Certification

Molecular Phylogeny and Origins of *Hordeum* Polyploid Species

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Abstract: The genus *Hordeum* in the tribe Triticeae comprises about thirty two species including diploids and polyploids. Although the phylogeny of diploid *Hordeum* species has been studied intensively, there have been incongruences between the datasets obtained from chloroplast and nuclear genes. Additionally, the origins of the polyploid species in the genus *Hordeum* have not been completely understood until now. In the present study, three chloroplast gene loci, trnT-trnF intergenic spacer, rps16 gene, and trnH-psbA intergenic spacer in addition to a single-copy nuclear gene, β-amylase gene, were used to explore the phylogeny and origins of *Hordeum* polyploid species. Eighty accessions from thirty two *Hordeum* species were used in this study. The present study supports previous suggestions on that *H. brachyantherum* ssp. *californicum* was one parent to the tetraploid species *H. brachyantherum* ssp. *brachyantherum*, *H. jubatum*, *H. guatemalense*, and *H. depressum*. Our nuclear DNA results suggest the diploid *H. roshevitzii* as one parent to tetraploid species *H. brachyantherum* ssp. *brachyantherum, H. jubatum,* and *H. fuegianum*. In addition, our results suggest *H. cordobense, H. brahcyantherum* ssp. *californicum, and H. roshevitzii* as the diploid genome donors to the hexaploid species *H. procerum*, the diploid species *H. pusillum* and *H. brachyantherum* ssp. *californicum* as genome donors to the hexaploid *H. lechleri*. Moreover, our study further confirms *H. pusillum* as a diploid parent to *H. arizonicum* and suggests *H. brachyantherum* ssp. *californicum* as another diploid genome donor to the hexaploid *H. arizonicum*.

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Table of Contents

1. Introduction

1.1 Phylogenetics

Phylogenetics represent the study of evolutionary relationships among operational taxonomic units at all levels (i.e., species, genus, family), and is a vital part of researching the evolutionary tree of life. The general aim is to resolve evolutionary relationships of various species. Biologists consider evolution as a branching process, where populations transform over periods of time and may possibly divide into distinct lineages, hybridize together or go extinct (Felsenstein, 2004). This is visualized by a phylogenetic tree, the typical tool to illustrate all these evolutionary processes in species history. Clarifying relationships among different populations represents an interesting challenge to evolutionists, which will furthermore lead to more findings of concealed evolutionary processes (Felsenstein, 2004). A reality is that different genes occasionally produce different trees, therefore presenting genetic conflicts in defining the phylogenetic relationship among lineages of interests. For that reason, we possibly could clarify historical relationships among species and better classify populations at all levels, by joining multiple gene datasets to explain the incongruences among distinct gene trees (Felsenstein, 2004).

With the progresses in molecular techniques over the last two decades, great interest has focused on investigating the evolutionary consequences of polyploid species in both genome size and contents (Wendel, 2000; Osborn *et al.*, 2003). Polyploid origins and evolution have also been the focus of plant evolutionists (Soltis and Soltis, 1999; Soltis *et al.*, 2003). Polyploidy is a substantial evolutionary event in the speciation process and history of plant evolution. The existence of more than two genomes per cell is referred to as polyploidy (Soltis and Soltis, 2000), which is a popular phenomenon particularly in plants. Polyploidy has been identified to happen in nearly seventy percent of all angiosperms (Masterson, 1994; Wendel, 2000). Various economically important crops, such as wheat, potato and cotton, are polyploids. Stebbins (1950) defined two distinct types of polyploids. Allopolyploids are created by joining two or more different genomes, whereas autopolyploids originated from duplicating of a single whole genome (Masterson, 1994; Soltis and Soltis, 1999, 2000). The history of plant evolution often involved interspecific hybridization and polyploidization, which have played an essential role in influencing plant divergence and speciation (Cui *et al.*, 2006).

Growing evidence has showed the complexity and dynamic characteristics of polyploids. Numerous polyploids are proved to be of multiple origins in

space and time (Soltis and Soltis, 1999; Soltis *et al.*, 2003), along with introgression (Mason-Gamer, 2004, 2008; Lihová *et al.*, 2006), whereas others are thought to have a single origin. Gene introgression has been reported to cause a sudden gene copies in a single genome, causing massive reticulate relationships in Triticeae species (Mason-Gamer, 2004, 2008). Furthermore, transposon elements can be activated by polyploidization, leading to enlarge the genome size, while, other mechanisms lead to genome downsizing (Kellogg and Bennetzen, 2004; Leitch and Bennett, 2004).

To explore the evolutionary relationships of related plant lineages, modern molecular phylogenetic analysis commonly use plastid DNA and nuclear markers to rebuild gene trees of related species. Regrettably, due to incongruences or conflicts between plastid and nuclear phylogenetic data, the effort to build a precise phylogenetic tree often fails (Galtier and Daubin, 2008). Such discrepancies of different gene phylogenies can occur as a result of three main evolutionary mechanisms: incomplete lineage sorting, hidden paralogy, and horizontal gene transfer (Galtier and Daubin, 2008). The most studied mechanism probably is incomplete lineage sorting, which results from retention and stochastic sorting of ancestral polymorphisms, and the complexities it imposes on interpreting the true

species tree have been well explained (Pamilo and Nei, 1988; Rosenberg, 2002; Maddison and Knowles, 2006; Meng and Kubatko, 2009).

1.2 The genus *Hordeum*

Triticeae is one tribe in the family of Poaceae, and includ barley and wheat, in addition to hundreds of related species. Intensive phylogenetic studies have been done on tribes of is grass family Poaceae, since they comprise a great number of economically significant crops and they have proven to have a reticulate evolutionary history (Wang and Sun, 2011). One of the important model genera for plant phylogenetic studies is *Hordeum* as it is considered as one the most economically important crops, barley, due to *Hordeum's* evolutionary history that involves hybridization, polyploidization and introgression. Thus, a better elucidation of the phylogeny of *Hordeum* species will make a significant impact on future plant phylogenetic study.

The genus *Hordeum* in Triticeae includes 32 species with a basic chromosome number of x=7, is dispersed disjunctly in southern South America, South Africa, and the northern hemisphere (von Bothmer *et al.*, 1995; Blattner, 2006). Morphology, meiotic chromosome pairing in

interspecific hybrids (von Bothmer *et al.*, 1986, 1987, 1988), karyotype and C- banding patterns (Linde-Laursen *et al.*, 1992, 1995), as well as nuclear and chloroplast DNA sequences have been used to reveal the phylogenetic relationship among *Hordeum* species (Doebley *et al.*, 1992; El-Rabey *et al.*, 2002; Nishikawa *et al.*, 2002; Petersen and Seberg, 2003; Wang and Sun, 2011). Karyotype analyses of chromosome types and meiotic chromosome pairing studies of hybrids (von Bothmer *et al.*, 1995; Linde-Laursen *et al*., 1992) have classified *Hordeum* species into four basic genome groups, H (*Hordeum bulbosum; Hordeum vulgare*), Xa (*Hordeum marinum*), and Xu (*Hordeum murinum*) and I (remaining species) following genome denomination by Blattner (2009). Isoenzyme analysis (Jørgensen, 1986), restriction site variation in chloroplast DNA (Baum and Bailey, 1991), restriction fragment length polymorphism with repetitive DNA (Svitashev *et al.*, 1994) and DNA sequence data (Petersen and Seberg, 2003; Blattner, 2004; Sun *et al.* 2009) supported the four basic genome groups. The largest group I genome includes 14 diploid species, 7 tetraploid species, 4 hexaploid species, and 2 species existing at three ploidy levels (2x, 4x, 6x). I genome species share a lot of morphological traits, while dispersed widely from central Asia to the American continent. It is believed that *Hordeum* diploid species originated from South-west Asia and dispersed into Europe and Central Asia (Blattner, 2006).

Accumulating evidences back up the monophyletic clade of western Asian and Mediterranean species of the H and Xu genome groups, along with another monophyletic clade of Eurasian *H. marinum* in Xa genome group and I genome taxa (Komatsuda *et al.*, 1999; Petersen and Seberg, 2003; Sun *et al.*, 2009). *Hordeum* species of the I genome group were divided into "New World" and "Old World" groups based on chloroplast DNA sequence data (Doebley *et al.*, 1992; Nishikawa *et al.*, 2002).

Several molecular phylogenetic studies have focused on the genus *Hordeum* (Petersen and Seberg, 1997; Seberg and Frederiksen, 2001; Blattner, 2004), but still the phylogeny of *Hordeum* is a subject of discrepancy. Due to the incongruence between chloroplast and nuclear data, the complete phylogenetic relationships among *Hordeum* species have not yet been fully revealed. Whereas the data obtained from nuclear genes of *Hordeum* species mostly deliver similar results (Petersen and Seberg, 2003; Blattner, 2004, 2006; Kakeda, 2009; Sun *et al.*, 2009), studies of chloroplast DNA in general resulted in conflicting conclusions (Doebley *et al.*, 1992; Nishikawa *et al.*, 2002; Petersen and Seberg, 2003; Jakob and Blattner, 2006). The most possible cause behind such discrepancy is incomplete lineage sorting (Petersen and Seberg, 2003; Jakob and Blattner, 2006, Wang and Sun, 2011)

Additional research is required to fully discover the origins of polyploids in *Hordeum*. Fluorescent *In Situ* Hybridization (FISH) and rDNA-RFLP patterns done by Taketa *et al.* (2001, 2005) suggested *H. roshevitzii* and *H. brachyantherum* ssp. *californicum* as the common ancestors of tetrapolyploid species *H. jubatum, H. fuegianum, H. tetraploidum* and *H. brachyantherum* ssp. *brachyantherum,* and identified a close relationship of the tetraploid species *H. jubatum* to the I genome hexaploid species. The suggestion that *H. roshevitzii* and *H. brachyantherum* ssp. *californicum* are the ancestors to *H. jubatum* was also reaffirmed by Blattner (2006). A recent study also suggested the diploid *H. brachyantherum* ssp. *californicum* as one parent to the polyploid species *H. arizonicum*, *H. brachyantherum* ssp. *brachyantherum*, *H. depressum*, and *H. procerum* (Wang and Sun, 2011). Wang and Sun (2011) also suggested the diploid *H. euclaston* as the other parent to *H. depressum* and the diploid *H. cordobense* as potential genome donor to the hexaploid *H. procerum.* The diploid *H. flexuosum* and tetraploid *H. tetraploidum* were identified as potential genome donors to hexaploid *H. parodii* (Wang and Sun, 2011). Additional studies are needed as the origins of some polyploid species still have not been fully revealed.

1.3 Molecular Genetics

1.3.1 Chloroplast Genes

The very commonly genetic marker to study plant phylogeny used to be chloroplast DNA (cpDNA). The major advantages of cpDNA rely on its relatively simple inheritance and the great number copies of cpDNA genes, which make it simple to achieve in restriction site examination in addition to gene amplification (Small *et al.*, 2004). On the other hand, cpDNA follows maternal inheritance, and uniparental inheritance allows uncovering only half of the parents in a hybrid or polyploid plants (Olmstead and Pamer, 1994; Soltis and Soltis, 1998).

1.3.2 Single Copy Nuclear Genes

Nowadays, single copy nuclear genes have been considered the preferred candidates for studying phylogenetics, particularly in revealing donors of hybrids or polyploids (Sang, 2002). Firstly, nuclear genes evolve and change faster than organelles genomes (Wolfe *et al.*, 1987; Gaut. 1998), thus they possess a higher detectable variation. Secondly, they are expected to have experienced independent evolution events like hybridization and introgression. Thirdly, single copy nuclear DNA is considerably less

susceptible to concerted evolution unlike ribosomal DNA (rDNA) (Small *et al.*, 2004), a feature particularly important in studying polyploid origins as polyploids are believed to possess several gene copies. Finally, nuclear genes follow biparental inheritance.

1.4 The Objectives of This Study

To help unravel the complicated evolutionary history of *Hordeum* species through using both chloroplast and nuclear genes sequencing data sets, three chloroplast gene loci, trnT-trnF intergenic spacer, rps16 gene, and trnH-psbA intergenic spacer in addition to one nuclear gene encoding enzymes usually linked with starch breakdown β-amylase gene were used in the present study. The main goal is to better understand the phylogeny and to elucidate origins *of Hordeum* polyploid species by using combined genetic data from both chloroplast and nuclear genes. Hopefully, this study could also provide additional information on understanding of evolutionary dynamic of *Hordeum* species in general.

2. Materials and Methods

2.1 Materials

Eighty accessions of thirty-two *Hordeum* species were used in this study. Species name, accession no., origin, genome and ploidy are listed in Table 1 and 2. The seeds used in this study were provided by the NordGen in Sweden and then germinated in sand-peat mixture in a greenhouse. Other sequences in Triticeae were downloaded from GenBank and included in the analysis (Table 2).

Table 1

Hordeum species used in this study. The species name, accession number, origins, genome and ploidy are showed. Sequences from the species with * were downloaded from GenBank.

