Proceedings of the 6th International Conference on Biological Engineering and Medical Science
DOI: 10.54254/2753-8818/2026.37376

CAPS Marker Development for the Tomato Late Blight Resistance Gene Ph-2

Jiahe Hu

Bathurst Future Agri-Tech Institute, Qingdao Agricultural University, Qingdao, China
fen2336602@outlook.com

Abstract. Tomato late blight, caused by the oomycete Phytophthora infestans, results in massive yield losses in tomato production worldwide. Traditional disease management strategies such as the application of fungicides, are ineffective against the frequent variation in P. infestans strains. Therefore, breeding late blight resistant cultivars is a sustainable strategy for disease control. The Ph-2 gene, which mainly has a cooperative effect with Ph-3 gene, provides resistance against most prevalent races in tomato. To detect Ph-2 gene in tomato plants of all growth stages, two Cleaved Amplified Polymorphic Sequence (CAPS) markers targeting the Ph-2 locus were designed based on sequence polymorphisms obtained from the Sol Genomics Network database. Two primer pairs, PP_Ph2_1 and PP_Ph2_2 were developed. Two CAPS marker, CAPS_Ph2_1and CAPS_Ph2_2 were designed basing on primers. CAPS_Ph2_1 pairs the restriction enzyme AseI, and CAPS_Ph2_2 pairs HinfI. Two expecting band patterns for homozygous-resistant, homozygous-susceptible and heterozygous genotypes are showed. These designed CAPS markers are aimed to support rapid genotyping of Ph-2 in tomato germplasm on late-blight resistance research

Keywords: Tomato Late Blight, Ph-2 Gene, CAPS Marker, Marker-assisted Selection, Disease-resistance Breeding

1. Introduction

Tomato late blight is a destructive disease of tomato plants caused by a type of oomycete-*Phytophthora infestans*. Its favorable weather conditions are temperature range of 15-18°C and relative humidity of above 90%. Its spores can be widely spread with airflow and cause infections rapidly [1]. In the early stage of infection, it causes latent infection that has no obvious external symptoms on plants, making it difficult for growers to detect and prevent the diseases in advance. Once the late blight breaks out in the field, tomatoes across the field will be severely infected within a few days. Oily, water-soaked lesion may appear on the leaves, disrupting the process of photosynthesis and then reducing the organic matter storage of fruit. The infection of late blight also causes direct damage to tomato fruit, making the fruit rot, color black and form white mycelium [2]. Furthermore, the populations of *P. infestans* are varied frequently, and scientists have detected many strains of *P. infestans* that resistant to existing widely used fungicides. These factors make tomato late blight a destructive and troublesome disease, significantly reducing the economic value of tomato fields once the disease occurred [3]. In the past, it has caused severe losses in tomato

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DOI: 10.54254/2753-8818/2026.37376

production, and one of the most typical cases happened in the eastern United States, 2009. Local farmers suffered great yield loss due to inefficient fungicide, symptomless infected tomato plant dealing and new pathogen variation-US-22. It eventually took many years to recover tomato production with constant financial supports [1].

Recently, breeding late blight-resistant tomato varieties has become the major strategy to stable tomato production. Most late blight resistance breeding resources are from wild tomato species. Among them, the red-fruited wild tomato *Solanum pimpinellifolium* and the green-fruited *Solanum habrochaites* serve as the most important resistance cultivars [4]. Those resistance genes including race-specific resistance genes (such as *Ph-1, Ph-2, Ph-3*) and race-nonspecific resistance quantitative trait loci (QTLs). The *Ph-1* was the first resistance gene that was found in the wild tomato relative *S. pimpinellifolium* [5]. However, it has been proved that new strains can resist the defense system controlled by *Ph-1*， and it is has not performed effectively towards predominant strains [6]. *Ph-3* is a particularly functional resistance gene of late blight and has been widely applied into tomato breeding [4]. However, *Ph-3* is also a qualitative gene， which are vulnerable to be overcame by varied strains [7, 8]. In fact, strains of *P. infestans* that can overcome *Ph-3*-mediated resistance have been verified [4].

