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Phylogenetic analysis of the maternal genome of tetraploid StStYY *Elymus* (Triticeae: Poaceae) species and the monogenomic Triticeae based on rps16 sequence data

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ABSTRACT

Although there have been several studies on the maternal genome donor of StStYY *Elymus* species, due to either the lack of inclusion of all the Triticeae diploid species or limited number of StY species used in the previous studies, it is necessary to investigate the origin of the maternal genome of the StStYY *Elymus* species with comprehensive samples of Triticeae diploid species. In this study we analyzed 18 tetraploid StStYY *Elymus* species along with 27 diploid Triticeae species using chloroplast gene encoding ribosomal protein S16 (rps16) in an attempt to identify the maternal genome donor of StStYY *Elymus* species. The rps16 data indicates that *Pseudoroegneria* is the maternal genome donor of StStYY *Elymus* species. A close relationship of the chloroplast of *Thinopyrum* and *Dasypyrum* with the St genome is found which leaves the possibility that *Thinopyrum* and *Dasypyrum* may have contributed to the chloroplast genome in StStYY species. We also analyzed the cpDNA phylogeny for the monogenomic genera. *Psathyrostachys juncea* is at the base of the Triticeae tree, while the rest of the Triticeae species forms a well supported clade with a 91% bootstrap value. *Triticum monococcum, Secale cereale, Heteranthelium piliferum* and all the *Aegilops* species form a clade. A very similar chloroplast genome between *Agropyron* and *Eremopyrum* is detected. Our rps16 data supports the placement of *Henrardia* within the Triticeae in a *Henrardia* + *Eremopyrum* clade.

1. Introduction

Triticeae is a monophyletic grass tribe that has global economic significance, as it includes important cereals such as barley (*Hordeum* L.), wheat (*Triticum* L.) and rye (*Secale* L.) [1–5]. It also includes the important foraging grass *Elymus* L. There are many autopolyploid and allopolyploid taxa within Triticeae and its pool of genes is essential for genetic enhancement of cereal crops [6–9]. The genus of the foraging grass *Elymus* includes approximately 150 perennial, allopolyploid species, which all contain at least one set of *Pseudoroegneria* (St) genomes [6,10–13]. The *Elymus* species contain this St genome in different combinations with a variety of other Triticeae genomes [11]. As a result of the allopolyploid nature of *Elymus*, it has close association with other genera of Triticeae [14]. Allopolyploidy arises from the unification of two completely divergent nuclear genomes from different donor species [15,16]. The diploid ancestors (e.g.: *Pseudoroegneria*) are known to be less resistant to heat, cold, and drought than their polyploid, *Elymus* descendants [13]. A rather elusive genome found in combination with the St genome of *Elymus*, is the Y-genome whose supposed diploid progenitor remains unknown [17].

The Y-genome is commonly found in species of *Elymus* throughout Central and Eastern Asia, with approximately 30 StStYY species located restrictedly in temperate Asia [8,9,14,18–21]. There has not been any Y-genome diploid species discovered, which adds to the question of where the Y-genome came from in StStYY *Elymus* species [6,13,22–25]. There has been much evidence to support the notion that the majority of *Elymus* species from North America have the genome combination, StStHH [e.g., 6,26–30].

Cytogenetic methods have previously been used to define the genus *Elymus*, but have proven less informative for the reconstruction of evolutionary relationships between genera [3,29–34]. Many current methods of phylogenetic analysis use molecular data that include nuclear and chloroplast DNA (cpDNA) gene sequences. A benefit to using chloroplast DNA for phylogenetic analysis is that it is clonally inherited from a maternal donor and it does not undergo recombination in higher plants [5,30].