Name of Species	Accession No.	Origin	Genome	Ploidy
Hordeum arizonicum	H 2144	Mexico		6x
Hordeum arizonicum	H 2313	USA		6x
Hordeum bogdanii	H 7476	China	I	2x
*Hordeum bogdanii**	GQ847675*		I	2x
Hordeum brachyantherum ssp. *brachyantherum*	H 2318	USA		4X
Hordeum brachyantherum ssp. *brachyantherum*	H 2348	USA		4X
Hordeum brachyantherum ssp. *californicum*	H 3317	USA	I	2x
Hordeum brachyantherum ssp. *californicum*	H 3319	USA	I	2x
Hordeum brevisubulatum	H 10056	Russia	I	2x
Hordeum brevisubulatum	H 8788	China	I	2x
Hordeum brevisubulatum ssp. *violaceum**	AY821713*		I	2x
Hodeum bulbosum	H 3878	Italy	H	2x
*Hordeum bulbosum**	AY821706*		H	2x
Hordeum capense	H 335	South Africa		4x
Hordeum capense	H 3923	Mexico		4x
Hordeum chilense	H 1819	Chile	I	2x
Hordeum comosum	H 10608	Argentina	I	2x
Hordeum cordobense	H 1702	Argentina	I	2x
Hordeum depressum	H 2008	USA		4x
Hordeum depressum	H 2089	USA		4x
Hordeum erectifolium	H 1150	Argentina	I	2x
Hordeum euclaston	H 1103	Argentina	I	2x
Hordeum euclaston	H 6045	Argentina	I	2x
Hordeum flexuosum	H 1112	Argentina	I	2x
Hordeum fuegianum	H 1376	Chile		4x
Hordeum fuegianum	H 1418	USA		4x
Hordeum guatemalense	H 2299	Guatemala		4x
Hordeum. intercedens	H 2310	USA	I	2x
Hordeum jubatum	H 1162	Argentina		4x
Hordeum jubatum	H 2013	USA		4x

*Hordeum jubatum**	AY821711*			4x
*Hordeum jubatum**	AY821708*			4x
Hordeum lechleri	H 1451	Chile		6x
Hordeum lechleri	H 6344	Argentina		6x
Hordeum marinum ssp. *gussoneanum*	H 160	Portugal	Xa	2x
Hordeum marinum ssp. *glaucum*	H 52	Jordan	Xu	2x
Hordeum marinum ssp. *marinum*	H 559	Spain	Xa	2x
*Hordeum marinum**	EU28225*		Xa	2x
Hordeum muticum	H 6470	Argentina	I	2x
Hordeum parodii	H 1444	Chile		6x
Hordeum parodii	H 1146	Argentina		6x
Hordeum parodii	H 1458	Argentina		6x
Hordeum patagonicum ssp. *magellanicum*	H 1363	Argentina	I	2x
Hordeum patagonicum ssp. *magellanicum*	H 1368	Chile	I	2x
Hordeum patagonicum ssp. *mustersii*	H 1358	Argentina	I	2x
Hordeum patagonicum ssp. *patagonicum*	H 1520	Argentina	I	2x
Hordeum patagonicum ssp. *santacrucense*	H 6054	Argentina	I	2x
Hordeum. patagonicum ssp. *santacrucense*	H 6243	Argentina	I	2x
Hordeum. patagonicum ssp. *santacrucense*	H 6249	Argentina	I	2x
Hordeum procerum	H 1166	Argentina		6x
Hordeum pubiflorum	H 1379	Chile	I	2x
Hordeum pusillum	H 2037	USA	I	2x
*Hordeum pusillum**	EU282261*		I	2x
Hordeum roshevitzii	H 10070	Russia	I	2x
Hordeum roshevitzii	H 7754	China	I	2x
Hordeum secalinum	H 231	Sweden		4x
Hordeum stenostachys	H 6439	Argentina	I	2x
Hordeum tetraploidum	H 6198	Argentina		4x
Hordeum vulgare	H 7405	China	H	2x
Hordeum vulgare ssp. *spontaneum*	H 3173	China	H	2x
Hordeum vulgare ssp. *spontaneum**	FJ936154*		H	2x
Hordeum vulgare ssp. *cultivar**	DQ889983*		H	2x

Table 2

The sequences from other species other than *Hordeum* downloaded from GenBank that were used in this study.

Name of Species	Accession No.	trnTF-FT	rps16	ß-amylase
*Aegilops bicornis**	AY821686*	-	-	Yes
*Aegilops comosa**	AY821696*	-	-	Yes
*Aegilops longissima**	PI 542196*	-	Yes	-
*Aegilops markgraffi**	AF519111*	Yes	-	-
*Aegilops markgraffi**	AY821687*	-	-	Yes
*Aegilops markgraffi**	AY821688*	-	-	Yes
*Aegilops markgraffi**	AY821689*	-	-	Yes
*Aegilops searsii**	PI 599150*	-	Yes	-
*Aegilops sharonensis**	PI 542237*	-	Yes	-
*Aegilops speltoides**	AF519112*	Yes	-	-
*Aegilops tauschii**	AF519113*	Yes	-	-
*Aegilops tauschii**	AY821695*	-	-	Yes
*Aegilops tauschii**	PI 486265*	-	Yes	-
*Aegilops tauschii**	PI 499261*	-	Yes	-
*Aegilops umbellulata**	PI 276994*	-	Yes	-
*Aegilops uniaristata**	AF519114*	Yes	-	-
*Aegilops uniaristata**	PI 554418*	-	Yes	-
*Agropyron cristatum**	AF519115*	Yes	-	-
*Agropyron cristatum**	AF519116*	Yes	-	-
*Agropyron cristatum**	AY821697*	-	-	Yes
*Agropyron fragile**	PI 598674*	-	Yes	-
*Agropyron mongolicum**	AF519117*	Yes	-	-
*Agropyron mongolicum**	PI 598460*	-	Yes	-
*Australopyrum retrofractum**	AF519118*	Yes	-	-
*Australopyrum retrofractum**	PI 533014*	-	Yes	-
*Australopyrum retrofractum**	PI 548363*	-	Yes	-
*Australopyrum velutinum**	AF519119*	Yes	-	-
*Bromus anomalus**	JF904751*	Yes	-	-
*Bromus catharticus**	DQ887428*	Yes	-	-
*Bromus catharticus**	EU036184*	Yes	-	-
*Bromus catharticus**	CN 32048*	-	Yes	-
*Bromus sterilis**	PI 229595	-	Yes	-
*Bromus suksdorfii**	EU036187*	Yes	-	-

*Bromus tectorum**	AY821734*	-	-	Yes
*Eremopyrum boneapartis**	AF519148*	Yes	-	-
*Eremopyrum boneapartis**	AF519149*	Yes	-	-
*Eremopyrum boneapartis**	AY821700*	-	-	Yes
*Eremopyrum boneapartis**	PI 203442*	-	Yes	-
*Eremopyrum distans**	AF519150	Yes	-	-
*Eremopyrum distans**	PI 193264*	-	Yes	-
*Eremopyrum orientale**	AF519151*	Yes	-	-
*Eremopyrum orientale**	PI 203440*	-	Yes	-
*Haynaldia villosa**	AF519128*	Yes	-	-
*Haynaldia villosa**	AF519129*	Yes	-	-
*Henrardia persica**	AF519152*	Yes	-	-
*Henrardia persica**	PI 577112*	-	Yes	-
*Heteranthelium piliferum**	AF519153*	Yes	-	-
*Heteranthelium piliferum**	PI 401354*	-	Yes	-
*Lophopyrum elongatum**	AF519166*	Yes	-	-
*Peridictyon sanctum**	AF519154*	Yes	-	-
*Psathyrostachys fragilis**	AY821715*	-	-	Yes
*Psathyrostachys juncea**	PI 406469*	-	Yes	-
*Pseudoroegnreria geniculate**	PI 632554*	-	Yes	-
*Pseudoroegnreria libanotica**	AF519156*	Yes	-	-
*Pseudoroegnreria libanotica**	PI 330688*	-	Yes	-
*Pseudoroegnreria spicata**	AF519157*	Yes	-	-
*Pseudoroegnreria spicata**	AF519158*	Yes	-	-
*Pseudoroegnreria spicata**	AF519159*	Yes	-	-
*Pseudoroegnreria spicata**	AF519160*	Yes	-	-
*Pseudoroegnreria spicata**	PI 506274*	-	Yes	-
*Pseudoroegnreria strigosa**	AF519155*	Yes	-	-
*Pseudoroegnreria strigosa**	EU282267*	-	-	Yes
Pseudoroegnreria	PI 420842*	-	Yes	-

strigosa ssp. *aegilopoides**				
*Pseudoroegnreria stipifolia**	PI 325181	-	Yes	-
*Secale cereale**	AF519162*	Yes	-	-
*Secale cereale**	AY821723*	-	-	Yes
*Secale cereale**	PI 573710*	-	Yes	-
*Secale montanum**	AF519161*	Yes	-	-
*Secale montanum**	AF519163*	Yes	-	-
*Taeniatherum caput-medusae**	AF519164*	Yes	-	-
*Taeniatherum caput-medusae**	AY821726*	-	-	Yes
*Taeniatherum caput-medusae**	AY821727*	-	-	Yes
*Taeniatherum caput-medusae**	AY821728*	-	-	Yes
*Taeniatherum caput-medusae**	AY821729*	-	-	Yes
Taeniatherum caput-medusae ssp. *asperum meldris**	PI 561091*	-	Yes	-
Taeniatherum caput-medusae ssp. *caput-medusae**	PI 208075*	-	Yes	-
Taeniatherum caput-medusae ssp. *caput-medusae**	PI 222048*	-	Yes	-
*Thinopyrum bessarabicum**	AF519165*	Yes	-	-
*Thinopyrum bessarabicum**	AY821730*	-	-	Yes
*Thinopyrum scirpeum**	AF519167*	Yes	-	-
*Triticum baeoticum**	AF519168*	Yes	-	-
*Triticum monococcom**	PI 191146*	-	Yes	-

2.2 Methods

2.2.1. DNA Extraction

Plant DNA extraction was performed by using GeneJET™ Plant Genomic DNA Purification Mini Kit (Fermentas, Lithuania). Plant tissue (young leaves) was placed into liquid nitrogen and grounded thoroughly with a mortar and a pestle. The tissue powder was transferred to 1.5 ml microcentrifuge tubes containing 350 µl of Lysis Buffer A and vortex for 10-20 seconds. Fifty microliters of Lysis Buffer B were added to the mixture in each tube. The mixture was incubated for 10 min at 65°C while shaking in a water bath. One hundred thirty microliters of Precipitation Solution were added to the mixture and mixed by inverting the tube 2-3 times. The samples were incubated on ice for 5 min and then centrifuged at ≥14,000 rpm for 5 min. The supernatant (usually 450-550 µl) was transferred to a clean new microcentrifuge tube. Four hundred microliters of Plant gDNA Binding Solution and 96% ethanol were added to the mixture and then mixed thoroughly. Half of the prepared mixture was transferred to a spin column and then centrifuged for 1 min at 8,000 rpm. The flow-through solution was discarded and the remaining half of the mixture was then applied onto the same column and centrifuged again at 8,000 rpm for 1 min. Five hundred microliters of Wash Buffer I (with ethanol added) were added to the spin column and then centrifuged for 1 min at 10,000 rpm. The

flow-through was discarded and the column was placed back into the collection tube. Five hundred microliters of Wash Buffer II (with ethanol added) were added to the column and then centrifuged for 3 min at maximum speed ≥14,000 rpm. The collection tube was emptied and the purification column was placed back into the tube was re-spun for 1 min at maximum speed of 14,000 rpm. The collection tube containing the flow-through then was discarded and the column was transferred to a 1.5 ml microcentrifuge tube. One hundred µl of the Elution Buffer were added to the centre of the column membrane to elute the plant genomic DNA and then incubated for 5 min at room temperature and centrifuged for 1 min at 10,000 rpm. A second elution step was performed using 100 µl of Elution Buffer. The purified DNA then was stored at -20°C. The DNA purity and concentration was assessed using spectrophotometry.

2.2.2. DNA Amplification

The gene sequence were amplified by polymerase chain reaction (PCR) with the primer pair of trnH-psbA-f/trnH-psbA-r (5'-CGCGCATGGTGGATTCACAAATC -3’/5'-TGCATGGTTCCTTGGTAACTTC-3'), rps16F/rps16R (5'-GTGGTAGAAAGCAACGTGCGACTT-3’/5’-TCGGGATCGAACATCAATTGCAAC-3') (Popp and Oxelman, 2007), trnTF/trnFT (5'-CATTACAAATGCGATGCTCT-3'/5'-ATTTGAACTGGTGACACGAG-3’), and 2a-for/5a-bac (5'-GCCATCATGTCRTTCCACCA-3’/5'- TCRGCTGCATGGTTTGGAAC-3'), following the protocols in Table 3. PCR products from diploids and chloroplast gene from both diploids and polyploids were sequenced directly. All sequencing was performed by the TaiHe Technology (Beijing, China). Both forward and reverse strands were sequenced separately to improve the sequencing quality.

