Molecular Phylogeny of *Elymus alaskanus* Complex

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Abstract

Elymus species have been ideal candidates for studying the evolution and polyploidy in plants because it has a worldwide distribution, great economic value, and has a complex genetic composition. Many studies have been done as part of an investigation mapping the speciation and analyzing genetic diversity in Eurasian *Elymus* species. Studying the molecular phylogeny of *Elymus alaskanus* complex is one part of this program. Even though many studies have focused on the genetic diversity of *E. alaskanus*, the information on the origin and phylogeny of *E. alaskanus* complex is lacking. In this study, seven populations including thirty-four *Elymus alaskanus* individuals from Canada, USA, and Greenland were analyze for the two chloroplasts genes (RPS16, RPOA) and one nuclear gene (RPB2). The objectives of this study were: 1) to identify whether *E. alaskanus* has single or multiple origins; and 2) to identify the potential maternal donor species in *E. alaskanus*. As a result, the potential maternal donor species in *E. alaskanus* was identified. Phylogenetic relationships within these seven populations were characterized. The outcome of this study can provide information for efficiently conservation and utilization of this species.

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Introduction:

Molecular phylogeny is a means to infer species evolutionary relationships by reconstructing gene trees based on sequence variation of related species. It contains both molecular and statistical techniques (Dowell, 2008). Because molecular variation provides a deeper insight into the evolutionary dynamics of nature, the study of relationships among plant and geographical variation was shifted from using the morphological variation to the use of molecular one. For comprehensive understanding of the history and microevolution of populations, it is important to study a large number of polymorphisms (Sun et al., 2003). DNA sequences change through evolutionary time, and these changes can be used to estimate species relationships and to produce species trees. There are many methods which can be used to produce phylogenetic trees; however, the most popular method is a maximum parsimony analysis. In this method, the species will be organized into a tree that requires the least amount of evolutionary change to explain observed differences (Ye, 2008). By using bootstrap analyses, the reliability of generated trees can be evaluated (Felsenstein, 1985; Aluru, 2006; Ye, 2008). Bootstrapping is a method that creates trees based on subsamples taking of the sites in an alignment. This process repeats multiple times; the preferred number is 1000. In nonstandard situations, the bootstrap is used to obtain estimates of error by resampling

the data set many times. By doing this, a distribution against which hypotheses could be tested will be provided and the results will be compiled to allow an estimate of a particular grouping reliability (Pamela et al., 2003; Soltis et al., 2003).

Taxonomy is the description, identification, nomenclature and classification of organisms. Plant systematics 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 natural hybridization causing morphological variation within and between species. Natural hybridization is a secondary contact that happens in nature between two populations that have evolved separately over a long period of time. Hybridization processes may occur because of the speciation that involves the establishment of many reproductive barriers which are incomplete. (Genovart, 2008). However, to further describe and classify species, comprehensive taxonomic and phylogenetic analyses must be performed to reveal relationships between and within these species. In the 18th century, Linnaeus, a systematicist, started to classify organisms according to their similarities and differences. Molecular phylogeny then derived from this traditional method for classifying organisms into a hierarchic series of taxonomic categories, starting with kingdom and progressing down through phylum, class, order, family and genus to species. This hierarchy was then linked to a 'tree of life', an analogy that was adopted by Darwin (1859). Therefore, the classificatory scheme devised by Linnaeus was redefined as a phylogeny indicating the similarities between species and their evolutionary relationships (Brown, 2002).

For the successful management of conservation programs, knowledge of genetic

diversity is necessary. Nucleotide diversity is the average number of different nucleotides per site and is a measure of genetic variation (population diversity). Genetic diversity provides insight into species and determines evolutionary relationships. High genetic variation is linked to survival, adaptation and evolution. An organism's ability to survive and reproduce could increase by some new alleles. This will ensure the survival of the allele in the population (Barrett and Schluter, 2007). Moreover, within a population, variation allows some individuals to adapt to the changing environment. Genetic variation is important for estimating population parameters. Moreover, it is critical for evolutionary studies of mating systems and relatedness (Sun et al., 2001). Phylogenies are important for addressing various biology questions such as relationships among genes, the origin and spread of viral infection, and the demographic changes and migration patterns of species. In addition, the most popular application is to describe relationships among groups of organisms in systematics and taxonomy, which are discovered through molecular sequencing data and morphological data matrices. However, plant systematics had faced an interesting challenge during the last decade. This challenge is to solve reticulate phylogenetic relationships at all levels and to uncover previous undetected evolutionary processes (Mason-Gamer et al., 2010).

The chloroplast is an organelle required for photosynthesis to produce energy for plant growth. Chloroplast DNA (cpDNA) is roughly 10 times larger and more variable than mitochondrial DNA. These characteristics make chloroplast DNA a good candidate for extensive use in plant phylogeny and genome evolution analyses (Sun et al., 2009). Due to their high variability, simple amplification and abundance of universal primers developed, non-coding regions can provide more information in phylogenetic studies at

the species level. Additionally, non-coding regions are variable in their content and arrangement, especially between related species and do not display recombination, thus are useful in studying phylogeny, evolution and genetic diversity of a species (Hollingsworth et al., 1999; Sun et al., 2002; Aluru, 2006). On the other hand, analyses of nuclear DNA may explain other aspects, for example, nuclear DNA allows an accurate level of genetic discrimination, at the population level in particular, of phylogenetic and evolutionary relationships because of its bi-parental inheritance.

A single copy nuclear gene has its advantages that make it a good choice in phylogenetic studies. One of these advantages is that it has relatively faster evolution rates compared to organelle genomes. It has been shown that synonymous substitution rates of nuclear genes are up to five times greater than those of chloroplast genes and 20 times than those of mitochondrial genes (Wendel, 2000). Moreover, each single copy nuclear gene provides an independent phylogenetic data set, which enhances both efficiency and accuracy of phylogenetic study. Another advantage is that single copy nuclear genes are able to reveal the full evolution history of both hybrid plants and polyploidy species (Wang, 2008). However, there are some disadvantages of the single copy nuclear genes. Only a few single copy nuclear genes were identified for use in phylogenetic investigations. Another challenge is associated with the low amount of target DNA derived from PCR amplification. Moreover, the use of single copy nuclear genes involves gene cloning and sequencing, and these techniques are expensive.

PCR is a technique used to amplify a single or few copies of piece of DNA across several orders of magnitude; and generate thousands to millions of copies of a particular DNA sequence. There are three steps in the PCR program: denaturing, annealing and

extension. PCR relies on thermal cycling which consist of cycles of repeated heating and cooling of the reaction for DNA melting and enzymatic replication of the DNA. Primers, short DNA fragments which contains sequences complementary to the target region along with a DNA polymerase are key components to enable selective and repeated amplification.

For a successful management of conservation programs, knowledge of genetic diversity and population genetic structure of a species is a prerequisite (Sun et al., 2002). It is important to know the life history characteristics of a species and to understand how the biotic and abiotic interactions within a community affect the species. It is also important to understand the specific threats (over-exploitation, habitat loss and degradation, climate change, pollution and diseases, introductions of alien species.) to the species to formulate an appropriate conservation strategy, and to obtain the genetic structure of the species (Falk, 1992).

The methods for detecting and analyzing genetic diversity have expanded over the years: from morphological and cytological methods, to statistical analyses of quantitative traits, to biochemical assays, and molecular assays. Therefore, molecular genetic markers have been developed to provide an opportunity to reveal DNA sequence polymorphisms, the existing of multiple forms of a single gene in an individual (Sun et al., 2002).

1. Polyploidization

A polyploidy is an entire chromosome set multiplied. In other words, polyploidy species comprise more than two sets of homologous chromosomes. Polyploidy arises through mitotic or meiotic mis-divisions, and frequently involves unreduced gametes and interspecific hybridization (Wendel, 2000). Based on the origin of the duplicated

genomes, it can be classified into allopolyploids or autopolyploids. While allopolyploids mean that the two sets of chromosomes are derived from different species, autopolyploids mean that they are derived from the same species (Tate et al., 2005). When replicating genes by polyploidy, various outcomes may be produced. These include the diversification of a gene function, regulation of proteins, or the silencing of genes that cause genetic recombination or conversion (Wendel, 2000). Polyploidy is considered a common process that contributed to both animals and plants diversity seen today. Polyploidy is widespread among flowering plants (Leitch et al., 2008) having occurred in 70% of all angiosperms (Masterson, 1994; Wendel, 2000). Over the past 150 years, several new species of plant are known to have evolved and established. Therefore, studying the origin, evolution, and consequences of polyploidy will give more of an understanding as to how it has contributed to the diversity of species seen today.

2. *Elymus* and its genome constitutions

2.1. Poaceae, Tririceae, and *Elymus*

The world's most important cereals, wheat and barley and other valuable species in cereal and forage are included in the tribe Triticeae, a monophyletic grass tribe in the grass family Poaceae. The tribe consist of a number of plants that are of economic importance (i.e., stabilizing eroded areas and vegetating metal-rich soils in reclaimed mines). Moreover, tribe have a complex evolutionary history. Therefore, the tribe of the grass family Poaceae is good research subject for studying evolution, phylogeny, and taxonomy (Yan et al., 2011). To illustrate the systematic relationships of the tribe Triticeae, specify the genera and clarify the ancestry of many polyploidy species, data from cytogenetic analyses have been used (Dewey, 1984; Love, 1984).

Elymus, was first described as a genus by Linnaeus (1753). It is a genus of perennial plants in the grass family "Poaceae". Moreover, it is the largest and most widely distributed genus in the tribe Triticeae, and encompasses approximately 150 species that occur worldwide. *Elymus* occur in the Arctic and temperate to subtropical regions even though it is predominately a northern temperate genus. Approximately 80 of all known *Elymus* species have their origins in Asia, which makes it the center for the origin and diversity of the *Elymus* species, while North America is the second most important area with about 50 species (Sun, 2007). Moreover, *Elymus* species can be found in various environments such as grasslands, mountainsides, semi-deserts, and valleys or in forests. *Elymus* has been an ideal candidate for studying the evolution and polyploidy in plants because it has a worldwide distribution, is of great economic value, and has a complex genetic composition (Yan et al., 2011). *Elymus* has been known for its extreme complexity when it comes to taxonomy due to the morphological variation within and between species, the polyploidy origin of the genus, and frequent spontaneous hybridizations between species (Sun et al., 2005). All these features make this genus a prime model for research on cytogenetics, genetic diversity, molecular genetics and phylogeny (Diaz et al., 1999)

Elymus species are known to possess useful genes for disease and pest resistance, stress tolerance, high protein content and diverse adaptation, which is valuable gene pool for improvement of crops, such as wheat, barley and rye. Many studies have been completed as part of an investigation to map the speciation and analyze the genetic diversity in Eurasian *Elymus* species. The overall objective of the program was to establish the prerequisites for efficient conservation strategies of the genetic diversity in

Elymus and related genera (Salomon, 1995; Salomon et al., 1996). Some *Elymus* species are known to contain good forage qualities, and high productivity under unfavorable climatic conditions and are widely used for grazing. Moreover, *Elymus* species are also used for revegetation, soil stabilization and erosion control (Dewey, 1984; Barkworth and Dewey, 1985; Diaz et al., 1999; Sun et al., 2002).

2.2. **Genome constitution of *Elymus***

Based on cytogenetic results, five basic genomes have been assigned to the genus *Elymus* which are: **St**, **H**, **Y**, **P**, and **W**. The **St** genome, that is originated from *Pseudoroegneria,* a genus in Triticeae (Poaceae), is the most important genome in the genus, and can be found in all allopolyploids combining with **H** (from *Hordeum* L.), **Y** (from an unknown donor), **P** (from *Agropyron* Gaertn.), and **W** (from *Australopyrum*) to form various tetraploid, hexaploid, and octoploid configurations (Dewey, 1984; Löve, 1984). However, the relationships among these genome combination groups are far from clear even though they are cytogenetically well defined (Sun, 2007).

Recent molecular phylogenetic studies on *Elymus* have added to our understanding of the origin of *Elymus* species. However, evolutionary dynamics and speciation of most species in *Elymus* are still unclear. By performing phylogenetic analysis, Mason-Gamer (2001) suggested that most of North American *Elymus* species contain both the **St** and **H** genomes. Moreover, the molecular data suggests a multiple origin of the **H** and **St** haplome in the **StH** genome tetraploid *Elymus* species (Sun et al., 2008; Sun and Komatsuda, 2010; Yan and Sun, 2012). For example, Yan and Sun (2012) reported multiple origins for the allopolyploid wheatgrass *E. caninus*. However, other studies suggest a single origin of **StH** genomic species (Mason Gamer et al., 2009). Previous

studies using chloroplast DNA sequence analyses indicated that **St** genomic *Pseudoroegneria* species was the maternal donor of *Elymus* species (Redinbaugh et al., 2000; McMillan and Sun, 2004).

Elymus alaskanus

Elymus alaskanus is a perennial, self-fertilizing and allotetraploid species (**StStHH**, 2n = 4x = 28), which grows on limestone outcrops, screes, moraines, dry meadows and similar low-competition habitats. It is an Artic-alpine species, found in the Nordic region, northern Russia, Siberia, Alaska, northern Canada and Greenland. There are about 15 taxa that have been described in this complex, either as species or subspecies (Sun et al., 2003). *Elymus alaskanus* is known as a morphologically variable species over its entire distribution area. However, it is usually morphologically homogenous within regions and populations. As a part of a larger investigation for mapping of species and analyzing genetic diversity in Eurasian *Elymus* species, genetic diversity of this species was characterized (Sun et al., 1998; Zhang et al., 2000, 2001). The study of Sun et al. (1998) focused on *E. alaskanus* and other on *E. caninus.* The overall objective was to establish the prerequisites for efficient conservation strategies of the genetic diversity in *Elymus* and related genera. Primer pairs were designed and evaluated for 18 selected microsatellites (DNA sequences with short repeated motifs less than 6 bp) from *E. alaskanus*. These primers pairs can be used to generate polymorphisms in *E. alaskanus* and other species within the genus. Moreover, the degree of polymorphism of 18 microsatellites was examined in a total of 18 accessions of *E. alaskanus* and other *Elymus* species. All loci displayed one or more alleles and the number of alleles found for each of these loci varied from 1 to 10. Gene diversity values ranged from 0.20 to 0.72, with an

average of 0.48. In *E. caninus*, seven of the eight primer pairs successfully amplified DNA from *E. alaskanus* and *E. mutabilis*. Based on these results, microsatellites appear to be useful markers in detecting variation in both *E. alaskanus* and *E. caninus*. The genetic variation and structure of 27 populations of *E. alaskanus* from different locations in Canada, USA, Greenland and Russia were examined (Zhang et al., 2000), the results indicated that *E. alaskunus* contains different levels of allozyme variation in its populations. The allozyme variation at the species level was higher in *E. alaskanus* than in other self-fertilizing plants. However, a low level of variation was found at the population level when comparing it with allozyme variation at the species level (Zhang et al., 2000). Another study using ten of the same populations, and random amplified polymorphic DNA (RAPD) markers to evaluate patterns of genetic diversity (Zhang et al., 2001) found that much higher genetic diversity values than allozymatic variation. Populations from Greenland and the USA (‘‘violaceus’’ and ‘‘latiglumis’’) were clearly separated, from the others (‘‘hyperarcticus’’, ‘‘komarovii’’ and ‘‘sajanensis’’) (Zhang et al., 2001). Sun et al. (2002) studied three local *E. alaskanus* populations from Norway to investigate genomic variation at seven microsatellite loci. This microsatellite variation was then compared with allozyme variation, the results showed that microsatellite loci have higher levels of variation than allozyme, and suggested that natural selection might be an important factor in shaping the genetic diversity in the three local *E. alaskanus* populations studied. Sun and Salomon (2003) reported that *E. alaskanus* is highly heterozygous and also highly variable. Moreover, there was no association between genetic distance and geographic distance of the populations. By comparing the genetic diversity profiles obtained in this study with microsatellite markers with those obtained

previously with allozyme markers (Zhang et al., 2000) and RAPD markers (Zhang et al. 2002), microsatellite based measures of diversity were higher than allozyme and RAPD estimates. Moreover, the Greenland populations were genetically similar to the American populations whereas a large genetic distance was found between the Canadian populations and the American populations.

Although many studies have focused on the genetic diversity of *E. alaskanus* (Sun et al., 1998; Zhang et al., 2000; Zhang et al., 2001; Zhang et al. 2002; Sun and Salomon, 2003; Gaudett et al., 2004; Stevens et al., 2007; Sun and Ma, 2008), the information on the origin and phylogeny of *E. alaskanus* complex is still lacking. Many studies have shown that the chloroplast gene encoding ribosomal protein S16 (RPS16) intron (Liden et al., 1997; Oxelman et al., 1999; Hodge et al., 2010), the chloroplast RNA polymerase alpha subunit (RPOA) (Petersen and Seberg, 1996), and the single low copy nuclear gene RNA polymerase II (RPB2) (Sun et al., 2007; Sun et al., 2008; Sun et al., 2009; Yan et al., 2011; Yan and Sun, 2012) are good genes for characterizing the origin and phylogeny of a species.

Objectives:

The objectives of this study were to use two chloroplast genes RPS16 and RPOA, and one nuclear gene RPB2 to: 1) to identify whether *E. alaskanus* has single or multiple origins; and 2) to identify the potential maternal donor species in *E. alaskanus*.

Materials:

Samples representing seven populations of *E. alaskanus* were collected from North America. The materials were tentatively divided into hyperarcticus, from arctic Canada; latiglumis, from the United States; and violaceus, from Greenland based on

morphological variation. The Canadian populations were further divided into "typical" and "tall" hyperarcticus. From separate plants from each of the seven populations, individual spikes were collected along a transect through the population. To ensure that the collected individuals represented different individuals, they were generally separated by at least 1 m. Appendix 1 listed species, accessions numbers and origins used in this research. Seeds were provided by the Swedish University of Agricultural Science. A single seed from each spike was then germinated and transplanted into individual pot containing soil mixture, and maintained in a greenhouse located at Saint Mary's University in Halifax, Nova Scotia with 19 ± 1 °C and 16 h light: 8 h dark cycle.

