Asia-Pacific Journal of Science and Technology: Volume: 29. Issue: 06. Article ID.: APST-29-06-11. Research Article

APST

Asia-Pacific Journal of Science and Technology

https://www.tci-thaijo.org/index.php/APST/index

Published by Research Department,
Khon Kaen University, Thailand

Characterization of a novel *Ty-2a* intragenic allele for marker development in tomato yellow leaf curl virus resistance breeding programs of tomato

Thananya Thongsanit[1,2], Ornubol Chomdej[1,2], Julapark Chunwongse[1,2,3] and Pumipat Tongyoo[1,2,*]

[1]Center for Agricultural Biotechnology, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom, Thailand
[2]Center of Excellence on Agricultural Biotechnology: (AG-BIO/MHESI), Bangkok, Thailand
[3]Department of Horticulture, Faculty of Agriculture at Kamphaeng Saen, Kasetsart University, Kamphaeng Saen Campus Nakhon Pathom, Thailand
[*]Corresponding author: pumipat.tong@ku.th

Received 4 October 2023
Revised 19 February 2024
Accepted 8 May 2024

Abstract

One of the most severe diseases for tomato plantations in Thai farming is the *Tomato Yellow Leaf Curl Virus* (TYLCV) that has challenged breeders to introduce resistance to the crop for decades. Several TYLCV resistance genes have been confirmed, such as *Ty-1*, *Ty*-2, and *Ty*-3, of which *Ty*-2 is the most common target in TYLCV resistance breeding. The gene is located on chromosome 11 as *TYNBS1*, encoding CC-NB-LRR proteins classified in the R gene family. A co-segregating T0302 marker has been used for tracing the *Ty-2* resistance allele. The marker illustrates three polymerase chain reaction (PCR) product fragments, classified as 800 bp, 900 bp, and 830 bp and representing the *ty-2* susceptible, *Ty-2* resistance, and *Ty-2a* resistance alleles, respectively. A novel intragenic resistance allele has now been demonstrated in *Solanum habrochaites* 'L06112' Clone No.1. DNA flanking the homozygous *Ty-2a TYNBS1* region in the *Solanum lycopersicum* TOMAC647 tomato line was compared with related genes in the family, resulting in the recognition of a conserved domain. The *Ty-2a* and *Ty-2* alleles were not significant for TYLCV resistance ability. We proposed *TYNBS1I* as the novel allele, showing 94% identify with *TYNBS1* in *Ty-2*. Furthermore, we designed DNA markers which detect the DNA sequence at the gene terminal and 3' downstream of *TYNBS1I* that were not in *TYNBS1,* with 164 bp of the PCR product only present on tomato lines that contained *Ty-2a*, allowing us to use these markers in TYLCV resistance programs.

Keywords: CC-NB-LRR, Marker-assisted selection, primer combination, allele identification, *Solanum habrochaites*

1. Introduction

One of the most severe threats in tomato production worldwide is the tomato yellow leaf curl disease (TYLCD) caused by *Tomato Yellow Leaf Curl Virus* (TYLCV). The virus belongs to the group of Begomoviruses that are single-stranded DNA plant viruses transmitted by whiteflies (*Bemisia tabaci*) [1]. Initially, the infected plants of the host exhibit signs of abnormal growth with chlorotic and upward-curled leaves. As the disease progresses, symptoms include severe stunting, substantial leaf size reduction, flower abscission, and significant yield reduction, finally resulting in up to 100% yield loss [2].

Six TYLCV resistance genes (*Ty-1*, *Ty-2*, *Ty-3*, *Ty-4*, *Ty-5*, and *Ty-6*), have been reported. *Ty-1* and *Ty-3* have been identified in the wild tomato, *Solanum chilense* 'LA1969' [3] and in 'LA2779, and 'LA1932' [4], respectively, and have been mapped in the long arm of tomato chromosome 6, being identified as allelic [5]. *Ty-2,* also known as *TYNBS1*, is an R gene introgressed from the *S. habrochaites* accession 'B6013' [6], located on the long arm of chromosome 11 and has been cloned [7]. *Ty-4* originates from *S. chilense* 'LA1932', located on the long arm of chromosome 3 [8]. *Ty-5* has been found in the *Solanum peruvianum* 'TY172' breeding line, located on chromosome 4 in the same region as recessive resistance, *ty-5* from the tomato cultivar Tyking [9], and lastly, the *Ty-6* gene conferring resistance to both monopartite TYLCV and bipartite *Tomato Mottle Virus* (ToMoV), derived from either *S. chilense* 'LA2779' or 'LA1938' [10] and mapped on chromosome 10 [11].