Table 3

The polymerase chain reaction (PCR) protocols of the four primer pairs used in this study.

Primers	**Initial**	**Exponential Amplification**				**Final**
	Denaturation	**Denaturation**	**Annealing**	**Elongation**		**Elongation**
rpsl6F/ rpsl6R	95 °C for 3 Min	95 °C for 40 Sec	63 °C for 40 sec	72 °C for 1 min	40x	72 °C for 10 min
trnTF/ trnFT	94 °C for 4 Min	94 °C for 1 Min	55 °C for 1 min	72 °C for 3 min	35x	72 °C for 10 min
trnH-psbA	94 °C for 3 Min	94 °C for 30 Sec	52 °C for 30 sec	72 °C for 2 min	35x	72 °C for 10 min
2a-for/ 5a-bac	95 °C for 4 Min	95 °C for 40 Sec	59-63 °C for 40 sec	72 °C for 2 min	40x	72 °C for 10 min

2.2.3. Cloning

PCR products of the nuclear gene amplified from polyploid *Hordeum* species were cloned using TOPO-TA kit from Invitrogen (Carlsbad, CA) following the manufacturer's protocol. Ten clones from each accession were randomly chosen for testing. Each colony was transferred to 150 µL of LB broth medium with antibiotics (0.1 mg-mL^{-1}) and then incubated for 1 hour at 37 °C before using 2 µL for PCR to confirm the existence of insert. 50 µL of positive clone solutions then transferred into 5 ml LB broth test tube (with 0.1 mg-mL^{-1} antibiotics) and incubated at 37 °C overnight while shaking at 250 rpm. Plasmid DNA was extracted by using Promega Wizard Plus Minipreps DNA Purification System (Promega Corporation, Madison, WI), following the manufacturer's instructions. Plasmid DNA was sequenced by the TaiHe Technology (Beijing, China). Both forward and reverse strands were sequenced separately to improve the sequencing results.

2.2.4. Data Analysis

ClustalX was used for multiple sequence alignments with default parameters (Thompson *et al.*, 1997). Phylogenetic analysis was performed using the maximum-parsimony (MP) method which was achieved with the computer program PAUP4.0 (Swofford, 2003). All characters were identified as unweighted and unordered. Heuristic search was done to obtain most-parsimonious tree using the Tree Bisection-Reconnection (TBR) option with MulTrees on. Characters analogy was assessed by the consistency index (CI), retention index (RI), rescaled consistency index (RC). Bootstrap values with 1000 replications (Felsenstein, 1985) were used to evaluate the robustness of the clades by performing a heuristic search using the TBR option with MulTrees on. In addition, maximum likelihood analysis was also performed. The approximate likelihood ratio test (ALR) value was used to evaluate robustness of the clades for ML phylogeny, which was achieved by using PHYML3.0 (Guindon *et al.*, 2010). Eight different substitution models were used (JC69, K80, F81, F84, HKY85, TN93, GTR and custom for nucleotides) for both chloroplast and nuclear data and finally the model with the highest log-likelihood value – GTR was used in our study.

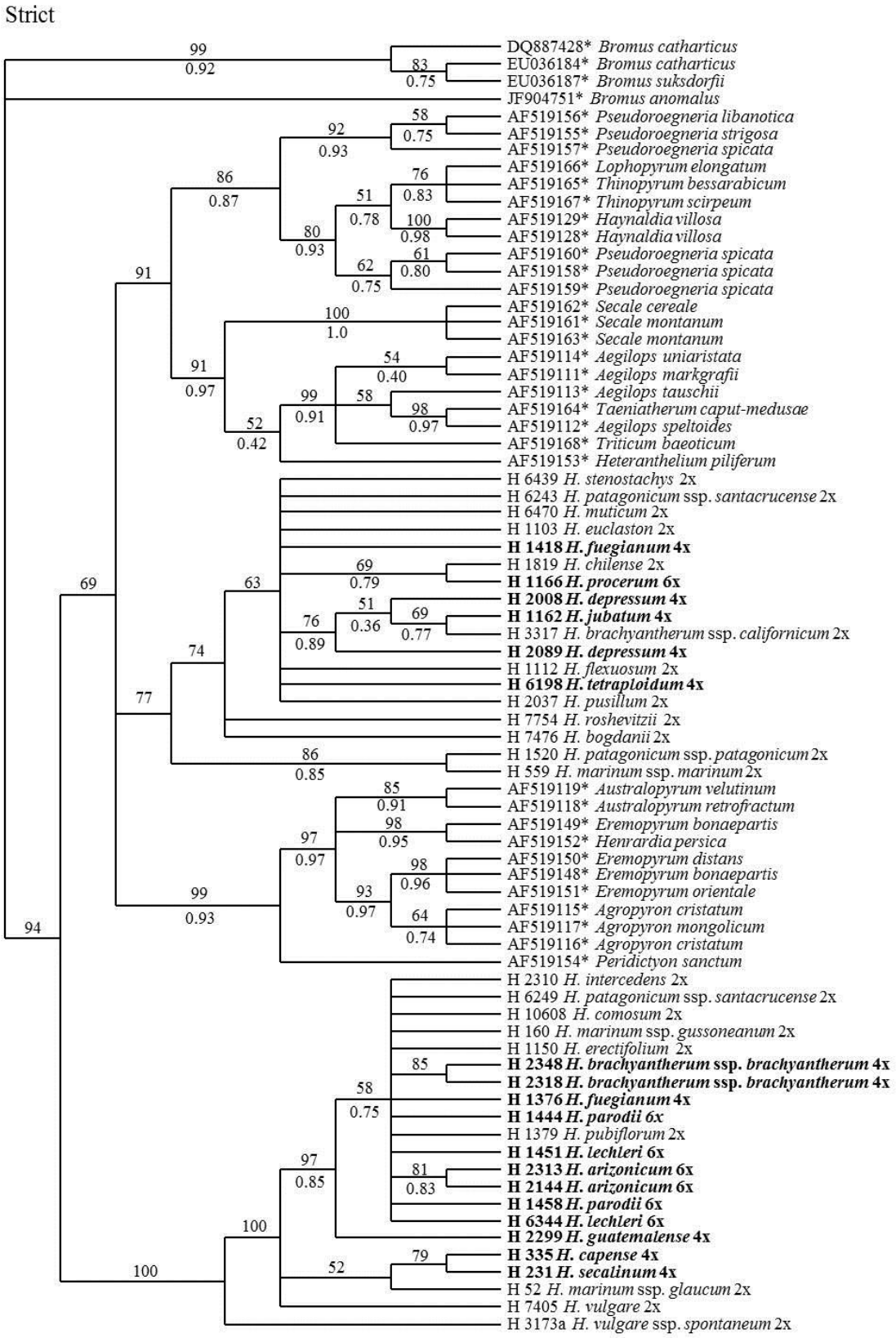
3. Results

3.1 Chloroplast DNA:

trnTF-trnFT: Seventy five trnTF-trnFT sequences were aligned. Thirty-nine sequences are from *Hordeum* species (twenty-one sequences from diploids and eighteen sequences from polyploids) and the remaining are sequences downloaded from GenBank for other species in the tribe Triticeae. Three sequences for *Bromus catharticus* and *Bromus suksdorfii* were used as outgroup. In total of 1632 characters were included in the final analysis; 549 characters were constant, 152 variable characters were parsimony-uninformative, and 931 characters were parsimony informative. Phylogenetic analysis based on trnTF-trnFT region sequences was done using the MP and ML methods. A strict consensus tree from the 1522 most-parsimonious trees is shown in Fig. 1, with consistency index = 0.873, retention index = 0.989, rescaled consistency index = 0.863. Both MP and ML analyses suggested that the diploid *H. brachyantherum* ssp. *californicum* is a potential maternal parent to the tetraploid *H. depressum*, and tetraploid *H. jubatum* with a bootstrap value of 76% and ALR value of 0.89. In addition, phylogenetic analysis suggested that the diploid *H. chilense* is a potential maternal parent to the hexaploid *H. procerum* with a bootstrap value of 69% and ALR value of 0.79.

Figure 1

A strict consensus tree obtained from the phylogenetic analysis of trnTF-trnFT intergenic spacer from 1522 most-parsimonious trees is shown. The numbers above the branches are bootstrap values from MP analysis and numbers below branches are approximate likelihood ratio (ALR) values from ML analysis. The * indicates the ones were downloaded from Genbank. Species written in bold are *Hordeum* polyploids.

