breeding programs (Baghizadeh and Khosravi, 2011). In last decades, the narrow genetic basis of modern wheat cultivars is well evident (Alnaddaf *et al*., 2011). Landraces and their wild relatives are an essential raw material source for genetic diversity maintenance and improvement programmes (Baghizadeh and Khosravi, 2011). As breeders prefer using either improved cultivars as parents or advanced breeding materials to accelerate the development of new cultivars. While in the beginning, selection was utilized to isolate pure lines from heterogeneous landraces or natural populations, today improved cultivars were used as parents in wheat breeding programs. 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).

Introduction of genes from *Aegilops* can contribute to favorable traits including yield, quality and resistance to biotic and abiotic stresses (Schneider *et al*., 2008). *Aegilops* species are potential reservoirs of genes for resistance to heat, drought (Zaharieva *et al*., 2001a; Molnár *et al*., 2004), salinity (Colmer *et al*., 2006) and cold (Monneveux *et al*., 2000). Many of the species contain valuable genes for resistance to various biotic pathogens: *Ae. neglecta*, *Ae. geniculata*, *Ae. biuncialis* and *Ae. speltoides* have shown rust resistance (Aghaee-Sarbarzeh *et al*., 2001; Schneider *et al*., 2008); *Ae. geniculata* is resistant to powdery mildew (Stoilova and Spetsov, 2006) and BYDV (Barley Yellow Dwarf Virus) (Zaharieva *et al*., 2001b); *Ae. variabilis* is resistant to nematode Heterodera avenae (Barloy *et al*., 2007). *Aegilops* species can also contribute to yield through modification of physiology of cultivated wheat from *Ae. tauschii* (D genome) by increasing the size of flag-leaf and thus biomass production (Monneveux *et al*., 2000). *Ae. speltoides* and *Ae. tauschii* have shown low activity of polyphenol oxidase (Fuerst *et al*., 2008), while *Ae. kotschyi* and *Ae. tauschii* possess high grain iron and zinc content (Chhuneja *et al*., 2006) which can have an impact on the wheat quality improvement. Therefore, it is important to understand the genetic relationships among *Aegilops* L. and

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Triticum L. species for the genetic improvement of these crops.

LITRATURE REVIEW

Triticum and *Aegilops*, the 2 important genera of the tribe *Triticeae* in the grass family *Poaceae*, have been used for a number of cytogenetic and taxonomic studies (Bandopadhyay *et al*., 2004). The genus *Triticum* L. is composed of diploid, tetraploid and hexaploid species including both domesticated and wild species (Cheniany *et al*., 2007). Of special cultural and economic importance are the tetraploid durum wheat *T. turgidum* L. and the hexaploid bread wheat (common wheat) *T. aestivum* L. (Baum *et al*., 2009). *T. aestivum* L. is by far the most important staple crop in the world feeding about 40% (nearly half) of the world population and providing 20% (one fifth) of total food calories and protein in human nutrition (Gupta *et al*., 2008).

The genus *Aegilops* consists of 22 species of which 10 are diploid, 10 tetraploid and 2 hexaploid with basic chromosome number x = n = 7 (van Slageren, 1994). *Ae.crassa* and *Ae. neglecta* include tetraploid and hexaploid types. Six different genomes were identified in diploid species: C, D, M, N, S and U. The species have been classified into the following five sections: *Aegilops* (8 species), *Comopyrum* (2 species), *Cylindropyrum* (2 species), *Sitopsis* (5 species) and *Vertebrata* (5 species).

The *Aegilops* genus includes the wild relatives of cultivated wheat. It can play an important role in broadening the cultivated wheat genepool and thus shows a potential interest for utilization in wheat improvement (Konstantinos and Bebeli, 2010).

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).

The various species of these genera easily hybridize with each other resulting in either a direct exchange of genetic material or polyploidy (Bhushan, 2001). Polyploid species in *Triticum* and its closely related genus *Aegilops* originated through the allopolyploidization process, i.e., interspecific hybridization and subsequent chromosome doubling (Mori *et al*., 2008). The genus *Aegilops* L. and *Triticum* L. have contributed 2 of the 3 (B and D) and one of the 3 (A) bread wheat genomes, respectively (Fig. 1).

