

# Technologies of DNA marking of target genes as a tool for selecting parental pairs in tomato breeding programs for resistance to *Fusarium oxysporum* f. ssp. *Lycopersici*.

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**Abstract.** Development of tomato varieties and hybrids with resistance to the pathogen *Fusarium oxysporum* f sp. *Lycopersici* is a priority in breeding of vegetable crops. Evaluation and selection of the initial material is important in effective breeding for resistance to a pathogen. To do this, along with classical methods in tomato breeding, the method of molecular marking is widely used. The purpose of this study was to screen the existing collection of tomatoes with molecular markers I-2/5, I2OH and At-2, which allow identification of resistance genes to the first and second races of the fusarium pathogen. As a result of the work, genotypes were identified that can be used as donors of resistance genes for further breeding work.

## 1 Introduction

Tomato is a widespread vegetable crop, which is cultivated both in open and protected ground. The presence of a large amount of nutrients, vitamins and trace elements in it makes this vegetable crop popular among the population [1]. The demand for quality products contributes to an increase in the diversity of tomato varieties and hybrids, and also stimulates the improvement of existing seed collections [2,3].

When developing new, modern forms of tomatoes, breeders are faced with the task of transferring and combining valuable economic traits in one genotype. Success in tomato breeding largely depends on the initial material. Knowing the exact characteristics of parental lines helps in solving this problem [4]. For a better assessment, the method of molecular marking is promising, which at times allows to reduce the time of breeding process. [5].

Molecular marking selection is a crop improvement tool in which a marker is used to look for desirable traits. When using markers, the choice of sample to serve as initial material is based only on genotype and is not influenced by environmental factors [3, 6].

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The basic principle of DNA marking is to identify the close linkage of a marker with a gene that controls an economically valuable trait [7]. The tomato was one of the first crops for which molecular markers were proposed as criteria for selection in breeding [8].

For breeders, one of the main features when developing a new breeding material is resistance to diseases, which are the main limiting stressor in obtaining high yields. The dominant disease on tomatoes is *Fusarium* (pathogen - *Fusarium oxysporum* f. ssp. *Lycopersici*) [2, 9]. Sources of resistance to the pathogen *Fusarium oxysporum* f. ssp. *Lycopersici* have been found in wild tomato species such as: *L. pimpinellifolium* and *L. Peruvianum* and cultivated tomato species *L. Pennellii* [10]. In each of these species, three resistance genes were identified that are responsible for resistance to three races of the pathogen: gene I, provides high resistance to race 1, gene I-2 was resistant to races 1 and 2, gene I-3 to provides resistance to races [1, 2, 3, 8, 10, 11].

The scientific literature presents markers for identification of all three genes [6, 9, 10, 12]. The I-2 gene is of the greatest interest for our study, as it has resistance to pathogen races common in our region. [13].

## 2 Materials and methods

As the material for the study we used samples from the tomato collection of the department of vegetable and potato growing of FSBSI “Federal Scientific Rice Centre” and farming enterprise “POISK” (used as control samples carrying the *Fusarium* wilt resistance gene).

The biological material serving as control samples was germinated on moistened filter paper in a thermostat maintaining a temperature of 26°C, then the seedlings of the tomato plant were taken into test tubes with a lysis buffer to isolate their DNA.

Tomato samples intended for crossing were also germinated in a thermostat, but with subsequent transplantation of plants into cassettes. Further, samples of tomato plants in the phase of two true leaves were transplanted into vessels, which were placed in artificial climate chambers (ACC) with the temperature regime maintained at 27-30°C.

The DNA of the analyzed tomato plants was extracted according to the Murray and Thompson method with modifications, using cetyltrimethylammonium bromide (CTAB) as the main lysis buffer [14].

The reaction mixture in an amount of 25 µl contained the following components: 0.05 mM dNTPs, 0.3 mM of each primer, 25 mM KCL, 60 mM Tris-HCL (pH 8.5), 0.1% Triton X-100, 10 mM 2-mercaptoethanol, 1.5 mM MgCL<sub>2</sub>, 1 unit of Taq polymerase.