The sequences for some diploid Triticeae species representing the **St**, **H**, **I**, **Xu**, **W**, **P**, **E**, **Ns**, **Ta**, **A**, **S**, **Xa**, **F**, **O**, **Q**, K, **V**, **U**, **R**, and **D** genomes along with *Bromus* were obtained from published data (Sun et al., 2008; Helfgott and Mason-Gamer, 2004), and included in the analyses. Plant materials with accession numbers, genomic constitutions, geographical origins, and Gen Bank identification numbers were presented in Appendix 2, 3 and 4.

Methods:

1. DNA Extraction:

The DNA was extracted from leaves of young plants using a GeneJet™ Plant Genomic DNA Purification Mini Kit (#K0792, Thermo Scientific) according to the manufacturer's instructions and used in the previous study (Sun and Salomon, 2003). Briefly, 100 mg of fresh plant tissue was crushed into a fine powder in liquid nitrogen using a mortar and pestle. The powder was transferred to a 1.5 mL centrifuge tube with 350 μL of Lysis Buffer A. 50 ml of Lysis Buffer B and 20 μL of RNase A were then

added. The tube was incubated at 65°C for 10 min, and then placed on ice for 5 min. Precipitation Solution (130 μL) was combined with the mixture, and the supernatant was collected and placed in a new 1.5 mL centrifuge tube with 400 μL of Plant gDNA Binding Solution and 400 μL of 95% Ethanol. After the solution was emulsified, it was centrifuged (1 min at 6,000x g). Wash Buffer 1 (500 μL) was added to the column after the flow through was discarded.

The column was then centrifuged (1 min at 3,000x g), and 500 μL of Wash Buffer 2 was added to the column to be centrifuged again at ≥ 20,000x g (3 min). 100 ml of Elution Buffer (10 mM Tris-HCl, 0.5 mM EDTA at a pH of 9.0) was added to the column that was incubated at room temperature (5 min), and then centrifuged at 8,000x g (1 min). The isolated genomic DNA was then stored at -20°C for use.

2. Polymerase Chain Reaction (PCR):

Polymerase Chain Reaction (PCR) amplification was carried out using high fidelity *Taq* polymerase and specific primers for each gene.

The primers sequence for the gene encoding ribosomal protein S16 (RPS16 F/ R), RNA polymerase alpha subunit (RPOA1/ 2) and the single low copy nuclear gene RNA polymerase II (RPB2 R/F) were given as follows:

Primers RPS16F (5'-GTGGTAGAAAGCAACGTGCGACTT-3'), and RPS16R (5'-TCGGGATCGAACATCAATTGCAAC-3') (Popp and Oxelman, 2007) were used to amplify the RPS16 gene within the chloroplast DNA which consists of 900 base pairs. Primers RPOA1 (5'-ACACCTATGCCGCATAATGG-3') and RPOA2 (5'GTTAGGTATTGGAGCAACAT-3') (Petersen and Seberg, 1997) were used to amplify the RPOA region gene which consists of 1300bp. Primers P6F: (5'

TGGGGAATGATGTGTCCTGC-3') and F6FR: (5' - CGAACCACACCAACTTCAGTGT-3') (Denton et al., 1998), were used to amplify the RPB2 nuclear gene which consists of 1000bp.

A master mix for amplifying the RPS16 contained 3 μL of forward primer, 3 μL of reverse primer (2 MM concentration), 1 μL DNA, 10 μL of *Taq* 2X Master Mix, an optimized ready-to-use solution containing *Taq* DNA Polymerase, dNTPs, $MgCl_2$, KCI and stabilizers, and 3 μL of autoclaved water in a 20 μL volume. The master mix for amplifying RPOA contained 0.3 μL of forward primer, 0.3 μL of reverse primer (10 MM concentration), 1 μL DNA, 10 μL of *Taq* 2X Master Mix, and 8.4 μL of autoclaved water in a 20 μL volume. To amplify the RPB2, a master mix contained 0.6 μL of forward primer, 0.6 μL of reverse primer (10 MM concentration), 1 μL DNA, 10 μL of *Taq* 2X Master Mix, and 7.8 μL of autoclaved water in a 20 μL volume. For each DNA, 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 (Zeng et al., 2010; Yan and Sun, 2011).

The amplification profile for the RPS16 gene was as follows: an initial denaturation at 95 °C (3 min) and 40 cycles of 95 °C (40 sec), 63 °C (40 sec), and 72 °C (90 sec). The cycling ended with 72 °C (10 min) and an infinite hold at 4°C. The PCR profile for amplifying RPOA gene was: an initial denaturation at 95 °C (3 min) and 40 cycles of 95 °C (40 sec), 55 °C (40 sec), and 72 °C (90 sec). The cycling ended with 72 °C (10 min) and an infinite hold at 4°C. For the RPB2, the PCR protocol was: one cycle at 95°C (4 min), 40 cycles at 95°C (40 sec), 40sec at 52°C and 1.5 min at 72°C, followed by 1 cycle at 72 °C (10 min) and an infinite hold at 4°C.

3. Gel Electrophoresis and Visualization:

Electrophoresis was performed using 1.5% 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 140 volts for 40 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.

4. Cloning:

For the nuclear gene (RPB2), the PCR products were cloned into the pGEM-easy T vector (Promega Corporation, Madison, WI, USA) according to the manufacturer's instructions. To transform *Escherichia coli* DH5, the resulting plasmids were used and at least 20 colonies for each accession were randomly selected for screening. Each colony was transferred to 10 μL of LB broth with 0.1 mg/ml ampicillin. The solutions were incubated at 37°C (30 min) before using 2 μL for PCR to check the presence of an insert using the same primers that were used for the original PCR amplification. When the solutions were confirmed to contain the insert, the remaining 8 μL of solution was transferred to 5 mL LB broth and incubated at 37°C overnight. Plasmid DNA was isolated using Promega Wizard ® *Plus*Minipreps DNA Purification System (Promega Corporation, Madison, WI, USA) according to manufacturer's instructions.

The PCR products amplified by primers RPS16R/F and RPOA1/2 were purified and then directly sequenced.

5. Sequencing and Data Analysis:

The plasmid DNA and PCR products were commercially sequenced by Taihe Biotechnology Company (Beijing, China). To enhance the sequence quality, both forward and reverse strands of PCR products were sequenced independently. To ensure the sequences from the gene desired were amplified, sequences were analyzed with the BLAST program (http://www.ncbi.nlm.nih.gov/). Multiple sequence alignments were performed using ClustalX (Thompson et al., 1997). To analyze the phylogenetic relationship of the species, the Phylogenetic Analysis Using Parsimony (PAUP) (Swofford, 2003) program was used. *Bromus* was used as an out-group, based on the previous study of phylogenetic analysis of Poaceae (Yan et al., 2012). Gaps were treated as missing data while all characters were considered un-weighted and ungrouped.

The most parsimonious (MP) trees were constructed by performing a heuristic search using the Tree Bisection-Reconnection (TBR) with the following parameters: MulTrees on and ten replications of random addition sequences with the stepwise addition option. Multiple parsimonious trees were combined to form a strict consensus tree. Overall character congruence was estimated by the consistency index (CI), and the retention index (RI). The consistency index was used as a measure of fit of a character within a phylogenetic tree and indicated presence of homoplasy while the retention index is a measure of how parsimonious the phylogenetic tree is, using the least evolutionary change to explain the observed data. To determine the clade consistency, bootstrap values (BS) with 1,000 replications (Felsenstein, 1985) were calculated by performing a heuristic search using the TBR option with Multree on, and a 50% majority rule consensus tree was created.

In addition to MP analysis, Bayesian analyses were also performed. Using jModelTest 2.1.10 (Darriba et al., 2012), the best-fitting model of sequence evolution was tested for each data set using default parameters. For each data, the Akaike information criterion (AIC) (Akaike, 1973), Bayesian Information Criterion (BIC) (Schwarz, 1978) and a decision-theoretic performance-based approach (DT) (Minin et al., 2003) were estimated. However, The BIC was used for model selection since it has high accuracy (Darriba et al., 2012). The GTR, K80, GTR models led to best BIC score for RPS16, RPOA and RPB2, respectively. Therefore, the GTR and K80 model were used in the Bayesian analysis using MrBayes v3.2 (Ronquist et al., 2012). MrBayes v3.2 was run with the program's standard setting of two analyses in parallel, each with four chains, one cold chain and three incrementally heated Markov Chain Monte Carlo (MCMC) chains, and estimates of convergence of results by calculating standard deviation of split frequencies between analyses. For both RPB2 and RPS16, samples were taken every 1000 generations under the GTR model with gamma-distributed rate variation across sites and a proportion of variable site. For RPOA, samples were taken every 1000 generations under the K80 model with gamma-distributed rate variation across sites and a proportion of variable site. For all analyses, the first 25% samples from each run were discarded as burn-in to ensure the stationary of the chains. Bayesian posterior probability (PP) values were obtained from a majority rule consensus tree generated from the remaining sampled trees.

Results:

The amplified products from each diploid species showed a single band for each of the three genes (RPS16, RPOA and RPB2) with sizes of approximately 900, 1300 and 1000bp, respectively. Figures 1, 2 and 3 showed the amplified products for the three

genes (RPS16, RPOA and RPB2) respectively. Sequence comparison of cloned PCR fragments identified two distinct copies of allele for each of the RPB2 genes from all *E. alaskanus* accessions analyzed.

Figure 1: Example of agarose gel electrophoresis after staining with ethidium bromide and visualization under UV light for *E. alaskanus* subspecies amplified with RPS16 primer. Lanes 1- 12 were DNA of *E. alaskanus* accessions, lane (C) was a control with no DNA present and lane (M) was DNA ladder (Kappa 1Kb DNA Ladder, kit code; KK6302).

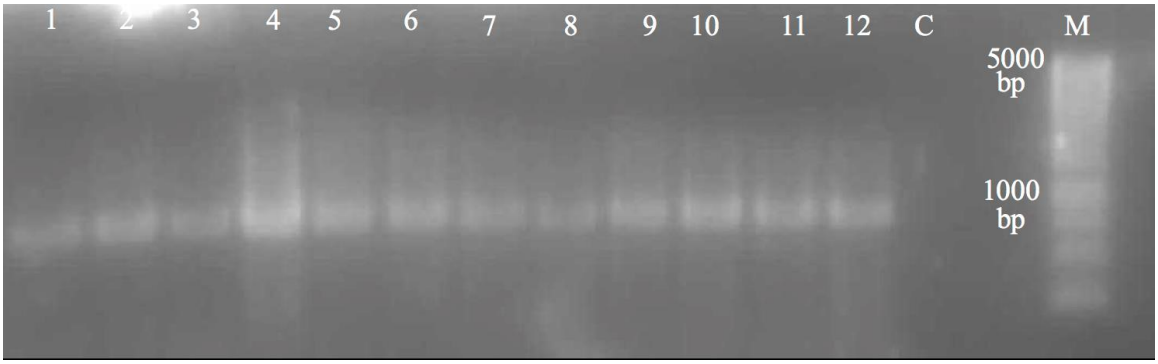


Figure 2: Example of agarose gel electrophoresis after staining with ethidium bromide and visualization under UV light for *E. alaskanus* amplified with RPOA primer. Lanes 1-8 were DNA of *E. alaskanus* accessions and lane (C) was a control with no DNA present.

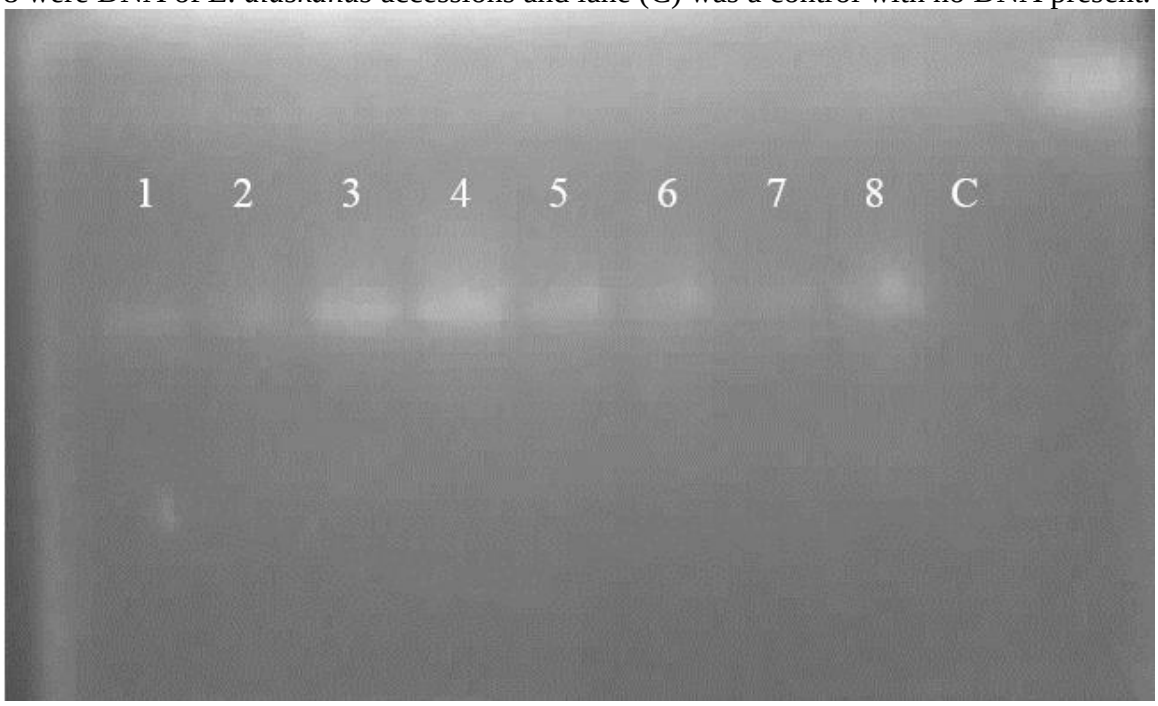
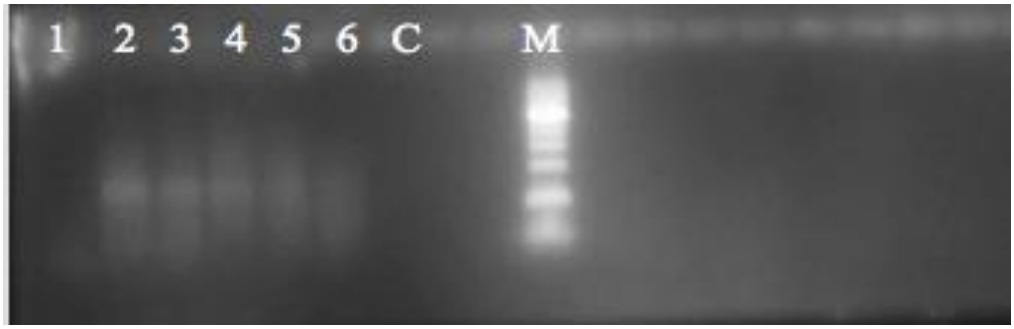


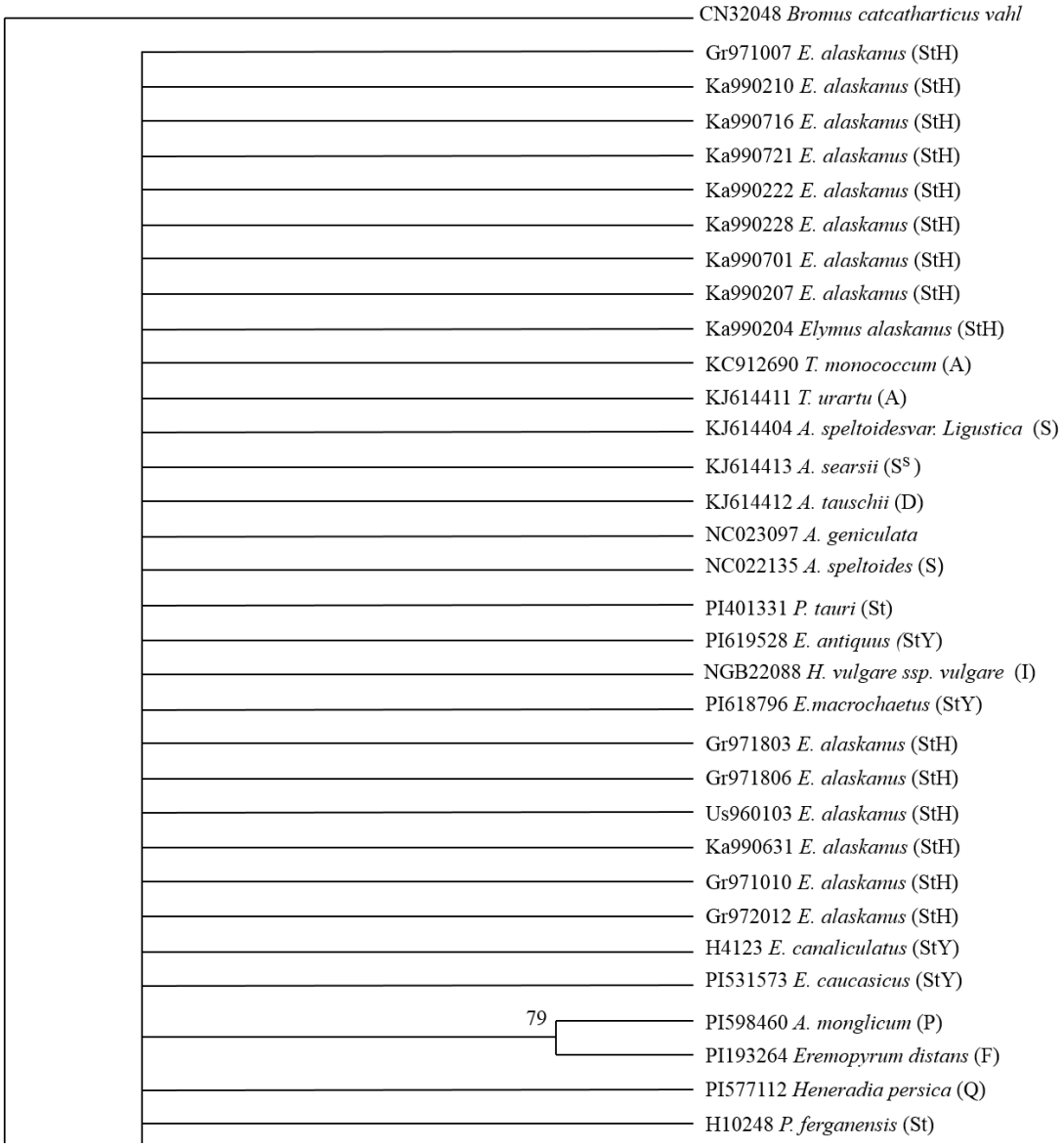
Figure 3: Example of agarose gel electrophoresis after staining with ethidium bromide and visualization under UV light for *E. alaskanus* amplified with RPB2 primer. Lanes 1-6 were DNA of *E. alaskanus* accessions, lane (C) was a control with no DNA present and lane (M) was DNA ladder (Kappa 1Kb DNA Ladder, kit code; KK6302).



