

Using molecular genetic testing and field evaluation methods to develop new tomato breeding material with complex resistance to diseases

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Motivation and aim: Molecular testing of tomato accessions from the Belarusian-Cuban collection for the presence of disease resistance genes using newly developed and known in the literature markers and the selection of material with a complex of economically valuable traits during field trials.

Materials and methods: The studies were conducted on the open ground tomato accessions (*Solanum lycopersicum*) of the Belarusian-Cuban collection: Zorka, Garant, Chirok, Bercut, Tamara, Irma, Redgrant, Dividend, Spazhitak, Istochnik, Zvezdopad, L-43, Daniel, Pablo, Lyto, Sile, LR-13, LRs-30, L 22/11, L 29/6, L33/1, L41/3 and Alty. Cuban selection accessions combining in the genotype of *Ty-1* and *Ty-3* alleles resistant to *tomato yellow leaf curl virus* (TYLCV) from *S. chilense* (LA 2779) and the *Sw-5* allele resistant to *tomato spotted wilt virus* (TSWV) from *S. peruvianum* were developed by way of interspecific crosses and the selection of material using the pedigree method until F₀ generation. The presence of disease resistance alleles was assessed using 18 markers presented in the literature (Babak et al. 2023) and 2 new markers associated with late blight resistance alleles. The new markers were developed based on the polymorphism identified as a result of *Ph2(Ch)* (LOC101246197, Zhi X, et al, 2021) and *Ph3* (KJ563933) genes' sequencing in the accessions exhibiting varying degrees of resistance to late blight in field trials.

Results: The *Ph2 (Ch)* gene (LOC101246197) was sequenced in Dividend, Irma, Pablo, Alty and Sile accessions. An allele, which, according to Chinese researchers (Zhi X, et al., 2021), causes resistance to late blight, was identified in the Irma variety. The field trials of accessions in the climatic conditions of Cuba and Belarus confirm the maximum resistance of this accession to late blight. The CAPS marker Ph-2(R)_HindIII CC-Ase for the typing of R/S alleles of this gene was developed.

Based on the DNA marking results on *Ph3* gene alleles using the NC-LB-9-6678 marker (Panthee D.R., 2015), as well as the phenotypic assessment of late blight traits, the sequencing of Irma, Alty, Sile and LR-13 forms was performed. The comparative analysis of obtained sequences showed that the *ph-3* allele of *S. lycopersicum* (NC_090808.1) has two 11 bp deletions at the beginning of the gene, which leads to a frameshift, the appearance of an early stop codon and the synthesis of a short non-functional protein sequence. At that, the resistant LR-13 accession (according to the NC-LB-9-6678 marker) was found to have one 11 bp deletion, which also leads to the formation of a non-functional protein and susceptibility to late blight. The SCAR marker ph3 del22/11, allowing the typing for the presence of the indicated deletions associated with susceptibility to late blight, was developed.

The DNA screening of the studied tomato breeding material was carried out for the presence of alleles resistant to a number of diseases: meloidoginosis (*Mi-1.2*, *Mi-9*), late blight (*Ph3*, *Ph2* and *Ph2(Ch)*), cladosporiosis (*Cf2*, *Cf4*, *Cf5* and *Cf9*), *fusarium* (*I-2*, *I-2C*, *I-3* and *I-7*), verticillium (*Ve*), bacterial spot (*Rx-4*, *Pto*), bacterial speck (*Pto*), yellow curl leaf (*Ty2*, *Ty3*), tomato mosaic (*Tm-1*, *Tm-2*) and spotted wilt (*Sw-5b*). Based on DNA typing results, among the accessions of the compiled collection, the forms that combine 7-9 disease resistance alleles were identified: Lyto (7 R), LRs-30 (7 R), Pablo (8 R), Alty (8 R), Sile (9 R) and LR-13 (9 R).

Conclusions: New molecular markers, allowing the ranking of accessions by resistance to late blight, have been developed. Based on the molecular testing for disease resistance, the best tomato accessions from the Belarusian-Cuban collection have been selected for further use in the breeding process.

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