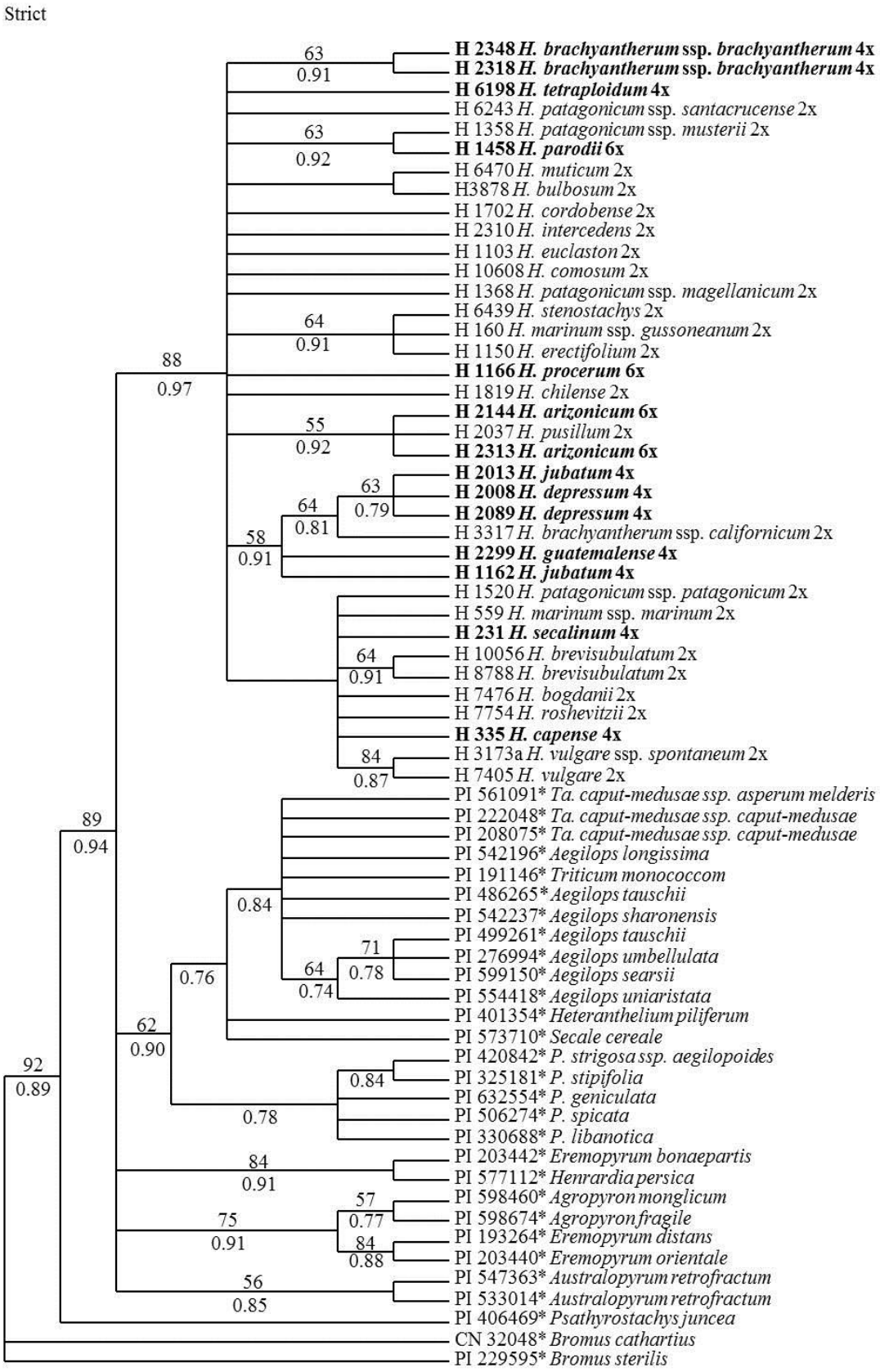

Strict
DQ887428* Bromus catharticus
EU036184* Bromus catharticus
EU036187* Bromus suksdorfii
JF904751* Bromus anomalus
AF519156* Pseudoroegneria libanotica
AF519155* Pseudoroegneria strigosa
AF519157* Pseudoroegneria spicata
AF519166* Lophopyrum elongatum
AF519165* Thinopyrum bessarabicum
AF519167* Thinopyrum scirpeum
AF519129* Haynaldia villosa
AF519128* Haynaldia villosa
AF519160* Pseudoroegneria spicata
AF519158* Pseudoroegneria spicata
AF519159* Pseudoroegneria spicata
AF519162* Secale cereale
AF519161* Secale montanum
AF519163* Secale montanum
AF519114* Aegilops uniaristata
AF519111* Aegilops markgrafii
AF519113* Aegilops tauschii
AF519164* Taeniatherum caput-medusae
AF519112* Aegilops speltoides
AF519168* Triticum baeoticum
AF519153* Heteranthelium piliferum
H 6439 H. stenostachys 2x
H 6243 H. patagonicum ssp. santacrucense 2x
H 6470 H. muticum 2x
H 1103 H. euclaston 2x
H 1418 H. fuegianum 4x
H 1819 H. chilense 2x
H 1166 H. procerum 6x
H 2008 H. depressum 4x
H 1162 H. jubatum 4x
H 3317 H. brachyantherum ssp. californicum 2x
H 2089 H. depressum 4x
H 1112 H. flexuosum 2x
H 6198 H. tetraploidum 4x
H 2037 H. pusillum 2x
H 7754 H. roshevitzii 2x
H 7476 H. bogdanii 2x
H 1520 H. patagonicum ssp. patagonicum 2x
H 559 H. marinum ssp. marinum 2x
AF519119* Australopyrum velutinum
AF519118* Australopyrum retrofractum
AF519149* Eremopyrum bonaepartis
AF519152* Henrardia persica
AF519150* Eremopyrum distans
AF519148* Eremopyrum bonaepartis
AF519151* Eremopyrum orientale
AF519115* Agropyron cristatum
AF519117* Agropyron mongolicum
AF519116* Agropyron cristatum
AF519154* Peridictyon sanctum
H 2310 H. intercedens 2x
H 6249 H. patagonicum ssp. santacrucense 2x
H 10608 H. comosum 2x
H 160 H. marinum ssp. gussoneanum 2x
H 1150 H. erectifolium 2x
H 2348 H. brachyantherum ssp. brachyantherum 4x
H 2318 H. brachyantherum ssp. brachyantherum 4x
H 1376 H. fuegianum 4x
H 1444 H. parodii 6x
H 1379 H. pubiflorum 2x
H 1451 H. lechleri 6x
H 2313 H. arizonicum 6x
H 2144 H. arizonicum 6x
H 1458 H. parodii 6x
H 6344 H. lechleri 6x
H 2299 H. guatemalense 4x
H 335 H. capense 4x
H 231 H. secalinum 4x
H 52 H. marinum ssp. glaucum 2x
H 7405 H. vulgare 2x
H 3173a H. vulgare ssp. spontaneum 2x

rps16: Phylogenetic analysis based on rps16 gene sequences was done using the MP and ML methods. Sixty six sequences for rps16 gene were aligned. Of which, thirty-seven sequences are from *Hordeum* species (twenty-three sequences from diploids and fourteen sequences from polyploids) and the remaining are sequences for other species in the tribe Triticeae. Three sequences for *Bromus catharticus* and *Bromus sterilis* were used as outgroup. Altogether 786 characters were used for the analysis; 711 characters were constant, 39 characters were parsimony-uninformative, and 36 characters were parsimony informative. A strict consensus tree from the 102 most-parsimonious trees is shown in Fig. 2 (consistency index = 0.843, retention index = 0.933, and rescaled consistency index = 0.786). MP and ML analyses resulted in highly similar phylogenetic trees. All *Hordeum* species were grouped together in one clade, with a bootstrap support value of 88% and ALR value of 0.97. Furthermore, both MP and ML trees suggested that the diploid *H. patagonicum* ssp. *musterii* is a potential maternal parent to the hexaploid *H. parodii*. In addition, both trees grouped the diploid *H. pusillum* with two different accessions of the hexploid *H. arizonicum*, with a bootstrap value of 55% and an ALR value of 0.92, suggesting that *H. pusillum* is a potential maternal parent to *H. arizonicum*. As well, all trees suggested that the diploid *H. branchyantherum* ssp. *californicum* is a potential maternal parent to the tetraploid *H. jubatum*,

tetraploid *H. depressum*, and tetraploid *H. guatemalense* with a bootstrap value of 58% and an ALR value of 0.91.

Figure 2

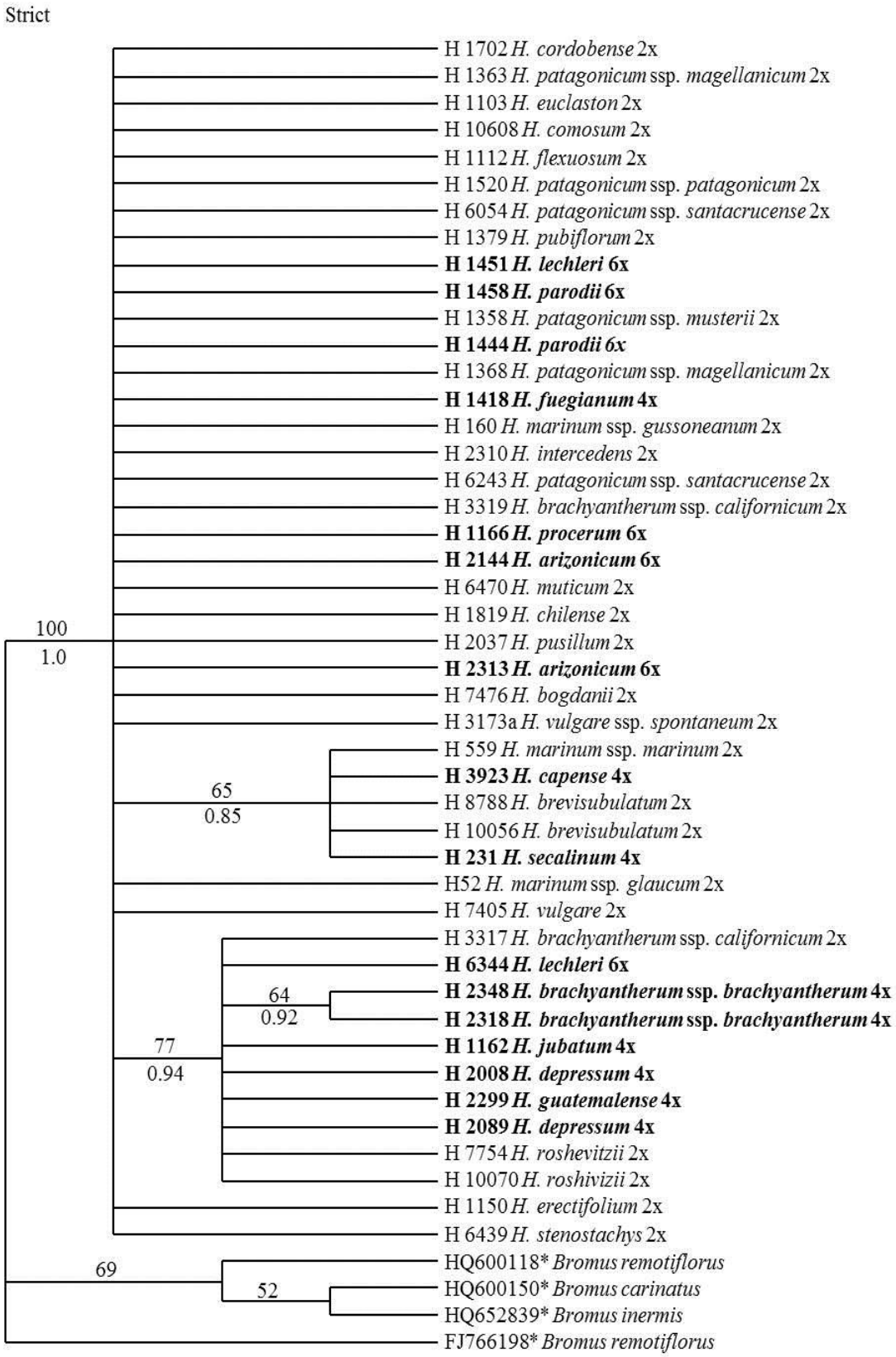
A strict consensus tree derived from 102 most-parsimonious trees based on rps16 gene is shown, with consistency index = 0.843 and retention index = 0.933. The numbers above the branches are bootstrap values from MP analysis and numbers below branches are approximate likelihood ratio (ALR) values from ML analysis. The * indicates the ones downloaded from GenBank. Species written in bold are *Hordeum* polyploids.


Strict
H 2348 H. brachyantherum ssp. brachyantherum 4x
H 2318 H. brachyantherum ssp. brachyantherum 4x
H 6198 H. tetraploidum 4x
H 6243 H. patagonicum ssp. santacrucense 2x
H 1358 H. patagonicum ssp. musterii 2x
H 1458 H. parodii 6x
H 6470 H. muticum 2x
H3878 H. bulbosum 2x
H 1702 H. cordobense 2x
H 2310 H. intercedens 2x
H 1103 H. euclaston 2x
H 10608 H. comosum 2x
H 1368 H. patagonicum ssp. magellanicum 2x
H 6439 H. stenostachys 2x
H 160 H. marinum ssp. gussoneanum 2x
H 1150 H. erectifolium 2x
H 1166 H. procerum 6x
H 1819 H. chilense 2x
H 2144 H. arizonicum 6x
H 2037 H. pusillum 2x
H 2313 H. arizonicum 6x
H 2013 H. jubatum 4x
H 2008 H. depressum 4x
H 2089 H. depressum 4x
H 3317 H. brachyantherum ssp. californicum 2x
H 2299 H. guatemalense 4x
H 1162 H. jubatum 4x
H 1520 H. patagonicum ssp. patagonicum 2x
H 559 H. marinum ssp. marinum 2x
H 231 H. secalinum 4x
H 10056 H. brevisubulatum 2x
H 8788 H. brevisubulatum 2x
H 7476 H. bogdanii 2x
H 7754 H. roshevitzii 2x
H 335 H. capense 4x
H 3173a H. vulgare ssp. spontaneum 2x
H 7405 H. vulgare 2x
PI 561091* Ta. caput-medusae ssp. asperum melderis
PI 222048* Ta. caput-medusae ssp. caput-medusae
PI 208075* Ta. caput-medusae ssp. caput-medusae
PI 542196* Aegilops longissima
PI 191146* Triticum monococcom
PI 486265* Aegilops tauschii
PI 542237* Aegilops sharonensis
PI 499261* Aegilops tauschii
PI 276994* Aegilops umbellulata
PI 599150* Aegilops searsii
PI 554418* Aegilops uniaristata
PI 401354* Heteranthelium piliferum
PI 573710* Secale cereale
PI 420842* P. strigosa ssp. aegilopoides
PI 325181* P. stipifolia
PI 632554* P. geniculata
PI 506274* P. spicata
PI 330688* P. libanotica
PI 203442* Eremopyrum bonaepartis
PI 577112* Henrardia persica
PI 598460* Agropyron monglicum
PI 598674* Agropyron fragile
PI 193264* Eremopyrum distans
PI 203440* Eremopyrum orientale
PI 547363* Australopyrum retrofractum
PI 533014* Australopyrum retrofractum
PI 406469* Psathyrostachys juncea
CN 32048* Bromus cathartius
PI 229595* Bromus sterilis
63
0.91
63
0.92
64
0.91
88
0.97
55
0.92
63
0.79
64
0.81
58
0.91
64
0.91
84
0.87
89
0.94
0.84
71
64
0.78
0.74
0.76
62
0.90
92
0.89
0.84
0.78
84
0.91
57
0.77
75
0.91
84
0.88
56
0.85

trnH-psbA: Forty-nine sequences were analyzed. Of the, forty-five sequences are from *Hordeum* species (twenty-nine sequences from diploid species and sixteen sequences from polyploid species) and the remaining are sequences downloaded from GenBank. *Bromus remotiflus*, *Bromus carinayus*, and *Bromus inermis* were used as outgroup. In total of 926 characters were included in the final analysis; 542 characters were constant, 33 characters were parsimony-uninformative, and 351 characters were parsimony informative. Phylogenetic analysis based on trnH-psbA sequences was done using the MP and ML methods. A strict consensus tree from the 408 most-parsimonious trees is shown in Fig. 3, with consistency index = 0.963, retention index = 0.987, rescaled consistency index = 0.950. MP and ML analyses resulted in similar phylogenetic trees, and suggested that either the diploid *H. brachyantherum* ssp. *californicum* or *H. roshevitzii* as a potential maternal parent to the hexaploid *H. lechleri*, tetraploid *H. brachyantherum* ssp. *brachyantherum*, tetraploid *H. jubatum*, tetraploid *H. depressum*, and *H. guatemalense* with a bootstrap value of 77% and ALR value of 0.94. In addition, Phylogenetic analyses grouped the diploid species *H. marinum* ssp. *marinum* and *H. brevisubulatum* with the tetraploid species *H. capense* and *H. secalinum*, with a bootstrap value of 65% and ALR value of 0.85.