1. Phylogenetic analyses of *RPS16* sequences:

Twenty-six accessions of *E. alaskanus* were analyzed together with an additional 49 *RPS16* sequences. MP analysis using *Bromus catharticus* as an outgroup was conducted (68 parsimony-informative characters, CI= 0.7456; RI = 0.7176). A 50% majority rule consensus tree with bootstrap values was shown in Figure 4. Bayesian analysis was also conducted using *Bromus catharticus* as the outgroup. (arithmetic mean= -8750.03; harmonic mean= -8838.55). A Bayesian analysis consensus tree with 95% majority rule was shown in Figure 5.

Figure 4: A 50% majority rule consensus tree was derived from the RPS16 intron sequences using the MP method for 75 accessions. Numbers above branches were bootstrap values. *Bromus catharticus* was used as an outgroup.



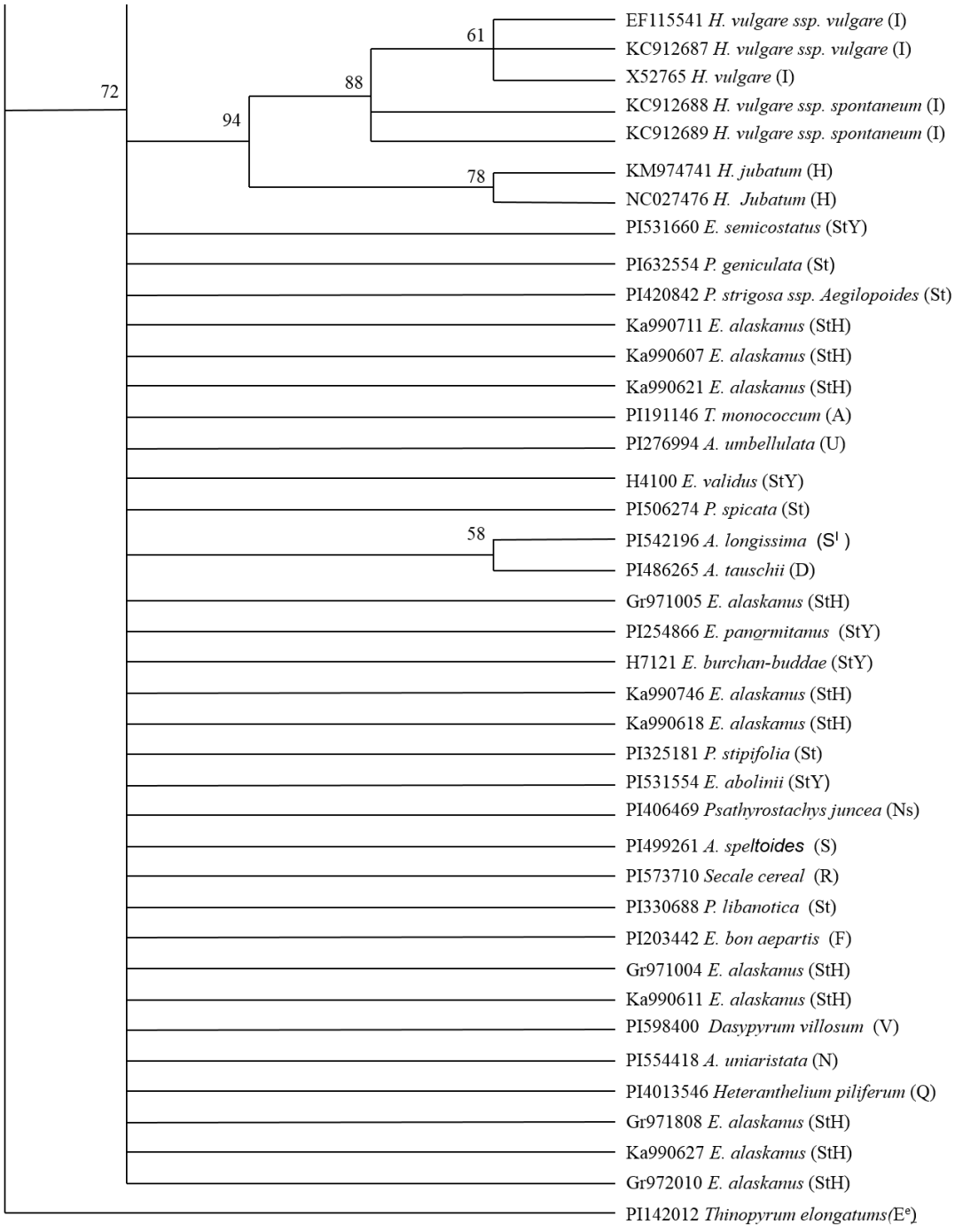
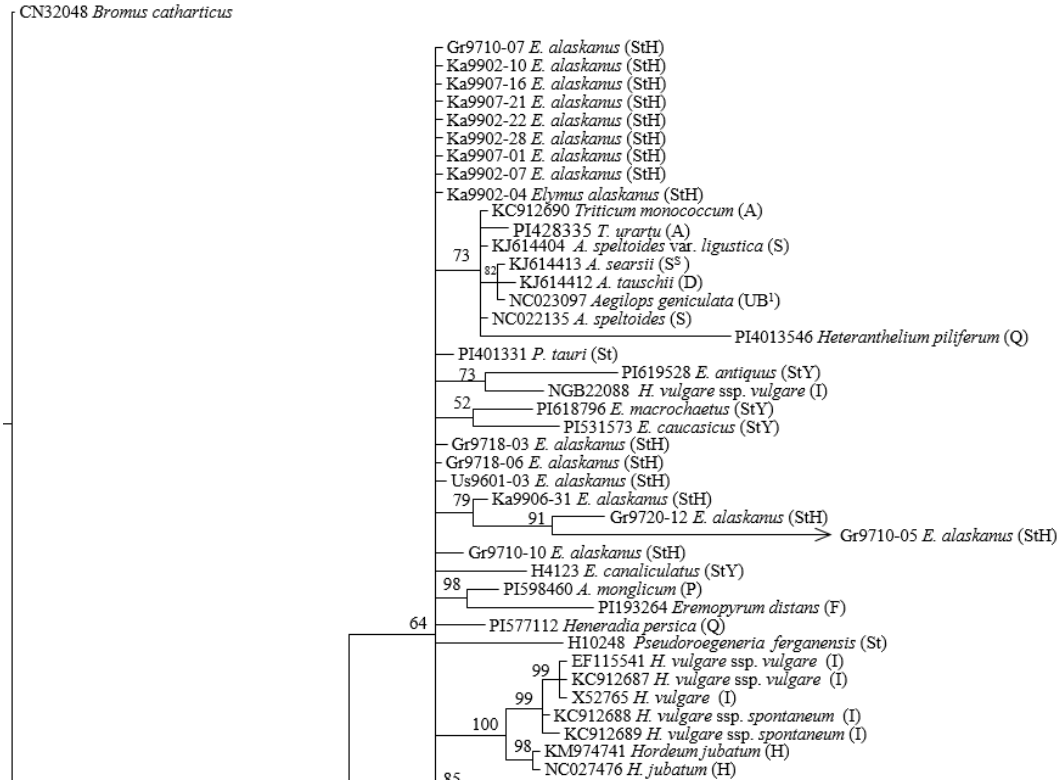
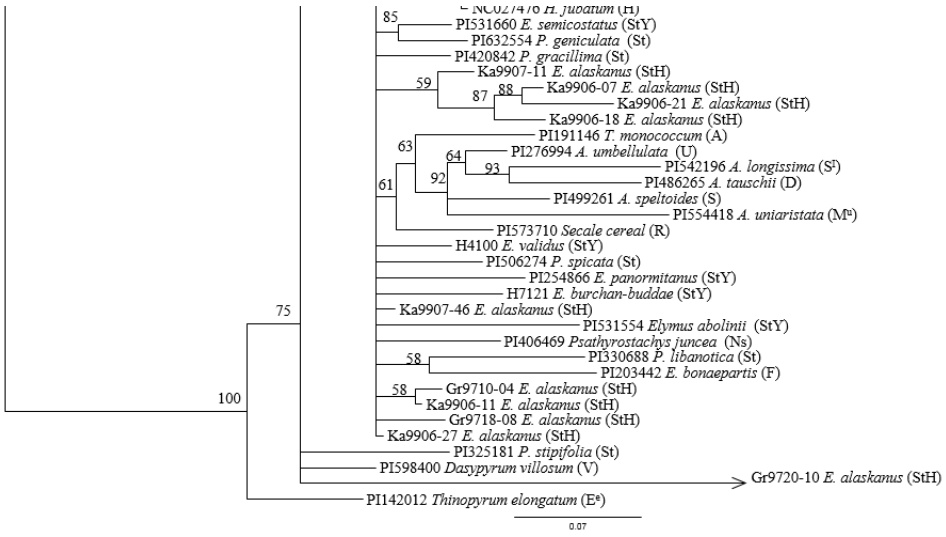

EF115541 H. vulgare ssp. vulgare (I)
KC912687 H. vulgare ssp. vulgare (I)
X52765 H. vulgare (I)
KC912688 H. vulgare ssp. spontaneum (I)
KC912689 H. vulgare ssp. spontaneum (I)
KM974741 H. jubatum (H)
NC027476 H. Jubatum (H)
PI531660 E. semicostatus (StY)
PI632554 P. geniculata (St)
PI420842 P. strigosa ssp. Aegilopoides (St)
Ka990711 E. alaskanus (StH)
Ka990607 E. alaskanus (StH)
Ka990621 E. alaskanus (StH)
PI191146 T. monococcum (A)
PI276994 A. umbellulata (U)
H4100 E. validus (StY)
PI506274 P. spicata (St)
PI542196 A. longissima (SI)
PI486265 A. tauschii (D)
Gr971005 E. alaskanus (StH)
PI254866 E. panormitanus (StY)
H7121 E. burchan-buddae (StY)
Ka990746 E. alaskanus (StH)
Ka990618 E. alaskanus (StH)
PI325181 P. stipifolia (St)
PI531554 E. abolinii (StY)
PI406469 Psathyrostachys juncea (Ns)
PI499261 A. speltoides (S)
PI573710 Secale cereal (R)
PI330688 P. libanotica (St)
PI203442 E. bon aepartis (F)
Gr971004 E. alaskanus (StH)
Ka990611 E. alaskanus (StH)
PI598400 Dasypyrum villosum (V)
PI554418 A. uniaristata (N)
PI4013546 Heteranthelium piliferum (Q)
Gr971808 E. alaskanus (StH)
Ka990627 E. alaskanus (StH)
Gr972010 E. alaskanus (StH)
PI142012 Thinopyrum elongatums(Ee)
61
88
72
94
78
58

Figure 5: Bayesian analysis generated consensus tree with 95% majority rule from the RPS16 sequence data using a GTR model. *Bromus catharticus* was used as an outgroup. Posterior probability (PP) values were shown above the branches. The arrows in the *Elymus alaskanus* (Gr971005 and Gr972010) indicated a long branch.





In both the MP and Bayesian tree, *Thinopyrum elongatums* (PI142012) was at the base of the Triticeae tree, while the rest of Triticeae species formed a well-supported clade with BS=72%, PP= 75%. Some minor difference between the trees was observed within the Triticeae clade in different analyzing methods. In the MP tree, one large group was formed consisting of the sequences from *Hordeum* species (**H** and **I** genomes) within the Triticeae clade in support of BS=94%. *Agropyron monglicum* (PI598460) showed a close relationship to *Eremopyrum distans* (PI193264) with BS=79%. Two *Aegilops* species (PI542196, PI486265) were placed in a group with BS=58%.

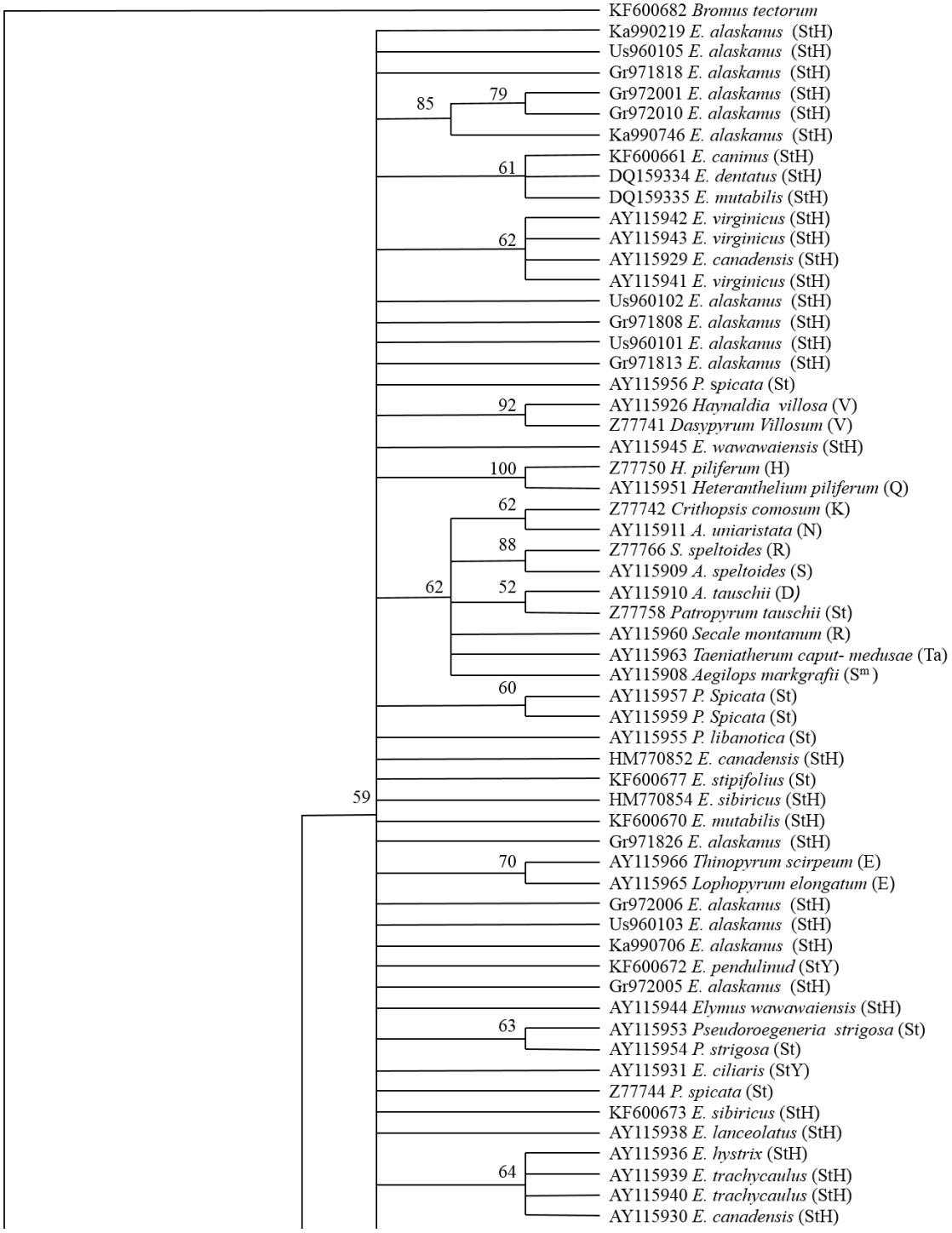
In the Bayesian tree, all the Triticeae species were clustered in one large group with PP= 64%. However, *P. stipifolia* (PI325181), *E. alaskanus* (Gr9720-10) and *Dasypyrum villosum* were separated from this large group. Within the large group, different sub clades were formed. The other *E. alaskanus* individual from the same population (Gr9720-12) was more closely related to (Gr9710-05) with PP=91%, and both were closely related to *E. alaskanus* Ka9906-31 with PP=79%. In support of PP= 73%, all of

the *Triticum* species examined formed a clade with some *Aegilops* species and *Heteranthelium piliferum*. *Triticum- monococcum* (PI191146) was grouped with the rest of *Aegilops* species in another clade (PP= 63%). *Agropyron- mongolicum* showed a close relationship to *Eremopyrum distans*, and they were clustered together with PP= 98%. While all the *Hordeum* species (**H**, **I** genome) were clustered together in one group with PP= 100%, with exception of *H. vulgare* ssp. *vulgare* (NGB22088). *H. vulgare* ssp. *vulgare* was grouped with *E. antiquus* in PP= 73%. Moreover, some accessions of *Elymus alaskanus* showed close relationship to each other more than to the others. While Ka9906-07 was grouped together with Ka9906-21 in support of PP= 88%, Ka9906-18 was sister to them with PP=87%. Ka9907-11 showed close relationship to this group. Gr9710-04 was grouped with Ka9906-11 (PP=58%).

2. Phylogenetic analyses of *RPOA* sequences:

Twenty-six *RPOA* sequences from *E. alaskanus* were analyzed together with 78 RPOA sequences from Triticeae species. An MP analysis was conducted with *Bromus tectorum* and *B. inermis* as the outgroups (114 parsimony-informative characters, CI= 0.8501; RI = 0.8191). A 50% majority rule consensus tree with bootstrap values was shown in Figure 6. Bayesian analysis was also conducted using *Bromus tectorum* and *B. inermis* as the outgroups (arithmetic mean= -4933.32; harmonic mean= -5018.18). A Bayesian analysis consensus tree with 95% majority rule was shown in Figure 7.

