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*Full Length Research Paper*

# Contribution of chloroplast DNA in the biodiversity of some *Aegilops* species

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**Four *Aegilops* species (*Aegilops longissima*, *Aegilops speltoides*, *Aegilops searsii* and Aegilops *caudata*) belonging to the family Poaceae were used in this study. 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 the earlier mentioned species. Maximum-parsimony and Neighbor-joining computational methods for tree building were applied to construct the relationship among the *Aegilops* species. In all trials, one parsimonious tree was obtained, in which, the *A. speltoides* was the oldest and was out of a cluster containing the other three *Aegilops* species. *A. searsii* and *A. caudata* were sisters to each other, while *A. longissima* was basal in this cluster. These findings did not agree with previous karyotypic studies in which *A. searsii* was the oldest, and *A. caudata* was recently originated, while both *A. longissima* and *A. speltoides* were intermediate. The present study therefore revealed the significance of molecular markers in clarifying the genetic diversity on the inter- and intra-specific levels. These markers can also be applied for taxonomic consequences and have an economic importance in the genetic amelioration programs.**

**Key words:** *Aegilops*, chloroplast DNA, biodiversity, systematic.

## INTRODUCTION

Several recent traditional and molecular studies reviewed the taxonomic consequences of the family to which *Aegilops* belongs. Kawahara (2009) stated that Triticeae is a taxonomically controversial group at both the species and genetic level. The author reviewed that one extreme is considering Triticum to be the only genus of Triticeae, and an opposite extreme is accepting a huge amount of monotypic genera. Kharazian (2007) determined the variation in leaf anatomical characters among different ploidy levels, to display the best anatomical characters for differentiating *Aegilops* species, and to determine the taxonomic relationships among these species. Cenkci et al. (2008) used 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*.

*Aegilops* is considered as one of the ancestors of the nowadays cultivated taxa of wheat. It is thought that the genome of this genus was incorporated in the genetic structure of other related taxa that have diploid, tetraploid and hexaploid chromosome numbers 2n = 14, 2n = 28 and 2n = 42, respectively (Riley and Chapman, 1958). Different species of *Aegilops* are distributed in the Mediterranean basin and grow well in the high rainy areas. Syria is considered as one of the main countries in which the different species of the genus are found with high density. Mouterde (1966) have recorded seventeen species of the *Aegilops* in very restricted area. This limited distribution facilitated the hybridization among the different species producing intermediate forms that are very difficult to be indentified morphologically.

Traditionally, identification of *Aegilops* species has relied heavily on morphological characters. These characters,

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**Abbreviations: MP,** Maximum-parsimony; **ML,** maximum-likelihood; **NJ,** Neighbor-Joining; **CI,** consistency index; **HI,** homology index; **RI,** retention index; **RC,** rescaled consistency index; **RFLP,** restriction fragment length polymorphism; **ETS,** external transcribed spacer; **RAPD,** random amplification of polymorphic DNA.

**Table 1.** The Genbank accession numbers for the chloroplast DNA segments used in this study for the different *Aegilops* species.

| Taxon | 5.8S rRNA | trnT-trnL | trnL-trnF |
|---|---|---|---|
| *A. longissima* | AF149196 | EU013858 | EU013619 |
| *A. searsii* | AF149194 | EU013893 | EU013654 |
| *A. caudata* | AF149199 | EU13691 | EU013456 |
| *A. spiltoides* | AJ301804 | EU13901 | EU013662 |
| *T. monococcom* | L11581 | EU13903 | EU013664 |

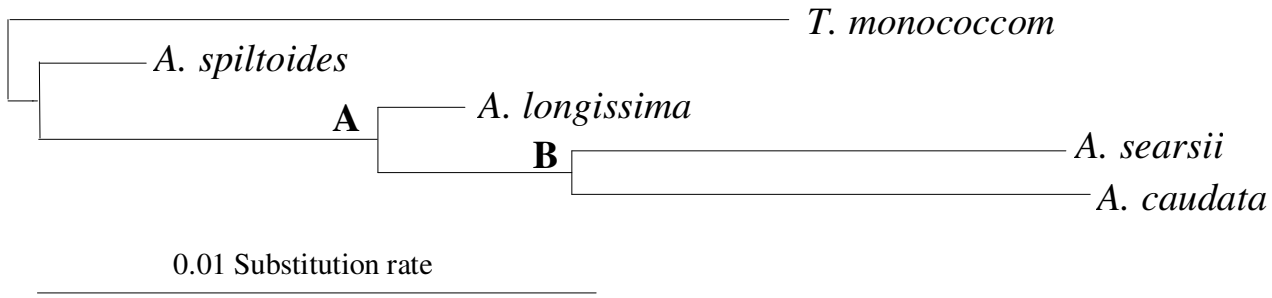


**Figure 1.** Maximum-likelihood tree for the relationship among the different *Aegilops* species that has been constructed by using different DNA combinations as shown in Table 2.