B genome: There are different views about which species donated the B genome. Intensive studies have been done on The species of section *sitopsis* because these species considered potential B genome donors to polyploid wheat. Sarkar and Stebbins (1956), Riley *et al*. (1958) and 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. 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. Kimber and Athwal (1972) and Gill and Kimber (1974) criticized this proposal using chromosome pairing data and C-banding patterns. 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 *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).

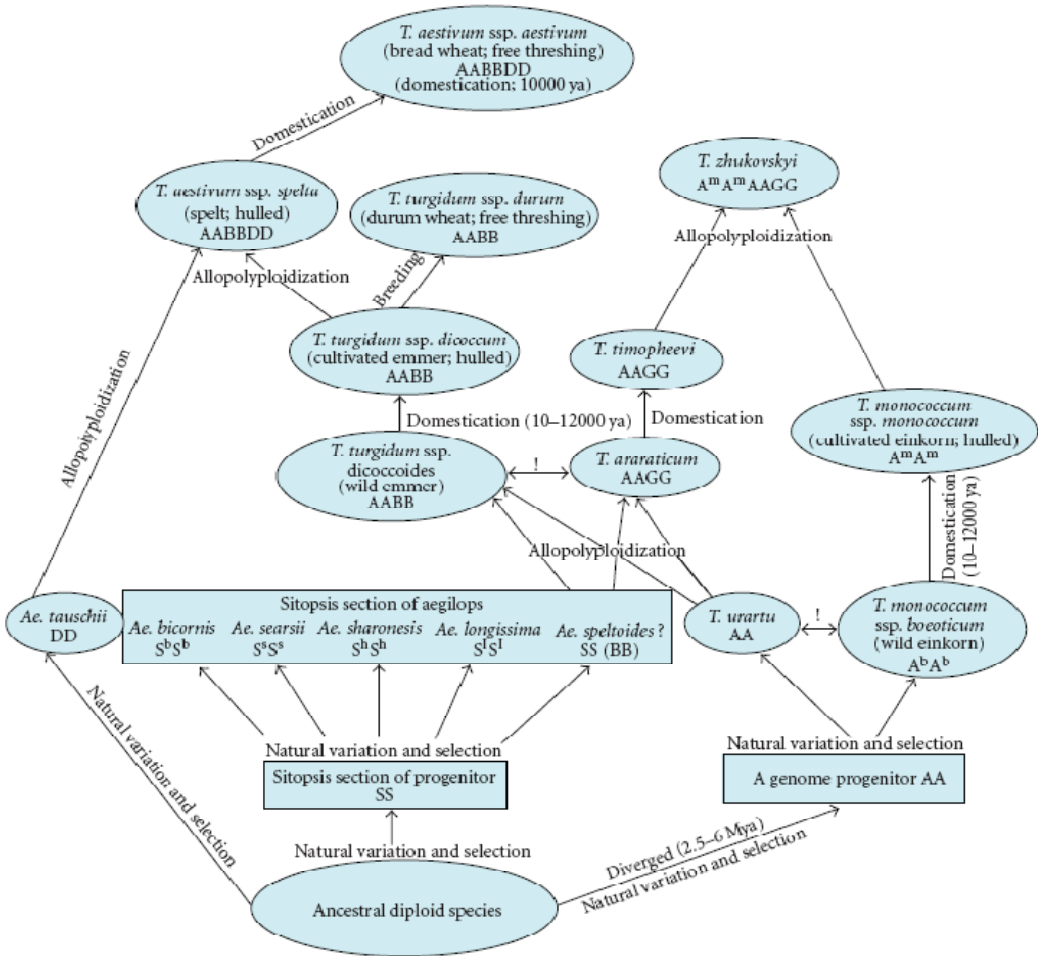

Fig. 1: Schematic representation of the evolutionary history of wheat species (*Triticum* and *Aegilops*) (Gupta *et al*., 2008)

Yen *et al*. (2005) observed the cytoplasm of *T. turgidum* L. is very similar to that in some races of *Ae. speltoides*.

