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ORIGINAL ARTICLE

Complete chloroplast genomes of *Aegilops tauschii* Coss. and *Ae. cylindrica* Host sheds light on plasmon D evolution

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Abstract Hexaploid wheat (*Triticum aestivum* L., genomes AABBDD) originated in South Caucasus by allopolyploidization of the cultivated Emmer wheat *T. dicoccum* (genomes AABB) with the Caucasian *Ae. tauschii* ssp *strangulata* (genomes DD). Genetic variation of *Ae. tauschii* is an important natural resource, that is why it is of particular importance to investigate how this variation was formed during *Ae. tauschii* evolutionary history and how it is presented through the species area. The D genome is also found in tetraploid *Ae. cylindrica* Host ($2n = 28$, CCDD). The plasmon diversity that exists in *Triticum* and *Aegilops* species is of great significance for understanding the evolution of these genera. In the present investigation the complete nucleotide sequence of plasmon D (chloroplast DNA) of nine accessions of *Ae. tauschii* and two accessions of *Ae. cylindrica* are presented. Twenty-eight SNPs are characteristic for both TauL1 and TauL2 accessions of *Ae. tauschii* using TauL3 as a reference. Four SNPs are additionally observed for TauL2 lineage. The longest (27 bp) indel is located in the intergenic spacer *Rps15-ndhF* of SSC. This indel can be used for simple determination of TauL3 lineage among *Ae. tauschii* accessions. In the case of *Ae. cylindrica* additionally 7 SNPs were observed. The phylogeny tree shows that chloroplast DNA of TauL1 and TauL2 diverged from the TauL3 lineage. TauL1 lineage is relatively older then TauL2. The position of *Ae. cylindrica* accessions on *Ae. tauschii* phylogeny tree constructed on chloroplast DNA variation data is intermediate between TauL1 and TauL2. The complete nucleotide sequence of chloroplast DNA of *Ae. tauschii* and *Ae. cylindrica* allows to refine the origin and evolution of D plasmon of genus *Aegilops*.

Keywords Chloroplast DNA · Illumina · Indels · Sequencing · SNP · *Aegilops*

Introduction

Hexaploid wheat (*Triticum aestivum* L., genomes AABBDD) originated in South Caucasus by allopolyploidization of the cultivated Emmer wheat *T. dicoccum* Schrank (genomes AABB) with the Caucasian *Ae. tauschii* ssp *strangulata* (Eig) Tzvelev (genomes DD) (Kihara 1944; McFadden and Sears 1946, cited according Wang et al. 2013; Dvorak et al. 1998; Dubcovsky and Dvorak 2007). *Ae. tauschii* Coss. (syn. *Ae. squarrosa* auct non L.) is a diploid ($2n = 14$, genome DD) goat-grass species which donated its genome D to common wheat, *T. aestivum* L. It is considered as the most important donor of agriculturally important genes for improvement of common wheat (Kimber and Feldman 1987). Genetic variation of *Ae. tauschii* is an important natural resource, that is why it is of particular importance to investigate how this variation was formed during *Ae. tauschii* evolutionary history and how it is presented through the species area. The D genome is also found in

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tetraploid *Ae. cylindrica* Host ($2n = 28$, CCDD). The parents of *Ae. cylindrica* are *Ae. caudata* L. ($2n = 14$, CC) and *Ae. tauschii*. *Ae. caudata* and *Ae. tauschii* overlap the area of *Ae. cylindrica*, but have no area in common (Nakai 1981). The term Plasmon is used for cytoplasmic (organellar) genomes—chloroplast and mitochondria (Tsunewaki et al. 2002; Gill and Friebe 2002). The plasmons of *Ae. tauschii* Coss.and *Ae. cylindrica* Host both belong to the D type. According to Tsunewaki et al. the plasmon diversity that exists in *Triticum* and *Aegilops* species is of great significance for understanding the evolution of these genera.

