Genetic relationship of *Elymus alaskanus, E. caninus, E. fibrosus*, and *E. mutabilis* revealed by chloroplast DNA sequences

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Abstract

The genetic diversity of a species measures its genetic variation, or population diversity, which determines evolutionary relationships; high genetic diversity is linked to survival, adaptation and evolution. In this study, nucleotide diversities of chloroplast genes of four *Elymus* species were determined. Phylogenetic relationship of these four species was obtained using PAUP computer software. The phylogenetic tree, using the rps16 primer pair, produced two distinct clades, one containing *Agropyron* and *Eremopyrum* while the other contained many more genera from the Triticeae tribe, including all four *Elymus* species being studied. The species formed distinct clades with the same populations of that species for most cases. Furthermore, results indicated that, with the rps16 cpDNA gene, an *E. caninus* population yielded the highest nucleotide diversity while two *E. alaskanus* populations yielded lowest diversity. Samples tested using the intergenic region trn-tw primer sequences found that a sample from the same *E. caninus* population as above produced the highest nucleotide diversity. The lowest nucleotide diversity was also detected in an *E. caninus* population. *E. caninus* populations demonstrate considerable genetic variability due to their genetic diversity.

March 24, 2014

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Introduction

Genetic Diversity

Phylogeny is the study and reconstruction of evolutionary relationships of taxa (Aluru, 2006). DNA sequences change through evolutionary time and can be used to estimate species relationships and to produce species trees. Phylogenetic trees can be deduced based on gene sequence. Many methods can be used to produce phylogenetic trees; however, the most popular method is a maximum parsimony analysis, which organizes the species into a tree that requires the least amount of evolutionary change to explain observed differences (Ye, 2008). The reliability of generated trees can be evaluated using bootstrap analyses (Felsenstein, 1985**;** Aluru, 2006; Ye, 2008). Morphological similarities are the most important data in determining phylogenetic relationships, but genome analysis can provide information (Salomon *et al*., 1992). Cytogenetic analyses examine the degree of chromosome pairing at meiosis in mother pollen cells, which indicate the overall degree of similarity and relatedness among genomes. This can lead to the proper classification of a species (Mason-Gamer *et al*., 2002).

Taxonomy is defined as the description, identification, nomenclature and classification of organisms. Plant systematics is a method that combines phylogenetic analyses and molecular data to provide a framework and tools to understand the relationships at all levels of the taxonomic hierarchy (Small *et al*., 2004). The taxonomy of some species, such as those used in this study, can be highly complex due to the morphological variation within and between species. To further describe and classify species, more taxonomic and phylogenetic analyses must be performed to reveal relationships between and within these species.

Knowledge of genetic diversity is necessary for the successful management of conservation programs. Nucleotide diversity, the average number of different nucleotides per site and measures of genetic variation (population diversity), examines variation and determines evolutionary relationships. High genetic variation is linked to survival, adaptation and evolution, and is important for estimating population parameters, such as evolutionary studies of mating systems and relatedness (Sun *et al*., 2001).

Chloroplast DNA

Chloroplasts, an organelle required for protein synthesis and production and utilization of energy, affect the performance of a plant. Most of their proteins are encoded in the cell nucleus and imported from the cytosol. Chloroplast DNA is roughly 10 times bigger than mitochondrial DNA; therefore it has been used extensively in plant phylogeny and genome evolution (Sun *et al*., 2009). Non-coding regions can provide more information in phylogenetic studies at the species level because of their high variability, simple amplification and abundance of developed universal primers. On the other hand, analyses of nuclear DNA may explain other aspects of phylogenetic and evolutionary relationships due to its bi-parental inheritance and easy detection, amplification and sequencing. The chloroplast genome is uniparentally inherited and haploid; therefore, intraspecific variation is reduced and this can be used to identify maternal lineages and genome donors (Xu & Ban, 2004). Furthermore, chloroplasts exhibit a non-Mendelian inheritance and a conservative coding region, as well as substitution rate, across different species and different genera. The non-coding regions, as mentioned above, are variable in their content and arrangement, especially between related species and do not display recombination and thus are useful in studying phylogeny, evolution and genetic diversity of a species (Aluru, 2006**;** Hollingsworth *et al*., 1999; Sun *et al*., 2002).

Triticeae

Triticeae is a monophyletic grass tribe within the Poaceae family comprised of over 300 economically important species. Roughly 250 of these species are perennials, including the staple crops such as wheat (*Triticum*), barley (*Hordeum*) and rye (*Secale*). Within this tribe, most species are polyploid; in other words, they comprise more than two sets of homologous chromosomes. Genes replicated by polyploidy can produce various outcomes, such as diversification of functions or regulations of proteins, silencing of genes and causing recombination or conversion, resulting in evolution (Wendel, 2000). These various combinations make it an excellent group for research in evolution, taxonomy, genetics, genetic diversity and speciation; nevertheless, this tribe has a complicated evolutionary history. Triticeae was classified based on morphological characteristics or key characteristics, such as number of spikelets per rachis node per plant. Reclassification of the Triticeae tribe using chromosome pairing defined boundaries of the genera; however, there is large morphological variation within and between species, which causes extensive instability in its taxonomy (Kellogg, 1994; McMillan & Sun, 2004; Salomon *et al*., 1992; Sun, 2007; Svitashev, 1997).

Elymus

Elymus is a genus within the tribe Triticeae, circumscribed by Dewey (1982, 1983), Löve (1982, 1984) and Tzvelev (1989) based on genome constitution. Morphological studies support the cytological data used in their classification; this is now used to provide species level taxonomic separation (Barkworth & Jacobs, 2011). Features, such as morphological variability, phenotypic plasticity, natural hybridization and polyploidy, make this genus a prime model for research on cytogenetics, genetic diversity, molecular genetics and phylogeny (Diaz *et al*., 1999). Some morphological

studies, as mentioned above, are not correlated with cytological studies or molecular data; however, some morphological characteristics examined do confirm these data and can be used in classification. Inconsistencies do exist within phylogenetic trees due to poorly supported clades. That being said, classification of this genus remains controversial due to genomic, physiological and ecological variation; little is known about the patterns, levels and distributions of genetic diversity within or between populations (Diaz *et al*., 1999).

This genus contains the main perennial cereal grains, known as wheatgrasses (Sun, 2007). It is the largest, most widely distributed and morphologically diverse genus, composed of over 150 allopolyploid species. These species are found in every continent, except Antarctica, and are best suited in temperate, subtropical areas, for example, Asia, North America, or New Zealand. They are found in various ecological niches such as grasslands, mountainsides, semi-deserts, and valleys or in forests; however, Asia is the center for the origin and diversity of the *Elymus* species (where 80 *Elymus* species originated), while North America is the second most important area for these species (50 *Elymus* species) (Sun, 2007). *Elymus* species are known to possess useful genes for disease and pest resistance, stress tolerance, high protein content and diverse adaptation. This genus therefore presents a valuable gene pool for improvement of crops, such as barley and rye, and has been utilized in breeding programs. Furthermore, some species are known to contain good forage qualities and high productivity under unfavourable climatic conditions and are used for grazing. *Elymus* species are also useful for revegetation, soil stabilization and erosion control (Barkworth & Dewey, 1985; Dewey, 1984**;** Diaz *et al*., 1999; Sun *et al*., 2002). *Elymus* species have an ability to adapt to various environmental pressures due to their variation and ability to cross-pollinate. Interspecific hybridization is common, resulting in increased ploidy and perhaps the

formation of new species when two or more of the distinct haplotypes within the Triticeae tribe hybridize.

Research demonstrates there are five fundamental genomes within *Elymus*: St, H, P, W, and Y. The St genome emerged from the *Pseudoroegneria* genus; the H genome was from the *Hordeum* genus; the P genome from *Agropyron*; the W genome from *Australopyrum*, such as a wheat fern; and the origin of the Y genome is unknown (Dewey, 1984; Jensen, 1990; Mason-Gamer *et al*., 2002; McMillan et al., 2004; Torabinejab & Mueller, 1993; Yan & Sun, 2011). A phylogenetic analysis performed by Mason-Gamer (2001) suggests that most of North American *Elymus* species contain both the St and H genomes. Moreover, chloroplast DNA sequence analyses indicated that *Pseudoroegneria* chloroplast genome was maternal donor in the speciation of *Elymus* species **(**McMillan *et al.*, 2004; Redinbaugh *et al*., 2000) and phylogenetic analyses using different chloroplast DNA data sets suggested the same results (Mason-Gamer *et al*., 2002; McMillan *et al*., 2004). In addition, a study by Sun, Salomon and von Bothmer (1998), as well as Díaz, Salomon and von Bothmer (1999) confirms that the four species used in this study all have SSHH genomes.