D genome: *Aegilops tauschii* is one of the ancestral species that has donated the D genome to common wheat and it serves as important genetic resources for wheat improvement (Wei *et al*., 2008).

A genome: *T. urartu*, *T. monococcum* belong to the *Einkorn* wheat group (Mizumoto *et al*., 2002). These 2 species proposed as the A genome doner to polyploid wheats (Dvorak *et al*., 1993; Takumi *et al*., 1993). *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). Whilst, 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*. Many studies have been carried out to elucidate the evolutionary relationships of the wild and domesticated species belonging to the genera *Triticum* L. and *Aegilops* L. Due to its close

relationships with *Aegilops*, the cultivated wheat *Triticum* has attracted attention for a long time. 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 no enough to assess the true relationships between these species (Gulbitti-Onarici *et al*., 2009).

The origin of *T. aestivum* and other polyploid wheat species has been subject of numerous studies. Salamini *et al*. (2002) state that understanding the origin of hexaploid wheat would further its genetic improvement. Moreover, knowledge about the role diploid species play in the evolution of polyploidy wheats is important for the development of artificial synthetic forms (Gulbitti-Onarici *et al*., 2009).

Phylogeny and evolution Earlier the theories of evolution were based on morphological and geographical variations between organisms (Sharma *et al*., 2008). The genetic information provided by morphological characters is often limited (Karcicio and Izbirak, 2003). These limitations have resulted in the deployment of biochemical techniques such as isozyme and protein electrophoresis (Gottlieb, 1977; Crawford, 1989). Electrophoretic surveys of proteins play an important role in the quantitative evaluation and management of genetic resources. This is because information concerning the geographical and taxonomic distribution of genetic variation provides guidelines for sampling strategies and germplasm preservation (Karcicio and Izbirak, 2003). In addition, high or low genetic diversity among and within natural populations can be deduced by using different isozyme patterns (Stuber *et al*., 1980; Price *et al*., 1984; Michaud *et al*., 1995).

But recently DNA markers have proved valuable in crop breeding, especially in studies on genetic diversity and gene mapping and study of the phylogenetic relationships among species, subspecies and cultivars (Landry *et al*., 1994) in many plant species including wheat and evolutionary biology in a wide range of crop species (Table 1).

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) (Table 1). The use of micro-satellites (Simple Sequence Repeats, SSRs) and Amplified Fragment Length Polymorphisms (AFLPs) and Random Amplified Polymorphic DNA (RAPD) are routine methods for quickly and efficiently estimating

Table 1: Comparison of different characteristics of most frequently used molecular markers techniques (Mondini *et al*., 2009)

Molecular Markers	RFLP	RAPD	AFLP	SSR	CAPS
Degree of polymorphism	M	M	M	M	L
Locus specificity	Y	N	N	N	Y
Dominance (D)/Co-dom. (C)	C	D	D	C	C
Ease of replication	H	L	H	M	H
Abundance	H	H	H	M	L
Sequence information required	Y	N	N	N	Y
Quantity of DNA required	H	L	M	L	L
Automation	N	Y	Y	Y	Y
Costs per assay	H	L	M	L/M	M
Technical requirement	H	L	M	L/M	H

H: High; M: Medium; L: Low; Y: Yes; N: No

relationships between lines and populations of many plant species and to evaluate variation at the DNA sequence level (Khalighi *et al*., 2008; Staub *et al*., 1996; Gupta and Varshney, 2000; Jones *et al*., 1997). 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).

