Molecular genetic analysis of alloplasmic recombinant lines (*Triticum dicoccum*) -*Triticum aestivum*

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Motivation and Aim: In connection with the task of increasing the genetic diversity of the main cereal crop, common wheat, *T. aestivum* L. (2n = 42; BBAADD), alloplasmic lines in which the nuclear genome of this species combines with the cytoplasm of an alien species are of great interest. Earlier, fertile alloplasmic wheat lines (*Triticum dicoccum*) -*Triticum aestivum* were obtained at the Institute of Plant Biology and Biotechnology (Almaty, Kazakhstan). The preliminary experiments showed the resistance of these lines to increased salt concentration, as well as to water deficiency [1]. Aim of this work is to analyze molecular genetic characteristics of the mitochondrial genome, which can determine the fertility and phenotypic variability of alloplasmic lines, as well as the search for genes associated with various manifestations of drought tolerance.

Methods and Algorithms: The plant material included 9 fertile alloplasmic wheat lines obtained from crossing (♀) *T. dicoccum* Schrank (2n = 28; BBAA) x (♂) *T. aestivum* L. cultivar Mironovskaya 808 (M808). Total DNA was isolated from 7-day old seedlings. PCR markers of the following, previously studied, genes were taken for the analysis of mtDNA: 1) *orf256* - chimeric reading frame near the cytochrome oxidase gene (associated with cytoplasmic male sterility (CMS); 2) *rps19-p*-pseudogene for a ribosomal protein. PCR products were separated on a 2% agarose gel with ethidium bromide. The *orf256* PCR product was excised from the gel, purified using QIAGEN kit (Germany) and sequenced using the Bigdye Terminator v3.1 cycle sequencing kit (Applied Biosystems, USA). Additionally, in the case of *orf256*, a CAPS marker was used, namely: the PCR product was digested with *Taq* I restriction endonuclease followed by electrophoresis on a 2% agarose gel. To search genes involved in drought tolerance we used the *Dreb-1* gene marker described in [2]. PCR products were analyzed on a 2% agarose gel. To confirm the single-nucleotide substitution specific for the *T. dicoccum Dreb-B1* gene, the PCR products were treated with restriction endonuclease *Bst*F5I (Sibenzyme, Novosibirsk) followed by separation of DNA fragments in the gel. A 635 bp fragment specific for *T. dicoccum* was excised from the gel and partially sequenced as described above. Analysis of all sequencing products was done at the "Genomika" Collective Use Center SB RAS.

Results: Analysis of the mtDNA of 9 alloplasmic wheat lines showed the spectra of PCR products and a CAPS marker identical to those of *T. aestivum* M808 (**Fig. 1,2**). Therefore, restoration of the fertility of alloplasmic recombinant lines (*T. dicoccum*) -*T aestivum* correlates with the displacement of sequences marking mtDNA of the female parent- *T. dicoccum*. An exception is the D-N-05 line, in which the spectra of PCR markers of the *rps19-p* and *orf256* genes corresponded to the latter species. Interestingly, the *orf256* sequence in this line has 100% homology with the analogous sequence of the CMS line of *T. timopheevii* (♀) x *T. aestivum* (X56186), however, unlike the latter, the D-N-05 line is fertile. In the future, we plan to identify fertility restoration genes within the nuclear genome of this line.

As previously shown, the greatest drought tolerance is characteristic of the lines D-d-05b, D-b-05, D-41-05 [1]. One of the important regulatory genes that may affect drought tolerance are the genes of the *Dreb* family (*Dehydration responsive element binding*), which are involved in the regulation of genetic cascades associated with various stress factors [3]. Using PCR- marker for one of the genes of this family, *Dreb-1*, we showed that in the nuclear genome of the drought-tolerant line D-41-05 there is a copy of the *Dreb-B1* gene of *T. dicoccum* (**Fig. 3**), which, according to the previous data [2], has a single nucleotide substitution relative to a similar gene in *T. aestivum*. Using sequencing (see above) we confirmed the presence of this substitution. Thus, it can be assumed that the increased drought tolerance of the D-41-05 alloplasmic line is associated with introgression of the *T. dicoccum* gene *Dreb-B1* in the genome of common wheat.

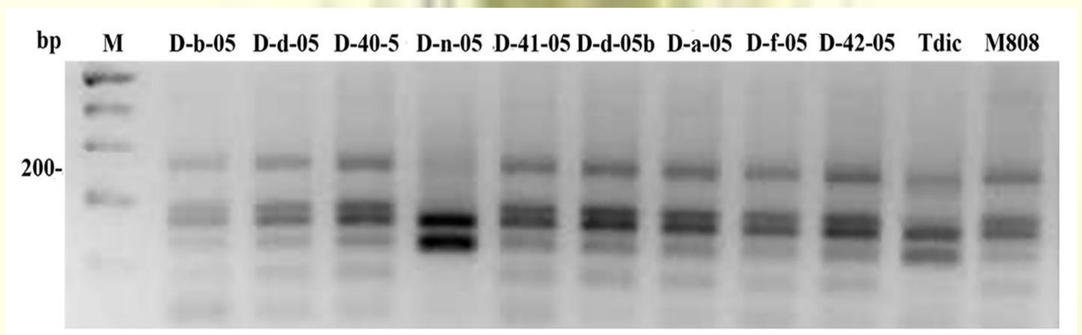

Fig. 1 Genotyping of alloplasmic lines with *orf256* CAPS -marker. The PCR products obtained using specific primers orf256f /orf256r were digested by *Taq* I-endonuclease. The "100 bp" ladder was used as a length marker.