Figure 3

A strict consensus tree obtained from 408 most-parsimonious trees based on trnH-psbA sequences is shown. The numbers above the branches are bootstrap values from MP analysis and numbers below branches are approximate likelihood ratio (ALR) values from ML analysis. The * indicates the ones were downloaded from Genbank. Species written in bold are *Hordeum* polyploids.


Strict
H 1702 H. cordobense 2x
H 1363 H. patagonicum ssp. magellanicum 2x
H 1103 H. euclaston 2x
H 10608 H. comosum 2x
H 1112 H. flexuosum 2x
H 1520 H. patagonicum ssp. patagonicum 2x
H 6054 H. patagonicum ssp. santacrucense 2x
H 1379 H. pubiflorum 2x
H 1451 H. lechleri 6x
H 1458 H. parodii 6x
H 1358 H. patagonicum ssp. musterii 2x
H 1444 H. parodii 6x
H 1368 H. patagonicum ssp. magellanicum 2x
H 1418 H. fuegianum 4x
H 160 H. marinum ssp. gussoneanum 2x
H 2310 H. intercedens 2x
H 6243 H. patagonicum ssp. santacrucense 2x
H 3319 H. brachyantherum ssp. californicum 2x
H 1166 H. procerum 6x
H 2144 H. arizonicum 6x
H 6470 H. muticum 2x
H 1819 H. chilense 2x
H 2037 H. pusillum 2x
H 2313 H. arizonicum 6x
H 7476 H. bogdanii 2x
H 3173a H. vulgare ssp. spontaneum 2x
H 559 H. marinum ssp. marinum 2x
H 3923 H. capense 4x
H 8788 H. brevisubulatum 2x
H 10056 H. brevisubulatum 2x
H 231 H. secalinum 4x
H52 H. marinum ssp. glaucum 2x
H 7405 H. vulgare 2x
H 3317 H. brachyantherum ssp. californicum 2x
H 6344 H. lechleri 6x
H 2348 H. brachyantherum ssp. brachyantherum 4x
H 2318 H. brachyantherum ssp. brachyantherum 4x
H 1162 H. jubatum 4x
H 2008 H. depressum 4x
H 2299 H. guatemalense 4x
H 2089 H. depressum 4x
H 7754 H. roshevitzii 2x
H 10070 H. roshivizii 2x
H 1150 H. erectifolium 2x
H 6439 H. stenostachys 2x
HQ600118* Bromus remotiflorus
HQ600150* Bromus carinatus
HQ652839* Bromus inermis
FJ766198* Bromus remotiflorus
100
1.0
65
0.85
64
0.92
77
0.94
69
52

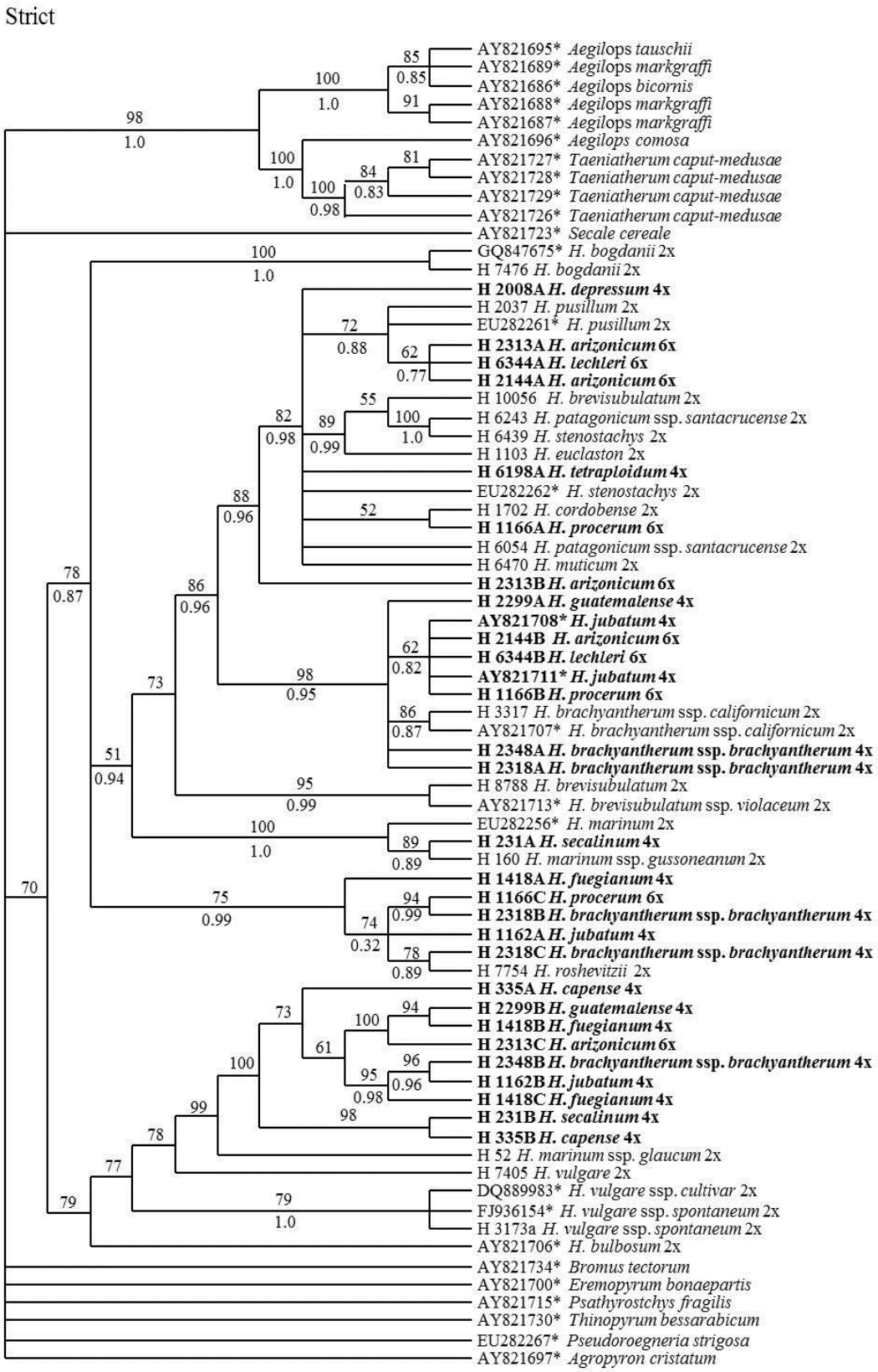
3.2 Nuclear DNA:

ß-amylase: Seventy-two ß-amylase sequences were analyzed, including twenty-nine *Hordeum* polyploid sequences, twenty-five *Hordeum* diploid species, and the remaining sequences are for other species in Triticeae except for *Bromus tectorum* as an outgroup. Overall 1448 characters were used in the analysis; 331 characters were constant, 191 variable characters were parsimony-uninformative, and 926 characters were parsimony-informative. Phylogenetic analysis was done using the MP, and ML methods using *Bromus tectorum* as an outgroup species. A strict consensus tree (Fig. 4) was obtained from 2337 most-parsimonious trees (consistency index=0.696, retention index=0.912, rescaled consistency index=0.635). MP and ML analyses resulted in similar phylogenetic tree. The MP tree suggested that diploid *H. cordobense* is a potential parent for the hexaploid *H. procerum* with bootstrap value of 52%. Also, MP and ML trees and suggested that the diploid *H. brachyantherum* ssp. *californicum* is a potential parent to the tetraploid *H. jubatum*, hexaploid *H. lechleri*, hexaploid *H. arizonicum*, tetraploid *H. branchyantherum* ssp. *brachyantherum*, tetraploid *H. guatemalense*, and hexaploid *H. procerum* with high bootstrap value of 98% and ALR value of 0.95. Furthermore, MP and ML trees grouped the diploid *H. roshevitzii* with tetraploid *H. jubatum*, tetraploid *H. branchyantherum* ssp. *branchyantherum*, hexaploid *H.*

procerum, and tetraploid *H. fuegianum* with a bootstrap value of 75% and ALR value of 0.99. In addition, the diploid *H. marinum* ssp. *gussoneanum* was grouped with tetraploid *H. secalinum* with a bootstrap value of 89% and ALR value of 0.89. Both MP and ML trees also suggests that the diploid *H. pusillum* is a potential parent to the hexaploid *H. lechleri* and hexaploid *H. arizonicum* with a bootstrap values of 72% and ALR value of 0.88. In total, thirty ß-amylase sequences were obtained for eleven polyploid species and were aligned using ClustalX. Only one copy of the gene was discovered for the tetraploid *H. depressum* and *H. tetraploidum*, while two different copies were found for the tetraploid species *H. guatemalense*, *H. jubatum*, *H. brachyantherum* ssp. *brachyantherum* (H 2348), *H. fuegianum*, *H. capense*, and *H. secalinum*. A third copy was identified for another accession (H 2318) of the tetraploid species *H. brachyantherum* ssp. *brachyantherum*. Three different copies were identified for the hexaploid species *H. arizonicum* and *H. procerum*, while only two copies were identified for the hexaploid *H. lechleri*.

Figure 4

A strict consensus tree constructed from a phylogenetic study of ß-amylase nuclear gene from the 2337 most parsimonious trees is shown, with CI = 0.696, RI = 0.912, and RC = 0.635. The tree topologies from MP and ML methods resulted in highly matching trees. Numbers of bootstrap values from MP analysis are placed above the branches and the other numbers below branches represent approximate likelihood ratio test (ALR) values from ML analysis. Species printed in bold are the polyploid species. The species *Bromus tectorum* is used as an outgroup. Species with * are downloaded from GenBank.


Strict
AY821695* Aegilops tauschii
AY821689* Aegilops markgraffi
AY821686* Aegilops bicornis
AY821688* Aegilops markgraffi
AY821687* Aegilops markgraffi
AY821696* Aegilops comosa
AY821727* Taeniatherum caput-medusae
AY821728* Taeniatherum caput-medusae
AY821729* Taeniatherum caput-medusae
AY821726* Taeniatherum caput-medusae
AY821723* Secale cereale
GQ847675* H. bogdanii 2x
H 7476 H. bogdanii 2x
H 2008A H. depressum 4x
H 2037 H. pusillum 2x
EU282261* H. pusillum 2x
H 2313A H. arizonicum 6x
H 6344A H. lechleri 6x
H 2144A H. arizonicum 6x
H 10056 H. brevisubulatum 2x
H 6243 H. patagonicum ssp. santacrucense 2x
H 6439 H. stenostachys 2x
H 1103 H. euclaston 2x
H 6198A H. tetraploidum 4x
EU282262* H. stenostachys 2x
H 1702 H. cordobense 2x
H 1166A H. procerum 6x
H 6054 H. patagonicum ssp. santacrucense 2x
H 6470 H. muticum 2x
H 2313B H. arizonicum 6x
H 2299A H. guatemalense 4x
AY821708* H. jubatum 4x
H 2144B H. arizonicum 6x
H 6344B H. lechleri 6x
AY821711* H. jubatum 4x
H 1166B H. procerum 6x
H 3317 H. brachyantherum ssp. californicum 2x
AY821707* H. brachyantherum ssp. californicum 2x
H 2348A H. brachyantherum ssp. brachyantherum 4x
H 2318A H. brachyantherum ssp. brachyantherum 4x
H 8788 H. brevisubulatum 2x
AY821713* H. brevisubulatum ssp. violaceum 2x
EU282256* H. marinum 2x
H 231A H. secalinum 4x
H 160 H. marinum ssp. gussoneanum 2x
H 1418A H. fuegianum 4x
H 1166C H. procerum 6x
H 2318B H. brachyantherum ssp. brachyantherum 4x
H 1162A H. jubatum 4x
H 2318C H. brachyantherum ssp. brachyantherum 4x
H 7754 H. roshevitzii 2x
H 335A H. capense 4x
H 2299B H. guatemalense 4x
H 1418B H. fuegianum 4x
H 2313C H. arizonicum 6x
H 2348B H. brachyantherum ssp. brachyantherum 4x
H 1162B H. jubatum 4x
H 1418C H. fuegianum 4x
H 231B H. secalinum 4x
H 335B H. capense 4x
H 52 H. marinum ssp. glaucum 2x
H 7405 H. vulgare 2x
DQ889983* H. vulgare ssp. cultivar 2x
FJ936154* H. vulgare ssp. spontaneum 2x
H 3173a H. vulgare ssp. spontaneum 2x
AY821706* H. bulbosum 2x
AY821734* Bromus tectorum
AY821700* Eremopyrum bonaepartis
AY821715* Psathyrostchys fragilis
AY821730* Thinopyrum bessarabicum
EU282267* Pseudoroegneria strigosa
AY821697* Agropyron cristatum