Among them, the *Ty-2* gene was identified as an Nucleotide-Binding Site-Leucine-Rich Repeat (NBS-LRR) and encodes Coiled-Coil-Nucleotide-Binding-Leucine-Rich Repeat (CC-NB-LRR) proteins containing an N-terminal coiled-coil domain [7] which is a dominant resistant gene which can confer resistance to monopartite begomoviruses [12]. Therefore, *TYNBS1*, or the *Ty-2* resistance gene, has been used in TYLCV resistance breeding programs for over three decades. SGN Marker T0302 (89 cM, physical distance 51.878 Mb, chromosome 11) has been found to co-segregate with *Ty-2* and is now being used in marker-assisted selection for TYLCV resistance [13]. This T0302 marker provides three different-sized PCR amplicons: 800 bp is the *Ty-2* susceptible allele, 900 bp belongs to the *Ty-2* resistance allele, and 830 bp is the *Ty-2a* resistance allele. Chomdej et al. (2016) [14] reported the *Ty-2* allele from S. *habrochaites* 'L06112' Clone No 1 (C1) and unofficially designated it as *Ty-2a*.

S. habrochaites accession 'L06112' C1 is highly resistant to the TYLCV-Thailand isolate, TYLCTHV [15]. The T0302 marker could detect 900 and 830 bp of the PCR products. Its BC1F1 generation (B10 line) generated by the backcross of the F1 of L06112 C1 with TOMAC463, a TYLCV susceptible near-isogenic line of commercial cultivar Seedathip3 that remains highly resistant to TYLCTHV. However, the T0302 marker amplified only one band of 830 bp and 800 bp of the susceptible allele [14]. In addition, the BC6F1 generation (B10-2-2-13-26-7) is selected by using T0302 makers to retain and show resistance to TYLCV at a lower level than the wild *S. habrochaites* 'L06112' C1 but at a higher resistance level than the commercial cultivar Seedathip3 that does not have the T0302 marker resistant allele [16].

In this study, we describe the development of specific markers for the *Ty-2a* allele which was inherited from *S. habrochaites* 'L06112' C1 that occurred during our breeding from TOMAC647, which is BC6F2 of L06112 C1 and TOMAC463. We detected the virus-resistance alleles by using a reported sequence upstream and downstream of the *Ty-2* region [7] and then validated the marker on tomato lines and hybrid lines containing *Ty-2*, *Ty-2a*, and *ty-2* as a strategy for tomato improvement. This specific marker detects at the gene terminal and 3' downstream of *TYNBS1I*.

2. Materials and methods

2.1 Plant material

The TOMAC647 tomato line was used as a source of the *Ty-2a* allele for data analysis, development, and validation as a specific marker. TOMAC648 and TOMAC646 are tomato lines containing homozygous *Ty-2*. The hybrids TOMAC463 x TOMAC648, TOMAC463 x TOMAC647, and TOMAC647 x TOMAC648 contain *ty-2/Ty-2*, *ty-2/Ty-2a*, and *Ty-2a/Ty-2*, respectively. TOMAC463 is a near isogenic line of Seedathip3 TYLCV susceptible (ty-2/ty-2) which contains the TMV resistance gene (*Tm-2*). All tomato lines and hybrids were genotyped with the T0302 marker and are shown in Table 1.

Table 1 Four tomato lines and three hybrids containing T0302 marker genotype.