In this study, four *Elymus* species were used: *Elymus alaskanus*, *E. caninus*, *E. fibrosus*, and *E. mutabilis*. Within these four species, 65 individuals were sampled from Sweden, Finland and Iceland. *E. alaskanus* is an artic-alpine species, found in northern Russia, Siberia, Alaska, northern Canada and Greenland. It is a self-pollinating, alloploid species that grows on limestone outcrops, screes, dry meadows, and other low competition habitats (Sun *et al*., 2002; Sun *et al*., 2003). It has been shown in studies that gene flow among these populations was low; genomic diversity was found within, rather than between species. This may be explained by populations dividing from a founder

population and, after isolation, acted upon by natural selection and may have implications for explaining evolutionary mechanisms and practical conservation (Díaz *et al*., 1999; Sun *et al*., 2002; Sun *et al*., 2003). *Elymus alaskanus* is a morphologically variable species over its whole distribution area, but is usually morphologically homogenous within regions and populations. There are approximately 15 taxa described under the *E. alaskanus* complex (Sun *et al*., 2003).

E. caninus has a wide distribution area, from Iceland to southern Siberia to the Mediterranean (Sun *et al*., 2001). It is commonly found in environments such as forest glades, among shrubs, as well as in subalpine meadows where the soil is moist and nutritious (Díaz *et al.,* 1999). It possesses a significant amount of genetic and morphological variation (Díaz *et al*., 1999; Sun *et al*., 2001). Sun (2001) has provided support to a hypothesis that *E. caninus* is self-pollinating, and has a high outcrossing rate, even though this species is supposed to be predominantly autogamous. Evidence of cross-pollination is observed due to the presence of interspecific hybrids; a high frequency of hybrids between *E. caninus* and *E. fibrosus* or *E. mutabilis* is found when these species are found neighbouring. Further, these examples of cross-pollination produce higher levels of variation, indicating that gene flow may occur between these species **(**Díaz *et al*., 1999; Sun *et al*., 2001).

E. fibrosus is also a predominantly self-pollinating and alloploid species, distributed in Russia and northern Scandinavia. It grows on wet meadows, riverside sand and pebbles, and among shrubs. It is usually found growing alone, however it can be found within populations of *E. caninus*, *E. sibiricus,* and *E. repens*. In Scandinavia, the number of the populations of this species is very low; its habitat preferences make it vulnerable to dam construction or river management implementation. Research suggests

that genetic variation within *E. fibrosus* is very limited within the population and the species; the mean of alleles per locus and the mean of polymorphic loci were low, indicating a high rate of selfing, a deficiency in heterozygosity and a potential bottleneck (Díaz *et al*., 2000; Sun *et al*., 2002). Knowledge of population genetic structure and genetic diversity of a species is required for successful organization of conservation programs (Sun *et al*., 1998).

E. mutabilis is self-pollinating and alloploid; it has a large, but sporadic, distribution area from northern Europe to certain parts of Asia, mostly in Norway, Sweden and Finland. It can be found alone, or in occurrence with *E. caninus*, *E. alaskanus*, or *E. jibrosus* (Díaz *et al*., 1999).

Objectives

This study utilized two chloroplast gene regions, the gene encoding ribosomal protein S16 (rps16) and an intergenic region of trn gene, to detect and analyze nucleotide diversity between and within four *Elymus* species, and to reveal the most likely phylogenetic relationship among these species.

Materials and Methods:

Plant Materials

The species used in this study included *Elymus alaskanus*, *E. caninus*, *E. fibrosus* and *E. mutabilis*. Table 1 provides a list of species, accessions numbers and origins used in this research. Seeds were provided by Dr. Salomon from the Swedish University of Agricultural Science; these seeds were germinated and transplanted to sand-peat mixtures, then were maintained in a greenhouse.

DNA Extraction

DNA was isolated using the Thermo Scientific GeneJet Plant Genomic DNA Purification Mini Kit. Briefly 100 mg of fresh plant tissue was crushed in liquid nitrogen using a mortar and pestle and transferred to a 1.5 mL tube with 350 µL of Lysis Buffer A. Fifty microliters of Lysis Buffer B and 20 µL of RNase A were added, and the tube was incubated at 65°C for 10 minutes. After 5 minutes of incubation on ice, 130 µL of Precipitation Solution was combined with the mixture. Afterwards, the supernatant was collected and placed in a new tube and 400 µL of Plant gDNA Binding Solution and 400 µL of 96% Ethanol and homogenized. This solution was centrifuged for 1 min at 6,000 x g, and then 500 µL of Wash Buffer 1 was added to the column after discarding the flow through. After centrifugation for 1 min at 3,000 x g, 500 µL of Wash Buffer 2 was added to the column to be centrifuged again at ≥ 20,000 x g for 3 minutes. One hundred microliters of Elution Buffer containing 10 mM Tris-HCL, 0.5 mM EDTA and at a pH of 9.0, was added and incubated at room temperature for 5 minutes, thereafter centrifuged for 1 min at 8,000 x g. A second elution step of 100 µL Elution Buffer was performed. DNA was then stored at -20°C.

Polymerase Chain Reaction (PCR)

Primers rps16F (5'-GTG GTA GAA AGC AAC GTG CGA CTT-3') and rps16R (5'-TCG GGA TCG AAC ATC AAT TGC AAC-3') (Popp and Oxelman (2007)) were used to amplify the rps16 gene within chloroplast DNA. Meanwhile, primers trn-tw (5'-CCC TTT TAA CTC AGT GGT AG-3') and trn-D (5'-ACC AAT TGA ACT ACA ATC CC-3') (Sun, 1997) were used to amplify non-coding chloroplast DNA between genes, which consists of 1,200 base pairs.

A master mix for both primer pairs included 6.88 µL of autoclaved water, 4 µL of deoxynucleotides (dNTP), 2 µL of 10X ThermoPol Reaction Buffer, 2 µL of forward primer, 2 µL of reverse primer, 0.12 µL of DNA *Taq* Polymerase and 3.0 µL DNA in a 20 µL volume. For each accession, two 20 µL samples were prepared for PCR amplification and later combined in order to reduce the chance of PCR selection or drift due to *Taq* Polymerase errors during the process (Yan & Sun, 2011; Zeng et al., 2010).

The polymerase chain reaction (PCR) program consists of three steps: denaturing, annealing and extension. For the rps16 primers, the PCR protocol was: one cycle at 95°C for 4 minute, 40 cycles of 40 seconds at 95°C, 40 seconds at 63°C, and 1 minute 30 seconds at 72°C, followed by one cycle of 10 minutes at 72°C and an infinite hold at 4°C. For the trn-tw/trn-D primers, the PCR protocol was: one cycle at 95°C for 3 minute, 40 cycles of 30 seconds at 95°C, 30 seconds at 54°C and 1 minute 45 seconds at 72°C, followed by one cycle of 10 minutes at 72°C and an infinite hold at 8°C. A Bio-Rad T100 Thermal Cycler was used for DNA amplification.

Gel Electrophoresis and Visualization

Electrophoresis was performed using 1.0% agarose gel and 1X TBE buffer, containing EDTA, Tris base, boric acid and water. After the gel hardened, 5 µL of DNA

sample and bromophenol blue loading dye was pipetted into the wells. The electrophoresis apparatus was turned on at 180 volts for 30 minutes, after which the gel was stained in ethidium bromide for 30 minutes. SynGene photographic equipment was used to visualize the DNA bands under UV light. Figure 1 shows an example of PCR products.

Data Analysis

Successful amplified DNA products were sent to the Taihe Biotechnology Company in Beijing, China, for purification and sequencing. Afterwards, sequences were analyzed with the BLAST program (http://www.ncbi.nlm.nih.gov/) to ensure the sequences from the gene we amplified. Multiple sequences were aligned using the Clustal X program, as demonstrated in Figure 2 (Thompson et al., 1997). The Phylogenetic Analysis Using Parsimony (PAUP) program was used to analyze phylogenetic relationships of these species. *Bromus catharticus* was used as an outgroup, based on previous studies of phylogenetic analysis of Poaceae (Hodge *et al*., 2010; Sun *et al*., 2009). A heuristic test was performed using 100 tree replications to produce the most parsimonious tree. Gaps were treated as missing data while all characters were considered unweighted and ungrouped. A maximum parsimony score was used to estimate character similarity by calculating consistency index (CI), rescaled consistency index (RC), retention index (RI) and homoplasy index (HI). The consistency index is used as a measure of fit of a character within a phylogenetic tree and indicates presence of homoplasy while the rescaled consistency index is a comparison of fit of characters. The retention index is a measure of how parsimonious the phylogenetic tree is, using the least evolutionary change to explain the observed data; meanwhile, the homoplasy index is a measure of shared traits by multiple species due to some causes other than common

ancestry (Farris, 1989). The heuristic search was also calculated in order to determine clade consistency using 1000 bootstrap replicates (Felsenstein, 1985). Nucleotide diversity was estimated by Watterson's (1975) θ, Fu and Li's F and D statistics, and Tajima's (1989) π statistics; Watterson's θ estimates population mutation rate; meanwhile, Fu and Li's F statistic is based on comparing polymorphisms observed in only one individual in the sample and the average number of nucleotide differences between sequences while Fu and Li's D statistic is based on mutations observed in only one individual and the total number of mutations observed within the sequences. Tajima's D statistic distinguishes between DNA sequences evolving randomly and those evolving non-randomly. A negative number indicates population expansion while a positive value indicates population decrease due to low levels or high levels of frequency polymorphisms. Further, the number of haplotypes and the number of polymorphic sites were examined. The DnaSP 4.0 program (Rozas et al., 2003) is used for the analysis of DNA polymorphism data; it provides information on both the evolutionary significance of DNA polymorphisms and the evolutionary histories of populations. It was used to calculate tests of neutral evolution, described by Fu and Li (1993) and Tajima (1989).