The material was evaluated by markers previously developed and presented in the literature. [8,9,15].

**Table 1.** Nucleotide sequence of markers for identifying I and I-2 genes.

Name	Sequence (5'↔3')	Literary source
I-2/5	F	Yu S.C., Zou Y.M. A co-dominant molecular marker of <i>Fusarium</i> wilt resistance gene I-2 derived from gene sequence in tomato.
	R	

AT-2	F	CGAATCTGTATATTACATCCGT	Arens P., Mansilla C., Deinum D., Cavellini L., Moretti A., Rolland S., H. van der Schoot, Calvache D., Ponz F., Collonnier C., Mathis R., Smilde D., Caranta C., Vosman B., Development and evaluation of robust molecular markers linked to disease resistance in tomato for distinctness, uniformity and stability testing.
	R	CGTGGTGAATACCGATCATAGTC GA	
I2OH	F	TGGAGAGTTCCTACACTTGAG	Popoola A.R., Ercolano M.R., Feriello F. CAPS markers TAO1 and TG105 in the identification of I2 resistant gene in Nigerian accessions of tomato, Solanum lycopersicum L.
	R	TTCTCTCAAGGTAGTTGGCAG	

The PCR conditions protocol is presented in Table 2.

**Table 2.** Protocols of PCR.

Marker name							
I-2/5, I-2OH				AT-2			
PCR stage number of cycles	T, °C	t, sec	PCR stage number of cycles	T, °C	t, sec		
1 Denaturation	94	120	1 Denaturation	94	300		
30x	2 Denaturation	94	30	35x	2 Denaturation	94	30
	3 Annealing	65	30		3 Annealing	55	30
	4 Extension	72	30		4 Extension	72	60
5 Final extension	72	420	5 Final extension	72	420		

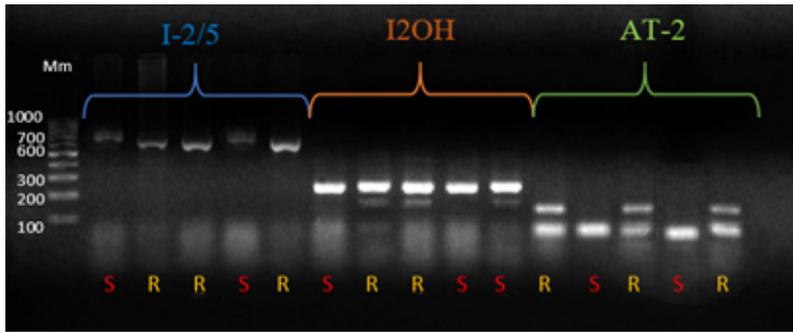
To visualize the PCR products, electrophoresis in 2% agar gel with the addition of BrEt was used. Visualization of the obtained products is carried out in ultraviolet light using the gel-documenting system Gel Doc XR + on Image Lab software.

### 3 Results and discussion

The use of DNA markers closely linked to loci determining agronomically valuable traits makes it possible to reliably conduct selection by genotype, excluding the environmental factor.

The selection of markers suitable for assessing the DNA polymorphism of tomato varieties that differ in resistance to fusarium was carried out on the material available in the collection of the Department of Vegetable and Potato Growing of FSBSI “Federal Scientific Rice Centre”.

To study the level of polymorphism of microsatellite loci between resistant and unstable tomato forms used as parental forms in breeding work, we tested the following markers: I-2/5, I2OH, AT-2.



**Fig. 1.** Results of PCR-analysis for the presence of fusarium resistance gene I and I-2.

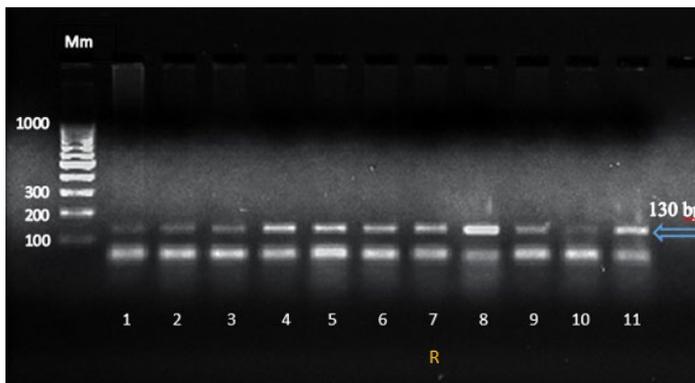
Note: Mm - molecular weight marker 100 bp + 1.5 Kb (supplier - Syntol, Russia). S - susceptible control sample; R - resistant control sample.