Bandopadhyay *et al*. (2004) were used 64 EST-SSRs in 18 species of *Triticum-Aegilops* complex to identify genus specific and genome specific EST-SSRs and to estimate the level of DNA polymorphism detected by them in these 18 species of the complex, any polymorphism detected using EST-SSRs, may reflect better the relationships among *Triticeae.* And they indicated that the SSRs derived from the functional portion of the genome of bread wheat may be successfully used in cultivated and wild relatives of wheat belonging to *Triticum-Aegilops* complex for comparative genomics studies such as genome analysis, localization of expressed genes, discrimination among different species, etc. Therefore, EST-SSRs may be used in studies on polymorphism, genetic diversity, gene mapping and synteny conservation across different species of *Triticeae*.

Naghavi *et al.* (2008) were used 21 simple microsatellite primers to determine the genetic

relationship of the D genome among hexaploid wheat *T. aestivum* and 3 *Aegilops* species *Ae. tauschii*, *Ae. cylindrica* and *Ae. crassa*. they reported that different genotypes of *Ae. tauschii* could be involved in the evolution of polyploid species. A high level of variation and also the highest number of unique alleles observed within *Ae. crassa* accessions, indicating that *Ae. crassa* is a good potential source of novel genes for bread wheat improvement. the conclusion of their study confirms the usefulness of SSR markers to study wheat genetic diversity. Additionally, the results obtained from their study could be useful for improving the understanding of diversity in and management of germplasm collections.

Cenkci *et al*. (2008) suggested that RAPD analysis can be used to distinguish wild *Triticum* and *Aegilops* species and wheat cultivars. In addition, RAPD technique can be used to develop genome-specific markers. Konstantinos and Bebeli (2010) were used Random Amplified Polymorphic DNA (RAPD) and Inter-Simple Sequence Repeat (ISSR) analyses to evaluate genetic variability and relationships of Greek *Aegilops* species.

Specific markers like STMS (sequence tagged microsatellite markers) ALPs (Amplicon length 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*., 2002 a, 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) is an extremely valuable molecule for studying phylogenetic relationships between closely related species (Palmer, 1987; Palmer *et al*., 1988; Clegg and Zurawski, 1991; Gielly and Taberlet, 1994) and has been used extensively to infer plant phylogenies at different taxonomic levels, because the small size of the chloroplast genome together with rapid progress in the molecular characterization of chloroplast encoded genes (Whitfeld and Bottomley, 1983) have combined to facilitate evolutionary investigations (Curtis and Clegg, 1984). And Chloroplast markers are useful for species determination and the study of hybrid evolution in plant taxa and seed dispersal (Zapiola *et al*., 2010) and to study many aspects of crop plant evolution such as documenting multiple events in the domestication of maize from its wild progenitor (Provan *et al*., 1999a) and the existence of cytoplasmic bottlenecks in the domestication of cultivated barley (Provan *et al.*, 1999b) and in the modern European cultivated potato (Provan *et al*., 1999c). Because of the uniparental mode of transmission (Ennos *et al*., 1999).

Powell *et al*. (1995) reported SSR variability in the chloroplast genome and suggested it may be a source of polymorphic markers for studies in plant population genetics and systematics. Chloroplast micro satellites (cpSSR) are now a high resolution tool for examining cytoplasmic variation in a wide range of species (Provan *et al*., 2001, 2004; McGrath *et al*., 2006).

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*. Kress *et al*. (2005) compared the complete chloroplast genomes of *Atropa* and *Nicotiana* to find an appropriate region for DNA barcoding in plants.

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*).

Sliai and Amer (2011) used Nucleotides of 1651 bp from 5.8 S rRNA gene and the intergenic spacers *trnT-trnL* and *trnL-trnF* from the chloroplast DNA were combined together in order to investigate the genetic diversity among Four *Aegilops* species (*Aegilops longissima*, *Aegilops speltoides*, *Aegilops searsii* and *Ae. caudata*).

In general, cpDNA has a low rate of nucleotide substitution, which facilitates comparison of variation in a wide range of plant taxa. Furthermore, uniparental inheritance lowers the impact of intermolecular recombination and helps to simplify theories of chloroplast genome evolution in most plant taxa. comparative analyses of grass cpDNA variation have complemented morphological studies and given us novel insights into grass evolution (Matsuoka *et al*., 2002).