Table 1: Species names, accession numbers and origin of *Elymus* species used in this study

Species	Accession	Origin
Elymus caninus	SV9714-7	Jamtland, Sweden
Elymus caninus	SV9714-15	Jamtland, Sweden
Elymus caninus	SV9714-64	Jamtland, Sweden
Elymus caninus	SV9714-48	Jamtland, Sweden
Elymus caninus	SV9714-56	Jamtland, Sweden
Elymus caninus	DK9604-5	Sjoelland, Denmark
Elymus caninus	DK9604-9	Sjoelland, Denmark
Elymus caninus	DK9604-21	Sjoelland, Denmark
Elymus caninus	DK9604-29	Sjoelland, Denmark
Elymus caninus	FI9310-5	Kittilan, Finland
Elymus caninus	FI9310-13	Kittilan, Finland
Elymus caninus	FI9309-51	Kittilan, Finland
Elymus caninus	NO0308-1	Finnmark, Norway
Elymus caninus	NO0308-3	Finnmark, Norway
Elymus caninus	NO0308-2	Finnmark, Norway
Elymus caninus	NO0308-15	Finnmark, Norway
Elymus caninus	FI9303-3	Pera-Pohjanmaa, Finland
Elymus caninus	FI9303-5	Pera-Pohjanmaa, Finland
Elymus caninus	FI9303-7	Pera-Pohjanmaa, Finland
Elymus caninus	FI9303-9	Pera-Pohjanmaa, Finland
Elymus alaskanus	NO9707-1	Oppdal Norway
Elymus alaskanus	NO9707-11	Oppdal Norway
Elymus alaskanus	NO9707-19	Oppdal Norway
Elymus alaskanus	NO9707-29	Oppdal Norway
Elymus alaskanus	NO9707-35	Oppdal Norway
Elymus alaskanus	IS9410-2	Skagafjardarsysla, Iceland
Elymus alaskanus	IS9410-10	Skagafjardarsysla, Iceland
Elymus alaskanus	IS9410-17	Skagafjardarsysla, Iceland
Elymus alaskanus	IS9410-24	Skagafjardarsysla, Iceland
Elymus alaskanus	IS9410-31	Skagafjardarsysla, Iceland
Elymus alaskanus	NO0307-1	Finnmark, Norway
Elymus alaskanus	NO0307-5	Finnmark, Norway
Elymus alaskanus	NO0307-7	Finnmark, Norway
Elymus alaskanus	NO0307-10	Finnmark, Norway
Elymus alaskanus	NO0307-16	Finnmark, Norway
Elymus alaskanus	NO9502-1	Troms, Norway
Elymus alaskanus	NO9502-8	Troms, Norway
Elymus alaskanus	NO9502-15	Troms, Norway
Elymus alaskanus	IS9401-27	Eyjafjardarsysla, Iceland
Elymus alaskanus	SV9505-1	Vasterbotten, Sweden
Elymus mutabilis	SV9318-3	Norrbotten, Sweden
Elymus mutabilis	SV9318-4	Norrbotten, Sweden
Elymus mutabilis	SV9318-5	Norrbotten, Sweden
Elymus mutabilis	SV9319-1	Norrbotten, Sweden
Elymus mutabilis	SV9319-2	Norrbotten, Sweden
Elymus mutabilis	SV9319-3	Norrbotten, Sweden

Elymus mutabilis	SV9319-4	Norrbotten, Sweden
Elymus mutabilis	SV9319-5	Norrbotten, Sweden
Elymus mutabilis	NO0305-1	Finnmark, Norway
Elymus mutabilis	NO0305-2	Finnmark, Norway
Elymus mutabilis	NO0305-3	Finnmark, Norway
Elymus mutabilis	NO0305-4	Finnmark, Norway
Elymus mutabilis	NO0305-51	Finnmark, Norway
Elymus mutabilis	FI9325-13	Pelkosniemi, Finland
Elymus mutabilis	FI9325-23	Pelkosniemi, Finland
Elymus mutabilis	FI9325-74	Pelkosniemi, Finland
Elymus mutabilis	FI9325-95	Pelkosniemi, Finland
Elymus fibrosus	FI9306-1	Pera-Pohjanmaa, Finland
Elymus fibrosus	FI9306-2	Pera-Pohjanmaa, Finland
Elymus fibrosus	FI9306-3	Pera-Pohjanmaa, Finland
Elymus fibrosus	FI9306-4	Pera-Pohjanmaa, Finland
Elymus fibrosus	FI9314-1	Kittilan, Finland
Elymus fibrosus	FI9314-2	Kittilan, Finland
Elymus fibrosus	FI9314-3	Kittilan, Finland
Elymus fibrosus	FI9314-4	Kittilan, Finland

Figure 1: Example of agarose gel electrophoresis after staining with ethidium bromide and visualization under UV light for *Elymus* species amplified with trn-tw/trn-D primers