As a result of testing the markers for tomato resistance to Fusarium wilt, we identified primer pairs that reliably show the presence of resistance and susceptibility alleles on contrasting forms. Markers: I-2/5 and I2OH are intended for the resistance gene I - 2, and the presence of the resistance gene I is identified using the AT2 marker (Figure 1).

The following are the results of PCR analysis for the detection of genes of interest in the initial material, which were carried out using approved markers.

Gene I was identified using the dominant marker AT-2. As a result of testing the AT-2 marker in the tomato genotype, an amplified PCR fragment with a size of 130 bps was detected, which indicates the presence of a dominant homozygous form of the gene.

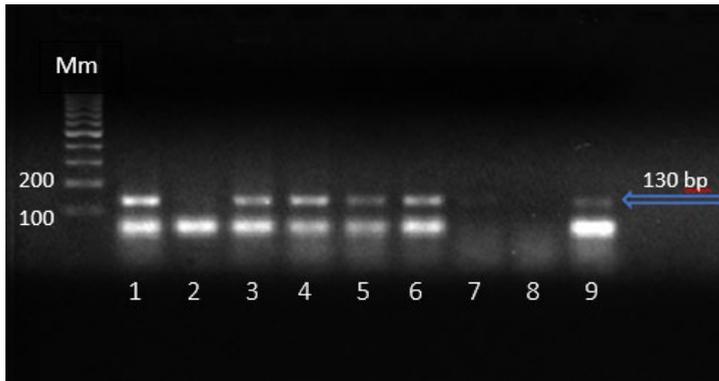
As a maternal component for the hybridization of tomato plants, we will use lines carrying the recessive ps-2 gene, which controls the non-opening of anthers.



**Fig. 2.** Results of PCR-analysis using the AT-2 molecular marker for the presence of the fusarium resistance gene I among the initial material of tomato plants.

Note: Mm - molecular weight marker 100 bp + 1.5 Kb (supplier - Syntol, Russia); 1 - 11 lines: 1, 2, 4, 5, 6(10), 4/1, Golden flow F1 (resistant control sample (R)), 7(9), 8, 9(7), 10(6) .

The foregram shows that in all the analyzed samples, a fragment with a product size of 130 bp is observed, from which it can be concluded that tomato lines are suitable for further hybridization (Figure 2).

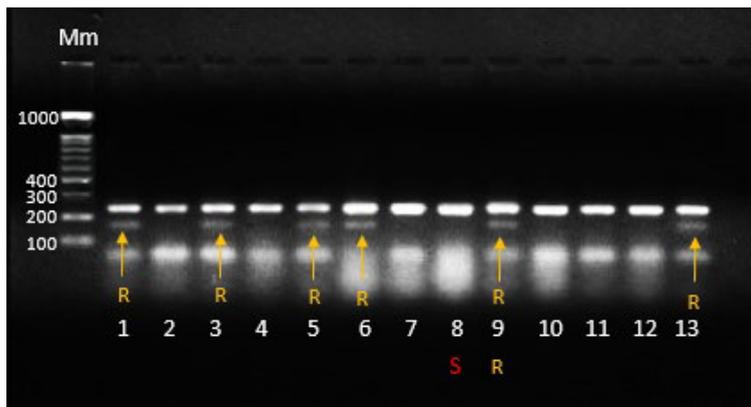


**Fig. 3.** Results of PCR-analysis using the molecular marker AT-2 for the presence of the fusarium resistance gene I among the initial material of tomato plants.

Note: Mm - molecular weight marker 100 bp + 1.5 Kb (supplier - Syntol, Russia); 1 - 9 lines: 4/1, 1/2, 2/4, 3/5, 6/6, 7, 8, 1/14, 7/20.

