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Molecular Relationships among Different Seryian *Aegilops* Species (Poaceae)

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ABSTRACT

Aegilops has been considered a complex genus with as many as 22 species in Syria. The current study has used 585 nucleotides from 5.8S nuclear ribosomal DNA gene and internal transcribed spacer 2 for these different species. These data were aligned manually and subjected to bioinformatics manipulation in order to construct the genetic relationship among these species. Three statistical methods (maximum-parsimony-MP, maximum-likelihood-ML and neighbor-joining-NJ) were used to execute the most likely relationship. The constructed genetic relationship showed homogeneinty in clustering of the species of the same plant type (A, B or C) with each other. A single NJ tree and a single ML tree were obtained with slight difference in topology within each plant type. Both trees disagreed with our previous finding in that *A. searsii*, *speltoides* and *A. longissima* clustered in one group and the first two species were sisters while *A. caudata* was out. Therefore, *A. speltoides* was not the oldest among them and these differences could be related to the difference in taxon sampling size. This study, however, supported our previous molecular finding and did not support the previous karyotypic study in that *A. searsii* was not the oldest, *A. caudata* was not recently originated and both *A. longissima* and *A. speltoides* were not intermediate. The molecular markers and taxon sampling size are therefore mandatory in clarifying the genetic diversity of closely related species, particularly, those which possess an economic importance like *Aegilops*.

Keywords: *Aegilops*; Molecular Relationship; Syria; Biodiversity

1. Introduction

The Mediterranean region, especially Syria, has been considered the main origin of plant genetic rescores [1] which constitutes the pillars of sustainable development. *Aegilops* is considered the main compound of the current cultivated wheat [2]. 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 [2].

Aegilops is distributed in a continuous landscape and is adapted with the climatic conditions of the Mediterranean basin. Syria, Palestine and other west Asian countries are considered as one of the main countries in which the different species of the genus are found with high density [1]. The author has recorded seventeen species in a very restricted ecological area of the region.

It is very difficult to classify the species of *Aegilops* morphologically because its species possess very similar morphological characteristics. Many phenotypes are found naturally which have been considered as hybrids or subspecies [3]. Because of these difficulties Boubes-Hammoud [3] and Silai *et al*. [4] conducted more deep studies on *Aegilops* karyotypically. Other molecular studies [5-10] were recently conducted for the same purpose.

Based on the abovementioned arguments, the present study aimed to revise the molecular relationship among different Syrian *Aegilops* species. We used the molecular data of 5.8S nuclear ribosomal DNA gene and internal transcribed spacer 2 (ITS2) for these different species to construct such relationship.

2. Materials and Methods

The sequences of 5.8S nuclear ribosomal DNA gene and internal transcriped spacer 2 for these different species were obtained from GenBank for 22 *Aegilops* species. The respective sequences for *Bymus tsukushiensis*, *Leymus cappadociocus* and *Leymus racemosus* from the same family (Poaceae) were used as outgroup due to their close relationship. **Table 1** shows the details of all these sequences including the GenBank accession numbers.

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Table 1. *Aegilops* species and their accession numbers for the genome data used in this study.

Species	Accession number
Aegilops bicornis	AF149192
Aegilops searsii	AF149194
Aegilops tauschii	AF149193
Aegilops longissima	AF149196
Aegilops sharonensis	AF149195
Aegilops speltoides speltoides	AY450268
Aegilops biuncialis	AF157003
Aegilops neglecta	AF157004
Aegilops peregrina	AF156996
Aegilops kotschyi	AF157002
Aegilops columnaris	AF156997
Aegilops geniculata	AF156998
Aegilops umbellulata	AF149197
Aegilops comosa	AF149198
Aegilops triuncialis	AF156994
Aegilops cylindrica	FR716085
Aegilops markgrafii	FR716108
Aegilops uniaristata	AF149200
Aegilops ventricosa	FR716128
Aegilops crassa	FR716079
Aegilops juvenalis	FR716104
Aegilops vavilovii	FR716127
Elymus tsukushiensis	FJ766143
Leymus cappadocicus	GQ373311
Leymus racemosus	GQ373316

The obtained sequences were aligned manually using DNASIS v.3 and MacClade v.4 programs. The unalienable and gap-containing sites were deleted and the unambiguous data were then concatenated so that 585 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 Neighbor-Joining (NJ) method with PAUP*4.0b10 [11]. We set the bootstrapping replicates to 1000 with simple additions. For the ML analysis, the general reversible model (GTR + I + G) and parameters optimized by Modeltest 3.0 [12] were used.