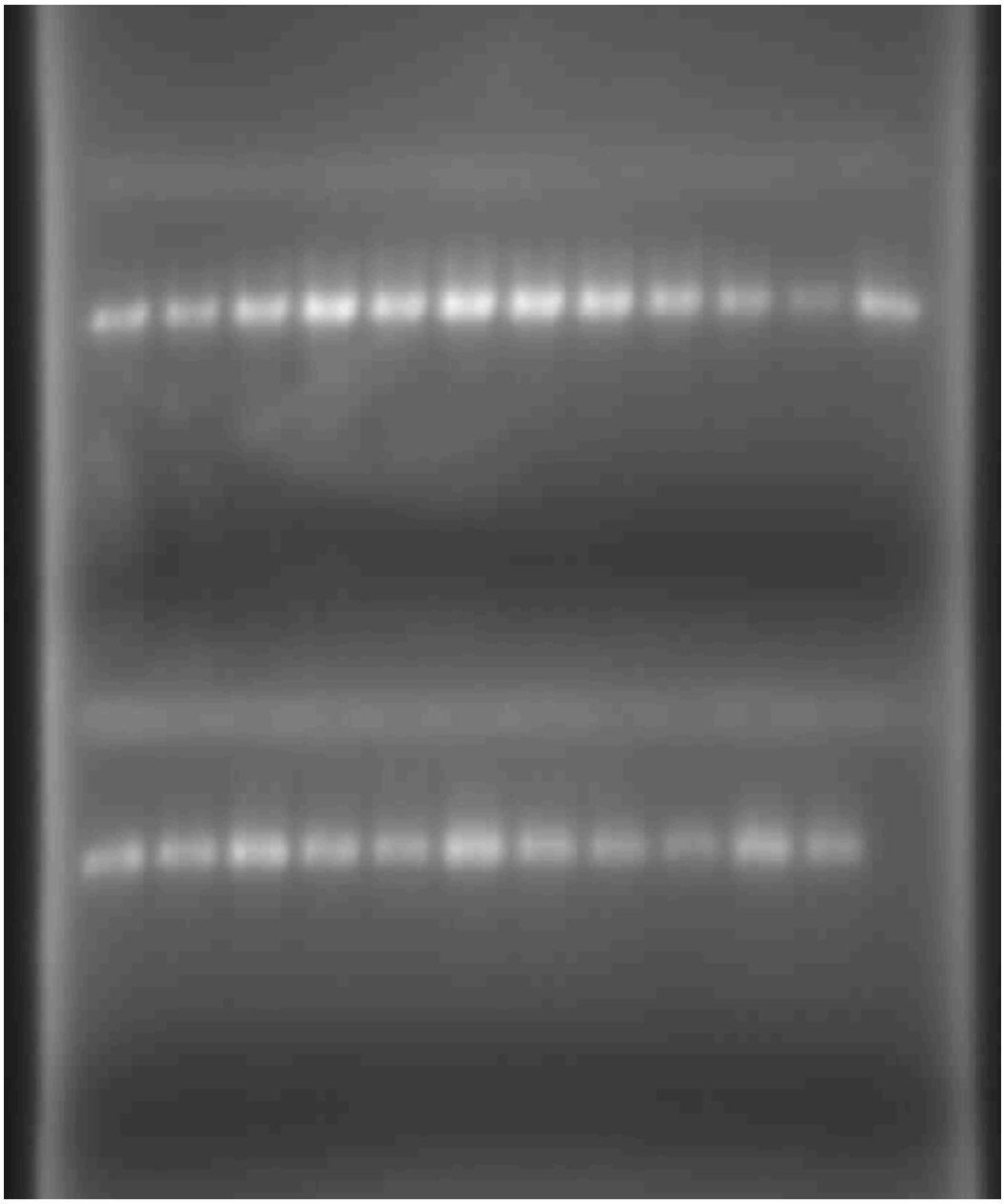
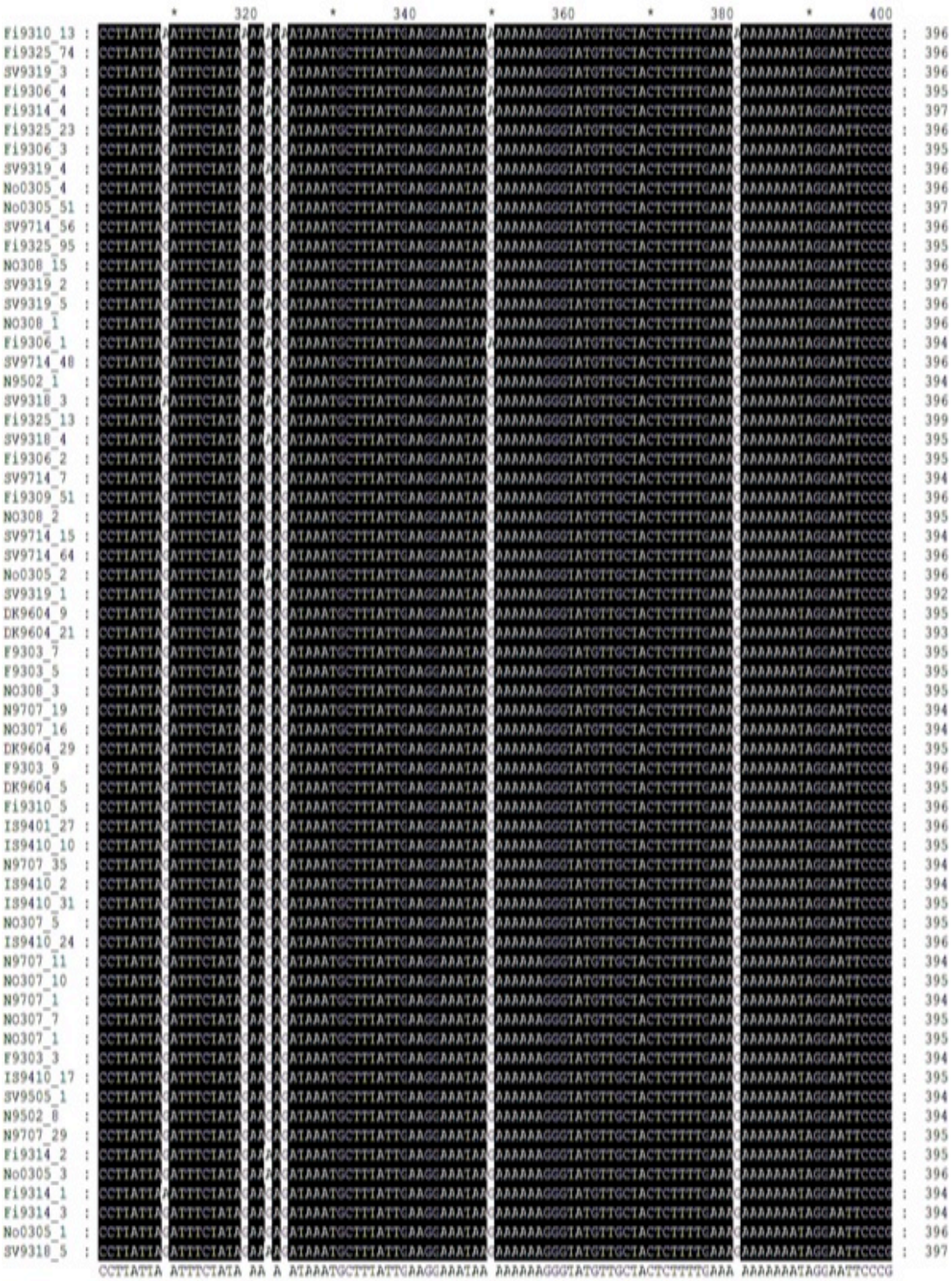


Figure 2: Representative of sequence alignment of rps16 sequences from *Elymus* populations