Ae. tauschii is presented by two subspecies, *Ae. tauschii* Coss. ssp *tauschii* and *Ae. tauschii* Coss. ssp *strangulata* (Eig) Tzvelev with cylindrical and moniliform types of spike, respectively (Eig 1929). In ssp *strangulata* a relict lineage "t-9^1s" was found which considerably differ genetically from other accessions of this subspecies (Dudnikov 1998, 2012; Pestsova et al. 2000). The three markedly different gene-pools of *Ae. tauschii*, i.e. those of ssp *tauschii*, "usual" ssp *strangulata*, and relict lineage "t-9^1s" of ssp *strangulata*, were designated by Matsuoka et al. (2013, 2015) as TauL1, TauL2 and TauL3, respectively. These designations were finally used after several investigations (Matsuoka et al. 2007, 2008, 2009, 2013, 2015; Mizuno et al. 2010). Chloroplast DNA variation in *Ae. tauschii* was studied by Matsuoka et al. (2007, 2008, 2009) and four major haplogroups, HG7, HG9, HG16 and HG17 were identified. From those, HG16, HG9 and HG17 belonged to ssp *tauschii*, ssp *strangulata* and relict lineage "t-9^1s", respectively; while haplogroup HG7 contained *Ae. tauschii* accessions from both ssp *tauschii* and ssp *strangulata* (Matsuoka et al. 2007, 2008, 2009). Analysis of AFLP polymorphism revealed two major gene-pools in *Ae. tauschii*: those of ssp *tauschii* and ssp *strangulata*, designated as L1 and L2 respectively; and *Ae. tauschii* accessions belonging to HG17 cpDNA (Chloroplast DNA) haplogroup had an intermediate position between L1 and L2 (Mizuno et al. 2010). Later on, the same three gene-pools, L1, L2 and HG17 were identified using DArT analysis (Matsuoka et al. 2015); they were renamed as TauL1, TauL2 and TauL3, respectively; and it was outlined that TauL3 is related to TauL2 (Matsuoka et al. 2013).

Ae. tauschii occupies the vast range, from Turkey to Kirgizia. The Georgian part of the area is of particular interest. Despite it is relatively very small, an essential part of *Ae. tauschii* genetic variation was pointed out here (Dudnikov 2000, 2012; Pestsova et al. 2000). Therefore Georgia is the only country where relict gene-pool TauL3 is rather common. Besides Georgia, it was pointed out only once, as a local population t-9^1s in Dagestan (Dudnikov 1998, 2012).

Traditionally, extranuclear DNA, such as cpDNA is considered as an effective tool of genealogic studies (Yamane and Kawahara 2005; Matsuoka et al. 2005; Tabidze et al. 2014; George et al. 2015; Kong and Yang 2015; Vieira et al. 2015; Oldenburg and Bendich 2015). Next-generation sequencing technologies, which have been developed in recent years, enable the determination of the complete nucleotide sequence of both, chloroplast and mitochondrial DNAs of many higher plants, including wheat and its relatives. Recently this technology was used in our lab to sequence cpDNA of three species of Zanduri wheat (*T. timopheevii*, *T. zhukovskyi*, *T. monococcum* var. *hornemannii*) as well as wild species *T. araraticum* (Gogniashvili et al. 2015). The new methodology of cpDNA sequencing was developed (see Tabidze et al. 2014; Gogniashvili et al. 2015). A study, in which complete cpDNA would be sequenced in a set of *Ae. tauschii* accessions originated from Georgia—the part of the area which is of particular importance for understanding the species evolution, seems to be of particular interest. Intraspecies divergency of *Ae. tauschii* was previously studied by Dudnikov (2012)—four regions of non-coding cpDNA, about 3000 bp in total, were sequenced in 112 accessions of *Ae. tauschii*. But the genealogy patterns obtained were complicated and rather contradictory. The root of phylogenetic tree was located between relict lineage t-9^1s of ssp. *strangulata* and the lineage "AE-725" of ssp *tauschii*. The latter was the ancestor for all the other lineages of both ssp *tauschii* and *stangulata* (Dudnikov 2012).

To date complete sequence of cpDNA of two ssp *strangulata* accessions and one accession of *Ae. cylindrica* is known (Middleton et al. 2014; Gornicki et al. 2014). In the present investigation we sequenced total cpDNA in nine *Ae. tauschii* and two *Ae.cylindica* accessions of Georgian origin. So, the data on eleven *Ae. tauschii* accessions was used for the analysis of its intraspecies phylogeny, and the data on tree accessions of *Ae. cylindrica* was used for investigation of peculiarities of plasmon origin of this spesies.

Materials and methods

Plant material

The seeds of *Ae. tauschii* Coss. and *Ae. cylindrica* Host were collected in East Georgia in 2010 (Jinjikhadze et al. 2010). In *Ae. tauschii*, the ssp determination was done according to Dudnikov (2000) on the basis of ssp index "SI", which is a ratio between spikelet glume width and rachis segment

width, and also on the basis of allozyme polymorphism of *Acph1* and *Got2* loci (Jaaska 1981; Dudnikov 2000).

DNA isolation, PCR analysis, Genomic DNA library preparation, sequencing on an Illumina MiSeq platform

The seeds were germinated in water at room temperature. Total genomic DNA was extracted from young leaves. The leaves were ground in liquid nitrogen, and DNA was isolated according to the modified Marmur's procedure (Beridze et al. 2011). Primers used for amplification of *Ae. tauschii* cpDNA fragment 101,130 −101,548: Forward—AATATGGGCCCTCAACACCC; Reverse—GGGTTAA CCGAACTCACGGA. The PCR conditions were as follows: 1 min denaturing at 94 °C; 30 cycles of 94 °C denaturing (1 min), 55 °C annealing (1 min) and 72 °C extension (2 min); followed by a final extension step at 72 °C (5 min).