Line	Genotype (T0302)	Source of tomato line
TOMAC647	*Ty-2a/Ty-2a*	Plant Biotechnology Laboratory at
TOMAC648	*Ty-2/Ty-2*	Centre of Agricultural Biotechnology,
F_1 (TOMAC463 x TOMAC648)	*ty-2/Ty-2*	Kasetsart University, Kamphaeng
F_1 (TOMAC463 x TOMAC647)	*ty-2/Ty-2a*	Saen Campus
F_1 (TOMAC647 x TOMAC648)	*Ty-2a/Ty-2*	
TOMAC646	*Ty-2/Ty-2*	
Seedathip3	*ty-2/ty-2*	[17]

2.2 DNA extraction and sequencing

DNA was extracted from all plant materials following the DNA extraction protocol described in [18]. Each DNA fragment was amplified using a PCR technique and then the PCR products were purified using a Nucleospin Gel and PCR Clean-up Kit (MACHEREY-NAGEL; Germany) before sending for sequencing at 1st BASE DNA Sequencing Services, (1st BASE Pte Ltd; Singapore).

The PCR cocktail per 10 µL of 1 DNA sample consisted of 1 µL 10X PCR buffer, 1 µL of 1mM dNTP, 0.25 µL of 10 mM of TYNBS1I2 primer, 0.8 µL of 25 mM Magnesium chloride ($MgCl_2$), 5.4 µL of Deionized Water (dH_2O), 0.3 µL of 5U Taq polymerase, and 1 µL of DNA template. For the primer combination between TYNBS1I2 and 20IY10, the PCR cocktail was added with 0.3 µL of 10 mM of 20IY10 primer and the dH_2O volume was decreased to 4.8 10 mM per DNA sample.

The PCR conditions started with pre-denaturation for 1 cycle at 95°C for 2 minutes, then denaturation at 95°C for 10 seconds, annealing at 56°C for 30 seconds, and extension for 30 cycles at 72°C for 30 minutes, followed by a final extension for 1 cycle at 72°C for 2 minutes. DNA amplicons were separated on 1.5% agarose gel electrophoresis at 100 V for 45 minutes with 0.5X Tris-Borate-EDTA (TBE) buffer; then, the gels were stained with ethidium bromide (EtBr) and inspected under UV light.

2.3 Data analysis

The National Center for Biotechnology Information's Basic Local Alignment Search Tool (NCBI-BLAST) and BioEdit v.7.0.5.3 [19] were used as DNA sequence alignment tools. The *Ty-2a* allele identities and similarities were analyzed by comparing DNA sequences of TOMAC647 with *TYNBS1* and a reference genome (SL3.0). The software was also used to compare *Ty-2a* to 14 other resistance proteins in the same R gene family (NB-LRR) with *TYNBS1* or *Ty-2* and then the protein domains were identified and the relationship inferred between *Ty-2a* and these resistance proteins. The relationship between *Ty-2a* and each resistance protein domain was also analyzed using BioEdit v.7.0.5.3.

2.4 Molecular marker development and validation

Specific markers were designed at polymorphisms region between *Ty-2* (*TYNBS1*) and *Ty-2a* (*TYNBS1I*) using BioEdit v.7.0.5.3 and Primer3. The developed markers were validated with four tomato lines and three hybrids genotyped using the T0302 marker, as shown in Table 1.

3. Results and discussion

We sequenced the DNA of tomato plants that had been selectively bred for resistance to TYLCV to identify any variations in the *Ty-2a* allele associated with resistance to TYLCV. Then, we analyzed the sequences to identify any new variants of the allele. Subsequently, we generated a specific DNA marker to detect this newly identified allele inherited from *S. habrochaites* accession 'L06112' C1 during our breeding for TYLCV resistance programs. To amplify the long-PCR product, we studied the *Ty-2a* allele located in the *Ty-2* or *TYNBS1* region based on a pair of primers from previously reported [7]. The *in-silico* PCR was done by blasting the forward and reverse primers to reference genome SL3.0 to estimate the product size and validate the specification of the primer. Only one region in SL3.0 could be amplified by this primer pair. The expected PCR product size was 11 kb. Primers for the long-PCR and *Ty-2a* allele gap close are illustrated in Figure 1. The primers are listed in Table S1.