320
340
360
380
400
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Fi9325_74 : CCTIATTA ATTTCIATA AA A ATAAATGCTTIATTGAAGGAAATAA AAAAAAGGGIATGTTGCTACTCTTTTGAAA AAAAAAATAGGAATTCCCG : 396
SV9319_3 : CCTIATTA ATTTCIATA AA A ATAAATGCTTIATTGAAGGAAATAA AAAAAAGGGIATGTTGCTACTCTTTTGAAA AAAAAAATAGGAATTCCCG : 396
Fi9306_4 : CCTIATTA ATTTCIATA AA A ATAAATGCTTIATTGAAGGAAATAA AAAAAAGGGIATGTTGCTACTCTTTTGAAA AAAAAAATAGGAATTCCCG : 395
Fi9314_4 : CCTIATTA ATTTCIATA AA A ATAAATGCTTIATTGAAGGAAATAA AAAAAAGGGIATGTTGCTACTCTTTTGAAA AAAAAAATAGGAATTCCCG : 397
Fi9325_23 : CCTIATTA ATTTCIATA AA A ATAAATGCTTIATTGAAGGAAATAA AAAAAAGGGIATGTTGCTACTCTTTTGAAA AAAAAAATAGGAATTCCCG : 396
Fi9306_3 : CCTIATTA ATTTCIATA AA A ATAAATGCTTIATTGAAGGAAATAA AAAAAAGGGIATGTTGCTACTCTTTTGAAA AAAAAAATAGGAATTCCCG : 395
SV9319_4 : CCTIATTA ATTTCIATA AA A ATAAATGCTTIATTGAAGGAAATAA AAAAAAGGGIATGTTGCTACTCTTTTGAAA AAAAAAATAGGAATTCCCG : 396
No0305_4 : CCTIATTA ATTTCIATA AA A ATAAATGCTTIATTGAAGGAAATAA AAAAAAGGGIATGTTGCTACTCTTTTGAAA AAAAAAATAGGAATTCCCG : 396
No0305_51 : CCTIATTA ATTTCIATA AA A ATAAATGCTTIATTGAAGGAAATAA AAAAAAGGGIATGTTGCTACTCTTTTGAAA AAAAAAATAGGAATTCCCG : 397
SV9714_56 : CCTIATTA ATTTCIATA AA A ATAAATGCTTIATTGAAGGAAATAA AAAAAAGGGIATGTTGCTACTCTTTTGAAA AAAAAAATAGGAATTCCCG : 396
Fi9325_95 : CCTIATTA ATTTCIATA AA A ATAAATGCTTIATTGAAGGAAATAA AAAAAAGGGIATGTTGCTACTCTTTTGAAA AAAAAAATAGGAATTCCCG : 395
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No0305_2 : CCTIATTA ATTTCIATA AA A ATAAATGCTTIATTGAAGGAAATAA AAAAAAGGGIATGTTGCTACTCTTTTGAAA AAAAAAATAGGAATTCCCG : 396
SV9319_1 : CCTIATTA ATTTCIATA AA A ATAAATGCTTIATTGAAGGAAATAA AAAAAAGGGIATGTTGCTACTCTTTTGAAA AAAAAAATAGGAATTCCCG : 392
DK9604_9 : CCTIATTA ATTTCIATA AA A ATAAATGCTTIATTGAAGGAAATAA AAAAAAGGGIATGTTGCTACTCTTTTGAAA AAAAAAATAGGAATTCCCG : 395
DK9604_21 : CCTIATTA ATTTCIATA AA A ATAAATGCTTIATTGAAGGAAATAA AAAAAAGGGIATGTTGCTACTCTTTTGAAA AAAAAAATAGGAATTCCCG : 393
F9303_7 : CCTIATTA ATTTCIATA AA A ATAAATGCTTIATTGAAGGAAATAA AAAAAAGGGIATGTTGCTACTCTTTTGAAA AAAAAAATAGGAATTCCCG : 395
F9303_5 : CCTIATTA ATTTCIATA AA A ATAAATGCTTIATTGAAGGAAATAA AAAAAAGGGIATGTTGCTACTCTTTTGAAA AAAAAAATAGGAATTCCCG : 395
NO308_3 : CCTIATTA ATTTCIATA AA A ATAAATGCTTIATTGAAGGAAATAA AAAAAAGGGIATGTTGCTACTCTTTTGAAA AAAAAAATAGGAATTCCCG : 395
N9707_19 : CCTIATTA ATTTCIATA AA A ATAAATGCTTIATTGAAGGAAATAA AAAAAAGGGIATGTTGCTACTCTTTTGAAA AAAAAAATAGGAATTCCCG : 394
NO307_16 : CCTIATTA ATTTCIATA AA A ATAAATGCTTIATTGAAGGAAATAA AAAAAAGGGIATGTTGCTACTCTTTTGAAA AAAAAAATAGGAATTCCCG : 394
DK9604_29 : CCTIATTA ATTTCIATA AA A ATAAATGCTTIATTGAAGGAAATAA AAAAAAGGGIATGTTGCTACTCTTTTGAAA AAAAAAATAGGAATTCCCG : 395
F9303_9 : CCTIATTA ATTTCIATA AA A ATAAATGCTTIATTGAAGGAAATAA AAAAAAGGGIATGTTGCTACTCTTTTGAAA AAAAAAATAGGAATTCCCG : 396
DK9604_5 : CCTIATTA ATTTCIATA AA A ATAAATGCTTIATTGAAGGAAATAA AAAAAAGGGIATGTTGCTACTCTTTTGAAA AAAAAAATAGGAATTCCCG : 395
Fi9310_5 : CCTIATTA ATTTCIATA AA A ATAAATGCTTIATTGAAGGAAATAA AAAAAAGGGIATGTTGCTACTCTTTTGAAA AAAAAAATAGGAATTCCCG : 396
IS9401_27 : CCTIATTA ATTTCIATA AA A ATAAATGCTTIATTGAAGGAAATAA AAAAAAGGGIATGTTGCTACTCTTTTGAAA AAAAAAATAGGAATTCCCG : 396
IS9410_10 : CCTIATTA ATTTCIATA AA A ATAAATGCTTIATTGAAGGAAATAA AAAAAAGGGIATGTTGCTACTCTTTTGAAA AAAAAAATAGGAATTCCCG : 395
N9707_35 : CCTIATTA ATTTCIATA AA A ATAAATGCTTIATTGAAGGAAATAA AAAAAAGGGIATGTTGCTACTCTTTTGAAA AAAAAAATAGGAATTCCCG : 394
IS9410_2 : CCTIATTA ATTTCIATA AA A ATAAATGCTTIATTGAAGGAAATAA AAAAAAGGGIATGTTGCTACTCTTTTGAAA AAAAAAATAGGAATTCCCG : 394
IS9410_31 : CCTIATTA ATTTCIATA AA A ATAAATGCTTIATTGAAGGAAATAA AAAAAAGGGIATGTTGCTACTCTTTTGAAA AAAAAAATAGGAATTCCCG : 395
NO307_5 : CCTIATTA ATTTCIATA AA A ATAAATGCTTIATTGAAGGAAATAA AAAAAAGGGIATGTTGCTACTCTTTTGAAA AAAAAAATAGGAATTCCCG : 395
IS9410_24 : CCTIATTA ATTTCIATA AA A ATAAATGCTTIATTGAAGGAAATAA AAAAAAGGGIATGTTGCTACTCTTTTGAAA AAAAAAATAGGAATTCCCG : 396
N9707_11 : CCTIATTA ATTTCIATA AA A ATAAATGCTTIATTGAAGGAAATAA AAAAAAGGGIATGTTGCTACTCTTTTGAAA AAAAAAATAGGAATTCCCG : 394
NO307_10 : CCTIATTA ATTTCIATA AA A ATAAATGCTTIATTGAAGGAAATAA AAAAAAGGGIATGTTGCTACTCTTTTGAAA AAAAAAATAGGAATTCCCG : 395
N9707_1 : CCTIATTA ATTTCIATA AA A ATAAATGCTTIATTGAAGGAAATAA AAAAAAGGGIATGTTGCTACTCTTTTGAAA AAAAAAATAGGAATTCCCG : 394
NO307_7 : CCTIATTA ATTTCIATA AA A ATAAATGCTTIATTGAAGGAAATAA AAAAAAGGGIATGTTGCTACTCTTTTGAAA AAAAAAATAGGAATTCCCG : 395
NO307_1 : CCTIATTA ATTTCIATA AA A ATAAATGCTTIATTGAAGGAAATAA AAAAAAGGGIATGTTGCTACTCTTTTGAAA AAAAAAATAGGAATTCCCG : 395
F9303_3 : CCTIATTA ATTTCIATA AA A ATAAATGCTTIATTGAAGGAAATAA AAAAAAGGGIATGTTGCTACTCTTTTGAAA AAAAAAATAGGAATTCCCG : 394
IS9410_17 : CCTIATTA ATTTCIATA AA A ATAAATGCTTIATTGAAGGAAATAA AAAAAAGGGIATGTTGCTACTCTTTTGAAA AAAAAAATAGGAATTCCCG : 395
SV9505_1 : CCTIATTA ATTTCIATA AA A ATAAATGCTTIATTGAAGGAAATAA AAAAAAGGGIATGTTGCTACTCTTTTGAAA AAAAAAATAGGAATTCCCG : 394
N9502_8 : CCTIATTA ATTTCIATA AA A ATAAATGCTTIATTGAAGGAAATAA AAAAAAGGGIATGTTGCTACTCTTTTGAAA AAAAAAATAGGAATTCCCG : 394
N9707_29 : CCTIATTA ATTTCIATA AA A ATAAATGCTTIATTGAAGGAAATAA AAAAAAGGGIATGTTGCTACTCTTTTGAAA AAAAAAATAGGAATTCCCG : 395
Fi9314_2 : CCTIATTA ATTTCIATA AA A ATAAATGCTTIATTGAAGGAAATAA AAAAAAGGGIATGTTGCTACTCTTTTGAAA AAAAAAATAGGAATTCCCG : 395
No0305_3 : CCTIATTA ATTTCIATA AA A ATAAATGCTTIATTGAAGGAAATAA AAAAAAGGGIATGTTGCTACTCTTTTGAAA AAAAAAATAGGAATTCCCG : 396
Fi9314_1 : CCTIATTA ATTTCIATA AA A ATAAATGCTTIATTGAAGGAAATAA AAAAAAGGGIATGTTGCTACTCTTTTGAAA AAAAAAATAGGAATTCCCG : 394
Fi9314_3 : CCTIATTA ATTTCIATA AA A ATAAATGCTTIATTGAAGGAAATAA AAAAAAGGGIATGTTGCTACTCTTTTGAAA AAAAAAATAGGAATTCCCG : 394
No0305_1 : CCTIATTA ATTTCIATA AA A ATAAATGCTTIATTGAAGGAAATAA AAAAAAGGGIATGTTGCTACTCTTTTGAAA AAAAAAATAGGAATTCCCG : 396
SV9318_5 : CCTIATTA ATTTCIATA AA A ATAAATGCTTIATTGAAGGAAATAA AAAAAAGGGIATGTTGCTACTCTTTTGAAA AAAAAAATAGGAATTCCCG : 397
CCTIATTA ATTTCIATA AA A ATAAATGCTTIATTGAAGGAAATAA AAAAAAGGGIATGTTGCTACTCTTTTGAAA AAAAAAATAGGAATTCCCG

Results

Nucleotide Diversity

A total of 64 samples of the 65 listed in Table 1 were amplified using the rps16F/R primer; meanwhile, 33 these samples were amplified using the trn-tw/D primer pairs. The highest nucleotide diversity observed for samples amplified with the rps16 primer was the Fi9310 *E. caninus* population. The lowest nucleotide diversity was observed in *E. alaskanus* populations, No0502 and No9707, and *E. caninus* population, Fi9303. This demonstrates that *E. caninus* has varied nucleotide diversity within the species. The *E. caninus* Fi9310 population had the highest level of polymorphic sites (17), and the *E. alaskanus* and *E. caninus* populations No0502 and No9707 had zero polymorphic sites. Watterson's θ calculations suggest that *E. caninus* population Fi9310 had the highest mutation rate, while the same three populations with no genetic diversity mentioned above have a θ-W value of zero, suggesting low mutation rates. None of the populations was significant regarding the Fu and Li and Tajima tests.

For the trn intergenic sequences, the highest nucleotide diversity was detected in the *E. caninus* population Fi9310 with 0.04476; however, the lowest diversity was detected in two *E. caninus* populations, DK9604 and Fi9303 (0.00000). The highest values of polymorphic sites, 44, were observed in *E. caninus* (Fi9306), *E. fibrosus* (Fi9310) and *E. mutabilis* (Fi9325) populations. Zero was the lowest number of polymorphic sites, observed in the populations of *E. caninus* with no detected nucleotide diversity. Watterson's θ calculations indicated that the *E. caninus* population Fi9310 yielded the highest mutation rate, followed by the *E. fibrosus* and *E. mutabilis* populations. The lowest mutation rates were observed in the populations with no genetic

diversity. Using this primer pair, none of the populations showed significant departure from neutrality with regards to the Fu and Li or Tajima statistical tests.