Figure 6: A 50% majority rule consensus tree using was derived from the RPOA sequences using the MP method for 104 accessions. Numbers above branches were bootstrap values. *Bromus tectorum* and *B. inermis* were used as outgroups.



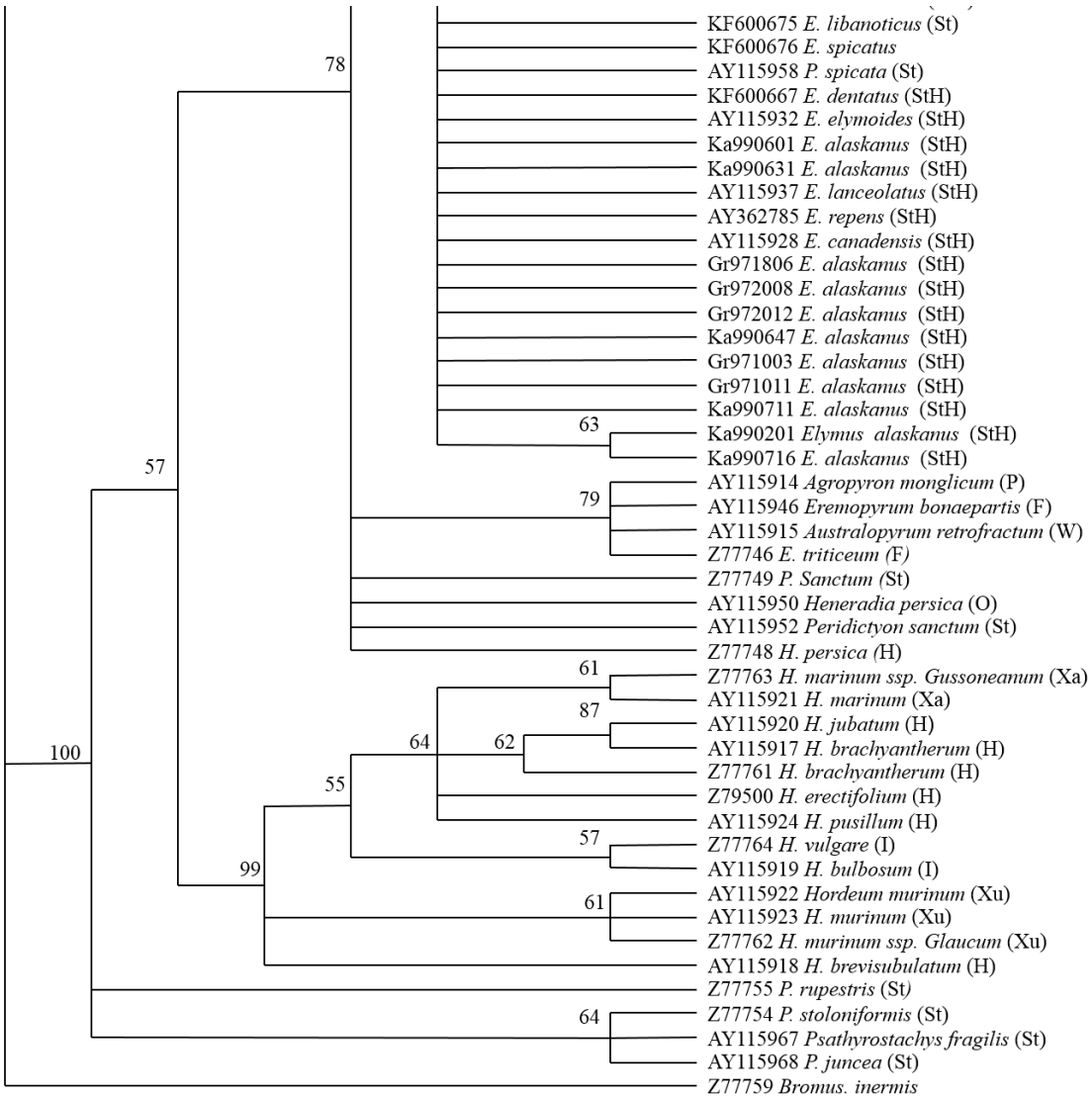
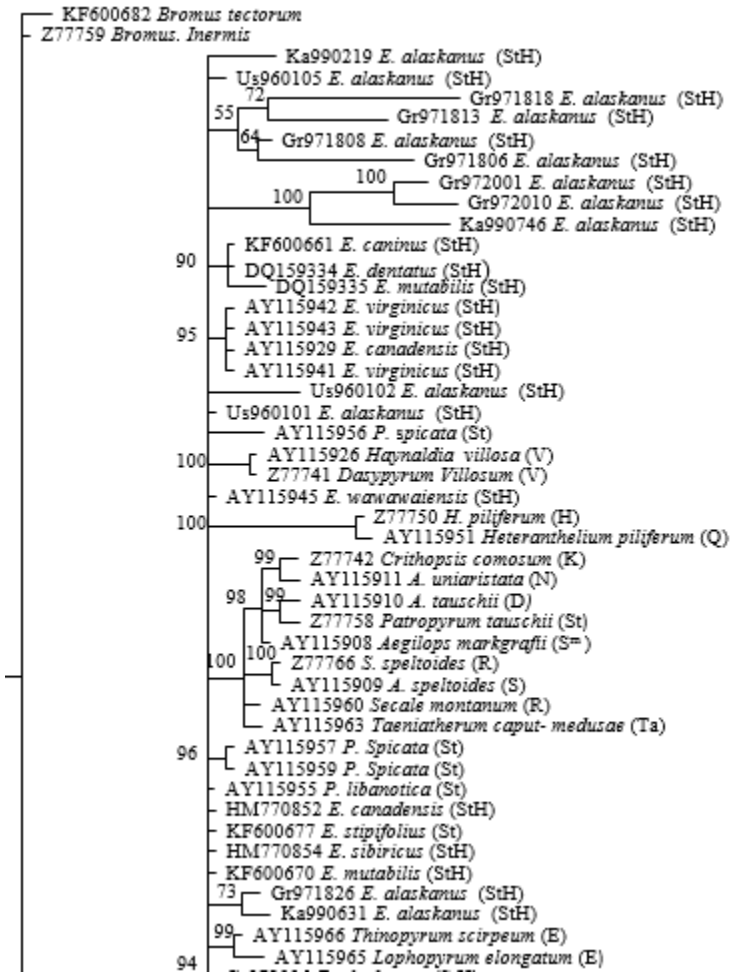
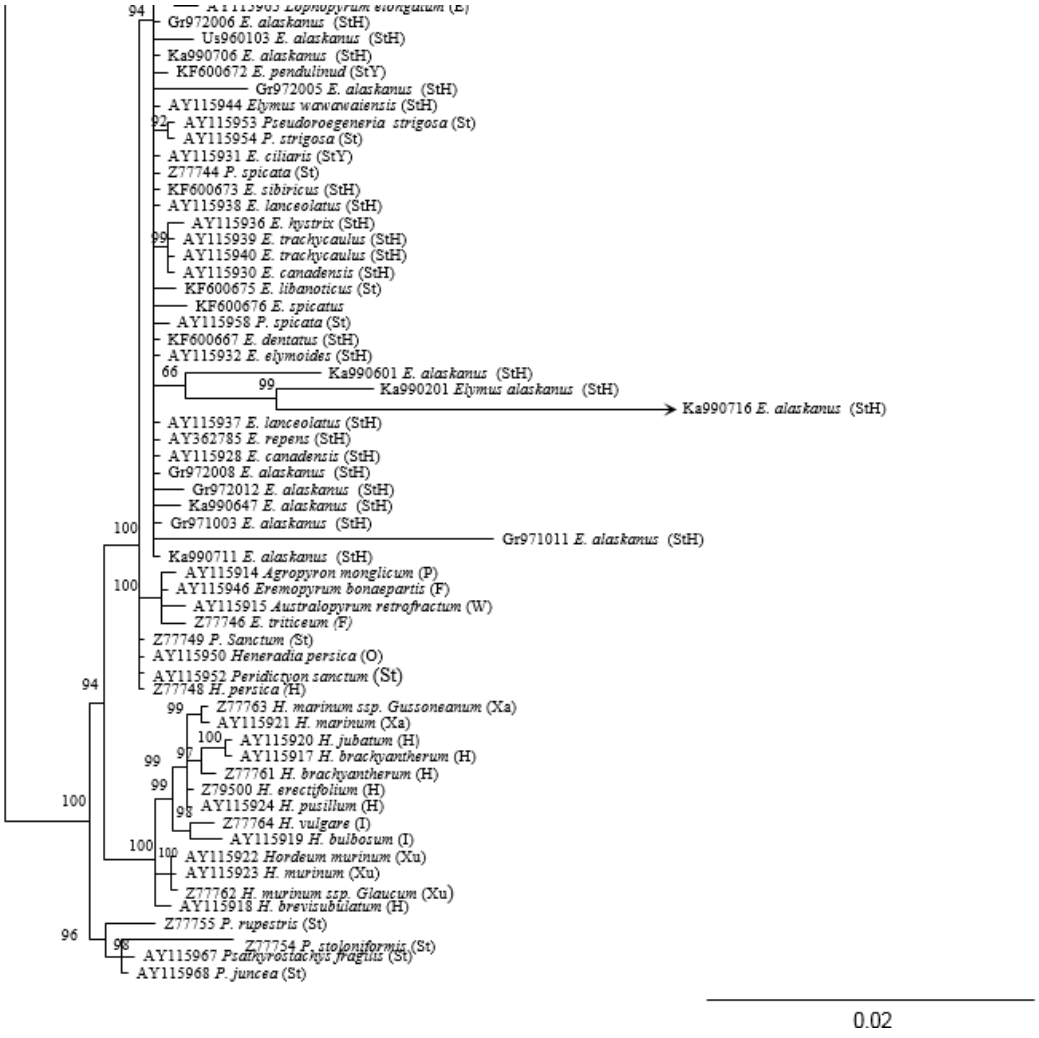

KF600675 E. libanoticus (St)
KF600676 E. spicatus
AY115958 P. spicata (St)
KF600667 E. dentatus (StH)
AY115932 E. elymoides (StH)
Ka990601 E. alaskanus (StH)
Ka990631 E. alaskanus (StH)
AY115937 E. lanceolatus (StH)
AY362785 E. repens (StH)
AY115928 E. canadensis (StH)
Gr971806 E. alaskanus (StH)
Gr972008 E. alaskanus (StH)
Gr972012 E. alaskanus (StH)
Ka990647 E. alaskanus (StH)
Gr971003 E. alaskanus (StH)
Gr971011 E. alaskanus (StH)
Ka990711 E. alaskanus (StH)
Ka990201 Elymus alaskanus (StH)
Ka990716 E. alaskanus (StH)
AY115914 Agropyron monglicum (P)
AY115946 Eremopyrum bonaepartis (F)
AY115915 Australopyrum retrofractum (W)
Z77746 E. triticeum (F)
Z77749 P. Sanctum (St)
AY115950 Heneradia persica (O)
AY115952 Peridictyon sanctum (St)
Z77748 H. persica (H)
Z77763 H. marinum ssp. Gussoneanum (Xa)
AY115921 H. marinum (Xa)
AY115920 H. jubatum (H)
AY115917 H. brachyantherum (H)
Z77761 H. brachyantherum (H)
Z79500 H. erectifolium (H)
AY115924 H. pusillum (H)
Z77764 H. vulgare (I)
AY115919 H. bulbosum (I)
AY115922 Hordeum murinum (Xu)
AY115923 H. murinum (Xu)
Z77762 H. murinum ssp. Glaucum (Xu)
AY115918 H. brevisubulatum (H)
Z77755 P. rupestris (St)
Z77754 P. stoloniformis (St)
AY115967 Psathyrostachys fragilis (St)
AY115968 P. juncea (St)
Z77759 Bromus. inermis
78
63
57
79
61
87
64
62
100
55
57
99
61
64

Figure 7: Bayesian analysis generated consensus tree with 95% majority rule from the RPOA sequences data using a K80 model. *Bromus tectorum* and *B. inermis* were used as outgroups. Posterior probability (PP) values were shown above the branches. The arrow that points toward *Elymus alaskanus* (Ka990716) indicated a long branch.





The phylogenetic tree in both analyses showed two distinct clades. The *Psathyrostachys* species (**Ns** genome) was grouped together in one clade (BS = 64%, PP = 98%). However, *Psathyrostachys rupestris* (Z77755) was separated from this clade but close to it. The rest of Triticeae species were grouped within one clade (BS = 57%, PP=94%), but were divided into two main sub-clades. The sequences from *Hordeum* species (**Xa, H**, **I** and **Xu** genomes) were clustered into a sub-clade (BS = 99%, PP =100%), while other species analyzed in this study were clustered together with *E. alaskanus* in one group. However, some minor differences between the Bayesian tree and

the MP tree were observed.

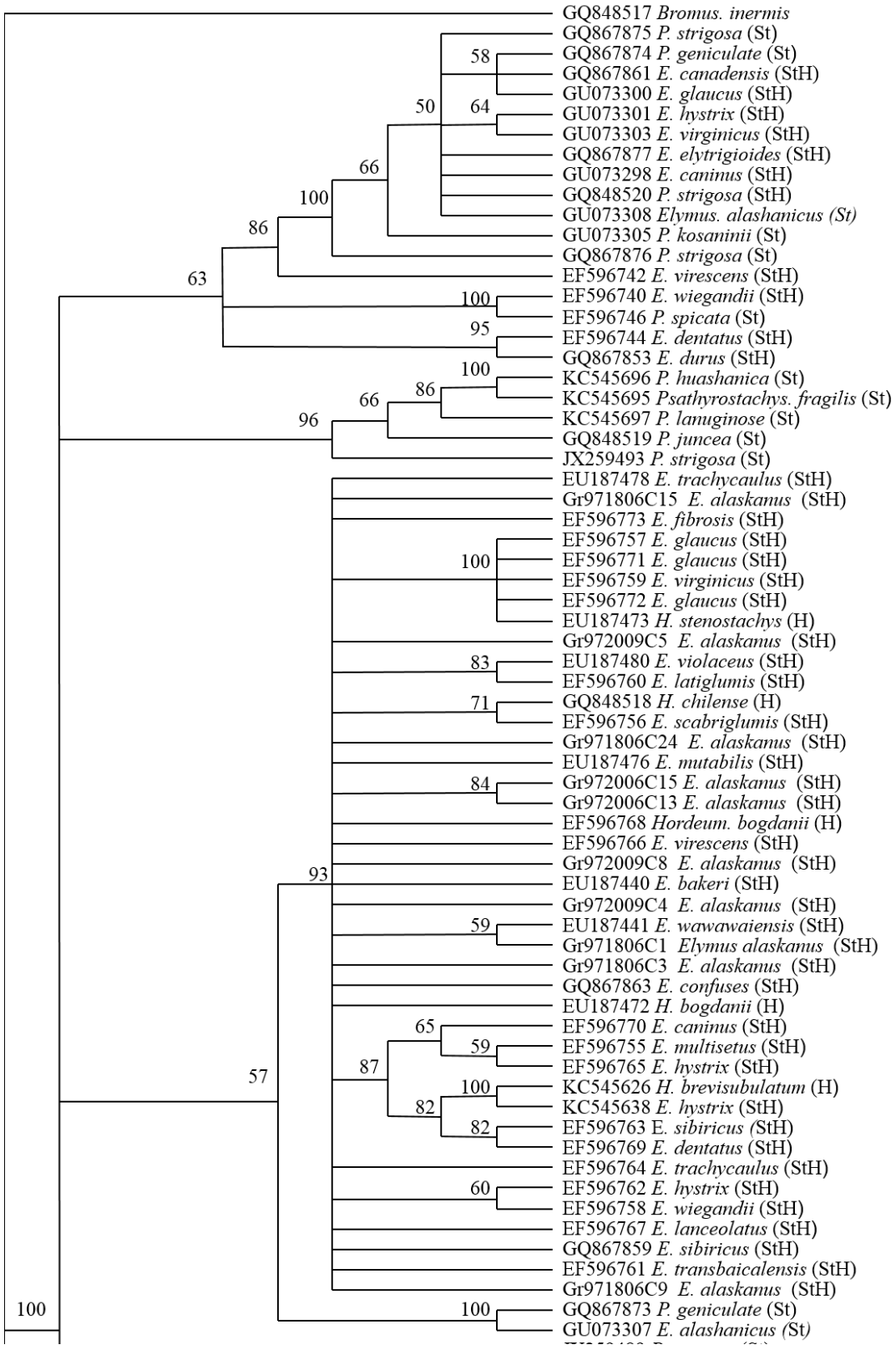
In the MP tree, all *E. alaskanus* were grouped in a clade with BS=59%. Two individuals from *E. alaskanus* (Gr9720-01 and Gr9720-10) were grouped together with BS=79%. Ka9907-46 was sister to this group with BS=85%. Moreover, Ka9902-01 and Ka9907-16 showed closer relationship to each other than to others with BS=63%.

In the Bayesian tree, all *E. alaskanus* were grouped in a clade with PP=94%. Where all the individuals from the (Gr9718) population were clustered in one clade with PP=55%, Gr9718-26 was grouped with Ka9906-31 in support of PP=73%. Two individuals (Gr9720-01 and Gr9720-10) were grouped in a clade with PP=100%, and showed a closer relationship to Ka9907-46. Moreover, Ka9902-01 and Ka9907-16 were clustered in one group with PP=99%, and showed close relationship to Ka9906-01 with PP=66%.

3. Phylogenetic analyses of *RPB2* sequences:

Five *RPB2* sequences from *E. alaskanus* were analyzed together with an additional 106 RPB2 sequences from *Elymus* and other diploid species in the tribe Triticeae. A MP analysis was conducted with *Bromus inermis* and *B. cathartharticus* as outgroups (853 parsimony-informative characters, CI= 0.5385; RI = 0.7640). A 50% majority rule consensus tree with bootstrap values was shown in Figure 8. Bayesian analysis was also conducted using *Bromus inermis* and *B. cathartharticus* as outgroups (arithmetic mean= -20648.12; harmonic mean= -20741.22). A Bayesian analysis consensus tree with 95% majority rule was shown in Figure 9.