3. Results and Discussion

In the present study, the dataset of 5.8S rRNA gene and ITS2 were aligned for 22 *Aegilops* species and three outgroups. Both ambiguousand gap-containing sites were deleted from the concatenated alignment and therfore 585 unambiguous sites were left and used to construct the relationship. The base frequencies of these datset were A = 20.80%, C = 33.10%, G = 27.90% and T = 18.20%. Of the 585 nucleotides, 505 were constant and 80 were variable. Thirty five of the variable sites were parsimony-uninformative and 45 were informative under parsimony criterion. Maximum-likelihood and neighbor-joining methods gave two trees with slight differences in the topology (**Figures 1** and **2**). With respect to the ML tree, the best-fit model that explained the dataset was GTR + G + I. Model parameters were as follows: substitution rate matrix R(a) = 1.000; R(b) = 1.685; R(c) = 1.000; R(d) = 3.479; R(e) = 1.000, assumed proportion of invariable sites = 0.675 and gamma shape parameter (alpha) = 0.749. This ML tree was found with a negative log likelihood score −lnL = 1,620,269. With respect to the NJ tree, the used distance measure was that of Tamura-Nei, branch-swapping algorithm was nearest-neighbor-joining (NNI) and the bootstrapping was 1000 replicates. The same algorithm and bootstrapping replicates were also applied for the maximum-parsimony method.

The studied species are belonging to three *Aegilops* clusters A, B and C. Clusters A and C constitue sections Siptosis and Vertebrata, respectively while cluster B constitues three sections (Cylindropyrum, Comopyrum and Polycides) [13]. The ML tree (**Figure 1**) and the NL tree (**Figure 2**) agreed in clustering the same plant type with each other. Both tree showed that *Aegilops* species of cluster A have been grouped together. Similarly, the species of cluster B have been grouped together and those of type C also. Using genome analysis, the tree topologies in the present study showed approximately general agreement with several recent molecular studies [10,14-17] for the species that have been encompassed by each cluster.

The cluster A species showed similar genome type as they have S genome (**Table 2**) and all of them possess similar chromosomal (7) and microsatellite (2) numbers. The difference in their positions in the trees could be attributed to the difference in their ecological habitats or to the type of soil in which they have been cultivated. This result can be supported by that the cluster A contains 6 species five of which represent Sitopsis section. This section contains five species: *Aegilops bicornis* (S^bS^b, $2n = 2x = 14$), *Ae*. *longissima* (S^lS^l, $2n = 2x = 14$), *Ae*. *sharonensis* ($S^{sh}S^{sh}$, $2n = 2x = 14$), *Ae*. *searsii*, (S^sS^s, $2n = 2x = 14$) and *Ae*. *speltoides* (SS, $2n = 2x = 14$) [18]. Previous reports on cytogenetic and genetic investigations indicated that *Aegilops* genomes from this section are closely related [19-22]. Our study therefore approved the homogeniety of this section on molecular basis. Contradictions of the relationship among the species of Sitopsis section still be found as our results disagreed with previous recent studies [9,10] regarding the position of *Ae*. *searsii*, *Ae*. *longissima* and *Ae*. *spiltiodes*. This discripancy could be attributed to that the molecular data used by the autours were not enough to resolve this contradiction. *Ae*. *tsuaschii* was found within this cluster in