4. Discussion

4.1 *Hordeum* Tetraploid species origins

The tetraploid *H. depressum* is an annual plant throughout the western region of United States. The origins of the ploylpoid *H. depressum* have been a subject of discussion for a while now. In previous studies, *H. depressum* was suggested to have an autolpoid origin due to its high autosyndetic pairing nature (Sakamoto, 1974; Petersen, 1991). On the other hand, other studies suggested the alloploid origin of *H. depressum* with *H. brachyantherum* ssp. *californicum* as one of the parents and either *H. pusillum* or *H. intercedens* as the other parent (Taketa *et al*., 2005), which supported the suggestions of Covas (1949) and Baum and Baily (1988). Wang and Sun (2011) also supported *H. brachyantherum* ssp. *californicum* as one ancestor and suggested the diploid *H. euclaston* as the other parent to *H. depressum*. Our chloroplast phylogenetic trees based on trnTF-trnFT, rps16, and trnH-psbA regions support that *H. brachyantherum* ssp. *californicum* is the maternal parent to *H. depressum* as in previous studies (Doebley *et al*., 1992; Jakob and Blattner, 2006). Phylogeny based on the trnTF-trnFT sequence grouped two different accessions of the tetraploid *H. depressum* with the diploid *H. brachyantherum* ssp. *californicum*, with a bootstrap value of 76% and ALR value of 0.89 (Fig. 1). In addition, rps16 data also placed *H. depressum* together with *H. brachyantherum* ssp.

californicum in a bootstrap value of 64% and an ALR value of 0.81 (Fig. 2). Although, the resolution of trnH-psbA region is not high enough to infer the maternal genome donor of *H. depressum*, it does not contradict with other phylogenetic data in this study. The trnH-psbA analysis grouped both accessions of *H. depressum* with the diploids *H. brachyantherum* ssp. *californicum* and *H. roshevitzii* and other polyploids *H. lecheri*, *H. brachyantherum* ssp. *brachyantherum*, *H. jubatum*, *H. guatemalense* with a bootstrap value of 77% and ALR value of 0.94 (Fig. 3), suggesting either *H. brachyantherum* ssp. *californicum* or *H. roshevitzii* as the potential maternal parent to *H. depressum*. Hence, all of our chloroplast DNA results further confirm that *H. brachyantherum* ssp. *californicum* as one parent to *H. depressum* (Covas, 1949; Baum and Bailey, 1988; Doebley *et al.*, 1992; Taketa *et al*., 2005; Jakob and Blattner, 2006).

Unfortunately, the resolution of ß-amylase phylogeny was not high enough to infer the other parent *to H. depressum*. Only one copy of *H. depressum* was identified, which was grouped with other polyploid species including *H. arizonicum*, *H. lechleri*, *H. tetraploidum*, and *H. procerum* and diploid species including *H. pusillum, H. brevisubuluatum, H. patagonicum* ssp. *santacrucense*, *H. euclaston*, *H. stenostachys*, *H. cordobense*, and *H. muticum* with a bootstrap value of 82% and ALR value of 0.98 (Fig. 4), suggesting any of these diploids as a potential parent to *H. depressum*.

Further research using nuclear DNA is needed to investigate the true paternal genome donor for *H. depressum*.

Tetraploid *H. brachyantherum* ssp. *brachyantherum* is a perennial plant. Previous studies suggested that *H. brachyantherum* ssp. *californicum* is one of the genome donors to *H. brachyantherum* ssp. *brachyantherum* using karyotype analysis (Linde-Laursen *et al.*, 1995), RFLP and FISH pattern (Taketa *et al.*, 2005) and nuclear DNA (Wang and Sun, 2011). Other studies supported that *H. brachyantherum* ssp. *californicum* as the maternal parent of *H. brachyantherum* ssp. *brachyantherum* using chloroplast DNA data (Nishikawa *et al.*, 2002; Jakob and Blattner, 2006). In this study, the ß-amylase phylogenetic tree grouped the first two copies of different accessions (H 2348, H 2318) of *H. brachyantherum* ssp. *brachyantherum* with the diploid *H. brachyantherum* ssp. *californicum* and other polyploids *H. jubatum*, *H. procerum*, *H. lechleri*, *H. arizonicum*, *H. guatmalense* with a high bootstrap value of 98% and ALR value of 0.95 (Fig. 4), thus further confirming that *H. brachyantherum* ssp. *californicum* as a genome donor to *H. brachyantherum* ssp. *brachyantherum*. A second and a third copy of *H. brachyantherum* ssp. *brachyantherum* was identified for the accession H 2318, which both were grouped with the diploid *H. roshevitzii* with 75% bootstrap support and ALR value of 0.99 (Fig. 4),

suggesting that Old World species *H. roshevitzii* as a possible parent to *H. brachyantherum* ssp. *brachyantherum* which supports RFLP and FISH pattern results of Taketa et al (2005) and Blattner (2004) results based on rDNA ITS sequences. The second copy of *H. brachyantherum* ssp. *brachyantherum* from H 2348 accession was grouped with the polyploid species *H. arizonicum, H. jubatum, H. fuegianum,* and *H. guatemalense* suggesting a common ancestor which could be *H. brachyantherum* ssp. *californicum* with bootstrap support of 61%. This second copy was grouped closely with *H. jubatum* with a bootstrap value of 96% and ALR value of 0.96 (Fig. 4), indicating that *H. brachyantherum* ssp. *brachyantherum* is mostly related to *H. jubatum*. More than two ß-amylase gene copies were found from this tetraploid species, which could be explained by gene introgression, as this was previously described in Triticeae genus *Elymus* (Mason-Gamer, 2004; Fortune *et al.*, 2008). However, trnTF-trnFT phylogenetic tree resolution based on a choloroplast DNA was not able to infer the maternal genome donor of *H. brachyantherum* ssp. *brachyantherum,* as it grouped two accessions (H 2318, H 2348) of *H. brachyantherum* ssp. *brachyantherum* with the diploid species *H. intercedens, H. patagonicum* ssp. *santaccrucense, H. comosum, H. erectifolium, H. pubiflorum, H. marinum* ssp. *gussoneanum* with a 58% bootstrap value and ALR value of 0.75 (Fig. 1), suggesting any of these diploid species as a potential maternal parent to *H. brachyantherum* ssp.

brachyantherum. The trnH-psbA tree grouped two accessions of *H. brachyantherum* ssp. *brachyantherum* and other polyploid species including *H. lechleri, H. jubatum, H. depressum*, *H. guatemalense* with the diploid species *H. brachyantherum* ssp. *californicum* and two different accession of (H 7754, H 10070) *H. roshevitzii* in a bootsrtap value of 77% and ALR value of 0.94 (Fig. 3), suggesting either *H. brachyantherum* ssp. *californicum* or *H. roshevitzii* as a maternal parent to *H. brachyantherum* ssp. *brachyantherum*, which does not contradict the results from the ß-amylase nuclear data. Due to low resolution, the rps16 phylogenetic tree wasn't able to infer the maternal parent to *H. brachyantherum* ssp. *brachyantherum*. Further studies needed to confirm the genome donor for *H. brachyantherum* ssp. *brachyantherum*.

The *H. jubatum* is a perennial tetraploid species. Previous studies suggested that *H. brachyantherum* ssp. *californicum* is one of the genome donors to *H. jubatum* using karyotype analysis (Linde-Laursen *et al.*, 1995) and was supported by RFLP and FISH pattern (Taketa *et al.*, 2005). Also, rDNA ITS sequences suggested that *H. roshevitzii* is a parent to the tetraploid *H. jubatum* (Blattner, 2004). In the present study, all chloroplast DNA results confirmed that *H. brachyantherum* ssp. *californicum* as a maternal parent to *H. jubatum*. In the rps16 phylogeny, two different

accessions (H 2013, H 1162) of *H. jubatum* were grouped together with *H. brachyantherum* ssp. *californicum* with 58% bootstrap value and 0.91 ALR value (Fig. 2). In addition, trnTF-trnFT tree also grouped *H. jubatum* with *H. brachyantherum* ssp. *californicum* with a bootstrap value of 69% and ALR value of 0.77 (Fig. 1). Furthermore, in trnH-psbA phylogeny *H. jubatum* was grouped with *H. brachyantherum* ssp. *californicum* with a bootstrap value of 77% and ALR value of 0.94 (Fig. 3), thus confirming that *H. brachyantherum* ssp. *californicum* the maternal genome donor of the tetraploid *H. jubatum*. In addition, our nuclear dataset strongly support *H. brachyantherum* ssp. *californicum* as a genome donor to *H. jubatum*. In the present study, two copies of ß-amylase gene were discovered for accession H 1162 and another two copies were downloaded from GenBank (AY821708 and AY821711). The ß-amylase phylogenetic tree placed one copy of *H. jubatum* (accession H 1162A) with other polyploid species including *H. fuegiaunm*, *H. procerum*, and *H. brachyantherum* ssp. *brachyantherum* and the diploid species *H. roshevitzii* with a bootstrap value of 75% and ALR value of 0.99 (Fig.4), suggesting *H. roshevitzii* as a parent to *H. jubatum*, thus supporting the results from rDNA ITS sequences (Blattner, 2004) and FISH pattern and RFLP profiles (Taketa *et al.*, 2005). The other copy of *H. jubatum* (H 1162B) was grouped with polyploid species *H. guatemalense*, *H. fuegianum*, *H. arizonicum*, *H. brachyantherum* ssp. *brachyantherum* with a bootstrap value of 61% (Fig. 4), suggesting

they share a common ancestor which could be *H. brachyantherum* ssp. *californicum*. As two copies of *H. jubatum* that were downloaded from GenBank (AY821708 and AY821711), were grouped with polyploid species *H. guatemalense*, *H. arizonicum*, *H. lechleri*, *H. procerum*, and *H. brachyantherum* ssp. *brachyantherum* and the diploid species *H. brachyantherum* ssp. *californicum* with high bootstrsap value of 98% and ALR value of 0.95 (Fig. 4), suggesting *H. brachyantherum* ssp. *californicum* as a parent to *H. jubatum*.

The tetraploid *H. fuegianum* is a perennial species. FISH pattern, RFLP profiles (Taketa *et al.*, 2005), and rDNA ITS sequences (Blattner, 2004) indicated the diploid *H. roshevitzii* as one parent to tetraploid *H. fuegianum*. This is supported by our ß-amylase results as it grouped one copy of *H. fuegianum* sequence with the diploid *H. roshevitzii* in a bootstrap value of 75% and ALR value of 0.99 (Fig. 4), suggesting *H. roshevitzii* as a potential parent to *H. fuegianum*. The second copy was grouped with other polyploid species *H. guatemalense*, *H. jubatum*, *H. arizonicum*, *H. brachyantherum* ssp. *brachyantherum* with a bootstrap value of 61% (Fig. 4), suggesting they share a common ancestor which could be *H. brachyantherum* ssp. *californicum.* In trnTF-FT phylogeny, the resolution was not high enough to infer the maternal parent to *H.*

fuegianum. One accession (H 1418) of *H. fuegianum* was grouped with diploid species, including *H. stenostachys*, *H. patagonicum* ssp. *santacrucense, H. muticum, H. euclaston, H. chilense, H. brachyantherum* ssp. *californicum, H. flexuosum H. pusillum, H. roshivitzii,* and *H. bogdanii* with a bootstrap value of 63% (Fig. 1), suggesting any of these diploids as a potential maternal parent to *H. fuegianum*. However, the other accession of *H. fuegianum* (H 1376) was grouped with the diploids *H. intercedens, H. patagonicum* ssp. *santacrucense, H. comsum, H. marinum* ssp. *gussoneanum, H. erectifolium*, and *H. pubiflorum* with a bootstrap value of 58% and ALR value of 0.75 (Fig. 1), suggesting one of these diploids as a potential maternal genome donor to *H. fuegianum*. Also, the trnH-psbA phylogeny resolution was not able to infer the direct maternal parent to *H. fuegianum*, due to a low level of variation in the gene. Nevertheless, these results are not enough to infer the other genome donor to *H. fuegianum*, further studies needed to discover the other parent to *H. fuegianum*.

Hordeum guatemalense is perennial tetraploid, which is distributed in northern Guatemala near Mexico. Previous study suggested *H. brahcyantherum* ssp. *californicum* as a maternal parent to *H. guatemalense* (Nishikawa *et al*., 2002). In our study, two distinct copies of ß-amylase sequences from *H. guatemalense* were encountered. One copy of *H.*

guatemalense was grouped closely with ploygploid species *H. fuegianum*, and *H. arizonicum* with a high bootstrap value of 100% (Fig. 4), suggesting they all share a common ancestor. While, the other copy of *H. guatemalense* was placed in a group with polyploid species *H. jubatum*, *H. procerum*, *H. brachyantherum* ssp. *brachyantherum*, *H. arizonicum*, *H. lechleri* and the diploid species *H. brachyantherum* ssp. *californicum* with a high 98% bootstrap value and 0.95 ALR value (Fig. 4), hence, suggesting *H. brachyantherum* ssp. *californicum* as one parent to *H. guatemalense*. Furthermore, this was confirmed by rps16 phylogeny as it grouped *H. guatemalnese* with *H. brachyantherum* ssp. *californicum* and other polyploids *H. depressum*, and *H. jubatum* with a 58% bootstrap value and ALR value of 0.91 (Fig. 2), suggesting *H. brachyantherum* ssp. *californicum* as a maternal genome donor to *H. guatemalense*. In addition, trnH-psbA phylogeny grouped *H. guatemalense* with the diploid species *H. brachyantherum* ssp. *californicum* and *H. roshevitzii* with a bootstrap value of 77% and ALR value of 0.94 (Fig. 3). However, trnTF-FT phylogenies resolution was not high enough to infer the direct maternal genome donor to *H. guatemalense*. Accordingly, further research is needed to confirm the paternal parent to *H. guatemalense*.