Figure 8: A 50% majority rule consensus tree was derived from the RPB2 sequences using the MP method for 118 accessions. Numbers above branches were bootstrap values. *Bromus inermis* and *B. catharticus* were used as outgroups.


GQ848517 Bromus. inermis
GQ867875 P. strigosa (St)
GQ867874 P. geniculate (St)
GQ867861 E. canadensis (StH)
GU073300 E. glaucus (StH)
GU073301 E. hystrix (StH)
GU073303 E. virginicus (StH)
GQ867877 E. elytrigioides (StH)
GU073298 E. caninus (StH)
GQ848520 P. strigosa (StH)
GU073308 Elymus. alashanicus (St)
GU073305 P. kosaninii (St)
GQ867876 P. strigosa (St)
EF596742 E. virescens (StH)
EF596740 E. wiegandii (StH)
EF596746 P. spicata (St)
EF596744 E. dentatus (StH)
GQ867853 E. durus (StH)
KC545696 P. huashanica (St)
KC545695 Psathyrostachys. fragilis (St)
KC545697 P. lanuginose (St)
GQ848519 P. juncea (St)
JX259493 P. strigosa (St)
EU187478 E. trachycaulus (StH)
Gr971806C15 E. alaskanus (StH)
EF596773 E. fibrosis (StH)
EF596757 E. glaucus (StH)
EF596771 E. glaucus (StH)
EF596759 E. virginicus (StH)
EF596772 E. glaucus (StH)
EU187473 H. stenostachys (H)
Gr972009C5 E. alaskanus (StH)
EU187480 E. violaceus (StH)
EF596760 E. latiglumis (StH)
GQ848518 H. chilense (H)
EF596756 E. scabriglumis (StH)
Gr971806C24 E. alaskanus (StH)
EU187476 E. mutabilis (StH)
Gr972006C15 E. alaskanus (StH)
Gr972006C13 E. alaskanus (StH)
EF596768 Hordeum. bogdanii (H)
EF596766 E. virescens (StH)
Gr972009C8 E. alaskanus (StH)
EU187440 E. bakeri (StH)
Gr972009C4 E. alaskanus (StH)
EU187441 E. wawawaiensis (StH)
Gr971806C1 Elymus alaskanus (StH)
Gr971806C3 E. alaskanus (StH)
GQ867863 E. confuses (StH)
EU187472 H. bogdanii (H)
EF596770 E. caninus (StH)
EF596755 E. multisetus (StH)
EF596765 E. hystrix (StH)
KC545626 H. brevisubulatum (H)
KC545638 E. hystrix (StH)
EF596763 E. sibiricus (StH)
EF596769 E. dentatus (StH)
EF596764 E. trachycaulus (StH)
EF596762 E. hystrix (StH)
EF596758 E. wiegandii (StH)
EF596767 E. lanceolatus (StH)
GQ867859 E. sibiricus (StH)
EF596761 E. transbaicalensis (StH)
Gr971806C9 E. alaskanus (StH)
GQ867873 P. geniculate (St)
GU073307 E. alashanicus (St)
50
64
58
66
100
86
63
100
95
100
86
66
96
100
83
71
84
93
59
65
59
87
57
100
82
82
60
100
100

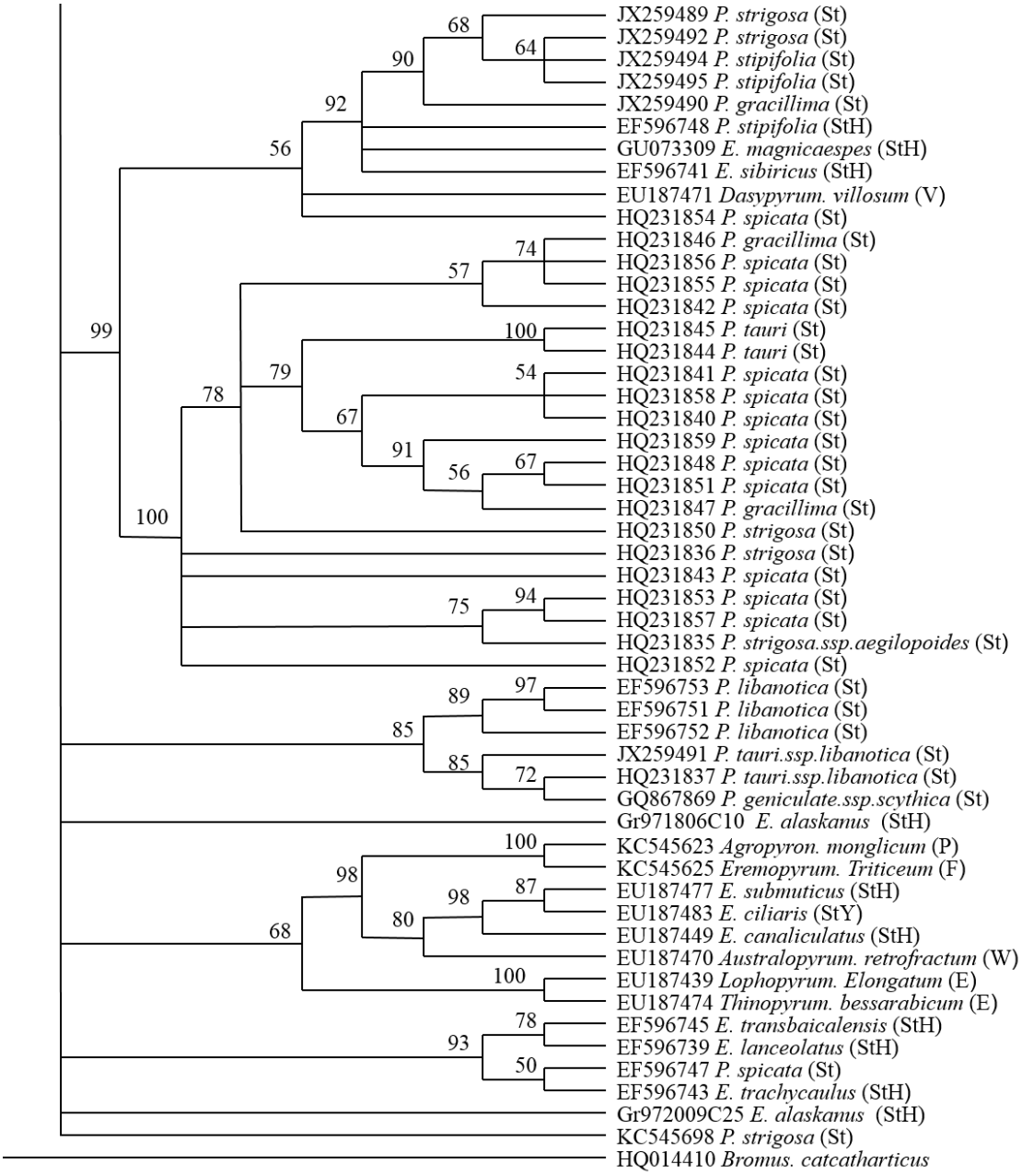
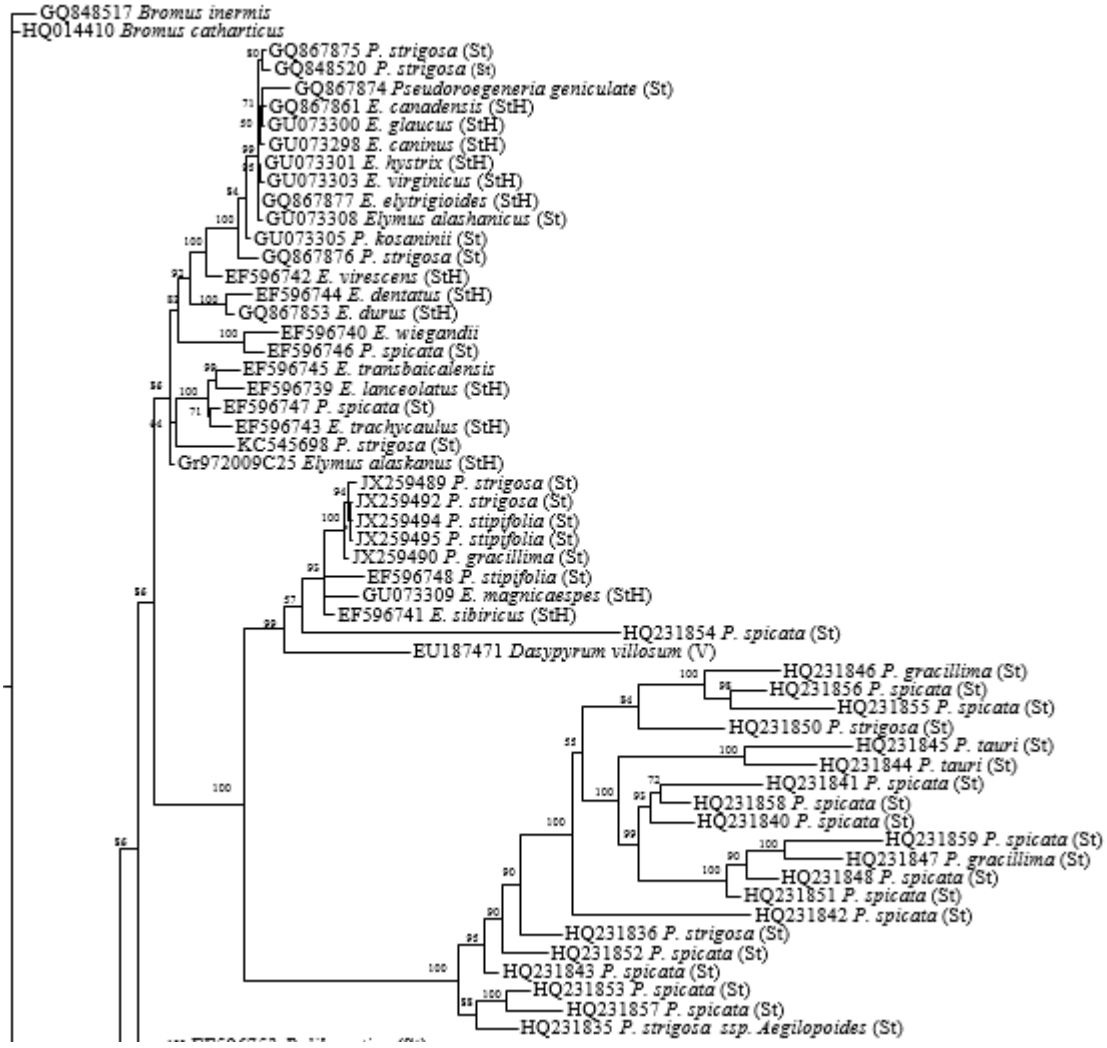
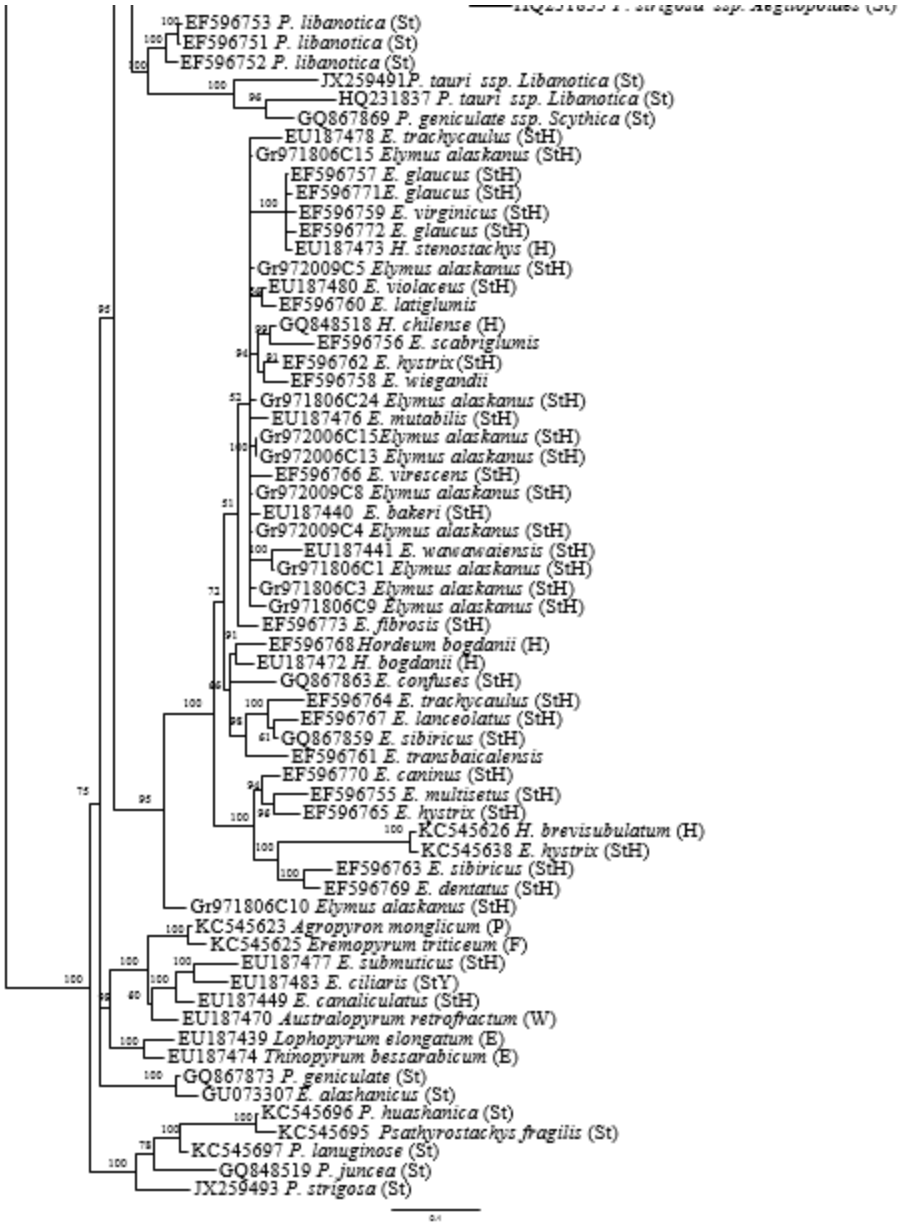

JX259489 P. strigosa (St)
JX259492 P. strigosa (St)
JX259494 P. stipifolia (St)
JX259495 P. stipifolia (St)
JX259490 P. gracillima (St)
EF596748 P. stipifolia (StH)
GU073309 E. magnicaespes (StH)
EF596741 E. sibiricus (StH)
EU187471 Dasypyrum. villosum (V)
HQ231854 P. spicata (St)
HQ231846 P. gracillima (St)
HQ231856 P. spicata (St)
HQ231855 P. spicata (St)
HQ231842 P. spicata (St)
HQ231845 P. tauri (St)
HQ231844 P. tauri (St)
HQ231841 P. spicata (St)
HQ231858 P. spicata (St)
HQ231840 P. spicata (St)
HQ231859 P. spicata (St)
HQ231848 P. spicata (St)
HQ231851 P. spicata (St)
HQ231847 P. gracillima (St)
HQ231850 P. strigosa (St)
HQ231836 P. strigosa (St)
HQ231843 P. spicata (St)
HQ231853 P. spicata (St)
HQ231857 P. spicata (St)
HQ231835 P. strigosa.ssp.aegilopoides (St)
HQ231852 P. spicata (St)
EF596753 P. libanotica (St)
EF596751 P. libanotica (St)
EF596752 P. libanotica (St)
JX259491 P. tauri.ssp.libanotica (St)
HQ231837 P. tauri.ssp.libanotica (St)
GQ867869 P. geniculate.ssp.scythica (St)
Gr971806C10 E. alaskanus (StH)
KC545623 Agropyron. monglicum (P)
KC545625 Eremopyrum. Triticeum (F)
EU187477 E. submuticus (StH)
EU187483 E. ciliaris (StY)
EU187449 E. canaliculatus (StH)
EU187470 Australopyrum. retrofractum (W)
EU187439 Lophopyrum. Elongatum (E)
EU187474 Thinopyrum. bessarabicum (E)
EF596745 E. transbaicalensis (StH)
EF596739 E. lanceolatus (StH)
EF596747 P. spicata (St)
EF596743 E. trachycaulus (StH)
Gr972009C25 E. alaskanus (StH)
KC545698 P. strigosa (St)
HQ014410 Bromus. catcatharticus

Figure 9: Bayesian analysis generated consensus tree with 95% majority rule from the RPB2 sequence data using a GTR model. *Bromus inermis* and *B. catharticus* were used as outgroups. Posterior probability (PP) values were shown above the branches.





The topology of MP tree obtained was similar to that of the Bayesian tree. In both trees, all the *Psathyrostachys* species were clustered within one clade (BS= 96%, PP=100%) with one *Pseudoroegeneria strigosa* from China (JX259493). One large clade included the sequences from **StH** (*Elymus*) and **H** (*Hordeum*) with (BS= 93%, PP=95%). Another large group contained the *Pseudoroegneria* (**St**) with the rest of *Elymus* (**StH**)

(BS= 99%, PP= 56%). For *E. alaskanus*, Gr9718-06, Gr9720-09 and Gr9720-06 were clustered in the **H** (*Hordeum + Elymus*) clade, indicating that they contained an **H** copy. However, in the MP tree, Gr9718-06 and Gr9720-09 were separated from each other. In the Bayesian tree, Gr9720-09 was grouped within the **St** (*Pseudoroegneria + Elymus*) clade, indicating that it is the (**St)** copy while Gr9718-06 was close to the (*Hordeum + Elymus*) clade indicating that it is the **H** copy.