Using chloroplast DNA data, Mason-Gamer et al. [30], showed that *Pseudoroegneria* is the maternal genome donor of most of the North American *Elymus* species, and one StStYY species, *Elymus ciliaris*; they suggested that *Thinopyrum* and *Dasypyrum* should not be ruled out as potential chloroplast donors to North American *Elymus* species. In a PCR–RFLP analysis using four chloroplast gene regions,

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trnD-trnT intron, trnK [tRNA-Lys (UUU) exon1]-trnK [tRNA-Lys (UUU) exon2], trnC-trnD, and rbcL, McMillan and Sun [23] indicated that *Pseudoroegneria* is the maternal genome donor of StStYY *Elymus* species. The trnL-F chloroplast sequences from StStYY *Elymus* suggested that *Pseudoroegneria* is the maternal genome donor of the StStYY *Elymus* species studied [9]. Due to either the lack of inclusion of large number of the Triticeae diploid species [9,23] or limited number of StY species used [30] in the aforementioned studies, it is necessary to investigate the origin of the maternal genome of the StStYY *Elymus* species with comprehensive samples of Triticeae diploid species.

In this study we used the chloroplast gene encoding ribosomal protein S16 (rps16), to investigate the maternal genome donor of tetraploid StStYY *Elymus* species. The results presented here are congruent with those of a small number of similar studies; however our study is the first of its kind to include comprehensive samples of diploid species in the Triticeae tribe. The objectives of this study were to investigate the maternal genome donor of StStYY *Elymus* species using rps16 chloroplast DNA in a phylogenetic comparison with all of the Triticeae diploid species and to see if the results are in agreement with previous studies that show *Pseudoroegneria* as the maternal genome donor. Here we show that the rps16 chloroplast data does support *Pseudoroegneria* as being the maternal genome donor of StStYY *Elymus* species.

2. Methods

2.1. Plant materials

The species names, accession numbers and genome symbols of the 46 different taxa used in this study are listed in Table 1. The assigned genome symbols are in accordance with Wang et al. [35]. Seeds of each accession were germinated and grown in a greenhouse for DNA extraction. DNA was extracted from 300 to 600 mg of young freeze-dried tissue collected from 5 to 10 plants of each accession using the method of Junghans and Metzlaff [36].

2.2. DNA amplification and sequencing

The rps16 primers were based on a study by Popp and Oxelman [37]. The forward, rps16F (GTGGTAGAAAGCAACGTGCGACTT), and reverse rps16R (TCGGGATCGAACATCAATTGCAAC), primers were devised to amplify the chloroplast DNA of the gene, rps16.

The mixture of components used for amplification in a 25 μl reaction vessel was 37.5 ng of template DNA, 0.25 μM of each primer, 0.25 mM of each deoxynucleotide (dCTP, dGTP, dATP, and dTTP), 1.875 mM of $MgCl_2$, 1 unit of *Taq* DNA polymerase (BioShop) and distilled deionized water to the final volume. A Biorad icycler® Thermal cycler was used to amplify the rps16 genes. The protocol for the PCR was: one 4 min cycle at 95 °C, 35 cycles of 1 min at 95 °C, 1 min at 60 °C, and 2 min at 72 °C, followed by 10 min at 72 °C. The PCR products were cleaned using ExoSAP-IT® (USB http://www.usbweb.com/) according to manufacture instructions.

Direct sequencing of the PCR amplicon from the accession PI 401354 was unsuccessful. The PCR product from this accession was cloned into the pGEM-easy T vector (Promega Corporation, Madison, WI, USA) according to the manufacturer's protocol. Plasmid DNA was isolated using GenEluteTM Plasmid Miniprep Kit (Sigma) according to manufacture instructions. All DNA were sequenced commercially at MACROGEN (Seoul, Korea). To increase the quality of the data, both forward and reverse strands were sequenced independently and repeated once.

2.3. Data analysis

Automated sequence outputs were visually inspected with chromatographs. The sequences from both strands of each clone were compared using the BLASTN program (http://www.ncbi.nlm.nih.gov/blast/) to find the overlap region, and was joined together to generate a full sequence of each clone.