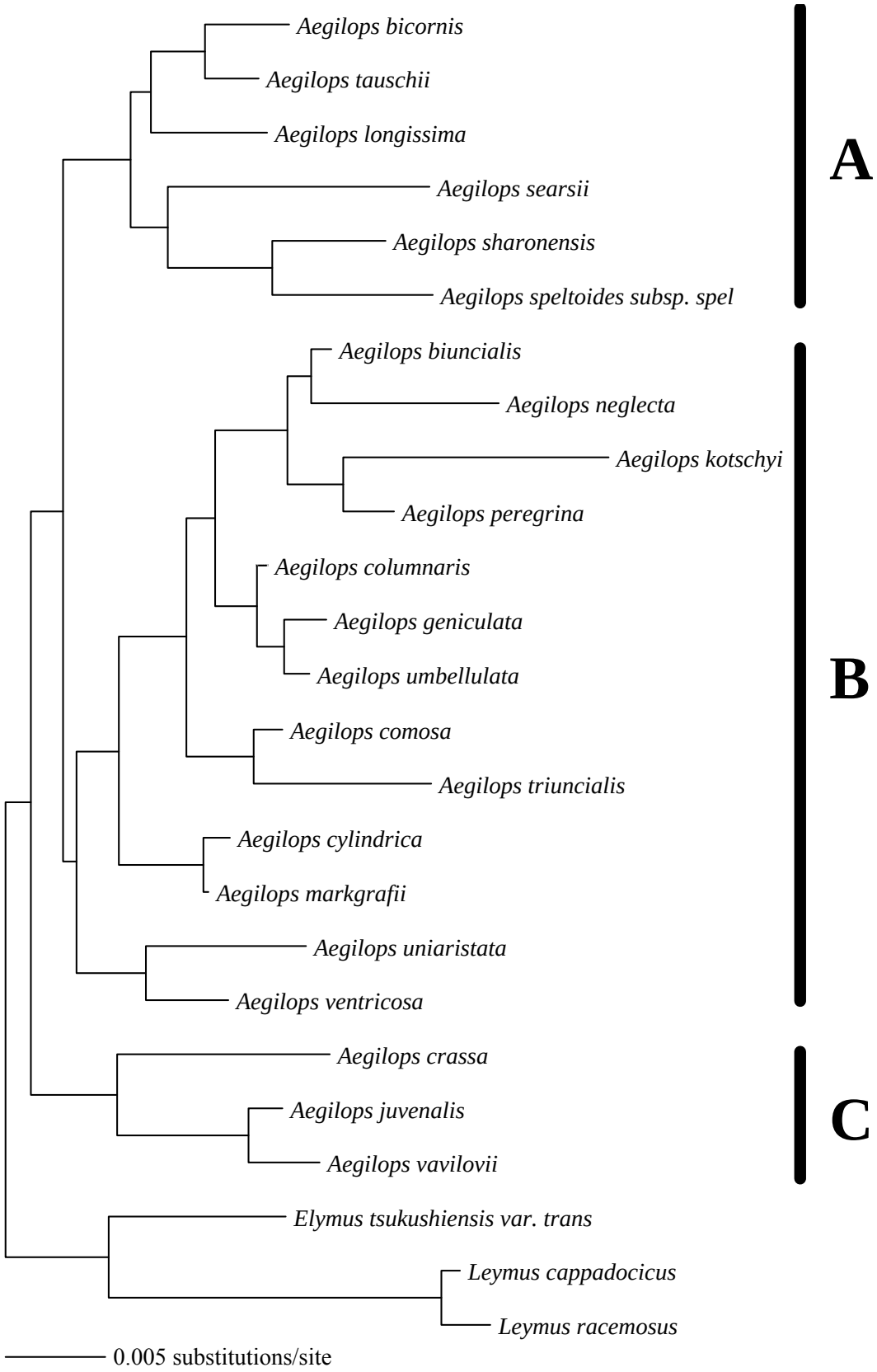

Figure 1. Neighbor-joining relationship between species belonging to the genus *Aegilops*, determined by aligning 585 sequences of the 5.8S gene and ITS2. *Elymus* and *Leymus* were used as outgroup.