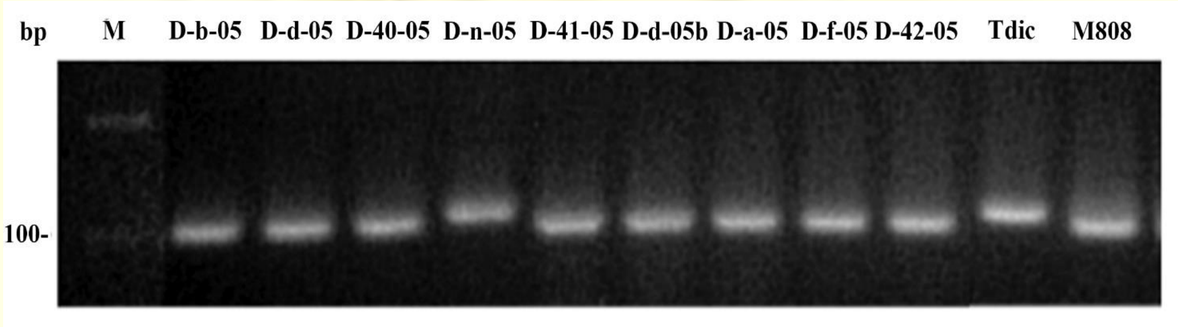

Fig. 2 Genotyping of alloplasmic lines with primers to rps19-p-pseudogene.

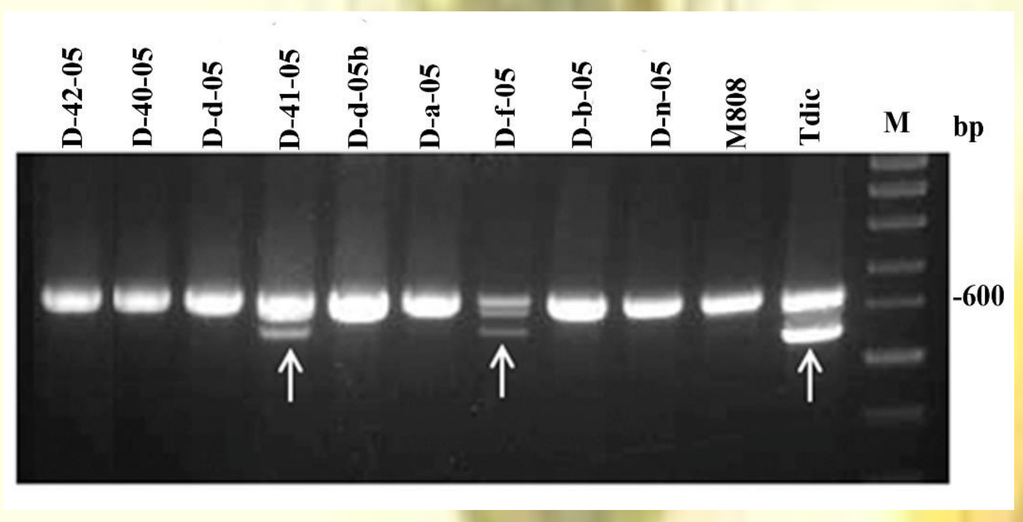

Fig. 3 PCR with a combination of primers P40/P18R for identification of *the Dreb-1* gene. The arrows indicate the common band for lines D-41-05, D-f-05, and *T. dicoccum*, corresponding to the *T. dicoccum*- specific allele of *Dreb-B1*. The upper double band corresponds to the *Dreb-A1* and *Dreb-D1* genes.

Conclusion: The obtained results showed the prospects of the studied alloplasmic lines for the analysis of molecular genetic effects that ensure drought tolerance and fertility restoration, the knowledge of which could be used in breeding programs for selection of useful genotypes of wild cereal species and combining them with the cultural ones.

References:

1. Terletskaya N.V., Shcherban A.B., Nesterov M.A., Perfil'ev R.N., Salina E.A., Altayeva N.A. and Blavachinskaya I.V. (2020) Drought stress tolerance and photosynthetic activity of alloplasmic lines *T. dicoccum* x *T. aestivum*. Int. J. Mol. Sci., 21, 3356; doi:10.3390/ijms21093356
2. Wei B., Jing R., Wang C. et al. (2009) *Dreb1* genes in wheat (*Triticum aestivum* L.): development of functional markers and gene mapping based on SNPs. Mol Breeding. 23:13–22.
3. Lucas S., Durmaz E., Akpınar B.A. (2011) The drought response displayed by a DRE-binding protein from *Triticum dicoccoides*. Plant Physiol. and Biochem. 49: 346e351.

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