The *Ph-2* gene, an incompletely dominant resistance gene found from *S. pimpinellifolium*-West Virginia 700 (WV 700), having resistance against the T1 race of *P. infestans* [4]. In 1998, this locus was detected by qualitative and quantitative analyses using restriction fragment length polymorphism markers（RFLP）technology and the *Ph-2* locus is located on the distal part of the long arm of chromosome 10 in an interval of 8.4 cM and flanked by markers CP105 and TG233 [9]. In 2021, the markers CAPS-1 and CC-Ase mapped *Ph-2* gene to a 141-kb sequence [10]. Compared with the *ph-2*， *which performing* as susceptible to late blight, the expression of *Ph-2* can induce hypersensitive response (HR) in *Nicotiana Benthamian*. Pan and his team claimed that the *Ph-2*-controlled protein is a prerequisite for triggering HR reaction [11]. In addition. There are investigations proved that tomato cultivars， which conferred both *Ph-2* and *Ph-3* resistance， performing highly effective resistance against most *P. infestans* races [4].

Marker-assisted selection has been widely applied to identify late blight-resistant tomato genes and QTLs, and to develop commercial tomato species [12]. By detecting corresponding molecular markers, scientists can determine whether plants carry target genes in all growth stages and make selection, without waiting for expression-traits that may weakly expressed and would consume extra time and resources on prolonged cultivation.

In this study, based on Sol Genomics Network databases, we designed two CAPS markers that can detect *Ph-2* gene, aiming to help the detection of *Ph-2* gene in different cultivars, expand existing research on the *Ph-2* resistance gene against late blight.

2. Materials and methods

2.1. Plant materials

Tomato materials used for in-silico genotype prediction were retrieved from public databases [13, 14]. Resistant and susceptible tomato cultivars collected from the Tomato Genetics Resource Center (TGRC) and Mountain Horticultural Crops Research and Extension Center (MHCREC). The WT *S. pimpinellifolium* WV 700 is designed as resistant material, which is the original source material of the *Ph-2* genotype. Several *S. lycopersicum* lines were used as susceptible controls.

All assessments in this study were performed based on published sequence data. Plant materials that were used to predict CAPS marker genotypes are presented in Table 1.

DOI: 10.54254/2753-8818/2026.37376

Table 1. Plant materials involved in this study

Name	Species	Origin
PI 224710	*S pimpinellifolium*	USDA-PGRU
PI 270443	*S. pimpinellifolium*	USDA-PGRU
NCEBR-2	*S. lycopersicum*	MHCREC
NC1CELBR	*S. lycopersicum*	MHCREC
Mountain Merit	*S. lycopersicum*	MHCREC
Plum Regal	*S. lycopersicum*	MHCREC
Moneymaker	*S. lycopersicum*	TGRC
M82	*S. lycopersicum*	TGRC

2.2. Primer design for Ph-2 genotyping

The genomic sequence of *Ph-2* and *ph-2* were retrieved form the Sol Genomics Network database. By comparing with the two sequences from the Tabel S4 of reference [11], we determined the polymorphisms between *Ph-2* and *ph-2* loci in a nearly-500-kb sequence within approximately 3000-kb total sequence length. To detect the genotype of *Ph-2 and ph-2* in various tomato varieties, we used the EMBOSS WATER tool to generate the pairwise local sequence alignments for primer design [15].

3. Result

3.1. Primers for CAPS candidates

Finally, two pairs of primers were designed to amplify *Ph-2* (Table 2). The Melting Temperature (T_m) of the forward PP-Ph2-1 is 57.69 ºC, GC Content (GC) is 50%，Hairpin is -1.69 kcal/mol and Self-dimer is -3.61 kcal/mol. For reverse primer of PP-Ph2-1，its T_m is 59.96 ºC, GC is 45.45%，Hairpin is 0.31 kcal/mol and Self-dimer is -3.61 kcal/mol. For forward primer of PP-Ph2-2，its T_m is 58.31ºC, GC is 47.62%，Hairpin is -0.06 kcal/mol and Self-dimer is -3.61 kcal/mol. For reverse primer of PP-Ph2-2，its T_m is 58.6 ºC, GC is 42.86%，Hairpin is -1.31 kcal/mol and Self-dimer is -5.36 kcal/mol.

Table 2. Primer sequences and amplicon lengths for CAPS markers targeting the tomato Ph-2 late blight resistance gene

	Forward primer	Reverse primers	Length of fragment
PP_Ph2_1	TGCGTAGCCTTATTGAGTGGA	TTGGGGCAGTGTTGTATTCG	371bp
PP_Ph2_2	TCTTAGGCTTGAGAAGTTGCG	AACTGAAATTGGGGCAGTGTT	360bp

3.2. Predicted gel patterns

Two CAPS markers were designed basing on two polymorphisms on *Ph-2* and *ph-2* respectively. CAPS Designer provided by Sol Genomics Network identified restriction enzymes that nuclease alleles. For the 371bp fragment amplified with primer pair PP_Ph2_1, amplicons from late blight susceptible plants (*ph-2*) can be recognized and cut by AseI into 243bp and 128bp fragments, while the 371bp fragments amplified from late blight-resistant plants (*Ph-2*) remains the intact. Enzyme