Discussion:

The origin of *E. alaskanus:*

It has been suggested that all *Elymus* species contain at least one set of *Pseudoroegneria* genomes combined with the genomes from one or more of several other Triticeae genera (Mason-Gamer et al., 2005). Moreover, it has been proposed that *Pseudoroegneria* (**St**) and *Hordeum* (**H**) species were genome donors of the allopolyploid *E. alaskanus* (Sun and Salomon, 2003). The chloroplast DNA data (both RPS16 and RPOA) in the present study indicated clearly that the potential maternal donor of *E. alaskanus* is not the **H** genome species. The results suggested that it may be the **St** genome species, which is consistent with the previous suggestion that *Pseudoroegneria* (**St**) is the maternal parent of some other *Elymus* species (Redinbaugh et al., 2000; Mason-Gamer et al., 2002; McMillan and Sun, 2004; Xu and Ban, 2004; Liu et al., 2006, Ni et al., 2011; Yan et al., 2014). Thirty-one diploid, allotetraploid, allohexaploid, and allo-octoploid Triticeae species were studied using the chloroplast gene sequence of the ndhF. The results showed that the ndhF DNA sequences were highly similar among Triticeae that contain the **St** nuclear genome. Moreover, there were five sets of accessions that had identical sequences among the **St** -genome-containing grasses. One of those sets

contained 13 *Elymus* and 3 *Pseudoroegneria* species (Redinbaugh et al., 2000). In an attempt to identify the maternal genome donor of *Elymus*, chloroplast DNA data from *Elymus* and from most of the genera of the Triticeae were analyzed. The results indicated that *Pseudoroegneria* is the maternal genome donor to all but one of the *Elymus* individuals (Mason-Gamer et al., 2002). The genetic relationships of 38 individuals from 13 *Elymus* tetraploid species, two *Pseudoroegneria* species and one *Hordeum* species strongly suggested that *Pseudoroegneria* species is the maternal donor to tetraploid *Elymus* species (McMillan and Sun, 2004). Xu and Ban (2004) studied the phylogenetic and evolutionary relationships between *Elymus humidus* and other *Elymus* species based on sequencing of non-coding regions of cpDNA and AFLP (amplified fragment length polymorphism) variation, and the results supported that *Pseudoroegneria* is the maternal genome donor to *Elymus*. In Xu and Ban study, the phylogenic tree resulting from the cpDNA sequence data revealed that all the species containing the **St** nuclear genome (**St**, **StH**, **StY**, and **StHY**) formed a well-supported clade that was remote from the *Hordeum* species (**H**). By analyzing 45 *Elymus* accessions containing various genomes with five *Pseudoroegneria* (**St**) and other accessions, the results suggested that the **St** genomes in polyploid *Elymus* are donated by *Pseudoroegneria*. Moreover, the trnL-F sequences revealed that *Pseudoroegneria* was closely related to all *Elymus* species examined (Liu et al., 2006). In addition, the phylogenetic analysis for 18 *Elymus* polyploid species, *Hordelymus europaeus* and other diploid ancestors showed that *Elymus* and *Pseudoroegneria* species formed a highly supported monophyletic group with 100 % bootstrap values. This result suggested that *Pseudoroegneria* is the maternal genome donor to polyploid *Elymus* species studied (Ni et al., 2011). Thirteen accessions of

tetraploid *Elymus pendulinus* were analyzed using two regions of chloroplast genome (Rps16 and trnD-trnT). The chloroplast DNA data identified two potential maternal genome donors (*Pseudoroegneria* and an unknown species outside *Hordeeae*) to this species (Yan et al., 2014).

Both *P. spicata* and *P. stipfolia* were suggested as potential **(St)** genome donors to *Elymus* by Sun (2014). However, the chloroplast DNA data results of our study did not give any clear support to this suggestion. Even though the *E. alaskanus* species analyzed were grouped together with all the *P. spicata* species studied in one large clade in the *RPOA* gene tree, but there was no *P. stipfolia* species analyzed. Therefore, we could not make a conclusion that the *Pseudoroegneria* species is the potential **(St)** genome donors to *Elymus.*

In general, the topology of the *RPOA* gene tree in our study is in agreement with previous studies (Petersen and Seberg, 1996) in which the *Psathyrostachys* species (**Ns** genome) was joined together in one group. Moreover, the sequences from *Hordeum* species (**H**, **Xa**, **I** and **Xu** genomes) were clustered into a clade. However, two *Hordeum* species were separated from the other **H**-genome species and grouped with other *Triticeae* species unexpectedly (*H. persica* Z77748 and *H. piliferum* Z77750).

No conclusion can be made as to whether *E. alaskanus* has single or multiple origins because of the lack of sequence data from *E. alaskanus* species analyzed in the RPB2 trees. However, a study done on *E. caninus* clearly indicated multiple origins of the **H** genome in by the RPB2 phylogenetic tree (Yan, 2011). One of them is Eurasian *H. bogdanii* and the other is American **H** genome *Hordeum* species. Moreover, the PepC phylogenetic tree supported Eurasian **H** genome species *H. bogdanii* as the **H** genome

donor, which suggested that *H. bogdanii* is more likely one of the major donors of the **H** copy in *E. caninus*. In present study, the **H** copy of the *E. alaskanus* individuals (Gr 9718-06, Gr 9720-09 and Gr 9720-06) showed a close relationship to *Hordeum* species in which one of them is *H. bogdanii* (EF596768) but still no conclusion can be made because of the lack of sequence data from the *E. alaskanus* species analyzed in the RPB2 trees.

There was no information provided about the genomes of the six *Elymus* species from the additional 106 RPB2 sequences in the tribe Triticeae analyzed here in the National center for biotechnology information (NCBI). However, these *Elymus* species were grouped, in the RPB2 phylogenetic tree in our study, in a way that could suggest their genomes. *E. latiglumis* (EF596760) showed a close relationship to **StH** genome where it clustered with *E. violaceus* with BS=83%, PP= 56%. Moreover, *E. scabriglumis* (EF596756) showed close relationship to **H** genome grouping with *H. chilense* with BS= 71 % and PP= 99%. *E. transbaicalensis (*Both EF596745 and EF596761) were closely related to **StH** genome clustering with *E. lanceolatus* and *E. sibiricus* with BS= 78%, PP= 99% and BS= 78%, PP= 61%, respectively. *E. wiegandii* EF596758 showed close relationship to **StH** genome (*E. hystrix)* with BS=60 %, PP= 91%, and EF596740 was close to (**St)** genome (*P. spicata)* with BS=60%, PP= 100%. Focusing on studying these *Elymus* species in the future may lead to a conclusion about their genomes constitution.

This study can provide information for efficiently conservation and utilization of *E. alaskanus* species. Our results indicated that *Pseudoroegneria* is the maternal genome donor to *E. alaskanus* species. *Pseudoroegneria* had been known for its cold tolerant, and extreme tolerant of fire. Knowing that it is the genome donor to *E. alaskanus* would help

scientist and even farmers to benefit from this when having *E. alaskanus* for grazing for example. *Elymus* species, on the other hand, had been known for possessing useful genes for disease and pest resistance, stress tolerance, high protein content and diverse adaptation. Therefore, we can use it as valuable gene pool for improvement of crops, such as wheat, barley and rye. One of these grasses will be *Pseudoroegneria*, the maternal donor of *E. alaskanus* identifying in our study. Moreover, an important challenge nowadays in world agriculture is due to climate change. Therefore, we need to ensure efficient conservation and utilization of genetic resources of wild cereals. A successful conservation program will prevent *E. alaskanus* species from genetic erosion or extinction. Moreover, understanding a specific threat that may affects both *E. alaskanus* and its maternal donor would give an opportunity to overcome this threat, and to use one of them to give better resistant to the other.

Conclusion:

Thirty-four individuals from *E. alaskanus* were analyzed using three genes, the gene encoding ribosomal protein S16 (RPS16), RNA polymerase alpha subunit (RPOA) and the single copy nuclear gene RNA polymerase II (RPB2) in order to identify whether *E. alaskanus* has single or multiple origins; and to identify the potential maternal donor species in *E. alaskanus*. The cpDNA sequence data indicated that the potential maternal donor of *E. alaskanus* is the **St** genome species. However, identifying whether *E. alaskanus* has single or multiple origins could not be shown by the results from our study due to the lack of RPB2 sequence data. Future studies are needed to evaluate other nuclear and chloroplast genes to support phylogenies present in this study. More chloroplast and nuclear genes will allow us to identify whether *E. alaskanus* has single or

multiple origins. For example, the non-coding regions in the chloroplast: the trnL-trnF (Bayer and Starr, 1998; Cros et al., 1998; Gielly and Taberlet, 1994; Mes and Hart, 1994; van Ham et al., 1994), the trnT-trnL (Böhle et al., 1994; Bohle et al., 1996; Small et al., 1998), the trnL intron (Bayer and Starr, 1998; Bayer et al., 2000; Kajita et al., 1998; Sang et al., 1997), the two nuclear genes PEPC (Yan and Sun, 2012), and PGK1 (Fan et al. 2012) can be used to characterize the molecular phylogeny and the origin of *E. alaskanus* complex in further study.

Reference List:

Aluru, S. (Ed.), 2006. Handbook of computational molecular biology. Boca Raton, FL: Chapman & Hall/CRC Taylor & Francis Group.17-19.

Anisimova, M., Gascuel, O., 2006. Approximate likelihood-ratio test for branches: A fast, accurate, and powerful alternative. Syst. Biol. 55, 539-552.

Antunes, A., Pontius, J., Ramos, M.J., Brien, S., Johnson, W., 2007. Mitochondrial introgressions into the nuclear genome of the domestic cat. J. Hered. 98, 414-420.

Barkworth, M., Dewey, D.R., 1985. Genomically based genera in the perennial Triticeae of North America: Identification and membership. Amer. J. Bot. 72, 767-776.

Bayer, R.J., Puttock, C.F., Kelchner, S.A., 2000. Phylogeny of South African Gnaphalieae (Asteraceae) based on two noncoding chloroplast sequences. Am. J. Bot. 87, 259–272.

Bayer, R.J., Starr, J.R., 1998. Tribal Phylogeny of the Asteraceae Based on Two Non-Coding Chloroplast Sequences, the trnL intron trnL/trnF intergenic spacer. Ann. Missouri Bot. Gard. 85, 242–256.

Böhle, U.-R., Hilger, H., Cerff, R., Martin, W.F., 1994. Non-coding chloroplast DNA for plant molecular systematics at the infrageneric level, in: Schierwater, B., Streit, B., Wagner, G.P., DeSalle, R. (Eds.), Molecular Ecology and Evolution: Approaches and Applications. Birkh{ä}user Basel, Basel, pp. 391–403.

Bohle, U.R., Hilger, H.H., Martin, W.F., 1996. Island colonization and evolution of the insular woody habit in Echium L. (Boraginaceae). Proc. Natl. Acad. Sci. 93, 11740–11745.

Brinkman, F. S. L., Leipe, D. D., 2001. Phylogenetic Analysis. 323-328.

Brokaw, J.M., Hufford, L., 2010. Origins and introgression of polyploid species in Mentzelia section *Trachyphytum* (Loasaceae). Amer. J. Bot. 97, 1457-1473.

Brown, T. A., Clayton, D. A., 2002. Release of replication termination controls mitochondrial DNA copy number after depletion with 2′, 3′-dideoxycytidine. Genomes 2. Oxford. 30(9), 2004-2010.

Buckler, E.S., Ippolito, A., Holtsford, T.P., 1997. The evolution of ribosomal DNA: Divergent paralogues and phylogenetic implications. Genet. 145, 821-832.

Chiang, T.Y., Chiang, Y.C., Chen, Y.J., Chou, C. H., Havanond, S., Hong, T. N., Huang, S., 2001. Phylogeography of *Kandelia candel* in east Asiatic mangroves based on nucleotide variation of chloroplast and mitochondrial DNAs. Mol. Ecol. 10, 2697-2710.

Collard, B.C.Y., Mackill, D.J., 2009. Start codon targeted (SCoT) polymorphism: A simple, novel DNA marker technique for generating gene-targeted markers in plants. Plant Mol. Biol. Rep. 27, 86–93.doi:10.1007/s11105-008-0060-5.

Cros, J., Combes, M.C., Trouslot, P., Anthony, F., Hamon, S., Charrier, A., Lashermes, P., 1998. Phylogenetic analysis of chloroplast DNA variation in Coffea L. Mol. Phylogenet. Evol. 9, 109–17. doi:10.1006/mpev.1997.0453

Cui, L.Y., Wall, P.K., Leebens-Mack, J.H., Lindsay, B. G., Soltis, D. E., Doyle, J. J., Soltis, P. S., Carlson, J. E., Arumuganathan, K., Barakat, A., Albert, V. A., Ma, H., dePamphilis, C. W., 2006. Widespread genome duplications throughout the history of flowering plants. Genome Res. 16, 738-749.

Curtis, S., Clegg, M., 1984. Molecular evolution of chloroplast DNA sequences. Mol. Biol. Evol. 1, 291–301.

De Bodt, S., Maere, S., Van de Peer, Y., 2005. Genome duplication and the origin of angiosperms. Trends Ecol. Evol. 20, 591-597.

Dewey, D.R., 1971. Synthetic hybrids of *Hordeum bogdanii* with *Elymus canadensis* and *Sitanion hystrix*. Amer. J. Bot. 58, 902-908.

Dewey, D.R., 1974. Cytogenetics of *Elymus sibiricus* and its hybrids with *Agropyron tauri*, *Elymu canadensis*, and *Agropyron caninum.* Bot. Gaz. 135, 80-87.

Dewey, D.R., 1982. Genomic and phylogenetic relationships among North American perennial Triticeae, in: J.R. Estes, Grasses and grasslands. Oklahoma University Press, Norman, Okla. 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: A guide to intergeneric hybridization with the perennial Triticeae. In: Gustafson JP, ed. Gene manipulation in Plant Improvement. New York, NY, USA: Plenum Press. 209-280.

Dewey, D.R., Asay K.H., 1982. Cytogenetic and taxonomic relationships among three diploid crested wheatgrasses. Crop Sci. 22, 645-650.

D.H. Barrett, R. and Schluter, D., 2007. Adaptation from standing genetic variation. Trends Ecol. Evol. 23(1), 38-44.

Dowell, K., 2008. Molecular phylogenetics, an introduction to computational methods and tools for analyzing evolutionary relationships. Technical report, University of Maine, Orono, USA. 168- 173.

Doyle, J.J., Doyle, J.L., Brown, A.H.D., 1999. Origins, colonization, and lineage recombination in a widespread perennial soybean polyploid complex. Proc. Natl. Acad. Sci. 96, 10741-10745.

Doyle, J.J., Doyle, J.L., Brown, A.H.D., Palmer, R. G., 2002. Genomes, multiple origins, and lineage recombination in the *Glycine tomentella* (Leguminosae) polyploid complex: Histone H3-D gene sequences. Evolution 56, 1388-1402.

Falk, D.A., 1992. From conservation biology to conservation practice: strategies for protecting plant diversity. In Conservation biology. Edited by P.L. Fiedler and S.K. Jain. Chapman and Hall, New York, N.Y. 397-431.

Fan, X., Sha, L.N., Zeng, J., Kang, H. Y., Zhang, H. Q., Wang, X. L., Zhang, L., Yang, R. W., Ding, C. B., Zheng, Y. L., Zhou, Y. H., 2012. Evolutionary dynamics of the *Pgk1* gene in the polyploid genus *Kengyilia* (Triticeae: Poaceae) and its diploid relatives. PLoS ONE 7(2), 1-10.

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

Felsenstein, J., 1981. Evolutionary trees from DNA sequences: A maximum likelihood approach. J. Mol. Evol. 17, 368-376.

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

Gaudett, M., Salomon, B., Sun G., 2005. Molecular variation and population structure in

Elymus trachycaulus and comparison with its morphologically similar *E. alaskanus*. Plant Syst. Evol. 250: 81–91.

Genovart, M., 2008. Natural hybridization and conservation. Biodivers Conserv. 1-5.

Gielly, L., Taberlet, P., 1994. The use of chloroplast DNA to resolve plant phylogenies: noncoding versus rbcL sequences. Mol. Biol. Evol. 11, 769–777.

Guindon, S., Gascuel, O., 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst. Biol. 52, 696-704.

Hall, B. G., 1942. Phylogenetic Trees Made Easy. A How-To Manual for Molecular Biologists. Sinauer Associates, Inc. 7-164.

Hamstra, T., 2016. Nucleotide diversity and genetic relationships of orchardgrass (*Dactylis glomerata*) inferred from two regions of the chloroplast. Honours thesis. Saint Mary's University. 1-37.

Hasegawa, M., Kishino, H., Yano, T., 1985. Dating of the human-ape splitting by a molecular clock of mitochondrial-DNA. J. Mol. Evol. 22, 160-174.

Hedren, M., Nordstrom, S., Stahlberg, D., 2008. Polyploid evolution and plastid DNA in the Dactylorhiza incarnata/maculata complex (Orchidaceae) in Scandinavia. Mol. Ecol. 17, 5075-5091.

Helfgott, D.M., Mason-Gamer, R.J., 2004. The evolution of North American *Elymus* (Triticeae, Poaceae) allotetraploids: Evidence from phosphoenolpyruvate carboxylase gene sequences. Syst. Bot. 29, 850-861.

Heuertz, M., Carnevale, S., Fineschi, S., Sebastiani, F., Hausman, J. F., Paule, L., Vendramin, G. G., 2006. Chloroplast DNA phylogeography of European ashes,

Fraxinus sp (Oleaceae): Roles of hybridization and life history traits. Mol. Ecol. 15, 2131-2140.

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

Hollingsworth, P.M., Bateman, R.M., Gornall, R.J. (Ed.). 1999. Molecular systematics and plant evolution. London: Taylor & Francis Limited. 1-54.

Hu, Q., Sun, G.L., 2017. Phylogenetic analysis of two single-copy nuclear genes revealed origin and complex relationships of Hordeum polyploids in Triticeae (Poaceae). Genome. 60 (6), 1-31.

Hu, Q., 2013. Origin and evolutionary dynamic of *Elymus ciliaris, E. pendulinus* and *E. longearistatus.* Master thesis. Saint Mary's University. 1-105.