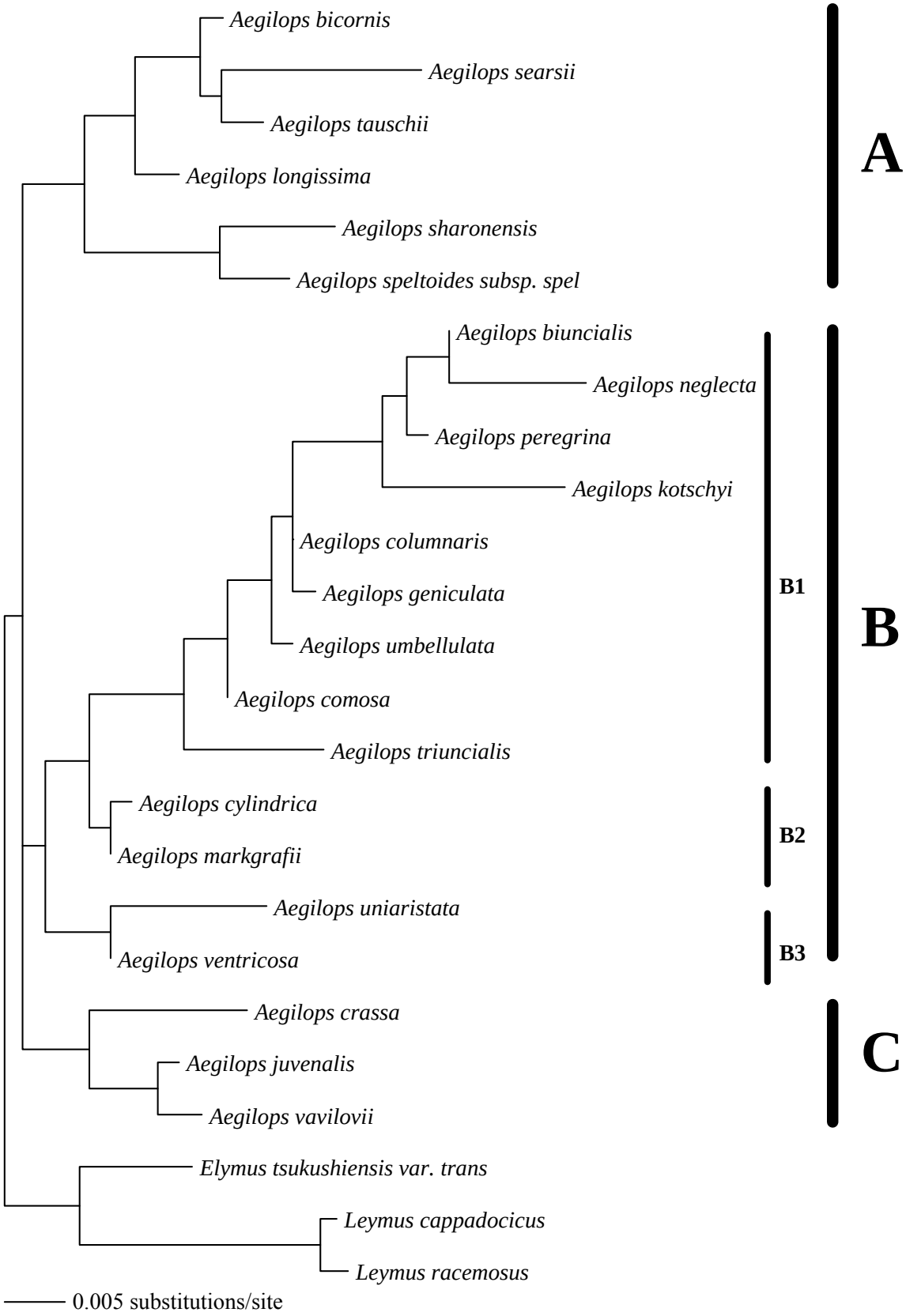

Figure 2. Maximum-likelihood relationship between species belonging to the genus *Aegilops*, determined by aligning 585 sequences of the 5.8S gene and ITS2. *Elymus* and *Leymus* were used as outgroup.

Table 2. Plant type, chromosomal number, genome constitution and origin of wild *Aegilops* species used in this study.