Several previous studies have proposed that European *H. secalinum* and South African *H. capense* are closely related (Stapf, 1900; von Bothmer and Jacobsen, 1979) and of allotetraploid origin, which share a common hybrid origin involving *H. marinum* ssp. *gussoneanum* and *H. brevisubulatum* (Petersen and Seberg, 2004). Baum and Johnson (2003) suggested the diploid *H. marinum* as a potential genome donor to *H. secalinum* (Svitashev *et al*., 1994; Komatsuda et al., 2001), and the diploid *H. muticum* as potential parent to *H. capense*. In the present study, two different copies of ß-amylase were discovered for *H. secalinum*. One copy of *H. secalinum* was grouped with two different accession of the diploid *H. marinum* ssp. *gussoneanum* in a high bootstrap value of 100% and ALR value of 1.0 (Fig. 4), strongly supporting that *H. marinum* ssp. *gussoneanum* as one parent to *H. secalinum*. While the second copy of *H. secalinum* was grouped closely with the tetraploid *H. capense* with a bootstrap value of 98% (Fig. 4), suggesting they probably share a common ancestor, which they were grouped with the diploids *H. marinum* ssp. *glaucum* and *H. vulgare*, suggesting that one of these diploids was the potential parent to *H. secalinum* and *H. capense*. The trnTF-FT phylogeny supported the nuclear DNA results as it grouped *H. capense* and *H. secalinum* with the diploid *H. marinum* ssp. *glaucum*, suggesting *H. marinum* ssp. *glaucum* as a maternal parent to *H. secalinum* and *H. capense*. However, In the rps16 phylogeny (Fig. 2), *H. secalinum* and *H.*

capense were grouped with other diploids *H. patagonicum* ssp. *patagonicum*, *H. marinum* ssp. *marinum, H. brevisubulatum*, *H. bogdanii*, *H. roshevitzii*, and *H. vulgare*. Yet, the rps16 results resolution is not high enough to infer the maternal parent to *H. secalinum* and *H. capense*. The trnH-psbA results grouped *H. secalinum* and *H. capense* with H. *marinum* ssp. *marinum*, and two accessions of H. *brevisubulatum* with a bootstrap value of 65% and ALR value of 0.85 (Fig. 3). Hence, our study suggest that *H. marinum* subspecies and *H. brevisubulatum* as possible genome donors to *H. capense* and *H. secalinum*, hence the close relatedness between *H. capense* and *H. secalinum*.

4.2 *Hordeum* Hexaploid species origins

4.2.1 Origins of the hexaploid *Hordeum lechleri*

The hexaploid *H. lechleri* is a perennial species which is distributed in South America. Previous studies suggested tetraploid *H. jubatum* as one of the genome donors to *H. lechleri* (Taketa *et al*., 2005) and *H. brevisubulatum* as a genome donor to *H. lechleri* (Wang and Sun, 2011). In the present study, only two distinct copies of ß-amylase for the hexaploid *H. lechleri* were identified. The first copy of *H. lechleri* was grouped with

two copies from two accessions of hexaploid *H. arizonicum* and the diploid *H. pusillum* with a bootstrap value of 72% and ALR value of 0.88 (Fig. 4), suggesting that *H. pusillum* as a potential genome donor to both *H. lechleri* and *H. arizonicum*. The second copy of *H. lechleri* was placed in a group with tetraploid *H. guatemalense*, tetraploid *H. jubatum*, hexaploid *H. arizonicum*, hexaploid *H. procerum*, tetraploid *H. brachyantherum* ssp. *brchyantherum*, and the diploid species *H. brachyantherum* ssp. *californicum* with a bootstrap value of 98% and ALR value of 0.95 (Fig. 4), suggesting that *H. brachyantherum* ssp. *californicum* is a potential genome donor to *H. lechleri*. This second copy was placed closely with the tetraploid *H. jubatu*m with a bootstrap value of 62% and ALR value of 0.82, which further support previous studies suggesting *H. jubatum* as a possible genome donor to *H. lechleri*. The trnTF-FT placed *H. lechleri* with two different accessions of hexaploid *H. parodii*, tetraploid *H. fuegianum*, and the diploids *H. intercedens*, *H. patagonicum* ssp. *santacrucense*, *H. comosum*, *H. marinum ssp. gussoneanum*, *H. erectifolium*, and *H. pubiflorum* with a bootstrap value of 58% and ALR value of 0.75 (Fig. 1), suggesting any of these species as a potential maternal genome donor to *H. lechleri*. The trnH-psbA phylogeny resolution was not able to infer the direct maternal parent to *H. lechleri*, due to a low level of variation in the gene.

4.2.2 Origins of the hexaploid *Hordeum arizonicum*

The hexaploid *H. arizonicum* is annual/biennial species, which is distributed in North America. Previous studies considered *H. arizonicum* to have an allopolyploidy origin from a tetraploid and a diploid species. They suggested that diploid *H. pusillum* and tetraploid *H. jubatum* are the genome donors to *H. arizonicum* (Rajhathy and Symko, 1996), which was supported by rDNA ITS data of Blattner (2004) and FISH and RFLP patterns of Taketa *et al*. (2005). Nishikawa *et al*. (2002), using cpDNA, suggested that the diploid *H. pusillum* could be the maternal genome donor to *H. arizonicum*. A recent study suggested *H. brachyantherum* ssp. *californicum* as one ancestor to *H. arizonicum* (Wang and Sun, 2011). In the present study, the rps16 phylogeny placed two different accessions of *H. arizonicum* in a group with diploid *H. pusillum* with a bootstrap value of 55% and ALR value of 0.92 (Fig. 2), further suggesting that *H. pusillum* is a potential maternal parent to *H. arizonicum*. Two different accessions (H 2313, H 2144) for *H. arizonicum* were used in the ß-amylase phylogeny. Three different copies were found for H 2313 and only two copies for H 2144 suggesting *H. arizonicum* was originated from three distinct genome donors. The first copy of both accessions were placed in a group with hexaploid *H. lechleri* and diploid *H. pusillum* with a bootstrap value of 72% and ALR value of 0.88 (Fig. 4), further confirming the results from

previous studies that suggest that *H. pusillum* is one parent to *H. arizonicum*. The second copy of H 2144 was grouped polyploid species *H. jubatum, H. procerum, H. lechleri, H. brachyantherum* ssp. *brachyantherum,* and *H. guatemalense* with the diploid *H. brachantherum* ssp. *californicum* with a high bootstrap value of 96% and ALR value of 0.96 (Fig. 4), suggesting that diploid *H. brachantherum* ssp. *californicum* is a potential second genome donor to *H. arizonicum*, thus supporting the suggestions by Wang and Sun (2011). The second copy position of H 2313 was placed in a clade with the diploid species *H. pusillum*, *H. brevisubulatum*, *H. patagonicum* ssp. *santacrucense*, *H. stenostachys*, *H. euclaston*, *H. cordobense*, and *H. muticum*, which was not clear enough to infer a direct genome donor to *H. arizonicum*. The third copy of H 2313 was grouped closely with other polyploid species *H. fuegianum* and *H. guatemalense* with a high bootstrap value of 100% (Fig. 4), suggesting that they share at least one common ancestor which could be the third genome donor to *H. arizonicum*. However, the trnTF-FT phylogeny results (Fig. 1) grouped *H. arizonicum* with other polyploid species and the diploid species *H. intercedens*, *H. patagonicum* ssp. *santacrucense*, *H. comosum, H. marinum ssp. gussoneanum, H. erectifolium*, and *H. pubiflorum*, with a bootstrap value of 58% and ALR value 0.75 (Fig. 1). The resolution of this clade was low and not enough to infer the direct maternal genome donor to *H. arizonicum*.

4.2.3 Origins of the hexaploid *Hordeum procerum*

The hexaploid *H. procerum* is a perennial species, which is distributed in southeastern South America, and considered to have an allopolyploid origin. Linde-Laursen *et al.* (1990) used C-banding pattern and morphology of SAT chromosomes proposed that the diploid *H. cordobense* is one genome donor to *H. procerum*, which was supported by Wang and Sun (2011). Blattner (2004) also supported the diploid species *H. cordobense* and tetraploid species *H. tetraploidum* as parents to *H. procerum*. Furthermore, *H. tetraploidum* was suggested as one of the ancestors to *H. procerum* by Taketa *et al*. (2005). *H. brachyantherum* ssp. *californicum* was suggested as another genome donor to *H. procerum* (Wang and Sun, 2011). In ß-amylase phylogeny, three different copies were discovered for the hexaploid species *H. procerum*. The first copy was grouped with the diploid species *H. cordobense* in a 52% bootstrap value (Fig. 4), supporting previous studies that suggested *H. cordobense* as a potential parent to *H. procerum*.

The second copy was placed in a group with other polyploids species *H. jubatum, H. lechleri, H. arizonicum, H. brachyantherum* ssp. *brachyantherum*, and *H. gutemalense* with the diploid *H. brachyantherum* ssp. *californicum*, with a high bootstrap value of 98% and ALR value 0.96 (Fig. 4), suggesting that *H. brachyantherum* ssp. *californicum* was another

parent to *H. procerum* supporting suggestion of Wand and Sun, 2011. The third copy was grouped with other polyploid species *H. jubatum, H. brachantherum* ssp. *brachyentherum*, *H. fuegianum* and the diploid species *H. roshevitzii* with a bootstrap value of 75% and high ALR value 0.99 (Fig. 4), thus, suggesting that *H. roshevitzii* as the third parent to *H. procerum*. In trnTF-trnFT phylogeny, *H. procerum* was grouped with the diploid species *H. chilense* with a bootstrap value of 69% and ALR 0.79 (Fig. 1), suggesting *H. chilense* as a maternal parent to *H. procerum*, which contradicts with the nuclear data results. Further study is needed to determine the maternal parent to *H. procerum*.

4.2.4 Origins of the hexaploid *Hordeum parodii*

The hexaploid *H. parodii* is a perennial species distributed in South America. C-banding pattern and marker SAT chromosomes morphology suggested that allopolyploidy origin of *H. parodii* was from the diploid species *H. muticum* and tetraploid species *H. tetraploidum* (Linde-Laursen *et al*., 1990). In addition, rDNA sequences (Blattner, 2004), and FISH and RFLP results (Taketa *et al,* 2005) supported that *H. tetraploidum* is a genome donor to *H. parodii*. Nuclear DNA study suggested the tetraploid *H. tetraploidum* and the diploid *H. flexuosum* as parents to *H. parodii* (Wang and Sun, 2011). In the present study, we were only able to amplify

cpDNA, which rps16 phylogeny grouped *H. parodii* with *H. patagonicum* ssp. *musterii* with a bootstrap value of 63% and a high ALR value of 0.92 (Fig. 2), suggesting that *H. patagonicum* ssp. *musterii* as a potential maternal parent to *H. parodii*. This contradicts Wang and Sun, 2011 suggestion of *H. flexuosum* as a potential maternal parent to *H. parodii*. On the other hand, trnTF-FT phylogeny grouped two different accessions of *H. parodii* with the diploid species *H. intercedens*, *H. patagonicum* ssp. *santacrucense*, *H. comosum*, *H. marinum* ssp. *gussoneanum*, *H. erectifolium*, and *H. pubiflorum* with a bootstrap value of 58% and ALR value 0.75 (Fig. 1), suggesting one of these diploid species as a possible maternal genome donor to *H. parodii*. Unfortunately, the resolution from trnH-psbA phylogeny was low and could not infer the direct maternal parent to *H. parodii*. More research is needed using nuclear DNA to confirm the other parents to *H. parodii*.

5. Conclusion

This study examined the origins of *Hordeum* polyploid species. For tetraploid species, our study support previous suggestions that *H. brachyantherum ssp. californicum* was one parent to *H. brachyantherum ssp. brachyantherum*, *H. jubatum*, and *H. guatemalense*. The nuclear DNA results also support previous studies suggesting that *H. roshevitzii* as the other parent to tetraploid *H. brachyantherum* ssp. *brachyantherum, H. jubatum,* and *H. fuegianum*. The study also confirms *H. marinum* subspecies as genome donors to the closely related tetraploid species *H. secalinum* and *H. capense*. Finally, the study suggests *H. brachyantherum* ssp. *californicum* and as the maternal diploid parents of *H. depressum*.