Genomic DNA libraries were constructed using the TruSeq DNA Sample prep kit (Illumina, San Diego, CA, USA). Genomic DNAs were quantified using the Qbit BR reagents (Qbit 2.0 Fluorometer, Life Technologies). Briefly, 1 μg of DNA was sheared into 300 bp fragments on a Covaris M220 focused ultrasonicator (Covaris Inc) using SonoLabTM 7.1 software for 250 cycles/burst, 20.0 Duty factor, 50.0 Peak power, in screw-cap microtubes. After shearing, the DNA was blunt-ended, 30-end A-tailed and ligated to indexed adaptors. The adaptor-ligated genomic DNA was size selected with AMPure-beads using the gelfree protocol described in the TruSeq DNA Sample Prep manual. Size-selected DNA was amplified by PCR to selectively enrich for fragments that have adapters on both ends. Final amplified libraries were run on an Agilent bioanalyzer DNA 2100 (Agilent, Santa Clara, CA, USA) to determine the average fragment size and to confirm the presence of DNA of the expected size range.

The libraries were pooled in equimolar concentration and loaded onto a flow cell for cluster formation and sequenced on an Illumina MiSeq platform. The libraries were sequenced from both ends of the molecules to a total read length of 150 nt from each end. The raw.bcl files were converted into demultiplexed compressed fastq files using Casava 1.8.2 (Illumina). Sequencing of wheat DNA samples was performed at the facilities of the National Centre for Disease Control and Public Health, Tbilisi, Georgia.

Assembly of chloroplast DNA

FASTAQ (a text-based format for storing nucleotide sequence) files were trimmed using the computer program Sickle, a windowed adaptive trimming tool for FASTQ files using quality (https://github.com/najoshi/sickle). The reads were filtered by standard parameters (quality reads—20, cutoff length—20). The reads containing ''N'' were discarded. The filtered chloroplast reads were assembled using the SOAPdenovo2 software program (version 127mer) (Li et al. 2009). The reads were first de novo assembled into contigs with k-mers 83–93. All contigs were aligned to the

Table 1 *Ae. tauschii* (Gt) and *Ae. cylindrica* (Gc) accessions of East Georgia used in the study

Accessions	Region	The length of cpDNA, (bp)	GenBank accession number
Gt_14	City: Tbilisi. District: Varketili. 519 m N 44.88; E 41.70	135,610	KU198481
Gt_15	Region: Kvemo Kartli. District: Gardabani. Village: Kojori. Wayside N 44.71; E 41.66	135,554	KU198482
Gt_17	Region: Kvemo Kartli. District: Gardabani. Village: Tsavkisi. Field. N 44.74; E 41.67	135,610	KU198483
Gt_19	City: Tbilisi. Village: Okrokana. Near the restaurant Kolkheti. Wayside, 714 m N 44.79; E 41.69	135,673	KU207223
Gt_24	Region: Kvemo Kartli. District: Tetritskaro. Village: Koda Field, 644 m N 44.78; E 41.58	135,620	KU198484
Gt_30	City: Tbilisi. District: Saburtalo. Ivane Javakhishvili Tbilisi State University N 44.72; E 41.72	135,654	KU207225
Gt_32	Region: Kvemo Kartli. District: Dmanisi. 865 m N 44.21; E 41.33	135,650	KU198485
Gt_34	City: Tbilisi. District: Didi Dighomi. Petre Iberi Avenue N 44.76; E 41.79	135,655	KU207222
Gt_40	Region: Kvemo Kartli. District: Tetritskaro. Field, 1122 m N 44.47; E 41.54	135,548	KU198486
Gc_27	City: Tbilisi. District: Vazisubani N 44.85; E 41.70	135,625	KU207224
Gc_31	Region: Mtskheta-mtianeti. City: Mtskheta. District: Tsitsamuri. 542 m N 44.73; E 41.86	135,622	KU207226