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Hinfl can recognize and cut the 360bp fragment amplified by primer pair PP_Ph2_2 on the resistant genotype into 216bp and 144bp fragment, whereas the amplicon of late blight-susceptible varieties is uncut. The designed PCR protocol was as follows: preheating at 94C° for 3 mins, denaturation at 94C° for 30s, annealing at 56C° for 30s, extension at 72C° for 30s, 35 cycles were carried out, final extension at 72C° for 5 min and storage at 4C°. The PCR system volume was 50 μL: Primers 4μL, cDNA 2μL, restriction endonuclease 25μL and ddH_2O 19μL. The information of enzymes and primers used for detection are presented in Figures 1 and 2.



Figure 1. Predicted banding patterns of CAPs_Ph2_1 marker



Figure 2. Predicted banding patterns of CAPs_Ph2_2 marker

3.3. Predicted genotype

Eight tomato varieties with *Ph-2* resistance were selected for in-silico genotyping using developed CAPS markers. Three *Ph-2* genotype could be detected by markers: homozygous resistant (R), homozygous susceptible (S), and heterozygous (HT). Three resistance materials, PI 224710, PI 270443 and NC1CELBR, were predicted to perform homozygous-resistant (R) with all five CAPS markers. Two commercial cultivars, Mountain Merit and Plum Regal, were predicted to be heterozygous (HT). Three susceptible strains, NCEBR-2, Moneymaker and M82, were predicted to perform homozygous-susceptible (S) genotypes for five markers. Complete predicted genotyping results are summarized in Table 3.

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Table 3. Predicted phenotype

Name	*Ph-2* resistance	CAPS 1	CAPS 2
PI 224710	Resistant	R	R
PI 270443	Resistant	R	R
NCEBR-2	Susceptible	S	S
NC1CELBR	Resistant	R	R
MountainMerit	Heterozygous	HT	HT
Plum Regal	Heterozygous	HT	HT
Moneymaker	Susceptible	S	S
M82	Susceptible	S	S

4. Discussion

4.1. How are these gene-based Ph-2 markers different from other Ph-2 markers

Traditional markers for *Ph-2* detection are mostly linked markers, using the characteristics of sequences around *Ph-2* allele to detection, in this study, we located the polymorphism between *Ph-2* and *ph-2* sequences to design the CAPs markers, which are gene-based markers. Gene-based markers have the potential to provide a direct association with the target gene and reduce the risk of marker misclassification caused by recombination between a linked marker and the target gene.

4.2. What are the limitation of in silico marker development？what kind of experiments would need to conducted

Although in silico marker designation provides a theoretically approach for mapping candidate markers, predicted result cannot confirm the reliability and efficiency of markers for practical applications. In this study, the candidate CAPS markers were designed by comparing *Ph-2* and *ph-2* sequences and restriction enzymes were picked according to its sequences in silico. However, the predicted amplification and digestion patterns have not been experimentally confirmed.

Furthermore, the polymorphism of sequences is not functionally responsible for late blight resistance. Therefore, the association of genotype with resistance should be evaluated using independent phenotypic disease-resistance assays. Combining molecular genotyping with late blight inoculation assays would make the relationship between the marker genotype and the resistance phenotype to be clearly.

5. Conclusion

In this study, two CAPS markers originating the *Ph-2* locus were developed through in silico according to the sequence polymorphisms between the *Ph-2* and *ph-2* alleles. Markers generated predicted banding patterns for homozygous-resistant, homozygous-susceptible, and heterozygous genotypes. In silico genotyping of eight tomato varieties also performed consistent genotype predictions, with PI 224710, PI 270443, and NC1CELBR predicted as homozygous-resistant, Mountain Merit and Plum Regal as heterozygous, and NCEBR-2, Moneymaker, and M82 as homozygous-susceptible. These results suggest that the developed CAPS markers have potential for

DOI: 10.54254/2753-8818/2026.37376

detecting *Ph-2* alleles and supporting marker-assisted selection in tomato late blight resistance breeding.

However, the markers developed in this study have merely been evaluated in silico, and their amplification and restriction digestion patterns have not yet been experimentally validated. In addition, the identified sequence polymorphisms cannot functionally confirm the relationship between the markers and late blight resistance. Therefore, future work should be conducted that using PCR and restriction digestion assays, followed by phenotypic evaluation through late blight inoculation assays.

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