Clustal × [38] was used, with default parameters, to complete a multiple sequence alignment of the 47 sequences. The sequence alignment was generally straightforward in spite of a 29 bp indel which was excluded in the phylogenetic analysis. The maximum parsimony method was used by the program, PAUP* version 4 beta 10 Win [39], to perform a phylogenetic analysis of the data set. A heuristic search using the Tree Bisection–Reconnection (TBR) option with MulTrees on and 10 replications of random addition sequence with the stepwise addition option, was used to produce the most parsimonious trees. All characters were considered unordered and unweighted, while the gaps were treated as missing data. The consistency index (CI), retention index (RI), and rescaled consistency index (RC) were used to estimate overall character similarity. Clade robustness was deduced using bootstrap values from 1000 replications [40], which were calculated through a heuristic search using the TBR option with MulTrees on. The outgroup, *Bromus catharticus*, was chosen based on a previous phylogenetic analysis of Poaceae [41]. In complement to parsimonious analysis, the neighbour-joining (NJ) method was also used for phylogenetic reconstruction.

Table 1
Taxa used in this study.

Species	Accession No.	Genome
Aegilops comosa Sibth. and Smith	PI 551032	MM
Aegilops longissima Schweinf. & Muschl.	PI 542196	S^lS^l
Aegilops searsii Feldman & Kislev	PI 599150	S^sS^s
Aegilops sharonensis Eig	PI 542237	S^lS^l
Aegilops speltoides Tausch	PI 499261	SS
Aegilops tauschii Coss.	PI 486265	DD
Aegilops umbellulata Zhuk.	PI 276994	UU
Aegilops uniaristata Vis.	PI 554418	NN
Agropyron fragile (Roth) P. Candargy	PI 598674	PP
Agropyron mongolicum Keng	PI 598460	PP
Bromus catharticus Vahl	CN32048	–
Dasypyrum villosum (L.) Candargy	PI 598400	VV
Elymus abolinii (Drobow) Tzvelev	PI 531554	StStYY
Elymus antiquus (Nevski) Tzvelev	PI 619528	StStYY
Elymus barbicallus (Ohwi) S.L. Chen	PI 504441	StStYY
Elymus burchan-buddae	H7121	StStYY
Elymus canaliculatus (Nevski) Tzvelev	H4123	StStYY
Elymus caucasicus (K. Koch) Tzvelev	PI 531573	StStYY
Elymus ciliaris (Trin.) Tzvelev	PI 531576	StStYY
Elymus fedtschenkoi Tzvelev	PI 564927	StStYY
Elymus gmelinii (Ledeb.) Tzvelev	PI 610898	StStYY
Elymus longearistatus (Boiss.) Tzvelev	PI 401280	StStYY
Elymus macrochaetus (Nevski) Tzvelev	PI 618796	StStYY
Elymus panormitanus (Parl.) Tzvelev	PI 254866	StStYY
Elymus pendulinus (Nevski) Tzvelev	H8986	StStYY
Elymus praeruptus	H10218	StStYY
Elymus semicostatus (Nees ex Steud.) Melderis	PI 531660	StStYY
Elymus strictus (Keng) Á. Löve	PI 499476	StStYY
Elymus tibeticus (Meld.) G. Singh	PI 639828	StStYY
Elymus validus (Meld.) B. Salomon	H4100	StStYY
Eremopyrum bonaepartis (Spreng.) Nevski	PI 203442	FF
Eremopyrum distans (C. Koch) Nevski	PI 193264	FF
Eremopyrum orientale (L.) Jaub. & Spach	PI 203440	FF
Henrardia persica (Boiss.) C.E. Hubb.	PI 577112	OO
Heteranthelium piliferum (Banks & Sol.) Hochst.	PI 401354	QQ
Psathyrostachys juncea (Fischer) Nevski	PI 406469	NsNs
Pseudoroegneria ferganensis Drobow	H10248	StSt
Pseudoroegneria geniculata (Trin.) Á. Löve	PI 632554	StSt
Pseudoroegneria gracillima (Nevski) Á. Löve	PI 420842	StSt
Pseudoroegneria libanotica (Hackel) D.R. Dewey	PI 330688	StSt
Pseudoroegneria spicata (Pursh) Á. Löve	PI 506274	StSt
Pseudoroegneria stipifolia (Czern. ex Nevski) Á. Löve	PI 325181	StSt
Pseudoroegneria tauri (Boiss. & Balansa) Á. Löve	PI 401330	StSt
Secale cereale L.	PI 573710	RR
Thinopyrum elongatum (Host) D.R. Dewey	PI 142012	E^eE^e
Triticum monococcum L.	PI 191146	A^MA^M