Table 2 SNPs specific for *Ae. tauschii* and *Ae. cylindrica*

Nucleotide position according Gt_30 (Taul3)	Locus		*Ae. tauschii*			*Ae. cylindrica*	Amino acid substitution
			TauL1	TauL2	TauL3	Gc_27, Gc_31	
			Gt_32, Gt_14, Gt_17	Gt_15, Gt_40, Gt_19, Gt_24	Gt_30, Gt_34		
1312	Intergenic *psbA-trnK-UUU*	LSC	C	C	T	C	
1554	Intron *trnK-UUU*	LSC	T	T	G	T	
1565	Intron *trnK-UUU*	LSC	C	C	G	C	
2127	Gene *matK*	LSC	T	T	T	C	S–G
2581	Gene *matK*	LSC	C	C	T	C	N–K
3517	Intron *trnK-UUU*	LSC	T	T	G	T	
4173	Intergenic *trnL-UUU-rps16*	LSC	A	A	A	T	
5826	Intergenic *Rps16-trnG-UUG*	LSC	A	A	C	A	
6633	Intergenic *trnG-UUG-psbK*	LSC	A	A	A	G	
11828	Gene *psbZ*	LSC	A	G	A	A	Syn
14801	Intergenic *trnT-GGU-trnG-UUC*	LSC	G	G	T	G	
17115	Intergenic *psbM-petN*	LSC	T	G	T	T	
18500	Intergenic *trnC-GCA-rpoB*	LSC	T	T	G	T	
18858	Intergenic *trnC-GCA-rpoB*	LSC	G	G	T	G	
18950	Intergenic *trnC-GCA-rpoB*	LSC	A	A	G	A	
19175	Intergenic *trnC-GCA-rpoB*	LSC	C	T	C	C	
19890	Gene *rpoB*	LSC	T	T	C	T	Syn
22347	Gene *rpoB*	LSC	A	A	A	C	Syn
22398	Gene *rpoB*	LSC	T	C	T	T	Syn
26001	Gene *rpoC2*	LSC	A	A	A	C	Syn
36711	Intergenic *rps14-psaB*	LSC	C	C	C	T	
41803	Intergenic *psaA-ycf3*	LSC	T	T	G	T	
44236	Intergenic *Ycf3-trnS-GGA*	LSC	T	T	G	T	
44384	Intergenic *Ycf3-trnS-GGA*	LSC	T	T	A	T	
53993	Intergenic *atpB-rbcL*	LSC	A	A	C	A	
59644	Gene *petA*	LSC	A	A	C	A	Syn
62139	Intergenic *psbE-petL*	LSC	G	G	G	A	
64139	Intergenic *psaJ-rpl33*	LSC	T	T	G	T	
65573	Intergenic *rps18-rpl20*	LSC	T	T	G	T	
65586	Intergenic *rps18-rpl20*	LSC	A	A	G	A	
71906	Intergenic *petB-petD*	LSC	T	T	G	T	
75839	Intergenic *infA-rps8*	LSC	A	A	G	A	
93763	Intergenic *trnV-GAC-rRNA-16S* ribosomal RNA	IR	T	T	G	T	
102127	Gene *ndhF*	SSC	C	C	A	C	I–M
102347	Gene *ndhF*	SSC	A	A	G	A	Syn
103292	Gene *ndhF*	SSC	C	C	T	C	L–V
103853	Intergenic *ndhF-rpl32*	SSC	T	T	G	T	
111640	Intron *ndhA*	SSC	A	A	G	A	
112254	Intron *ndhA*	SSC	A	A	T	A	

Table 3 Indels in cpDNA of *Ae. tauschii* and *Ae. cylindrica* accessions

Nucleotide position according t_30 (TauL3)	Locus		*Ae. tauschii*			*Ae. cylindrica* (2 accessions)
			TauL3 (2 accessions)	TauL1 (3 accessions)	TauL2 (6 accessions)	
11211	Intergenic *psbC-trnS-UGA*	LSC	AAAAAAT	AAAAAAT	–	–
18827	Intergenic *trnC-GCA-rpoB*	LSC	CCCCCCCCCCC (11)	CCCCCCCC (8)	CCCCCCCC (8)	CCCCCCCCC (9)
33160	Intron *atpF*	LSC	TATTAG	–	TATTAG	–
45717	Intergenic *Rps4-trnT-UGU*	LSC	TTTTTTGT	–	–	–
72026	Intergenic *petB-petD*	LSC	ACAA	–	–	–
75762	Intergenic *infA-rps8*		–	–	–	18 bp duplication TTTTTTTTTTCTG TCATA
101322	Intergenic *rps15-ndhF*	SSC	TAGGATAGTT ATTTTTGCA AGAGACTT	–	–	–
105394	Intergenic *Rpl32-trnL-UAG*	SSC	TTATT	–	–	–
112521	Intron *ndhA*	SSC	AATAT	–	–	–

reference chloroplast genome sequence using BLASTN (http://www.ncbi.nlm.nih.gov). Merging of large overlapping contigs was performed according to EMBOSS 6.3.1: merger (Rice et al. 2000). Totally, 2,446,306 reads were generated for Gt_30 accession. Number of reads mapping at kmer 89 was 237,690. Nine contigs 780–75,970 bp length were used for chloroplast genome assembly with the coverage 17.6.

Automatic annotation of cpDNA was performed by CpGAVAS (Liu et al. 2012). For detection of SNP (single nucleotide polymorphism) and Indels (insertion/deletion) and phylogeny tree construction computer programs Mafft and Blast were used (Katoh et al. 2002; Altschul et al. 1990).