Although the analysis of cpDNA has contributed significantly to our understanding of grass evolution, most of the conclusions drawn so far are based on nucleotide sequence variation in a single chloroplast gene or gene intron such as *rbcL* (Doebley *et al*., 1990),

ndhF (Clark *et al*., 1995), *rpoC2* (Cummings *et al*., 1994), *rps4* (Nadot *et al*., 1994), *matK* (Hilu and Alice, 1999) and *rpl16* intron (Zhang, 2000).

In contrast to widespread studies done with the single gene-based approach, there have been only a few studies that addressed grass evolution or grass chloroplast gene evolution based on multiple chloroplast gene sequences (Wolfe *et al*., 1987; Wolfe *et al*., 1989; Gaut and Clegg, 1993). The entire chloroplast genome structure of wheat was reported recently (Ogihara and Isono, 2002) and the fully sequenced cpDNAs of 3 cereals, maize (Maier *et al*., 1995) rice (Hiratsuka *et al*., 1989) and wheat, now provide a unique opportunity to investigate grass evolution based on whole-genome comparison.

Cleaved Amplified Polymorphic Sequence (CAPS) is a combination of the PCR and RFLP and it was originally named PCR-RFLP (Maeda *et al*., 1990). The technique involves amplification of a target DNA through PCR, followed by digesting with restriction enzymes (Konieczny and Ausubel, 1993; Jarvis *et al*., 1994; Michaels and Amasino, 1998). Hence, CAPS markers rely on differences in restriction enzyme digestion patterns of PCR fragments caused by nucleotide polymorphism between samples. Critical steps in the CAPS marker approach include DNA extraction, PCR conditions and the number or distribution of polymorphic sites. CAPS markers have several advantages. First, since analysis of restriction fragment length polymorphisms is based on PCR amplification, it is much easier and less time-consuming than analyzing alternative types of markers that require southern hybridizations. Second, CAPS primers developed from ESTs are more useful as genetic markers for comparative mapping study than those markers derived from non-functional sequences such as genomic microsatellite markers. Third, CAPS markers are inherited mainly in a co-dominant manner (Matsumoto and Tsumura, 2004; Semagn *et al*., 2006)

Restriction endonucleases were first employed to study chloroplast DNA evolution by Atchison *et al*. (1976). A variety of studies have accumulated since 1976 that indicate relatively low rates of nucleotide substitution, either between individuals within species or between related species (Timothy *et al*., 1979; Kung *et al*., 1982; Bowman *et al*., 1983; Palmer and Zamir 1982; Tsunewaki and Ogihara, 1983).

Then Restriction enzyme digestion of chloroplast DNA has become a new means for studying interspecific, phylogenetic relationship in plants (Vedel *et al*., 1978; Kung *et al*., 1982; Gordon *et al*., 1982). Bowman *et al*. (1981) constructed a physical map of wheat ctDNA by use of this technology and was able to locate a few genes on it. Ogihara and Tsunewaki (1982) compared ctDNA from 29 species (37 strains in total) of 2 related genera *Triticum* and *Aegilops* by the restriction enzyme technique and demonstrated its usefulness for clarifying interspecific relationships.

Table 2: Special accessions according to the results of CAPS according to (Alnaddaf *et al*., 2012)

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	

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.*

Alnaddaf *et al*. (2012) used 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.* From the results of their study it is concluded that they can distinguish 16 accessions from *Aegilops* and *Triticum* species in 5 loci (Table 2).

RESULTS AND DISCUSSION

Revealing genetic relationships between economically important cultivated species and closely related wild species is valuable for designing conservation program. Further, it facilites the selection of genetic resources to be used in improvement programs of cultivated species (Buso *et al*., 2001). It is

also useful for plant breeders in determination of the primary, secandary and tertiary gene pools for cultivated species to be improved (Pillay, 1995).