Table 2: Estimates of nucleotide diversity of the rps16 gene of cpDNA in the *Elymus* species studied

Populations	N	S	h	π	θ	Fu and Li F	Fu and Li D	Tajima D
DK9604 *E. caninus*	4	2	3	0.00152	0.00142	0.50356	0.59158	0.59158
Fi9303 *E. caninus*	4	0	1	0.00000	0.00000	0.00000	0.00000	0.00000
Fi9306 *E. fibrosus*	4	4	4	0.00302	0.00283	0.60044	0.65010	0.65010
Fi9310 *E. caninus*	2	17	2	0.02205	0.02205	0.00000	0.00000	0.00000
Fi9314 *E. fibrosus*	4	6	4	0.00411	0.00424	-0.30226	-0.31446	-0.31446
Fi9325 *E. mutabilis*	4	2	3	0.00130	0.00142	-0.60427	-0.70990	-0.70990
IS9410 *E. alaskanus*	5	1	2	0.00052	0.00062	-0.77152	-0.81650	-0.81650
No0307 *E. alaskanus*	5	1	2	0.00052	0.00062	-0.77152	-0.81650	-0.81650
No0308 *E. caninus*	4	4	3	0.00281	0.00283	-0.06004	-0.06501	-0.06501
No9502 *E. alaskanus*	2	0	1	0.00000	0.00000	0.00000	0.00000	0.00000
No9707 *E. alaskanus*	5	0	1	0.00000	0.00000	0.00000	0.00000	0.00000
No0305 *E. mutabilis*	5	1	2	0.00078	0.00062	1.15728	1.12247	1.22474
SV9318 *E. mutabilis*	3	1	2	0.00086	0.00086	0.00000	0.00000	0.00000
SV9319 *E. mutabilis*	5	2	3	0.00156	0.00125	1.43161	1.45884	1.45884
SV9714 *E. caninus*	5	3	4	0.00208	0.00188	0.70126	0.69900	0.69900

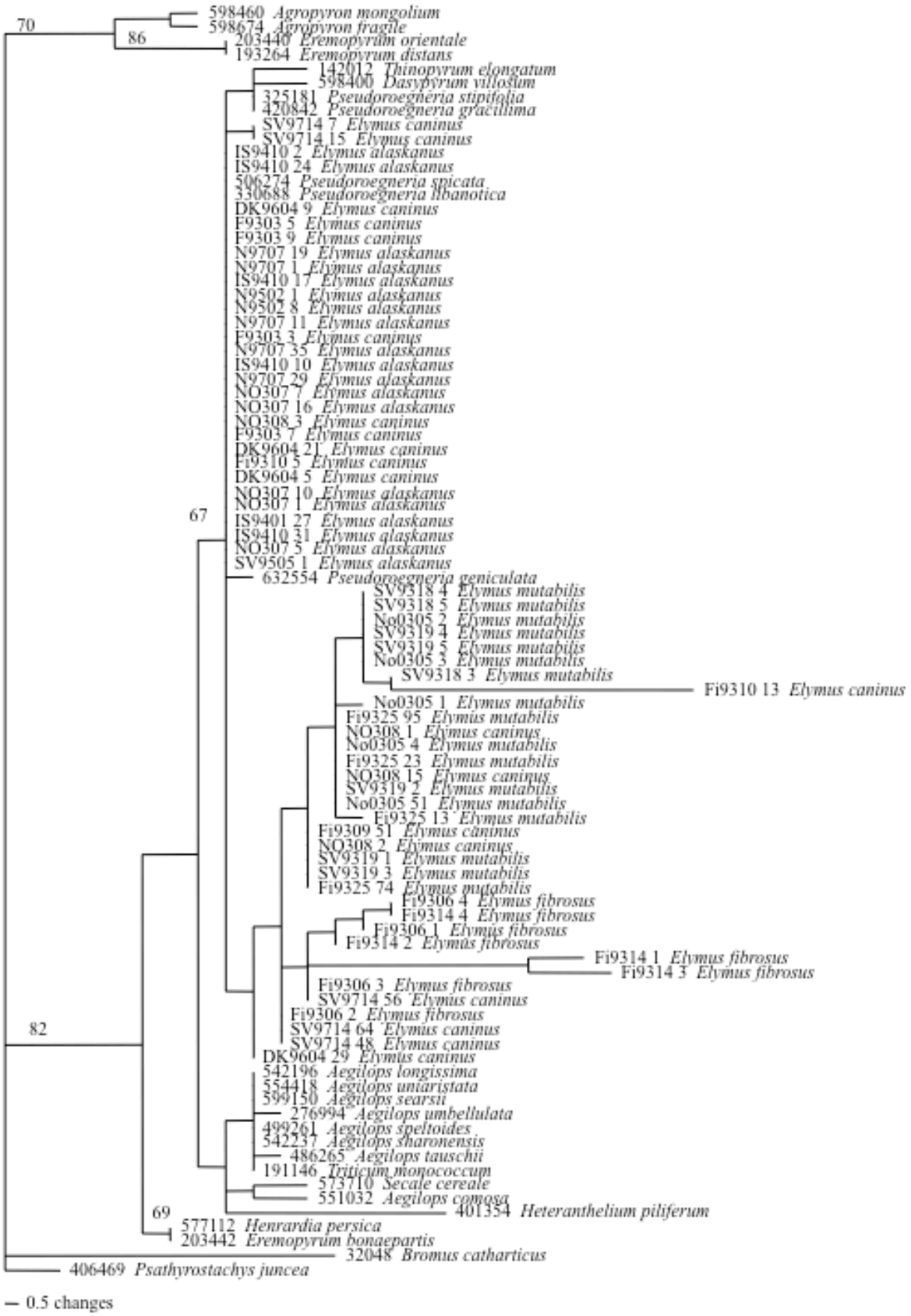
Table 3: Nucleotide diversity estimates of the trn-tw intergenic region of cpDNA in the *Elymus* species studied

Populations	N	S	h	π	θ	Fu and Li F	Fu and Li D	Tajima
DK9604 *E. caninus*	3	0	1	0.00000	0.00000	0.00000	0.00000	0.00000
Fi9303 *E. caninus*	3	0	1	0.00000	0.00000	0.00000	0.00000	0.00000
Fi9306 *E. fibrosus*	4	44	4	0.02311	0.02429	-0.53528	-0.50525	-0.50525
Fi9310 *E. caninus*	2	44	2	0.04476	0.04476	0.00000	0.00000	0.00000
Fi9325 *E. mutabilis*	4	44	4	0.02311	0.02429	-0.53528	-0.50525	-0.50525
IS9410 *E. alaskanus*	4	2	3	0.00099	0.00108	-0.60427	-0.70990	-0.70990
No0307 *E. alaskanus*	3	3	3	0.00199	0.00199	0.00000	0.00000	0.00000
No0308 *E. caninus*	3	22	3	0.01492	0.01492	0.00000	0.00000	0.00000
No9707 *E. alaskanus*	5	1	2	0.00040	0.00048	-0.77152	-0.81650	-0.81650

Phylogenetic Tree

The parsimony analysis of rps16 sequences, with *Bromus catharticus* as an outgroup, yielded 88 parsimonious trees with a consistency index (CI) of 0.807, a retention index (RI) or 0.917 and a rescaled consistency index (RC) of 0.740. One of the most parsimonious trees with bootstrap values is shown in Figure 3. *Psathyrostachys juncea* is observed at the base of the Triticeae phylogenetic tree with an 82% bootstrap, while two main clades form with 69% and 70% bootstrap values. In the first clade, two groups are formed, consisting of *Agropyron* and *Eremopyrum*, respectively. Concurrently, the second clade consists of *Elymus*, *Pseudoroegneria*, and *Aegilops* species with a 67% bootstrap value. This clade also included *Thinopyrum elongatum*, *Dasypyrum villosum*, *Triticum monococcum*, *Secale cereale*, *Heteranthelium piliferum*, *Henrardia persica* and *Eremopyrum bonaepartis*. *Thinopyrum elongatum* and *D. villosum* formed a group, while *H. persica* and *E. bonaepartis* formed a separate clade. Within the phylogenetic divisions, the *Elymus* species examined form clades with other *Elymus* species, as opposed to other genera within the Triticeae tribe; *Elymus alaskanus* populations formed a distinct and supported clade, as did *E. mutabilis* and *E. fibrosus*, respectively. On the other hand, *Elymus caninus* didn't form an individual clade. Population samples examined within the *Elymus* genus for this study are grouped together with other samples from the same populations and appear to form clades based on the species.