Results and discussion

Tauschii accessions

Nine accessions of *Ae. tauschii* and two accessions of *Ae. cylindrica* used in the study are listed in Table 1. *Ae. tauschii* ssp attribution is distinct in all the accessions studied. Accessions Gt_14, Gt_17, Gt_30, Gt_32, Gt_34 have $Acph1^{95}$ and $Got2^{105}$ alleles and spike morphology characteristic for ssp *strangulata*, while Gt_15, Gt_19, Gt_24 and Gt_40 have $Acph1^{100}$ and $Got2^{100}$ alleles and spike morphology characteristic for ssp *tauschii*. Accessions G_30 and G_34 was found to belong to the relict lineage "t-9^1-s" (TauL3) of ssp *strangulata* (Dudnikov 2012).

SNP and indel analysis

Using Gt_30 as a reference (TauL3), 33 SNPs can be identified in *Ae. tauschii* lineages TauL1 and TauL2 (Table 2). 28 SNPs are characteristic for both TauL1 and TauL2 accessions. 4 SNPs are additionally characteristic for TauL2. 20 SNPs are in the intergenic regions, 5—in introns; 26 SNPs are located in LSC (Long Single Copy section of chloroplast DNA), 6—in SSC (Short Single Copy section of chloroplast DNA), 1—in IR (Inverted Repeat). Eight SNPs were found into the genes. Three SNPs were observed in *ndhF* gene, 2 in *rpoB* and one in each *matK*, psbZ and *petA*. Five coding substitutions are synonymous, which does not alter the amino acid. In genes *matK* and *ndhF* amino acid substitutions were observed (Table 2). 19 bp inversion in *psbA-trnL-UUU* intergenic region with 3 bp loop and 8 bp stem were found.

Eight indels longer than one bp, were observed (Table 3). They are located in intergenic spacers, as well as within the introns. The most interesting is the 27 bp indel. It is located in the intergenic spacer *rps15-ndhF* of SSC. This sequence is present in analyzed TauL3 accessions and is absent in TauL1 and TauL2 (Fig. 1). This indel can be used for simple determination of TauL3 lineage. Blast analysis demonstrated that all plasmons of *Aegilops* and *Triticum* (D, B, G, S, A, M) contain 27 bp sequence

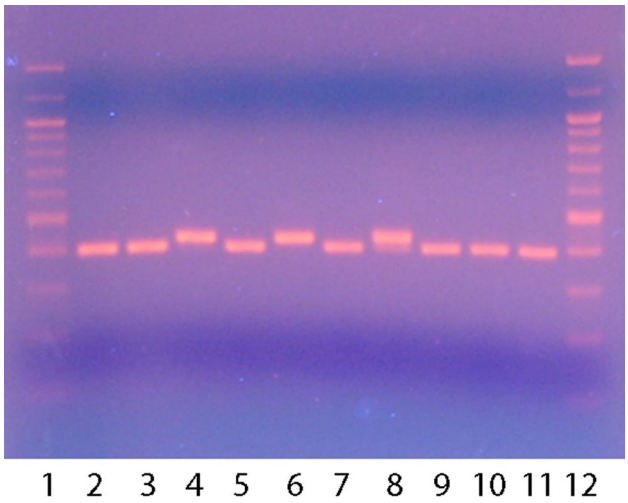

Fig. 1 2 % agarose gel electrophoresis of PCR-amplified *Ae. tauschii* cpDNA fragment 101,130–101,548. *Lanes*: *2* Gt_25, *3* Gt_26, *4* G-28, *5* Gt_29, *6* Gt_30, *7* Gt_33, *8* Gt_34, *9* Gt_35, *10* Gt_36, *11* - Gt_37; *Lanes 1*, 12–100 bp DNA marker

of *rps15-ndhF* intergenic region except *Ae. tauschii* ssp *tauschii, Ae. tauschii* ssp *strangulata* and *Ae. cylindrica* (all of them contain plasmon D).

In the case of *Ae. cylindrica* additionally 7 SNPs were observed, 4 in intergenic sequences and three in genes (*matK, rpoB* and *rpoC2*). Amino acid substitution is observed in *matK* and synonimous in *rpoB* and *rpoC2*. In the intergenic sequence *infA-rps8* 18 bp duplication was detected.