There is still controversy regarding the number of species that belong to the genus *Triticum* and on combinig the genus with the genus *Aegilops* (Haider *et al*., 2010). Stebbins (1956) and Morris and Sears (1967) suggested merging both genera as one genus *Triticum*. Bowden (1959) argued that more studies should support this merger. Some other authors apposed this suggestion **(**MacKay, 1968). in 1990s Van Slageren (1994) and Kellogg *et al.* (1996) believed that the 2 genera are different and they should be kept as sparate genera. In a recent study, Yamane and Kawahara (2005) analyzes intra- and inter-specific variation in chloroplast DNA (cpDNA) in diploid *Triticum-Aegilops* species, in their study *Aegilops* species should be included in *Triticum.* Also Haider *et al*. (2010) suggest the merger of the 2 genera based on the 3 phylogenetic trees of seed storage proteins, ISSRs and RAPDs.

Wang *et al*. (1997) which used (PCR-SSCP) To investigate phylogenetic relationships among plasmons in *Triticum* and *Aegilop*. And based on 2 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).

Inaddition Alnaddaf *et al*. (2012) 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.* And they observed that *T. monococcum* and *T. urartu* have a similar restriction profiles with *T. dicoccoides, T. durum, T. turgidum, T. dicoccon, T.aestivum* except 2 loci (*psbE&psbF, trnT-L).*

Tsunewaki and Ogihara (1983) found no differences between *T. monococcum* and *T. urartu* in their chloroplast DNA. This is in agreement with (Alnaddaf *et al*., 2012) van Slageren (1994) classified the species of genus *Aegilops* into the following five sections:

Aegilops: *Ae. biuncialis, Ae. Ovata = Ae. geniculata, Ae. neglecta, Ae. peregrine, Ae. umbellulata, Ae. triuncialis, Ae. kotschyi.* Alnaddaf *et al*. (2012) found These species have identical restriction profiles except 2 species which they can distinguish its by 3 loci [*Ae. peregrine* (*trnK* intron), *Ae.triuncialis* (*orf62, trnL-F*)].

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 *et al.* (2010) reported that the 2 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.

Al-ahmar *et al.* (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 results of study (Alnaddaf *et al*., 2012) *Ae. searsii* differed in 2 loci *orf62*, *trnL-F* compored with *Ae. kotschyi*.

Konstantinos and Bebeli (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 the study of (Alnaddaf *et al*., 2012) *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.

Using genome analysis and anatomical studies, Lang and Jochemsen (1992a, b) and Kharazian (2007) proved that *Ae. triuncialis* was related to *Ae. umbellulata*. Al-ahmar *et al.* (2010) showed the 2 species *Ae. triuncialis* and *Ae. umbellulata* as sister species in The tree diagram.

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. These evidences have been observed in (Alnaddaf *et al*., 2012) the high similarity in cpDNA obsereved between *Ae. triuncialis* and *Ae. caudata* and differed from *Ae. umbellulata* with 2 loci *orf62, trnL-F*. (*Comopyrum*: *Ae. comosa*, *Ae. uniaristata*. *Cylindropyrum*: *Ae. caudate*, *Ae. cylindrica*. *Vertebrata*: *Ae. Juvenalis*, *Ae. tauschii*, *Ae. crassa*, *Ae. ventricosa*).

Comopyrum has been characterized by Jauberst and Spach in (1850-1853) and included only *Ae. comosa*. Later, Zhukkovsky (1928) classifed *Ae. caudata*, *Ae. comosa*, *Ae. uniaristata* in the section based on morphological characters. Based on genome analysis, Kihara (1954) referred to the close genetic relationship between *Ae. comosa*, *Ae. uniaristata*. He also excluded *Ae. caudata* from the section and assigned the MM symbol to *Ae. comosa* genome and M^uM^u to *Ae. uniaristata*.