Figure 3: One of the most parsimonious trees derived from rps16 sequence data using heuristic search with respective bootstrap values


70
86
82
67
69
598460 Agropyron mongolium
598674 Agropyron fragile
203440 Eremopyrum orientale
193264 Eremopyrum distans
142012 Thinopyrum elongatum
598400 Dasypyrum villosum
325181 Pseudoroegneria stipifolia
420842 Pseudoroegneria gracillima
SV9714 7 Elymus caninus
SV9714 15 Elymus caninus
IS9410 2 Elymus alaskanus
IS9410 24 Elymus alaskanus
506274 Pseudoroegneria spicata
330688 Pseudoroegneria libanotica
DK9604 9 Elymus caninus
F9303 5 Elymus caninus
F9303 9 Elymus caninus
N9707 19 Elymus alaskanus
N9707 1 Elymus alaskanus
IS9410 17 Elymus alaskanus
N9502 1 Elymus alaskanus
N9502 8 Elymus alaskanus
N9707 11 Elymus alaskanus
F9303 3 Elymus caninus
N9707 35 Elymus alaskanus
IS9410 10 Elymus alaskanus
N9707 29 Elymus alaskanus
NO307 7 Elymus alaskanus
NO307 16 Elymus alaskanus
NO308 3 Elymus caninus
F9303 7 Elymus caninus
DK9604 21 Elymus caninus
Fi9310 5 Elymus caninus
DK9604 5 Elymus caninus
NO307 10 Elymus alaskanus
NO307 1 Elymus alaskanus
IS9401 27 Elymus alaskanus
IS9410 31 Elymus alaskanus
NO307 5 Elymus alaskanus
SV9505 1 Elymus alaskanus
632554 Pseudoroegneria geniculata
SV9318 4 Elymus mutabilis
SV9318 5 Elymus mutabilis
No0305 2 Elymus mutabilis
SV9319 4 Elymus mutabilis
SV9319 5 Elymus mutabilis
No0305 3 Elymus mutabilis
SV9318 3 Elymus mutabilis
Fi9310 13 Elymus caninus
No0305 1 Elymus mutabilis
Fi9325 95 Elymus mutabilis
NO308 1 Elymus caninus
No0305 4 Elymus mutabilis
Fi9325 23 Elymus mutabilis
NO308 15 Elymus caninus
SV9319 2 Elymus mutabilis
No0305 51 Elymus mutabilis
Fi9325 13 Elymus mutabilis
Fi9309 51 Elymus caninus
NO308 2 Elymus caninus
SV9319 1 Elymus mutabilis
SV9319 3 Elymus mutabilis
Fi9325 74 Elymus mutabilis
Fi9306 4 Elymus fibrosus
Fi9314 4 Elymus fibrosus
Fi9306 1 Elymus fibrosus
Fi9314 2 Elymus fibrosus
Fi9314 1 Elymus fibrosus
Fi9314 3 Elymus fibrosus
Fi9306 3 Elymus fibrosus
SV9714 56 Elymus caninus
Fi9306 2 Elymus fibrosus
SV9714 64 Elymus caninus
SV9714 48 Elymus caninus
DK9604 29 Elymus caninus
542196 Aegilops longissima
554418 Aegilops uniaristata
599150 Aegilops searsii
276994 Aegilops umbellulata
499261 Aegilops speltoides
542237 Aegilops sharonensis
486265 Aegilops tauschii
191146 Triticum monococcum
573710 Secale cereale
551032 Aegilops comosa
401354 Heteranthelium piliferum
577112 Henrardia persica
203442 Eremopyrum bonaepartis
32048 Bromus catharticus
406469 Psathyrostachys juncea
0.5 changes

Discussion

A variety of genetic markers have been used to evaluate *Elymus* species population structures; these include allozyme, random amplified polymorphic DNA, restriction fragment length polymorphism, and microsatellite markers. Allozyme studies have provided little information due to low levels of allozyme variation detected within *Elymus* species (Díaz *et al*., 1999). Random amplified polymorphic DNA has revealed significant variation in *Elymus alaskanus* species (Zhang *et al*., 2002). On the other hand, microsatellites proved to be highly polymorphic in a study by Sun and Salomon (2003). Microsatellites detected higher genetic variability in *E. alaskanus* populations; these repetitive DNA sequences are important in plants to adapt to environmental pressures and changes (Rogers & Bendich, 1987). Further, a study by Sun *et al*. (1998) demonstrates that *E. alaskanus* populations are more variable than *E. fibrosus* populations, which was supported by the studies of Díaz *et al*. (2000), who revealed greater allozyme and RAPD variation in *E. alaskanus* when compared to *E. fibrosus*. This can be attributed to geographic range; widely distributed plant species have to adapt to their large distributions which gives rise to high genetic diversity. Further, low genetic variation has been found on islands due to small founding populations and inbreeding; founder effects play a considerable role in governing patterns of genetic variability (Sun & Salomon, 2003). In addition, a study by Sun *et* al. (2002) showed that diversity in *E. alaskanus* populations was observed within rather than between populations, which was attributed to the populations originated from one founder population. Another study by Sun *et al*. (2001) demonstrates that *E. caninus* populations are mainly self-pollinating; low gene flow can indicate a predominantly inbreeding mating system. Self-pollination typically results in increased levels of homozygosity, thereby reducing genetic diversity (Foxe *et*

al., 2010). That being said, outcrossing may occur between this species and other *Elymus* species. The authors reported that higher levels of variation were found when *E. caninus* was observed growing together with *E. mutabilis* or *E. fibrosus*. Despite that, Sun *et al*. (1998) demonstrated that *E. fibrosus* populations contained very low genetic variation; however, the authors suggest sampling *E. fibrosus* populations from a main distribution area, such as Russia, to observe a different pattern of genetic diversity. Another study demonstrates that *E. fibrosus* is expected to have a restricted gene-pool due to a high number of homozygote individuals (Díaz *et al*., 2000). This study suggests that the low levels of variation of these populations could be due to a bottleneck event or a founder effect. Selfing species may be able to fix beneficial allele combinations in these conditions to achieve uniformity (Díaz *et al*., 2000). In this study, the highest levels of diversity were found in Finland for both rps16 and trn intergenic sequences; meanwhile, the lowest levels of nucleotide diversity were observed in Norway for the rps16 sequences, and in Denmark and Finland. Within *E. alaskanus* populations, the highest rps16 nucleotide diversity was 0.00052 while the lowest was 0; for *E. caninus,* level of nucleotide diversity was the overall highest at 0.02205, while the lowest was 0; *E. fibrosus* populations showed that the highest level of diversity for these populations is 0.0041, while the lowest is 0.0030. Finally, the *E. mutabilis* populations showed high diversity at 0.00156, and low diversity of 0.00078. For the intergenic region of cpDNA, only one population of each *E. fibrosus* and *E. mutabilis* was calculated for nucleotide diversity, each at a level of 0.02311, which was the second highest level of diversity. For *E. caninus,* the overall highest level of diversity was observed (0.04476), meanwhile it was also the smallest level of nucleotide diversity observed (0). *E. alaskanus* populations resulted in a high diversity level of 0.00199 and a low level at 0.00040. High levels of

variability in *E. caninus* populations support that this species possesses considerable genetic variation (Sun *et al,* 1999, 2006). Our study supports the results of Díaz *et al*. (1999) that *E. caninus* is more variable than *E. alaskanus* and *E. fibrosus* and that *E. caninus* contained the highest amount of polymorphic sites. The low levels of diversity for *E. fibrosus* populations can be explained due to the low sample sizes available for this study. Moreover, the phylogenetic tree indicated that most individual samples form clades with their own populations.

In summary, *E. caninus* displayed the highest nucleotide diversity while *E. fibrosus* was the least diverse of the species. The intergenic regions of the chloroplast DNA would yield a higher resolution in the phylogenetic tree, perhaps dividing *E. caninus* into a clade of its own. Classification of the Triticeae tribe is very complicated; future studies should evaluate other nuclear and chloroplast genes to support present and past phylogenetic and evolutionary relationships. Moreover, data determined by evaluating different modes of inheritance using molecular markers provides different types of information; combining this information can be used to characterize populations and support phylogenies.

References

Aluru, S. (Ed.). 2006. Handbook of computational molecular biology. Boca Raton, FL: Chapman & Hall/CRC Taylor & Francis Group.

Barkworth, M., Dewey, D.R. 1985. Genomically based genera in the perennial Triticeae of North America: Idenfitication and membership. American Journal of Botany. 72, 767-776.

Dewey, D.R. 1982. Genomic and phylogenetic relationships among North American perennial Triticeae, in: J.R. Estes (Ed.), Grasses and grasslands. Oklahoma University Press, Norman, Okla. Pp. 51-88.

Dewey, D.R. 1983. New nomenclatural combinations in the North American perennial Triticeae. Brittonia. 35, 30-33.