Phylogeny tree

At the present time *Ae. tauschii* accessions are grouped into three intraspecific lineages: TauL1, TauL2, and TauL3 (Matsuoka et al. 2015). In the present investigation the complete nucleotide sequence of cpDNA of 9 accessions of *Ae. tauschii* and 2 accessions of *Ae. cylindrica* are presented. To illustrate the evolutionary relationship among sequenced cpDNA of both *Aegilops* species

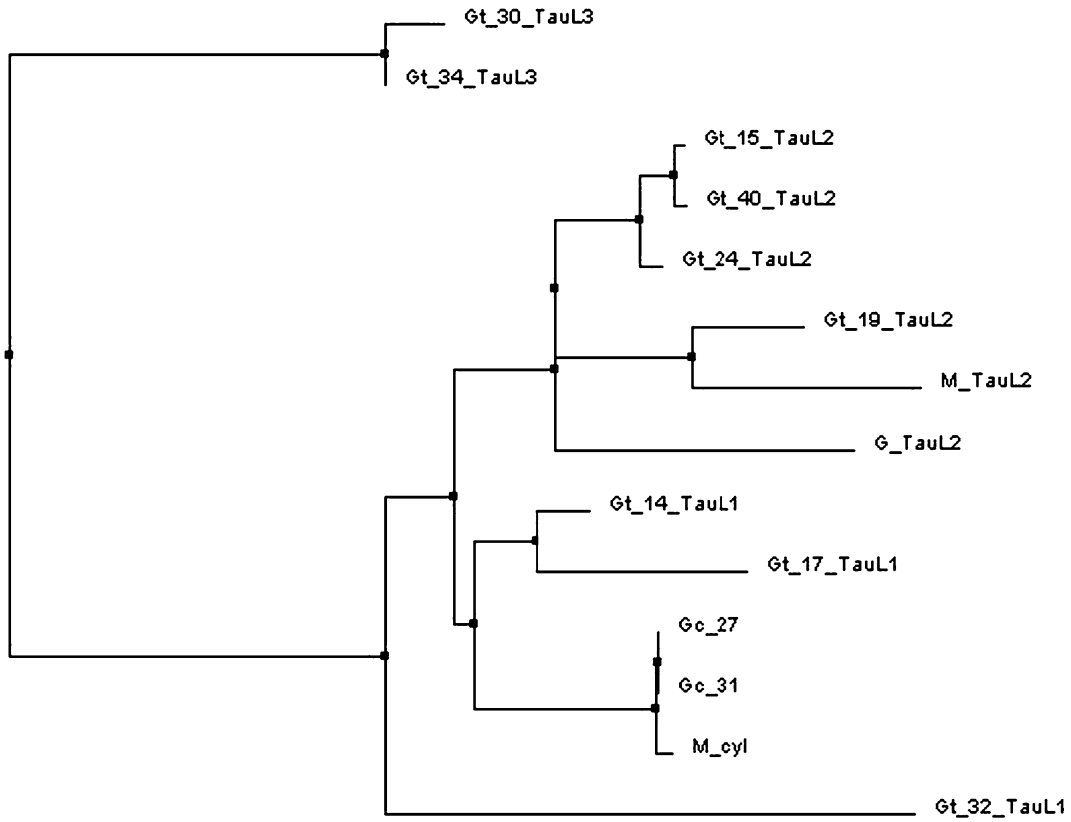

Fig. 2 Complete chloroplast genome phylogeny of *Ae. tauschii* and *Ae. cylindrica* accessions, Neighbour joining tree using PID (Waterhouse et al. 2009). The GenBank accessions used for the analyses are: *G* (*TauL2*) KJ614412.1 (Gornicki et al. 2014), *M* (*TauL2*) NC_022133.1 (Middleton et al. 2014), *M_cyl* (*Ae. cylindrica*) NC_023096.1 (Middleton et al. 2014)

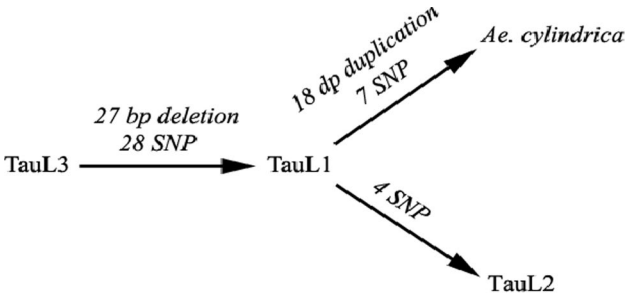

Fig. 3 The scheme reflecting the differences in single nucleotide polymorphisms (SNP) and indels between cpDNA of *Ae. tauschii* lineages and *Ae. cylindrica*

accessions a neighbor-joining phylogenetic tree was constructed based on multiple alignments using Jalview version 2 (Waterhouse et al. 2009) (Fig. 2). The tree was drawn using also two published cpDNA sequence of ssp *strangulata* (Middleton et al. 2014; Gornicki et al. 2014).

The phylogeny tree shows that TauL3 diverged from other *Ae. tauschii* in ancient times. TauL1 lineage is relatively older then TaulL2, and was an ancestor one to the latter (Fig. 2). The data also reveal relatively high sequence variation within TauL1 and TauL2 lineages. According the branch length sequence divergence is quite high also within each of the two TauL1 and TauL2.