however, are either not variable enough among species or too plastic to be used for identification at the species level. Boubes-Hammoud (1986) and Silai et al. (1999) therefore, conducted more deep studies on *Aegilops* karyotypically. The authors clarified some confused taxonomy of the genus and hypothesized the historical origin of its species. Other molecular studies (Meimberg et al., 2009; Arrigo et al., 2010; Sepsi, 2010; Thomas and Bebeli, 2010) were recently conducted for the same purpose.

The genus *Aegilops* is a very important genetic resource for the breeding of bread wheat. Therefore, an accurate and easy identification of *Aegilops* species is required. Molecular markers are more stable within species, therefore, could be the alternative strategy towards an accurate identification. Since the chloroplast DNA has a lower level of variation when compared to the nuclear genome, an attempt was made in this study to investigate polymorphism in the chloroplast DNA among four *Aegilops* species (including *Aegilops longissima*, *Aegilops speltoides*, *Aegilops searsii* and *Aegilops caudata)*. Similar study by Haider and Nabulsi (2008) was conducted on twenty one Syrian *Aegilops* species.

## MATERIALS AND METHODS

The sequences of 5.8S rRNA gene and the intergenic spacers trnT-trnL and trnL-trnF from chloroplast DNA were obtained from GenBank for four *Aegilops* species (*A. longissima, A. speltoides, A. searsii* and *A. caudata*). The respective sequences for *Triticum monococcom* from the same family, Triticeae was used as an out-group due to close relationship between *Aegilops* and *Triticum*. Table 1 shows the details of all these sequences including the GenBank accession numbers.

The obtained sequences were aligned separately and manually using MacClade v.4. The unalignable and gap-containing sites were deleted and the aligned data were then concatenated so that 1655 bp were left for the analyses. The aligned nucleotide sequences can be obtained from author for correspondence upon request. The tree analyses were done by maximum-parsimony (MP), maximum-likelihood (ML) and Neighbor-Joining (NJ) methods with PAUP* 4.0b10 (Swofford, 2003) by heuristic searches with the TBR branch swapping and 10 random taxon additions, respectively. We set the bootstrapping replicates to 1000 with simple additions for the three methods. For the ML analysis, the general reversible model (GTR+I+G) and parameters optimized by Modeltest 3.0 (Posada and Crandall, 1998) were used.

Different combinations of the obtained sequences were analyzed separately using the same computational methods after excluding the gap-containing and unalignable sites. Therefore, 1397 bp from both 5.8S rRNA gene and the intergenic spacer trnT-trnL and 855 bp from both 5.8S rRNA gene and trnL-trnF spacer were used separately for constructing the genetic relationship among the *Aegilops* species. The same sequence of *T. monococcom* was also used as an out-group.

## RESULTS AND DISCUSSION

In the present study, three datasets (5.8S rRNA+trnT-trnL; 5.8S rRNA+trnL-trnF; 5.8S rRNA+ trnT-trnL + trnL-trn-F) were used separately. In the analyses of the three datasets 855, 1397 and 1655, characters were unambiguous sites used, respectively, to construct the ML tree topology of Figure 1. The base frequencies of the 1655 sites for the studied *Aegilops* were A = 30.8%, C =

**Table 2.** Statistical supports at the nodes of Figure 1 that have been obtained by different statistical methods for three combinations of DNA segments at 1000 bootstrap replications for each.

| Method | Bootstrap replication | 5.8S+(T-L) spacer | | 5.8S+(L-F) spacer | | 5.8S+(T-L)+(L-F) spacers | |
|---|---|---|---|---|---|---|---|
| | | A | B | A | B | A | B |
| 1- MP-BP | 1000 | 91 | - | 88 | 76 | 88 | 77 |
| 2-NJ-BP | 1000 | 91 | - | 82 | 71 | 83 | 69 |
| 3-ML-BP | 1000 | 97 | - | 94 | 83 | 95 | 72 |