Hu, Q., Yan, C, Sun, G., 2013. Phylogenetic analysis revealed reticulate evolution of allotetraploid *Elymus ciliaris*. Science Direct. 69, 805-813.

Huang, S.X., Sirikhachornkit, A., Faris, J.D. Su, X., Gill, B. S., Haselkorn, R., Gornicki, P., 2002. Phylogenetic analysis of the *Acetyl-CoA carboxylase* and *3-phosphoglycerate* kinase loci in wheat and other grasses. Plant Mol. Biol. 48, 805-820.

Huson, D. H., Bryant, D., 2005. Application of phylogenetic networks in evolutionary studies. Mol. Biol. Evol. 23(2), 254-267.

Jensen, K.B., 1990. Cytology, fertility, and morphology of *Elymus-kengii* (Keng) Tzvelev and *Elymus-grandiglumis* (Keng) A Löve (Triticeae, Poaceae). Genome. 33, 563-570.

Jensen, K.B., Salomon, B., 1995. Cytogenetics and morphology of *Elymus -panormitanus* var. *heterophyllus* and its relationship to *Elymus-panormitanus* var. *panormitanus* (Poaceae, Triticeae). Int. J. Plant Sci. 156, 731-739.

Jensen, K.B., Wang, R.C., 1991. Cyto genetics of *Elymus caucasicus* and *Elymus longearistatus* (Poaceae, Triticeae). Genome. 34, 860-867.

Jiang, F., Miao, Y., Liu, B., Shen, X., Ren, J., He, F., 2009. Mitochondrial Introgressions into the Nuclear Genome of the Domestic Horse. Zoological Res. 29(6), 577−584.

Jiao, Y.N., Wickett, N.J., Ayyampalayam, S., Chanderbali, A. S., Landherr, L., Ralph, P. E., Tomsho, L. P., Hu, Y., Liang, H., Soltis, P. S., Soltis, D. E., Clifton, S. W., Schlarbaum, S. E., Schuster, S. C., Ma, H., Leebens-Mack, J., dePamphilis, C. W., 2011. Ancestral polyploidy in seed plants and angiosperms. Nature. 473, 97-113.

Jones, T.A., Redinbaugh, M.G., Zhang, Y., 2000. The western wheatgrass chloroplast genome originates in *Pseudoroegneria*. Crop Sci. 40, 43-47.

Joyce, A., 2014. Genetic relationship of *Elymus alaskanus, E. caninus, E. fibrosus, and E. mutabilis* revealed by chloroplast DNA sequences. Honours thesis. Saint Mary's University. 1-31.

Kajita, T., Kamiya, K., Nakamura, K., Tachida, H., Wickneswari, R., Tsumura, Y., Yoshimaru, H., Yamazaki, T., 1998. Molecular phylogeny of Dipetrocarpaceae in Southeast Asia based on nucleotide sequences of matK, trnL intron, and trnL-trnF intergenic spacer region in chloroplast DNA. Mol. Phylogenet. Evol. 10, 202–9.

Kakeda, K., Ibuki, T., Suzuki, J., Tadano, H., Kurita, Y., Hanai, Y., Kowyama, Y., 2008. Molecular and genetic characterization of the S locus in *Hordeum bulbosum* L., a wild self-incompatible species related to cultivated barley. Mol. Genet. Genom. 280, 509-519.

Kakeda, K., Taketa, S., Komatsuda, T., 2009. Molecular phylogeny of the genus *Hordeum* using thioredoxin-like gene sequences. Breeding Sci, *59*, 595-601.

Kelchner, S., 2000. The evolution of non-coding chloroplast DNA and its applications in plant systematics. Ann. Missouri Bot. Gard. 87, 482–498.

Kimura, M., 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J. Mol. Evol. 16, 111-120.

Komatsuda, T., Tanno, K., Salomon, B., Bryngelsson, T., Bothmer, R., 1999. Phylogeny in the genus *Hordeum* based on nucleotide sequences closely linked to the vrs1 locus (row number of spikelets). Genome. 42, 973-981.

Lanave, C., Preparata, G., Saccone, C., Serio, G., 1984. A new method for calculating evolutionary substitution rates. J. Mol. Evol. 20, 86-93.

Li, G., Quiros, C.F., 2001. Sequence-related amplified polymorphism (SRAP), a new marker system based on a simple PCR reaction: Its application to mapping and gene tagging in Brassica. Theor. Appl. Genet. 103, 455–461. doi:10.1007/s001220100570

Liu, Q., Ge, S., Tang, H., Zhang, X., Zhu, G., Lu, B. R., 2006. Phylogenetic relationships *Elymus* (Poaceae: Triticeae) based on the nuclear ribosomal internal transcribed spacer and chloroplast *trnL-F* sequences. New Phytol. 170, 411-420.

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

Löve, Á., 1984. Conspectus of the Triticeae. Feddes Reper. 95, 425 -521.

Lu, B.R., Bothmer, V., 1989. Cytologicalgical studies of a dihaploid and hybrid from intergeneric cross *Elymus-shandongensis* x *Triticum-aestivum*. Hereditas. 111, 231-238.

Lu, B.R., Bothmer, V., 1993. Meiotic analysis of *Elymus caucasicus*, *E. longearistatus*, and their interspecific hybrids with 23 *Elymus* Species (Triticeae, Poaceae). Plant Syst. Evol. 185, 35-53.

Mallet, J., 2005. Hybridization as an invasion of the genome. Trends Ecol. Evol. 20, 229-237.

Martinsen, G.D., Whitham, T.G., Turek, R.J., Keim, P., 2001. Hybrid populations selectively filter gene introgression between species. Evolution. 55, 1325-1335.

Mason-Gamer, R.J., 2001. Origin of North American *Elymus* (Poaceae: Triticeae) allotetraploids based on granule-bound starch synthase gene sequences. Syst. Bot. 26, 757-768.

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.

Mason –Gamer, R.J., Burns, M.M., Naum, M., 2005. Polyploidy, introgression, and complex phylogenetic patterns within *Elymus*. Czech J. Genet. Plant Breed. 41, 21-26.

Mason-Gamer, R. J., Burns, M. M., Naum, M., 2009. Phylogenetic relationships and reticulation among Asian *Elymus* (Poaceae) allotetraploids: Analyses of three nuclear gene trees. Molecular Phylogenetics and Evolution. 54, 10-22.

Mason-Gamer, R., Burnsa, M., Naum, M., 2010. Reticulate Evolutionary History of a Complex Group of Grasses: Phylogeny of *Elymus* StStHH Allotetraploids Based on Three Nuclear Genes. PLoS ONE. 5, 1-15.

Masterson, J. 1994. Stomatal size in fossil plants: evidence for polyploidy in majority of angiosperms. Science. 264, 421-424.

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

Mes, T.H.M., Hart, H. 't, 1994. Sedum surculosum and S. jaccardianum (Crassulaceae) share a unique 70 bp deletion in the chloroplast DNA trnL (UAA)-trnF (GAA) intergenic spacer. Plant Syst. Evol. 193, 213–221.

Ni, Y., Asamoah-Odei, N., Sun, G.L., 2011. Maternal origin, genome constitution and evolutionary relationships of polyploid *Elymus* species and *Hordelymus europaeus*. Biol. Plant. 55, 68-74.

Olmstead, R.G., Palmer, J.D., 1994. Chloroplast DNA systematics: A review of methods and data analysis. Amer. J. Bot. 81, 1205–1224.

Otto, S.P., 2007. The evolutionary consequences of polyploidy. Cell 131, 452 -462.

Otto, S.P., Whitton, J., 2000. Polyploid incidence and evolution. Annu. Rev. Genet. 34, 401-437.

Palmer, J.D., 1987. Chloroplast DNA evolution and biosystematic uses of chloroplast DNA variation. Am. Nat. 130, Suppl, S6–S29. doi:http://www.jstor.org/stable/2460163

Petersen, G., Seberg, O., 1996. Phylogenetic analysis of the Triticeae (Poaceae) based on rpoA sequence data. Mol. Phylogenet. Evol. 7, 217-230.

Ramsey, J., Schemske, D.W., 1998. Pathways, mechanisms, and rates of polyploid formation in flowering plants. Ann. Rev. Ecol. S. 29, 467-501.

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

Ronquist, F., Huelsenbeck, J.P., 2005. Bayesian analysis of molecular evolution using MrBayes. In: Nielsen R, ed. Statistical methods in molecular evolution. Springer-Verlag Press, 183-232.

Ronquist, F., Teslenko, M., Van Der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A., Huelsenbeck, J.P., 2012. Mrbayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Syst. Biol. 61, 539–542. doi:10.1093/sysbio/sys029

Sang, T., 2002. Utility of low-copy nuclear gene sequences in plant phylogenetics. Crit. Rev. Biochem. Mol. Biol. 37,121-147.

Sang, T., Crawford, D., Stuessy, T., 1997. Chloroplast DNA phylogeny, reticulate evolution, and biogeography of Paeonia (Paeoniaceae). Am. J. Bot. 84, 1120–1136.

Sambrook, J., Fritsch, E. F., Maniatis, T., 1989. Gel Electrophoresis of DNA. Molecular cloning, a laboratory manual. Cold Spring Harbor Laboratory Press. 2-22.

Sharma, H.C., Gill, B.S., 1983. Current status of wide hybridization in wheat. Euphytica 32, 17-31.

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

Small, R.L., Ryburn, J.A., Cronn, R.C., Seelanan, T., Wendel, J.F., 1998. The tortoise and the hare: choosing between noncoding plastome and nuclear Adh sequences for phylogeny reconstruction in a recently diverged plant group. Am. J. Bot. 85, 1301–1315.

Soltis, D.E., Soltis, P.S., 1993. Molecular data and the dynamic nature of polyploidy. Crit. Rev. Plant Sci. 12, 243-273.

Soltis D.E., Soltis P.S., 1999. Polyploidy: recurrent formation and genome evolution. Trends Ecol. Evol. 14, 348-352.

Soltis, P. S., Soltis, D. E., 2003. Applying the Bootstrap in Phylogeny Reconstruction. Statist. Sci. 18. 256-267.

Soltis, D.E., Soltis, P.S., Tate, J.A., 2004. Advances in the study of polyploidy since plant speciation. New Phytol. 161, 173-191.

Stebbins, G.L., 1947. Types of polyploids: Their classification and significance. Adv. Gen1. 403-429.

Stebbins, G.L., 1995. Variation and evolution in plants. New York: Columbia University Press.

Stevens, L., Salomon, B., Sun, G., 2007. Microsatellite variability and heterozygote excess in *Elymus trachycaulus* populations from British Columbia in Canada. Biochem. Syst. Ecol. 35 725-736.

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

Sun, G.L., Salomon, B., Bothmer, R. V., 1998. Characterization and analysis of microsatellite loci in *Elymus caninus* (Triticeae: Poaceae). Theor. Appl. Genet. 96, 676–682.doi:10.1007/s001220050788

Sun, G., 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 Syst. Evol. 227, 235-244.

Sun, G., 2002. Interspecific polymorphism at non-coding regions of chloroplast, mitochondrial DNA and rRNA IGS region in *Elymus* species. Hereditas. 137, 119–124. doi:10.1034/j.1601-5223.2002.01547.

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

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

Sun, G., Li, W., 2005. Molecular diversity of species of the *Elymus trachycaulus* complex and their relationships to non-North American taxa. Plant Syst. Evol. 179-191.

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

Sun, G., Ni, Y., Daley, T., 2008. Molecular phylogeny of RPB2 gene reveals multiple

origin, geographic differentiation of H genome, and the relationship of the Y genome to other genomes in *Elymus* species. Mole. Phylogenet. Evol. 46, 897-907.

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

Sun, G., Pourkheirandish, M., Komatsuda, T., 2009. Molecular evolution and phylogeny of the RPB2 gene in the genus *Hordeum*. Ann. Bot. 103, 975–983.

Sun, G.L., Salomon, B., 2009. Molecular evolution and origin of tetraploid *Elymus* species. Breed. Sci. 59, 487- 491.

Sun, G., 2014. Molecular phylogeny revealed complex evolutionary process in *Elymus* species. J. Syst. Evol. 52, 1-6.

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

Swofford, D.L., 2003. PAUP*. Phylogenetic analysis using parsimony (*and other methods). Version 4. Sunderland, MA, USA: Sinauer Associates.

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

Tate, J.A., Soltis, D.E., Soltis, P.S., 2005. Polyploidy in plants. In the evolution of the genome (T. R. Gregory, ed.)

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., Higgins, D.G., 1997. The CLUSTAL X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucl. Acids. Res. 25, 4876-4882.

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

van Ham, R.C.H.J., Hart, H., Mes, T.H.M., Sandbrink, J.M., 1994. Molecular evolution of noncoding regions of the chloroplast genome in the Crassulaceae and related species. Curr. Genet. 25, 558–566.

Wang, H., Sun, D., Sun, G., 2011. Molecular phylogeny of diploid *Hordeum* species and incongruence between chloroplast and nuclear datasets. Genome. 54, 986-992.

Wang, H., Sun, G., 2011. Molecular phylogeny and reticulate origins of several American polyploidy *Hordeum* species. Botany. 89, 405-415.

Weir, B. S., 1996. Nature of discrete genetic data. Genetic data analysis II, methods for discrete population genetic data. Sinauer Associates, Inc. 1-30

Weir, B. S., 1996. Sequence data. Genetic Data Analysis II, Methods for Discrete Population Genetic Data. Human Biol. 291- 340.

Weir, B. S., 1996. Phylogeny reconstruction. Genetic Data Analysis II, Methods for Discrete Population Genetic Data. Human Biol. 341- 375.

Weir, B. S., 1996. Population structure. Genetic Data Analysis II, Methods for Discrete Population Genetic Data. Sinauer Associates, Inc. 161- 199.

Weissmann, S., Feldman, M., Gressel, J., 2005. Sequence evidence for sporadic intergeneric DNA introgression from wheat into a wild *Aegilops* species. Mol. Biol. Evol. 22, 2055-2062.

Wendel, J.F., 2000. Genome evolution in polyploids. Plant Mol. Biol. 42, 225-249.

Xu, D.H., Ban, T., 2004. Phylogenetic and evolutionary relationships between *Elymus humidus* and other *Elymus* species based on sequencing of noncoding regions of

cpDNA and AFLP of nuclear DNA. Theor. Appl. Genet. 108, 1443–1448. doi:10.1007/s00122-004-1588-x

Yan, C., 2011. Molecular characterization of the St genome in *Pseudoroegneria* species and their relationships to the Y genome in *Elymus* species, and origin of *Elymus caninus*. Master thesis. Saint Mary's University. 1-87.

Yan, C., Sun, G., 2011. Nucleotide divergence and genetic relationships of *Pseudoroegneria* species. Biochem. Syst. Ecol. 39, 309-319.

Yan, C., Sun, G., 2012. Multiple origins of allopolyploid wheatgrass *Elymus caninus* revealed by *RPB2*, *PepC* and *TrnD*/*T* genes. Mol. Phylogenet. Evol. 64, 441-451.

Yan, C., Hu, Q., Sun, G., 2014. Nuclear and chloroplast DNA phylogeny reveals complex evolutionary history of *Elymus pendulinus*. Genome. 57, 97–109

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

Zhang, X-Q., Salomon, B., Bothmer, R. V., Diaz, O., 2000. Patterns and levels of genetic differentiation in North American populations of the Alaskan wheatgrass complex. Hereditas. 133, 123-132.

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

Appendix

Appendix 1: List of species, accessions numbers and origins used in this research.

Populations designation (abbreviation)	Morphological type	Geographical origin (accession No.)
Ka9902 (Ka2)	Tall *Elymus hyperarcticus*	Tuktoyaktuk airport, Mackenzie river delta, North West Territories, Canada. (H10613)
Ka9906 (Ka6)	Typical *Elymus hyperarcticus*	Calcareous silt plane (10 m), Cape Bathurst peninsula, North West Territories, Canada. (H10619)
Ka9907 (Ka7)	Typical *Elymus hyperarcticus*	Riverbank campsite (40 m), Parker River Valley, northeast of Banks Island, North West Territories, Canada. (H10621)
Us9601 (Us1)	*Elymus Latiglumis*	Loose fell field, Chowder ridge, Mt. Baker wilderness, Whatcom Co., Washington. USA (H10476)
Gr9710 (Gr10)	*Elymus Violaceus*	Western valleyan (or) desert, Sdr. Igaliku, Julianehab, Greenland. (H10621)
Gr9718 (Gr18)	*Elymus Violaceus*	Reservoir and harbor, Narssarssuag, Julianehab, Greenland. (H10596)
Gr9720 (Gr20)	*Elymus Violaceus*	Airport at Sdr. Stromfjord, Greenland. (H10598)

Appendix 2: Taxa of *Elymus alaskanus* with species from *Bromus, Aegilops, Dasypyrum, Elymus, Eremopyrum, Heneradia, Heteranthelium, Hordeum, Psathyrostachys, Pseudoroegneria, Secale, Thinopyrum*, and *Triticum* used in this study for RPS16 gene.