Conclusion

The simplified scheme based on SNP and indel data of *Ae. tauschii* lineages and *Ae. cylindrica* was constructed (Fig. 3). According to this scheme plasmon (cpDNA) of TauL1 has an intermediate position between TauL3 on one hand, and TauL2 and *Ae.cylindrica*—on the other. It is known that the cytoplasm of *Ae. cylindrica* was contributed by *Ae. tauschii* (Maan 1976; Tsunewaki 1989). The position of *Ae. cylindrica* accessions on *Ae. tauschii* phylogeny tree constructed on cpDNA variation data is intermediate between TauL1 and TauL2. Thus, the complete nucleotide sequence of cpDNA of *Ae. tauschii* and *Ae. cylindrica* allows to refine the origin and evolution of D plasmon of genus *Aegilops*.

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References

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215:403–410

Beridze T, Pipia I, Beck J, Hsu SC, Gamkrelidze M, Gogniashvili M, Tabidze V, This P, Bacilieri R, Gotsiridze V, Glonti M, Schaal B (2011) Plastid DNA sequence diversity in a worldwide set of grapevine cultivars (*Vitis vinifera* L. subsp. *vinifera*). Bull Georgian National Acad Sc 5:98–103

Dubcovsky J, Dvorak J (2007) Genome plasticity a key factor in the success of polyploidy wheat under domestication. Science 316:1862–1866

Dudnikov AJ (1998) Allozyme variation in Transcaucasian populations of *Aegilops squarrosa*. Heredity 80:248–258

Dudnikov AJ (2000) Multivariate analysis of genetic variation in *Aegilops tauschii* from the world germplasm collection. Genet Resour Crop Evol 47:185–190

Dudnikov AJ (2012) Chloroplast DNA non-coding sequences variation in *Aegilops tauschii* Coss.: evolutionary history of the species. Genet Resour Crop Evol 59:683–699

Dvorak J, Luo MC, Yang ZL, Zhang HB (1998) The structure of the *Aegilops tauschii* genepool and the evolution of hexaploid wheat. Theor Appl Genet 97:657–670

Eig A (1929) Monographisch-kritische Ubersicht der Gattung Aegilops. Repert Spec Nov Regni Veg Beih 55:1–228

George B, Bhatt BS, Awasthi M, George B, Singh AK (2015) Comparative analysis of microsatellites in chloroplast genomes of lower and higher plants. Curr Genet 61:665–677. doi:10.1007/s00294-015-0495-9

Gill BS, Friebe B (2002) Cytogenetics, phylogeny and evolution of cultivated wheats (2002). In: Curtis BC, Rajaram S, Macpherson HG (eds) Bread wheat; FAO plant production and protection series (FAO), vol 30. FAO, Rome, pp 71–88

Gogniashvili M, Naskidashvili P, Bedoshvili D, Kotorashvili A, Kotaria N, Beridze T (2015) Complete chloroplast DNA sequences of Zanduri wheat (*Triticum* spp.). Genet Resour Crop Evol 62:1269–1277. doi:10.1007/s10722-015-0230-x

Gornicki P, Zhu H, Wang J, Challa GS, Zhang Z, Gill BS, Li W (2014) The chloroplast view of the evolution of polyploid wheat. New Phytol 204:704–714

Jaaska V (1981) Aspartate aminotransferase and alcohol dehydrogenase enzymes: intraspecific differentiation in *Aegilops tauschii* and the origin of the D genome polyploids in the wheat group. Pl Syst Evol 137:259–273

Jinjikhadze T, Maisaia I, Shanshiashvili T, Togonidze N (2010) Distribution of *Aegilops* L.—wheat ancestor species in Georgia, Rustaveli Foundation Report. Grant 1-8/43 **(in Georgian)**

Katoh K, Misawa K, Kuma KI, Miyata T (2002) MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucl Acids Res 30:3059–3066

Kihara H (1944) Discovery of the DD-analyser, one of the ancestors of *Triticum vulgare* (Japanese). Agriculture and Horticulture (Tokyo) 19: 13–14 *cylindrica*, and their molecular organization. Ann Bot 116:189–200

Kimber G, Feldman M (1987) Wild wheat, an introduction. Columbia (MO): College of Agriculture. University of Missouri Special Report. 353:1–146

Kong W, Yang J (2015) The complete chloroplast genome sequence of Morus mongolica and a comparative analysis within the Fabidae clade. Curr Genet. doi:10.1007/s00294-015-0507-9

Li R, Yu C, Li Y, Lam TW, Yiu SM, Kristiansen K, Wang J (2009) SOAP2: an improved ultrafast tool for short read alignment. Bioinformatics 25:1966–1967

Liu C, Shi Y, Zhu Y, Chen H, Zhang J, Lin X, Guan X (2012) CpGAVAS, an integrated web server for the annotation, visualization, analysis, and GenBank submission of completely sequenced chloroplast genome sequences. BMC Genom 13:715

Maan SS (1976) Cytoplasmic homology between *Aegilops squarrosa* L., and *A. cylindrica* Host. Crop Sci 16:757–761