Species	Plant type	Chromosome No.		Genome type	Satellites No.	Chromosome charge	Distribution	Soil type
		n	2*n*					
Aegilops bicornis	A	7 + 1B	14	S^b	**	3 (40)	Common	Sandy desert
Aegilops searsii		7	14	S	***	2 (34.4)	S Syria	Steppes
Aegilops tauschii		7	14	S	*	2	N Syria	Sandy desert
Aegilops longissima		7	14	S^l	**	3 (48.6)	S Syria	Steppes
Aegilops sharonensis		7	14	S^l	***	3 (40.9)	S Syria	Sandy dune
Aegilops speltoides		7	14	S	**	2 (33.7)	N and NW Syria	Stoney slopes
Aegilops biuncialis	B	14 + 1f	28	CM	**	1 (30.1)	N and NW Syria	Sandy hills
Aegilops neglecta		14, 21	28, 42	CM	**	1 (33)	N and NW Syria	Wooded
Aegilops peregrina		14	28	CS	****	1 (32.9)	N Syria	Sand lands
Aegilops kotschyi		14	28	CS	**	1 (32.9)	NW Syria	Not cultivated
Aegilops columnaris		14	28	CM	**	2 (33.5)	N and NW Syria	Wooded areas
Aegilops geniculata		14	28	CM	**	1 (29.1)	N and S Syria	All lands
Aegilops umbellulata		7 + 1f	14	C	***	2 (31.6)	N and S Syria	Steppes, dry hills
Aegilops comosa		7	14	M	***	2 (39.5)	N Syria	Humid land
Aegilops triuncialis		14 + 1f	28	CC	**	1 (34.7)	N and S Syria	All lands
Aegilops cylindrica		14	28	DC	**	1 (28.6)	S Syria	Pastures
Aegilops markgrafii		14	28	C	***	1 (28.4)	N and S Syria	Uncultivated
Aegilops uniaristata		7	14	M	*	1 (33.1)	N Syria	Uncultivated
Aegilops ventricosa		14	28	DM	*	1 (29.9)	Uncommon	Wet land
Aegilops crassa	C	14, 21	28, 42	DD^uM^{cr}	**	2 (32.5, 30.3)	N and S Syria	Fertile dry land
Aegilops juvenalis		21	42	DC^uM^j	**	2 (28)	Uncommon	Stoney land
Aegilops vavilovii		21	42	DM	****	3 (36.6)	N and S Syria	Humid

*1 pair equipped with satellites; **2 pair equipped with satellites; ***1 pair equipped with large satellite 1 pair with small satellite; ****3 pair equipped with satellites. N = north; S = south; W = west.

spite of being belong to section Vertebrata. The molecular analysis of Petersen *et al*. [23] showed section *Sitopsis* as the sister to *Ae*. *tauschii* (and the D genome of *T*. *aestivum*).

The cluster B showed a group of *Aegilops* species which possess M, D, C and S genomes or mixures of these types. The karyotypic number of Syrian taxa within this cluster was 14 chromosomes. Because of the complicated genome type and the difference in both number of microsatellites (2 or 3) and habitat, both trees showed great difference within this cluster. Konstantinos and Bebeli [17] observed *Ae*. *triuncialis* (genome UC) grouped in the same subgroup with *Ae*. *markgrafii* (C), which is its progenitor male parent. These evidences have been observed in our study by clustering of both species in the same group. In section B in which *Aegilops* contains *Ae*. *ovata* = *Ae*. *geniculata*, *Ae*. *neglecta*, *Ae*. *umbellulata*, *Ae*.*triuncialis*, *Ae*. *Kotschyi*, Alnaddaf *et al*. [10] found these species have identical restriction profiles. Konstantinos and Bebeli [17] noticed that *Ae*. *kotschyi*-SU, *Ae*. *peregrina*-SU) grouped closer to the male parent (*Ae*. *umbellulata*-U) than to *Ae*. *searsii*-S. This is agree with the study of Alnaddaf *et al*. [10] where *Ae*. *kotschyi*, *Ae*. *umbellulata* have identical restriction profiles.

Aegilops species of cluster C showed identical topology in both ML and NJ trees. The three species of this cluster poccess karyotypic number of 42 chromosome, DCM genome and 2 or 3 microsatellites. Because they did not show clear differences in these data, their topology was fixed in both trees. The section Vertebrata consists of five species which are *Aegilops tauschii*, *Aegilops ventricosa*, *Aegilops crassa*, *Aegilops juvenalis* and *Aegilops vavilovii*. The chromosomic numbers for these species are commonly 42 chromosomes with exceptions for *Aegilops tauschii* (2*n* = 14 chromosome) and *Aegilops ventricosa* (28 chromosome). *Aegilops tauschii* clustered with section Siptosis while *Aegilops ventricosa* clustered with cluster B. Molecular evidence for the sister relationship of *Aegilops tauschii* to section Siptosis [23] and clustering of *Aegilops ventricosa* with taxa of cluster B were possible.

4. Conclusion

The constructed genetic relationship showed homogeneinty in clustering of the species of the same plant type (either A, B or C) with each other. Using genome analysis, the constructed trees showed approximately general topological agreement with several molecular investiga-

tions for the species that have been encompassed by each cluster. The present study raised the significance of the size of both molecular data and taxon sampling in clarifying the genetic diversity of closely related species, particularly, those which possess an economic importance like *Aegilops*.

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