**Table 3.** Pairwise genetic distances that have been determined for Triticeae.

| Species | *A. longissima* | *A. searsii* | *A. caudata* | *A. spiltoides* |
|---|---|---|---|---|
| *A. longissima* | | | | |
| *A. searsi* | 0.0120 | | | |
| *A. caudata* | 0.0121 | 0.0157 | | |
| *A. spiltoides* | 0.008 | 0.0151 | 0.016 | |
| *T. monococcom* | 0.018 | 0.020 | 0.021 | 0.013 |

20.77%, G = 19.7% and T = 28.7%. Of the 1655 nucleotides, 1604 were constant and 51 were variable. Thirty one of the variable sites were parsimony-uninformative and 20 were informative under parsimony criterion. The tree that was constructed showed consistency index (CI = 0.854), homology index (HI = 0.2903), retention index (RI = 0.55) and rescaled consistency index (RC = 0.472). The best-fit model that explained both datasets was GTR+I. Model parameters were as follow: Substitution rate matrix R(a) = 1.000; R(b) = 1.939; R(c) = 1.000; R(d) = 3.511; R(e) = 1.000 and proportion of invariable sites (I) = 0.963. A single ML tree was found with a negative log likelihood score –lnL = 2677,909.

Single ML tree (Figure 1) was obtained from all datasets with strong statistical support for the different computational methods (Table 2). The tree topology has grouped *A. longissima* with *A. searsii* and *A. caudata* in one cluster (statistical supports of BP were), while *A. speltoides* was the oldest of this group.

As shown in Table 3, the smallest genetic distance (D = 0.008) was found between *A. speltoides* and *A. longissima* and the latter species was nearly equally distant from both *A. searsii* (D = 0.0120) and *A. caudata* (D = 0.0121). Although *A. searsii* and *A. caudata* were sister to each other (Figure 1), they exhibited slightly large distance (D = 0.0151) than expected. The interpretation of this may be attributed to the ambiguity of the dataset of 5.8S rRNA+ trnT-trnL. The statistical supports for the tree topology that has been constructed by these data were very weak and missed in Table 2.

Morphological study (Boubes-Hammoud, 1986) placed *A. spiltoides* and *A. longissima* in one taxonomic group called *Sitopsis*. Karyotypic study (Sliai et al., 1999) has proved that the two species are entirely different and have placed them in different groups. The authors have suggested that *A. searsii* is more primitive than *A. longissima*. Previous studies have observed that *A. longissima* and *A. searsii* are sympatric and both *A. spiltoids* and *A. longissma* are also sympatric. However, the hybrids of these species were found to be sterile.

The topology of the tree in the current study, therefore, agreed with the morphological relationship of both *A. spiltoides* and *A. longissima* and disagreed with the karyotypic study (Sliai et al., 1999). Discrepancy between the two studies was shown for *A. speltoides*: Sliai et al. (1999) hypothesized close similarity between this species and *A. searsii* with the latter being the oldest. The present study revealed large distance between both species and suggested that *A. speltoides* was the oldest one. The karyotypic relationship (Sliai et al., 1999) was similar to the current study in showing close similarity between the three species of *A. longissima*, *A. searsii* and *A. caudata*. Using restriction fragment length polymorphism (RFLP) on the chloroplast DNA and nuclear genome, Mason-Gamer and Kellog (1996) agreed with our results regarding the group A of Figure 1 with strong bootstrap support. In their study, the authors also revealed the exclusion of *A. spiltiodes* from the *Triticum-Aegilops* group. Yamane and Kawahara (2005) by using chloroplast DNA have shown similar conclusion with the present study. The authors revealed that *A. speltoides* does not form a monophyletic clade with other *Sitopsis* species and that *A. speltoides* is distant from the other four *Sitopsis* species. This finding is supported by various molecular data (PCR-SSCP, Wang et al., 1997; Provan et al., 2004; chloroplast microsatellites, the entire 5' external transcribed spacer (ETS) region of the 18S rRNA gene, Sallares and Brown, 2004). In most of these studies, differences between *A. speltoides* and the remaining *Sitopsis* species have been detected.

In conclusion, the present study has revealed that *A.*

*speltiodes* is not in the other *Sitopsis* group. *A. longissima* is sister to both *A. searsii* and *A. caudara*. The present study therefore revealed the significance of molecular markers in clarifying the genetic diversity on the inter- and intra-specific levels. These markers can also be applied for taxonomic consequences and have an economic importance in the genetic amelioration programs. The study must be extended to include more *Aegilops* species and more molecular markers in order to clarify the taxonomic situation of the genus accurately.

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