Matsuoka Y, Mori N, Kawahara T (2005) Genealogical use of chloroplast DNA variation for intraspecific studies of *Aegilops tauschii* Coss. Theor Appl Genet 111:265–271

Matsuoka Y, Takumi S, Kawahara T (2007) Natural variation for fertile triploid F1 hybrid formation in allohexaploid wheat speciation. Theor Appl Genet 115:509–518

Matsuoka Y, Takumi S, Kawahara T (2008) Eurasian wild wheat *Aegilops tauschii* Coss.: genealogical and ecological framework. PLoS One 3:e3138

Matsuoka Y, Nishioka E, Kawahara T, Takumi S (2009) Genealogical analysis of subspecies divergence and spikelet-shape diversification in central Eurasian wild wheat *Aegilops tauschii* Coss. Plant Syst Evol 279:233–244

Matsuoka Y, Nasuda S, Ashida Y, Nitta M, Tsujimoto H, Takumi S, Kawahara T (2013) Genetic basis for spontaneous hybrid genome doubling during allopolyploid speciation of common wheat shown by natural variation analyses of the paternal species. PLoS One 8:e68310

Matsuoka Y, Takumi SH, Kawahara T (2015) Intraspecific lineage divergence and its association with reproductive trait changeduring species range expansion in central Eurasianwild wheat Aegilops tauschii Coss. (Poaceae). BMC Evol Biol 15:213. doi:10.1186/s12862-015-0496-9

McFadden ES, Sears ER (1946) The origin of *Triticum spelta* and its free threshing hexaploid relatives. J Hered 37:81–89

Middleton CP, Senerchia N, Stein N, Akhunov ED, Keller B, Wicker T, Kilian B (2014) Sequencing of chloroplast genomes from wheat, barley, rye and their relatives provides a detailed insight into the evolution of the Triticeae tribe. PLoS One 9:e85761

Mizuno N, Yamasaki M, Matsuoka Y, Kawahara T, Takumi S (2010) Population structure of wild wheat D-genome progenitor *Aegilops tauschii* Coss.: implications for intraspecific lineage diversification and evolution of common wheat. Mol Ecol 19:999–1013

Nakai Y (1981) D genome doners for *Aegilops cylindrica* (CCDD) and *Triticum aestivum* (AABBDD) deduced from esterase isozyme analysis. Theor Appl Genet 60:11–16

Oldenburg DJ, Bendich AJ (2015) The linear plastid chromosomes of maize: terminal sequences, structures, and implications for DNA replication. Curr Genet. doi:10.1007/s00294-015-0548-0

Pestsova E, Korzun V, Goncharov NP, Hammer K, Ganal MW, Roder MS (2000) Microsatellite analysis of *Aegilops tauschii* germplasm. Theor Appl Genet 101:100–106

Rice P, Longden I, Bleasby A (2000) EMBOSS: the European molecular biology open software suite. Trends Genet 16(6):276–277

Tabidze V, Baramidze G, Pipia I, Gogniashvili M, Ujmajuridze L, Beridze T, Hernandez AG, Schaal B (2014) The complete chloroplast DNA sequence of eleven grape cultivars. Simultaneous resequencing methodology. J Int Sci Vigne Vin 48(2):99–109

Tsunewaki K (1989) Plasmon diversity in *Triticum* and *Aegilops* and its implication in wheat evolution. Genome 31:143–154

Tsunewaki K, Wang GZ, Matsuoka Y (2002) Plasmon analysis of *Triticum* (wheat) and *Aegilops*. 2. Characterization and classification of 47 plasmons based on their effects on common wheat phenotype. Genes Genet Syst 77:409–427

Vieira LN, Anjos KG, Faoro H, Fraga HPF, Greco TM, Pedrosa FO, Souza EM, Rogalski M, Souza RF, Guerra MP (2015) Phylogenetic inference and SSR characterization of tropical woody bamboos tribe Bambuseae (Poaceae: bambusoideae) based on complete plastid genome sequences. Curr Genet. doi:10.1007/s00294-015-0549-z

Wang J, Luo MCh, Chen Z, You FM, Wei Y, Zheng Y, Dvorak J (2013) Aegilops tauschii single nucleotide polymorphisms shed light on the origins of wheat D-genome genetic diversity and pinpoint the geographic origin of hexaploid wheat. New Phytol 198:925–937

Waterhouse AM, Procter JB, Martin DMA, Clamp M, Barton GJ (2009) Jalview version 2: a multiple sequence alignment and analysis workbench. Bioinformatics 25(9):1189–1191

Yamane K, Kawahara T (2005) Intra- and interspecific phylogenetic relationships among diploid *Triticum-Aegilops* species (Poaceae) based on base-pair substitutions, indels, and microsatellites in chloroplast noncoding sequences. Am J Bot 92(11):1887–1898