Dewey, D.R. 1984. The genomic system of classification as a guide to intergeneric hybridization with the perennial Triticeae, in: Gene Manipulation in Plant Improvement, 16th Stadler Genetics Symposium, edited by J.P. Gustafson, Plenum Publishing Crop., New York. pp.209-280

Díaz, O., Salomon, B., von Bothmer, R. 1999. Genetic diversity and structure in populations of *Elymus caninus* (L.) L. (Poaceae). Hereditas. 131, 63-74.

Díaz, O., Sun, G.L., Salomon, B., von Bothmer, R. 2000. Levels and distribution of allozyme and RAPD variation in populations of *Elymus fibrosus* (Schrenk) Tzvel. (Poaceae). Genetic Resources and Crop Evolution. 47, 11-24.

Farris, J.S. 1989. The retention index and the rescaled consistency index. Cladistics. 5, 417-419.

Felsenstein, J. 1985. Confidence limits on phylogenies: An approach using bootstrap. Evolution. 39 (4), 783-791.

Foxe, J.P., Stift, M., Tedder, A., Haudry, A., Wright, S.I., Mable, B.K. 2010. Reconstructing origins of loss of self-incompatibility and selfing in North American *Arabidopsis lyrata*: A population genetic context. Evolution. 64(12); 3495-3510.

Fu, Y.X., Li, W.H.1993. Statistical tests of neutrality of mutations. Genetics. 133, 693-709.

Hodge, C.D., Wang, H., Sun, G.L. 2010. Phylogenetic analysis of the maternal genome of tetraploid StStYY *Elymus* (Titriceae: Poaceae) species and the monogenomic Triticeae based on rps16 sequence data. Plant Science. 178, 463-468.

Hollingsworth, P.M., Bateman, R.M., Gornall, R.J. (Ed.). 1999. Molecular systematics and plant evolution. London: Taylor & Francis Limited.

Jensen, K. 1990. Cytology and taxonomy of *Elymus kengii*, *E. grandiglumis*, *E. alatavicus* and *E. batalinii* (Triticeae: Poaceae). Genome. 33, 668-673.

Kellogg, E.A., 1994. Systematics of the Triticeae: Problems and Progress, in: R.R.C. Wang, K.B. Jensen, C. Jaussi (Eds.), Proceedings of the 2nd International Triticeae Symposium, Logan, UT, USA, The Utah State University Press, pp. 207-214.

Löve, Á. 1982. Genetic evolution of the Wheatgrasses. New Zealand Journal of Botany. 20, 169-186.

Löve, Á. 1984. Conspectus of the Triticeae. Feddes Repertorium. 95, 468-473.

Mason-Gamer, R.J. 2001. Origin of North American *Elymus* (Poaceae: Triticeae) allotetraploids based on granule-bound starch synthase gene sequences. Systematic Botany. 26, 757-758.

Mason-Gamer, R.J., Orme, N.L. Anderson, M.A. 2002. Phylogenetic analysis of North American *Elymus* and the monogenomic Triticeae (Poaceae) using three chloroplast DNA data sets. Genome. 45, 991-1002.

McMillan, E., Sun, G.L. 2004. Genetic relationship of tetraploid *Elymus* species and their genomic donor species inferred from polymerase chain reaction restriction length polymorphism analysis of chloroplast gene regions. Theoretical and Applied Genetics. 108, 535-542.

Popp, M., Oxelman, B. 2007. Origin and evolution of North American polyploid *Silene* (Caryophyllaceae). Amercian Journal of Botany. 94 (3), 330-349.

Redinbaugh, M.G., Jones, T.A., Zhang, Y. 2000. Ubiquity of the St chloroplast genome in St-containing Triticeae polyploids. Genome. 43, 846-852.

Rogers, S.O., Bendich, A.J. 1987. Ribosomal RNA genes in plants: variability in copy number and in the intergenic spacer. Plant Molecular Biology. 9, 509-520.

Rozas, J., Sánchez-DelBarrio, J.C., Messeguer, X., Rozas, R. 2003. DnaSP, DNA polymorphism analyses by the coalescent and other methods. Bioinformatics. 19, 2496-2497.

Salomon, B., Lu, B.R. 1992. Genomic groups, morphology, and sectional delimination in Eurasian *Elymus* (Poaceae: Triticeae). Plant Systematics and Evolution. 180, 1-13.

Small, R.L., Cronn, R.C., Wendel, J.F. 2004. Use of nuclear genes for phylogeny reconstruction in plants. Australia Systematics Botany. 17, 145-170.

Sun, G.L., Daley, T., Ni, Y. 2007. Molecular evolution and genome divergence at *RPB2* gene of the St and H genome in *Elymus* species. Plant Molecular Biology. 64, 645-655.

Sun, G.L., Díaz, O., Salomon, B., von Bothmer, R. 1998. Microsatellite variation and its comparison with allozyme and RAPD variation in *Elymus fibrosus* (Schrenk) Tzvel. (Poaceae). Hereditas. 129, 275-282.

Sun, G.L., Díaz, O., Salomon, B., von Bothmer, R. 1999. Genetic diversity in *Elymus caninus* as revealed by isozyme, RAPD, and microsatellite markers. Genome. 42, 420-431.

Sun, G.L., Díaz, O., Salomon, B., von Bothmer, R. 2001. Genetic diversity and structure in a natural *Elymus caninus* population from Denmark based on microsatellite and isozyme analyses. Plant Systematics and Evolution. 227, 235-244.

Sun, G.L., Ma, X. 2009. Nucleotide diversity and minisatellite in chloroplast Asp(GUC)-Thr(GGU) region in *Elymus trachycaulus* complex, *Elymus alaskanus* and *Elymus caninus*. Biochemical Systematics and Ecology. 37, 67-75.

Sun, G.L., Salomon, B., von Bothmer, R. 1997. Analysis of tetraploid *Elymus* species using wheat microsatellite markers and RAPD markers. Genome. 40, 806-814.

Sun, G.L., Salomon, B., von Bothmer, R. 2002. Microsatellite polymorphism and genetic differentiation in three Norwegian populations of *Elymus alaskanus* (Poaceae). Plant Systematics and Evolution. 234, 101-110.

Sun, G.L., Salomon, B. 2003. Microsatellite variability and heterozygot deficiency in the arctic-alpine Alaskan wheatgrass (*Elymus alaskanus*) complex. Genome. 46, 729-737.

Sun, G.L., Shee, J., Salomon, B. 2006. Molecular diversity and relationship among *Elymus trachycaulus, E. subsecundus, E. virescens, E. violaceus,* and *E. hyperarcticus* (Poaceae: Triticeae) as determined by amplified fragment length polymorphism. Genome. 49, 1160-1169.

Svitashev, S., 1997. Analysis of Triticeae genomes using DNA markers. Acta Universitatis Agriculturae Sueciae. Agraria 61.

Tajima, F. 1989. Statistical method for testing the neutral mutation of hypothesis by DNA polymorphism. Genetics. 123, 585-595.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., Higgins, S.G. 1997. The CULSTAL_X windows interface: flexible strategies for multiple sequence alignment aided y quality analysis tools. Nucleic Acids Research. 25 (24), 4876-4882.

Torabinejab, J., Mueller R. 1993. Genome constitution of the Australian hexaploid grass, *Elymus scabrous* (Poaceae: Triticeae). Genome. 21, 112-125.

Tzvelev, N.N. 1989. The system of grasses (Poaceae) and their evolution. Botanical Review. 55, 141-204.

Watterson, G.A. 1975. On the number of segregation sites in genetical models without recombination. Theoretical Population Biology. 7, 256-276.

Wendel, J.F. 2000. Genome evolution in polyploids. Plant Molecular Biology. 42, 225-249.

Xu, D.H., Ban, T. 2004. Phylogenetic and evolutionary relationships between *Elymus humidus* and other *Elymus* species based on sequencing of non-coding regions of cpDNA and AFLP of nuclear DNA. Theoretical and Applied Genetics. 108, 1443-1448.

Yan, C., Sun, G. 2011. Nucleotide divergence and genetic relationships of *Pseudoreogneria* species. Biochemical Systematics and Ecology. 39-319

Ye, S.Q. (Ed.) 2008. Bioinformatics a practical approach Boca Raton, FL: Chapman & Hall/CRC Taylor & Francis Group.

Zeng, J., Fan, X., Zhang, L., Wang, X., Zhang, H., Kang, H., Zhou, Y. 2010. Molecular phylogeny and maternal progenitor implication in the genus Kengylia (Triticeae: Poaceae): Evidence from COXII intron sequences. Biochemical Systematics and Ecology. 38, 202-209.

Zhang, X.Q., Salomon, B., von Bothmer, R. 2002. Application of random amplified polymorphic DNA markers to evaluate intraspecific genetic variation in the *Elymus alaskanus* complex (Poaceae). Genetic Resources and Crop Evolution.49, 397-407.