In Yamane and Kawahara (2005) study *Ae. comosa* and *Ae. uniaristata* were clustered as sister species in 1 clade in the trees based on SSRs and cpDNA. this agrees with classification of Zhukkovsky (1928) and Eig (1929) that treated these species as sister species in the section *Comopyrum*. In the results of (Alnaddaf *et al*., 2012) *Ae. comosa* and *Ae. uniaristata* have similar restriction profiles except one loci (*trnL-F*-HinfI) *Ae. comosa* has unique restriction profile in this loci compered with *Ae. uniaristata*. 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. likewise Wang *et al*. (1997) observed the plasmon of *Ae. umbellulata* is closely related to the plasmons of *Ae. comosa*.

Kimber and Sears (1987) mentioned that *Ae. caudata* is distinct from others species in the section *Comopyrum* and there is doubt over the inclusion of this species in the section based on analysis of genomes of those species. Dvorak and Zhang (1992) and Sasanuma *et al*. (1996-2004) confirmied the close relationship between *Ae. caudata* and *Ae. umbellulata* since they appeared as sisters in those trees. Likewise (Alnaddaf *et al*., 2012) indicated that *Ae. caudata* and *Ae. umbellulata* have similar restriction profiles except 2 loci. *Ae. caudata* has unique restriction profile in (*trnL-F*-HinfI) loci and negative restriction profile in (*orf62*-HindII) compared with *Ae. umbellulata*. Yamane and Kawahara (2005) mentioned that genes may transfer from *Ae. umbellulata* to *Ae. caudata*. therefore, they suggested transferring *Ae. caudata* to another section. the section of *Ae. umbellulata* is the highly nominated section.

Vertebrata: *Ae. Juvenalis*, *Ae. tauschii*, *Ae. crassa*, *Ae. ventricosa*. (Alnaddaf *et al*., 2012) observed that the restriction analysis of the chloroplast genome 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 (Alnaddaf *et al*., 2012) observed that *Ae. crassa*, *Ae. bicornis*, *Ae. sharonensis*, *Ae. kotschyi* have identical cpDNA, but *T. monococcum* in (*TrnT-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 3 tetraploids, *Ae. cylindrica*, *Ae. crassa* and *Ae. ventricosa* (Wan *et al*., 2002). Also according to Queen *et al*. (2004) The 3 D genome-containing *Aegilops* species, comprising the 2 members of Section *Vertebrata* (*Ae. ventricosa*, *Ae. tauschii*; 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 (Alnaddaf *et al*., 2012) study *Ae. triuncialis* has negative restriction profile in (*orf62*-HindII) and unique restriction profile in (*TrnL-F*-HinfI) compared with *Ae. tauschii*. And *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*., (2002) 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.

Alnaddaf *et al.* (2012) results showed 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).

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

Alnaddaf *et al*. (2012) mentioned that The high similarity in cpDNA observed for *Ae. sharonensis*, *Ae. longissima* and *Ae. bicornis*. this is in agreement with Wang *et al*. (1997). 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 *Ae. 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 (Alnaddaf *et al*., 2012) *Ae.* searsii differed on 2 chloroplast regions (*orf62*, *trnL-F*) compared with another species in the same section. Whereas *Ae*. speltoides differed in (*psbE&psbF*) loci. Eig (1929) recognizes 2 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). Yen *et al*. (2005) suggested that the S-genome species share a same basic genome but each has a unique variety. 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 Alnaddaf *et al*. (2012) study showed identical restriction profiles between *Ae. speltoides* and (*T. durum*, *T. turgidum*, *T. dicoccon*, *T. aestivum*) in all loci except one loci they 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.

Inaddition *Ae. searsii* and *Ae. caudate* have identical chloroplast type. Also Sliai and Amer (2011) reported that 2 species were sisters to each.

CONCLUSION

Triticum and *Aegilops*, the 2 important genera of the tribe *Triticeae* in the grass family *Poaceae*, are important genetic and economic resources because they have an evolutionary relationship with the 2 main agricultural crops *T. aestivum* and *T. durum*. so, it is important to understand the genetic relationships among the cultivated wheat species and their wild relatives because The high polymorphism found in wild gene resources of cultivated crops can be important for agricultural crop improvement/breeding purposes. Molecular markers are the best choice and most reliable means to study these relationships accurately.

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