Figure 3 shows that the resistance allele is observed in 6 samples, which are planned to be used for the transfer of the I gene in the next stages of breeding.

Using the SCAR marker I-2OH, sterile samples were analyzed, which will be used in our breeding scheme as a maternal component, as they are characterized by the presence of functional male sterility of the Vrbciany type.

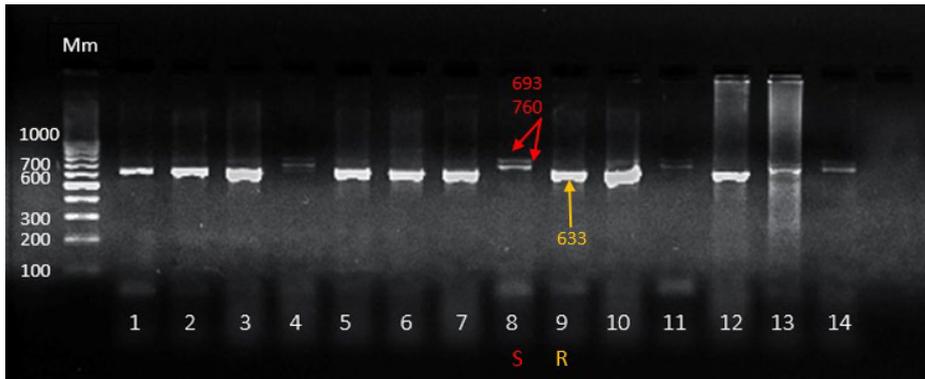


**Fig. 4.** Results of PCR-analysis using the molecular marker I2OH for the presence of the fusarium resistance gene I - 2 among the initial material of tomato plants.

Note: Mm - molecular weight marker 100 bp + 1.5 Kb (supplier - Syntol, Russia); 1-13 lines: 2, 4, 5, 6 (10), 4/1, 3/5, 4/1, 6/6 (susceptible control (S)), Golden flow F1 (resistant control (R)), 7(9), 9(7), 10(6), 8.

According to Figure 4, samples No. 1, 3,5,6,13 are characterized by the presence of fragments indicating the presence of resistance alleles to the pathogen *Fusarium oxysporum* f. sp. *Lycopersici*.

For the analysis of tomato forms, the pollen of which is planned to be used for pollination, the I-2/5 marker was used. Amplification with this marker makes it possible to obtain 633 bp fragments, which characterize the presence of the resistance allele, and 693 bp fragments combined with 760 bp characterizing the susceptibility allele.



**Fig. 5.** Results of PCR-analysis using the molecular marker I2OH for the presence of the fusarium resistance gene I-2 among the initial material of tomato plants.

Note: Mm - molecular weight marker 100 bp + 1.5 Kb (supplier - Syntol, Russia); 1-14 lines: 1/2, 2/4, 3/5, 6/6, Casseopeia F1, Raspberry Dessert F1, Torry, 4/1 (susceptible control (S)), Golden flow F1 (resistant control (R)), Indent Sw5, Indent to Alt, 7/20, 663, 868.

After analyzing the result shown in Figure 5, we concluded that it would be appropriate to use lines carrying the resistance gene I - 2 as the paternal parental component, namely samples Nos. 1, 2, 3, 5, 6, 7, 10, 12.

## 4 Conclusion

As a result of the study, we identified 17 tomato genotypes carrying I gene and 13 genotypes carrying I – 2 gene, of which 8 samples had a combination of these two genes for resistance to Fusarium wilt of tomatoes in their genotype.

Thus, lines 4/1, 1/2, 2/4, 3/5 and 7/20 are planned to be used as paternal forms for transferring genes of interest to progeny. Among the sterile forms, 3 lines can be distinguished that have these two genes. The selected lines were recommended as parental forms for the development of tomato varieties and hybrids resistant to Fusarium oxysporum. And the tested markers can be used to screen collections and search for new sources of I-2 gene.

## Acknowledgement

The research was carried out with the financial support of the Kuban Science Foundation and the Organization in the framework of the scientific project № MFI-P-20.1/1.

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