For hexaploid species, our study further support *H. cordobense* as one parent to the hexaploid species *H. procerum*. Also, the nuclear DNA results showed that *H. brahcyantherum* ssp. *californicum* and *H. roshevitzii* was the other genome donors to *H. procerum*. In addition, results suggest the diploids *H. pusillum*, and *H. brachyantherum* ssp. *californicum* as diploid genome donors to *H. lechleri* and support *H. jubatum* as a tetraploid genome donor to *H. lechleri*. The study further confirms *H. pusillum* as the diploid parent to *H. arizonicum* and suggests *H. brachyantherum* ssp. *californicum* as another diploid genome donor to *H. arizonicum*, and futher suggests *H. jubatum* as a tetraploid genome donor to *H. arizonicum*.

Finally, this study suggests *H. patagonicum* ssp. *musterii* as possible maternal genome donor to *H. parodii*.

6. References

Baum, B.R., and Bailey, L.G. 1988. A taxanomic study of the annual *Hordeum depressum* and related species. *Canadian Journal of Botany* **66:** 401-408.

Baum, B.R., and Bailey, L.G. 1991. Relationships among native and introduced North American species of *Hordeum*, based on chloroplast DNA restriction site variation. *Canadian Journal of Botany* **69:** 2421-2426.

Baum, B.R., Johnson, D.A., Baily, L.G. 2001. Defining orthologous groups among multicopy genes prior to inferring phylogeny, with special emphasis on the Triticeae (Poaceae). *Hereditas* **135:** 123–138.

Blattner, F.R. 2004. Phylogenetic analysis of *Hordeum (Poaceae)* as inferred by nuclear rDNA ITS sequences. *Molecular Phylogenetics and Evolution* **33:** 289-299.

Blattner, F.R. 2006. Multiple intercontinental dispersals shaped the distribution area of *Hordeum* (Poaceae). *New Phylogist* **169:** 603-614.

Blattner, F.R. 2009. Progress in phylogenetic analysis and a new infrageneric classification of the barley genus *Hordeum* (Poaceae:Triticeae). *Breed Science* **59(5):** 471–480.

von Bothmer, R., and Jacobsen, N. 1979. A taxonomic revision of *Hordeum secalinum* and *H. capense*. *Botanisk Tidsskrift* **74:** 223–235.

von Bothmer, R., Fink, J., Landstrom, T. 1986. Meiosis in interspecific *Hordeum* hybrids. I. Diploid combinations. *Canadian Journal of Genetics and Cytology* **28:** 525-535.

von Bothmer, R., Fink, J., Landstrom, T. 1987. Meiosis in interspecific *Hordeum* hybrids. II. Triploid hybrids. *Evolutionary Trends in Plants* **1:** 42-50.

von Bothmer, R., Fink, J., Landstrom, T. 1988. Meiosis in interspecific *Hordeum* hybrids. IV. Tetraploid (4x X 4x) hybrids. *Genome* **30:** 479-485.

von Bothmer, R., Jacobsen, N., Baden, C., Jorgensen, R.B., Linde-Laursen, I. 1995. An ecogeographical study of the genus *Hordeum*. *Second edn. Rome*: IBPGR.

Covas, G. 1949. Taxonomic observations on the North American species of *Hordeum*. *Madorono* **10:** 1-21.

Cui, L., Wall, P.K., Leebcns-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 Research* **16:** 738-749.

Doebley, J., von Bothmer, R., Larson, S. 1992. Chloroplast DNA variation and the phylogeny of *Hordeum* (Poaceae). *American Journal of Botany* **79:** 576-584.

El-Rabey, H.A., Badr, A., Scafer-Pregl, R., Martin, W., Salamini, F. 2002. Speciation and species separation in *Hordeum* L. (Poaceae) resolved by discontinuous molecular markers. *Plant Biology* **4:** 567-575.

Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39:** 783-791.

Felsenstein J. 2004. Inferring phylogenies. Massachusetts: *Sinauer Associates, Incorporated, Inc.* ISBN 0878931775, 9780878931774.

Fortune, P.M., Pourtau, N., Viron, N., Ainouche, M.L. 2008. Molecular phylogeny and reticulate origins of the polyploid *Bromus* species from section *Genea* (Poaceae). *American Journal of Botany* **95:** 454-464.

Galtier, N., and Daubin, V. 2008. Dealing with incongruence in phylogenetic analyses. *Philosophical transactions of the royal society B.* **363:** 4023-4029.

Gaut, B.S. 1998. Molecular clocks and nucleotide substitution rates in higher plants. *Evolutionary Biology* **30:** 93-120.

Guindon, S., Dufayard, J.F., Lefort, V., Anisimova, M., Hordijk, W., Gascuel, O. 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0, *Systematic Biology*. **59:** 307-321.

Jakob, S.S., and Blattner, F.R. 2006. A chloroplast genealogy of *Hordeum* (Poaceae): Long-term persisting haplotypes, incomplete lineage sorting, regional extinction, and the consequences for phylogenetic inference. *Molecular Biology and Evolution* **23:** 1602-1612.

Jørgensen, R.B. 1986. Relationships in the barley genus (*Hordeum*): an elecfrophloretic examination of proteins. *Hereditas* **104:**165-221.

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

Kellogg, E.A., Bennetzen, J.L. 2004. The evolution of nuclear genome structure in seed plants. *American Journal of Botany* **91:** 1709-1725.

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

Komatsuda, T., Salomon, B., Bryngelsson, T., von Bothmer, R. 2001. Phylogenetic analysis of *Hordeum marinum* Huds. Based on nucleotide sequences linked to the *vrsl* locus. *Plant Systematics and Evolution* **227:**137-144.

Leitch, I.J., and Bennett, M.D. 2004. Genome downsizing in polyploid plants. *Biological Journal of Linnean Society* **82:** 651-663.

Lihová, J., Shimizu, K.K., Marhold, K. 2006. Allopolyploid origin of *Cardamine asarifolia* (Brassicaceae): Incongruence between plastid and nuclear ribosomal DNA sequences solved by a single-copy nuclear gene. *Molecular Phylogenetics and Evolution* **39:** 759-786.

Linde-Laursen, I., von Bothmer. R., Jacobsen, N. 1990. Giemsa C-banded karyotypes of South and Central American *Hordeum (Poaceae)*. II. 6 polyploid taxa. *Hereditas* **111:** 93-107.

Linde-Laursen, I., von Bothmer, R., Jacobsen, N. 1992. Relationships in the genus *Hordeum*: Giemsa C-banded karyotypes. *Hereditas* **116:** 111-116.

Linde-Laursen, I., von Bothmer, R., Jacobsen, N. 1995. Karyotype differentiation and evolution in the genus *Hordeum* (Poaceae). In: Brandham PE, Bennett MD, eds. *Kew chromosome Conference IV*. Kew, UK: Royal Botanic Gardens 233-247.

Maddison, W.P., and Knowles, L.L. 2006. Inferring phylogeny despite incomplete lineage sorting. *Society of Systematic Biologists* **55:** 21-30.

Mason-Gamer, R.J. 2004. Reticulate evolution, introgression, and intertribal gene capture in an allohexaploid grass. *Systematic Biology* **53 (1):** 25-37.

Mason-Gamer, R.J. 2008. Allohexaploidy, introgression, and the complex phylogenetic history of *Elymus repens* (Poaceae). *Molecular Phylogenetics and Evolution* **47:** 598-611.

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

Meng, C., and Kubatko, L.S. 2009. Detecting hybrid speciation in the presence of incomplete lineage sorting using gene tree incongruence: A model. *Theoretical Population Biology* **75:** 35-45.

Nishikawa, T., Salomon, B., Komatsuda, T., von Bothmer, R., Kadowaki, K. 2002. Molecular phylogeny of the genus Hordeum using three chloroplast DNA sequences. *Genome* **45:** 1157-1166.

Olmstead, R.G., and Pamer, J.D. 1994. Chloroplast DNA systematic: a review of methods and data analysis. *American Journal of Botany* **81:** 1205-1244.

Osborn, T.C., Pires, J.C., Birchler, J.A., Auger, D.L., Chen, Z.J., Lee, H.S., Comai, L., Madlung, A., Doerge, R.W., Colot, V., Martienssen, R.A. 2003. Understanding mechanisms of novel gene expression in polyploids. *Trends in Genetics* **19:** 141-147.

Pamilo, P., and Nei, M. 1988. Relationships between gene trees and species trees. *Molecular Biology and Evolution* **5:** 568-583.

Petersen, G. 1991. Intergeneric hybridization between *Hordeum* and *Secale*. *U* Analysis of meiosis in hybrids. *Hereditas* **114:**141-159.

Petersen, G., and Seberg, O. 1997. Phylogenetic analysis of the Triticeae (Poaceae) based on rpoA sequence data. *Molecular Phylogenetics and Evolution* **7:**217-30.

Petersen, G., and Seberg, O. 2003. Phylogenetic analyses of the diploid species of *Hordeum* (Poaceae) and a revised classification of the genus. *Systematic Botany* **28:** 293-306.

Petersen, G., and Seberg, O. 2004. On the origin of the tefraploid species *Hordeum capense* and *H. secalicum* (Poaceae). *Systematic Botany* **29:** 862-873.

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

Rajhathy, T., and Symko, S. 1966. The synthesis of a species: *Hordeum arizonicum*. *Canadian Journal of Botany* **44:** 1224-1228.

Rosenberg, N.A. 2002. The probability of topological concordance of gene frees and species trees. *Theoretical Population Biology* **61:** 225-247.

Sakamoto, S. 1974. Intergeneric hybridization among three species of *Heteranthelium, Eremopyrum* and *Hordeum,* and its significance of the genetic relationships within the tribe Triticeae. *Newphytologist* **73:** 341-350.

Sang, T. 2002. Utility of low-copy nuclear gene sequences in plant phylogenetics. *Critical Reviews in Biochemistry and Molecular Biology* **37:** 121-147.

Seberg, O., and Frederiksen, S. 2001. A phylogenetic analysis of the monogenomic Triticeae (Poaceae) based on morphology. *Biological Journal of Linnean Society* **136:**75-97.

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

Soltis, D.E., and Soltis, P.S. 1998. Choosing an approach and an appropriate gene for phylogenetic analysis. In "Molecular systematic of plants II: DNA sequencing". Kluwer Academic Publisher, Boston: 1-42.

Soltis, D.E., and Soltis, P.S. 1999. Polyploidy: Recurrent formation and genome evolution. *Trends in Ecology and Evolution* **14:** 348-352.

Soltis, D.E., and Soltis, P.S. 2000. The role of genetic and genomic attributes in the success of polyploids. *The proceedings of National Academy of Science* **97:** 7051-7057.

Soltis, D.E., Soltis, P.S., Tate, J.A. 2003. Advances in the study of polyploidy since plant speciation. *New Phytologist* **161:**173-191.

Stapf, O. 1900. Gramineae. Pp. 310-750 in *Flora Capensis 7*, ed.W.T. Thiselton-Dyer. London: Lovell Reeve.

Stebbins, G.L. 1950. Variation and Evolution in Plants (Columbia Univ. Press, New York).

Sun, G. 1997. Analysis of tetraploid *Elymus* species using wheat microsatellite markers and RAPD markers. *Genome* **40:** 806-814.

Sun, G., Pourkheirandish, M., Komatsuda, T. 2009. Molecular evolution and phylogeny of the *RPB2* gene in the genus *Hordeum*. *Annals of Botany* **103:** 975-983.

Svitashev, S., Bryngelsson, T., Vershinin, A., Pedersen, C., Sail, T., von Bothmer, R. 1994. Phylegenetic analysis of the genus *Hordeum* using repetitive DNA sequences. *Theoretical and Applied Genetics* **89:** 801-810.

Swofford, D.L. 2003. PAUP beta version 4.0b2. *Phylogenetic Analysis using Parsimony,*Version 4. Sunderland, MA: Sinaeur Associates.

Taketa, S., Ando, H., Takeda, K., von Bothemer, R. 2001. Physical locations of 5S and 18S-25S rDNA in Asian and American diploid *Hordeum* species with the I genome. *Heredity* **86:** 522-530.

Taketa, S., Ando, H., Takeda, K., Ichii, M., von Bothmer, R. 2005. Ancestry of American polyploid *Hordeum* species with the I genome inferred from 5S and 18S-25S rDNA. *Annals of Botany* **96:** 23-33.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmouguin, F., Higgins, D.G. 1997. The CLUSTALX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* **25:** 4876-4882.

Wang, H., and Sun, G. 2011. Molecular phylogeny and reticulate origins of several American polyploid *Hordeum* species. *Botany* **89(6):** 405-415.

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

Wolfe, K.H., Li, W.H., Sharp, P.M. 1987. Rates of nucleotide substitution vary greatly among plant mitochondrial, chloroplast, and nuclear DNAs. *Proceedings of National Academy of Science U.S.A.* **84:** 9054-9058.