Species	Accession No.	Genome	Origin	GenBank Accession No.
Bromus catharticus	-	-	-	CN32048
Aegilops geniculata	-	UB[1]	Switzerland	NC023097
A. longissima	PI 542196	S^I	Turkey, Izmir	-
A. monglicum	PI 598460	P	China	-
A. searsii	TA 1926	S^S	Brookings, USA	KJ614413
A. speltoides	SPE 0661	S	Switzerland	NC022135
A. speltoides	PI 499261	S	China	
A. speltoides var. *ligustica*	AE 918	S	Brookings, USA	KJ614404
A. tauschii	-	D	Brookings, USA	KJ614412
A. tauschii	PI 486265	D	Turkey, Hakkari	-
A. umbellulata	PI 276994	U	Turkey	-
A. uniaristata	PI 554418	M^u	Former Soviet Union	-
Dasypyrum villosum	PI 598400	V	Greece	-
Elymus abolinii	PI 531554	StY	Xinjiang, China	-
E. antiquus	PI 619528	StY	-	-
E. burchan-buddae	H 7121	StY	-	-
E. canaliculatus	H 4123	StY	-	-
E. caucasicus	PI 531573	StY	Estonia	-
E. macrochaetus	PI 618796	StY	Kazakhstan	-
E. panormitanus	PI 254866	StY	Iraq	-
E. semicostatus	PI 531660	StY	Pakistan	-
E. validus	H 4100	StY		

Eremopyrum distans	PI 193264	F	Afghanistan	-
E. bonaepartis	PI 203442	F	Turkey	-
Heneradia persica	PI 577112	Q	Turkey	-
Heteranthelium piliferum	PI 4013546	Q	-	-
Hordeum jubatum	-	H	Dekalb, USA	KM974741
H. jubatum	-	H	Dekalb, USA	NC027476
H. vulgare	-	I	USA	x52765
H. vulgare ssp. *vulgare*		I		NGB22088
H. vulgare ssp. *vulgare*	-	I	Orlando, USA	EF115541
H. vulgare ssp. *vulgare*	-	I	Switzerland	KC912687
H. vulgare ssp. *spontaneum*	-	I	Switzerland	KC912688
H. vulgare ssp. *spontaneum*	-	I	Switzerland	KC912689
Psathyrostachys juncea	PI 406469	Ns	Former Soviet Union	-
Pseudoroegeneria ferganensis	H 10248	St	Gissar mtns, Tadzhikistan	-
P. geniculata	PI 632554	St	Uzbekistan	-
P. libanotica	PI 330688	St	Sirak-Sar, Iran	-
P. spicata	PI 506274	St	Wasington, United States	-
P. gracillima	PI 420842	St	Soviet Union, Former	-
P. stipifolia	PI 325181	St	Stavropol, Russian Federation	-
P. tauri	PI 401331	St		
Secale cereal	PI 573710	R	Georgia	-
Thinopyrum elongatum	PI 142012	E[e]	Soviet Union, Former	-
Triticum monococcum	-	A	Switzerland	KC912690
T. monococcum	PI 191146	A	Spain	
T. urartu	PI 428335	A	Lebanon, El	KJ614411

			Beqaa	
Elymus alaskanus	Ka 9902-04	StH	Mackenzie river	-
	Ka 9902-07	StH	delta, N.W.T.	
	Ka 9902-10	StH		
	Ka 9902-22	StH		
	Ka 9902-28	StH		
	Ka 9906-07	StH	Cape Bathurst peninsula, N.W.T.	
	Ka 9906-11	StH		
	Ka 9906-18	StH		
	Ka 9906-21	StH		
	Ka 9906-27	StH		
	Ka 9906-31	StH		
	Ka 9907-01	StH	Northeast of Banks Island, N.W.T.	
	Ka 9907-11	StH		
	Ka 9907-16	StH		
	Ka 9907-21	StH		
	Ka 9907-46	StH		
	Gr 9710-04	StH	Julianehab, Greenland	
	Gr 9710-05	StH		
	Gr 9710-07	StH		
	Gr 9710-10	StH		
	Gr 9718-03	StH	Julianehab, Greenland	
	Gr 9718-06	StH		
	Gr 9718-08	StH		
	Gr 9720-10	StH	Stromfjord, Greenland	
	Gr 9720-12	StH		
	Us 9601-03	StH	Whatcom Co., Wash	

Appendix 3: Taxa of *Elymus alaskanus* with species from *Bromus, Aegilops, Agropyron, Australopyrum, Crithopsis, Dasypyrum, Elymus, Eremopyrum, Haynaldia, Heneradia, Heteranthelium, Hordeum, Lophopyrum, Peridictyon, Psathyrostachys, Pseudoroegneria, Secale, Taeniatherum* and *Thinopyrum* used in this study for RPOA gene.

Species	Accession No.	Genome	Origin	GenBank Accession No.
Bromus inermis	OSA 414	-	Copenhagen K, DENMARK	Z77759
Bromus tectorum	Brotec1	-	Chicago, USA	KF600682
Aegilops markgrafii	-	S^m	Chicago, USA	AY115908
A. tauschii	-	D	Chicago, USA	AY115910
A. speltoides	-	S	Chicago, USA	AY115909
A. uniaristata	G 1297	M^u	Chicago, USA	AY115911
Agropyron monglicum	D 2774	P	Chicago, USA	AY115914
Australopyrum retrofractum	-	W	Chicago, USA	AY115915
Crithopsis comosum	-	K	-	Z77742
Dasypyrum villosum	H 5561	V	Copenhagen K, DENMARK	Z77741
Elymus wawawaiensis	PI 285272	StH	Chicago, USA	AY115944
E. wawawaiensis	PI 598812	StH	Chicago, USA	AY115945
E. virginicus	PI 436945	StH	Chicago, USA	AY115941
E. virginicus	PI 490361	StH	Chicago, USA	AY115942
E. virginicus	RJMG 163	StH	Chicago, USA	AY115943
E. trachycaulus	PI 372500	StH	Chicago, USA	AY115939
E. trachycaulus	PI 452446	StH	Chicago, USA	AY115940
E. spicatus	Psespi6	St	Chicago, USA	KF600676
E. elymoides	PI 531606	StH	Chicago, USA	AY115932
E. canadensis	H 3994	StH	Denmark	HM770852
E. canadensis	-	StH	Chicago, USA	AY115928
E. canadensis	PI 578675	StH	Chicago, USA	AY115929
E. canadensis	PI 531568	StH	Chicago, USA	AY115930
E. caninus	Elydog1	StH	Chicago, USA	KF600661
E. lanceolatus	-	StH	Chicago, USA	AY115937

E. lanceolatus	PI 531623	StH	Chicago, USA	AY115938
E. ciliaris	D 2811	StY	Chicago, USA	AY115931
E. hystrix	-	StH	Chicago, USA	AY115936
E. pendulinus	-	StY	-	KF600672
E. dentatus	PI 531599	StH	Chicago, USA	KF600667
E. dentatus	PI 628702	StH	Chicago, USA	DQ159334
E. mutabilis	PI 499449	StH	Chicago, USA	KF600670
E. mutabilis	PI 628704	StH	Chicago, USA	DQ159335
E. repens	-	StStH	Chicago, USA	AY362785
E. sibiricus	H 10072	StH	DENMARK	HM770854
E. sibiricus	PI 628699	StH	Chicago, USA	KF600673
E. libanoticus	PI 228392	St	Chicago, USA	KF600675
E. stipifolius	PI 313960	St	Chicago, USA	KF600677
Eremopyrum bonaepartis	H 5554	F	Chicago, USA	AY115946
E. triticeum	H 5553	F	Denmark	Z77746
Haynaldia villosa	PI 470279	V	Chicago, USA	AY115926
Heneradia persica	H 5556	O	Chicago, USA	AY115950
Heteranthelium piliferum	PI 402352	Q	Chicago, USA	AY115951
Hordeum murinum	PI 247054	Xu	Chicago, USA	AY115922
H. murinum	CIho 15683	Xu	Chicago, USA	AY115923
H. murinum ssp. *glaucum*	H 801	Xu	Copenhagen K, DENMARK	Z77762
H. marinum	PI 304346	Xa	Chicago, USA	AY115921
H. marinum ssp. *gussoneanum*	H 299	Xa	Copenhagen K, DENMARK	Z77763
H. persica	-	H	-	Z77748
H. piliferum	-	H	-	Z77750
H. brachyantherum	-	H	Copenhagen K, DENMARK	Z77761
H. brachyantherum	PI 531764	H	Chicago, USA	AY115917
H. jubatum	RJMG 106	H	Chicago, USA	AY115920
H. pusillum	CIho 15654	H	Chicago, USA	AY115924
H. bulbosum	PI 440417	I	Chicago, USA	AY115919
H. erectifolium	H 1150	H	Copenhagen K, DENMARK	Z79500
H. vulgare	H 3139	I	Copenhagen K, DENMARK	Z77764
H. brevisubulatum	PI 401387	H	Chicago, USA	AY115918
Lophopyrum elongatum	PI 531719	E	Chicago, USA	AY115965
Patropyrum tauschii	H 6668	St	Denmark	Z77758
Peridictyon sanctum	KJ 248	St	Chicago, USA	AY115952
P. sanctum	H 5575	St	Denmark	Z77749

Psathyrostachys fragilis	C-46-6-16	Ns	Chicago, USA	AY115967
P. juncea	PI 206684	Ns	Chicago, USA	AY115968
P. rupestris	H 6703	Ns	Denmark	Z77755
P. stoloniformis	H 9182	Ns	Denmark	Z77754
Pseudoroegeneria strigosa	MA 109-31-50	St	Chicago, USA	AY115953
P. strigosa	PI 531755	St	Chicago, USA	AY115954
P. spicata	PI 236681	St	Chicago, USA	AY115956
P. spicata	H 9082	St	Denmark	Z77744
P. spicata	PI 610986	St	Chicago, USA	AY115957
P. spicata	D 2844	St	Chicago, USA	AY115958
P. spicata	D 2839	St	Chicago, USA	AY115959
P. libanotica	PI 228391	St	Chicago, USA	AY115955
Secale montanum	PI 440654	R	Chicago, USA	AY115960
S. speltoides	-	R	-	Z77766
Taeniatherum caput-medusae	-	Ta	Chicago, USA	AY115963
Thinopyrum scirpeum	-	E	Chicago, USA	AY115966
Elymus alaskanus	Ka 9902-01	StH	Mackenzie river delta, N.W.T.	
	Ka 9902-19	StH		
	Ka 9906-01	StH	Cape Bathurst peninsula, N.W.T.	
	Ka 9906-31	StH		
	Ka 9906-47	StH		
	Ka 9907-06	StH	Northeast of Banks Island, N.W.T.	
	Ka 9907-11	StH		
	Ka 9907-16	StH		
	Ka 9907-46	StH		
	Gr 9710-03	StH	Julianehab, Greenland	
	Gr 9710-11	StH		
	Gr 9718-06	StH	Julianehab, Greenland	
	Gr 9718-08	StH		
	Gr 9718-13	StH		
	Gr 9718-18	StH		
	Gr 9718-26	StH		
	Gr 9720-01	StH	Stromfjord, Greenland	
	Gr 9720-05	StH		

	Gr 9720-06	StH	
	Gr 9720-08	StH	
	Gr 9720-10	StH	
	Gr 9720-12	StH	
	Us 9601-01	StH	Whatcom Co., Wash
	Us 9601-02	StH	
	Us 9601-03	StH	
	Us 9601-05	StH	

Appendix 4: Taxa of *Elymus alaskanus* with species from *Bromus, Agropyron, Australopyrum, Dasypyrum, Elymus, Eremopyrum, Lophopyrum. Hordeum, Psathyrostachys, Pseudoroegneria* and *Thinopyrum* used in this study for RPB2 gene.

Species	Accession No.	Genome	Origin	GenBank Accession No.
Bromus catharticus	CN 32048	-	Halifax, Canada	HQ014410
Bromus inermis	PI 618974	-	China	GQ848517
Agropyron monglicum	PI 531543	P	China	KC545623
Australopyrum retrofractum	PI 547363	W	Halifax, Canada	EU187470
Dasypyrum villosum	PI 368886	V	Halifax, Canada	EU187471
Elymus alashanicus	PI 531573	St	China	GU073308
E. alashanicus	ZY 2003	St	China	GU073307
E. bakeri	PI 610985	StH	Halifax, Canada	EU187440
E. canadensis	PI 531576	StH	China	GQ867861
E. canaliculatus	H 4123	StH	Halifax, Canada	EU187449
E. caninus	PI 499413	StH	China	GU073298
E. caninus	H 3169	StH	Halifax, Canada	EF596770
E. ciliaris	PI 564917	StY	Halifax, Canada	EU187483
E. confuses	W 621505	StH	China	GQ867863
E. dentatus	PI 628702L	StH	Halifax, Canada	EF596769
E. dentatus	PI 628702U	StH	Halifax, Canada	EF596744
E. durus	Y 2119	StH	China	GQ867853
E. elytrigioides	ZY 2001	StH	China	GQ867877
E. fibrosis	H 10339	StH	Halifax, Canada	EF596773
E. glaucus	PI 232258	StH	Halifax, Canada	EF596772
E. glaucus	PI 232258	StH	Halifax, Canada	EF596771
E. glaucus	PI 232259	StH	China	GU073300

E. glaucus	PI 232258	StH	Halifax, Canada	EF596757
E. hystrix	PI 372546	StH	China	GU073301
E. hystrix	H 5495	StH	Halifax, Canada	EF596762
E. hystrix	H 5495b	StH	Halifax, Canada	EF596765
E. hystrix	PI 531616	StH	China	KC545638
E. lanceolatus	PI 236663U	StH	Halifax, Canada	EF596739
E. lanceolatus	PI 236663L	StH	Halifax, Canada	EF596767
E. latiglumis	H 10476	-	Halifax, Canada	EF596760
E. magnicaespes	Y 0756	StH	China	GU073309
E. multisetus	W6-209631	StH	Halifax, Canada	EF596755
E. mutabilis	PI 564949	StH	Halifax, Canada	EU187476
E. scabriglumis	PI 331168L	-	Halifax, Canada	EF596756
E. sibiricus	-	StH	China	GQ867859
E. sibiricus	PI 499461L	StH	Halifax, Canada	EF596763
E. sibiricus	PI 499461U	StH	Halifax, Canada	EF596741
E. submuticus	PI 499480	StH	Halifax, Canada	EU187477
E. trachycaulus	PI 537323	StH	Halifax, Canada	EU187478
E. trachycaulus	H 3526L	StH	Halifax, Canada	EF596764
E. trachycaulus	H 3526U	StH	Halifax, Canada	EF596743
E. transbaicalensis	H 10391U	-	Halifax, Canada	EF596745
E. transbaicalensis	H 10391L	-	Halifax, Canada	EF596761
E. violaceus	H 10588	StH	Halifax, Canada	EU187480
E. virescens	H 10584U	StH	Halifax, Canada	EF596742
E. virescens	H 10584	StH	Halifax, Canada	EF596766
E. virginicus	PI 436946	StH	Halifax, Canada	EF596759
E. virginicus	PI 490361	StH	China	GU073303
E. wawawaiensis	PI 506262	StH	Halifax, Canada	EU187441
E. wiegandii	PI 531708U	-	Halifax, Canada	EF596740
E. wiegandii	PI 531708L	-	Halifax, Canada	EF596758
Eremopyrum triticeum	PI 502364	F	China	KC545625
Lophopyrum elongatum	142012	E	Halifax, Canada	EU187439
Hordeum bogdanii	PI 499498	H	Halifax, Canada	EF596768
H. bogdanii	PI 499645	H	Halifax, Canada	EU187472
H. brevisubulatum	H 1954	H	China	KC545626
H. chilense	PI 531781	H	China	GQ848518
H. stenostachys	H 6439	H	Halifax, Canada	EU187473
Psathyrostachys fragilis	Y 882	Ns	China	KC545695
P. huashanica	ZY 3157	Ns	China	KC545696
P. juncea	PI 430871	Ns	China	GQ848519

P. lanuginose	H 8803a	Ns	China	KC545697
Pseudoroegeneria geniculate	PI 565009	St	China	GQ867874
P. geniculate	PI 565009	St	China	GQ867873
P. geniculate ssp. *scythica*	PI 502271	St	China	GQ867869
P. gracillima	PI 440000	St	Halifax, Canada	HQ231847
P. gracillima	PI 420842	St	Halifax, Canada	HQ231846
P. gracillima	-	St	China	JX259490
P. kosaninii	PI 237636	St	China	GU073305
P. libanotica	PI 330688	St	Halifax, Canada	EF596751
P. libanotica	PI 401274	St	Halifax, Canada	EF596752
P. libanotica	PI 330687	St	Halifax, Canada	EF596753
P. spicata	PI 506274	St	Halifax, Canada	EF596746
P. spicata	PI 610986	St	Halifax, Canada	EF596747
P. spicata	PI 539873	St	Halifax, Canada	HQ231853
P. spicata	PI 232128	St	Halifax, Canada	HQ231840
P. spicata	PI 598822	St	Halifax, Canada	HQ231858
P. spicata	PI 516184	St	Halifax, Canada	HQ231848
P. spicata	PI 537379	St	Halifax, Canada	HQ231851
P. spicata	PI 286198	St	Halifax, Canada	HQ231843
P. spicata	PI 537389	St	Halifax, Canada	HQ231852
P. spicata	PI 563872	St	Halifax, Canada	HQ231857
P. spicata	PI 563869	St	Halifax, Canada	HQ231856
P. spicata	PI 619445	St	Halifax, Canada	HQ231859
P. spicata	PI 547162	St	Halifax, Canada	HQ231855
P. spicata	PI 232134	St	Halifax, Canada	HQ231841
P. spicata	PI 236669	St	Halifax, Canada	HQ231842
P. spicata	PI 547154	St	Halifax, Canada	HQ231854
P. stipifolia	PI 325181	St	Halifax, Canada	EF596748
P. stipifolia	-	St	China	JX259494
P. stipifolia	-	St	China	JX259495
P. strigosa	PI 499637	St	China	GQ848520
P. strigosa	PI 531752	St	China	GQ867875
P. strigosa	PI 531752	St	China	GQ867876
P. strigosa	14049	St	Halifax, Canada	HQ231836
P. strigosa	PI 531752	St	Halifax, Canada	HQ231850
P. strigosa	-	St	China	JX259489
P. strigosa	-	St	China	JX259492
P. strigosa	PI 499493	St	China	KC545698
P. strigosa	-	St	China	JX259493
P. strigosa ssp. *aegilopoides*	13089	St	Halifax, Canada	HQ231835
P. tauri	PI 401324	St	Halifax, Canada	HQ231844
P. tauri	PI 401326	St	Halifax, Canada	HQ231845

P. tauri ssp. *libanotica*	PI 228389	St	Halifax, Canada	HQ231837
P. tauri ssp. *libanotica*	-	St	China	JX259491
Thinopyrum bessarabicum	PI 531712	E	Halifax, Canada	EU187474
Elymus alaskanus	Gr 9718-06	StH	Julianehab, Greenland	
	Gr 9718-06	StH		
	Gr 9720-06	StH	Stromfjord, Greenland	
	Gr 9720-09	StH		
	Gr 9720-09	StH		