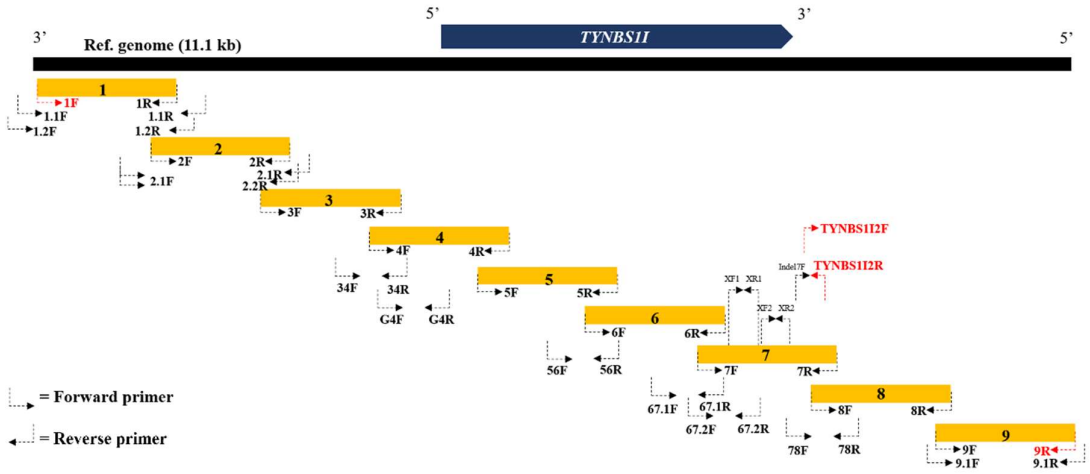

Figure 1 Illustrated primer pairs for identifying *TYNBS1I* aligned to genomic structure of reference tomato genome (SL3.0) and *Ty-2a*-resistant allele.

We proposed a 4,071 bp DNA sequence from TOMAC647 as TYNBS1I, located in the range of the 4th and 7th primers of the reference genome (SL3.0). The *TYNBS1I* DNA sequence was further compared with other *Ty-2* DNA sequences (*TYNBS1* and *TYNBS2*). Notably, we identified a unique 69 bp insertion at nucleotide position 3,484 of *TYNBS1I* (*Ty-2a* allele) translated into 23 amino acids, as shown in the alignment results in Figure 2 which shows an overview of DNA sequence variation in these genes, highlighting the unique features of *TYNBS1I* Figure 2(A). While Figure 2(B) provides a detailed visual representation of the 69 bp insertion in *TYNBS1I* compared to *TYNBS1* and *TYNBS2*, demonstrating the insertion's specificity to *TYNBS1I*. Finally, Figure 2(C) focuses on the aligned nucleotide sequence in the region of the 69 bp insertion, emphasizing the precise location and sequence of this unique genetic feature. Additionally, the annotation of *TYNBS1I* using the NCBI domain search tool [20] showed that the amino acids were in the CC-NBS-LRR domain, which is associated with innate immunity in plants [21], similar to other resistant genes such as *TYNBS1*, *I2* and *R3a* (tomato yellow leaf curl

virus, tomato fusarium wilt, and potato late blight, respectively) as shown in Figure 3 and Table2. The comparison, based on protein blast, between *TYNBS1I* and 14 other resistant proteins to observe the *TYNBS1I* structure and its relationship with other R genes in the same NB-LRR family produced 96% identity with *TYNBS2*, 94% identity with *TYNBS1*, 67% identity with *I2* and *R3a*, and less than 50% identity with *N*, *Bs4*, *R1, Rx*, *Sw5-a*, *Sw5-b*, *Tm-2*, *RCY1*, *CYR1*, and *Ph-3*, as shown in Table 3. The results showed that *TYNBS1I* was closer to *TYNBS2*, *TYNBS1*, *I2*, and *R3a* than to the other 10 R genes (less than 50%), especially the TNL subfamily (N and Bs4, with 23% identity), because they encode proteins in the same subfamily (CLN or CC-NBS-LRR).

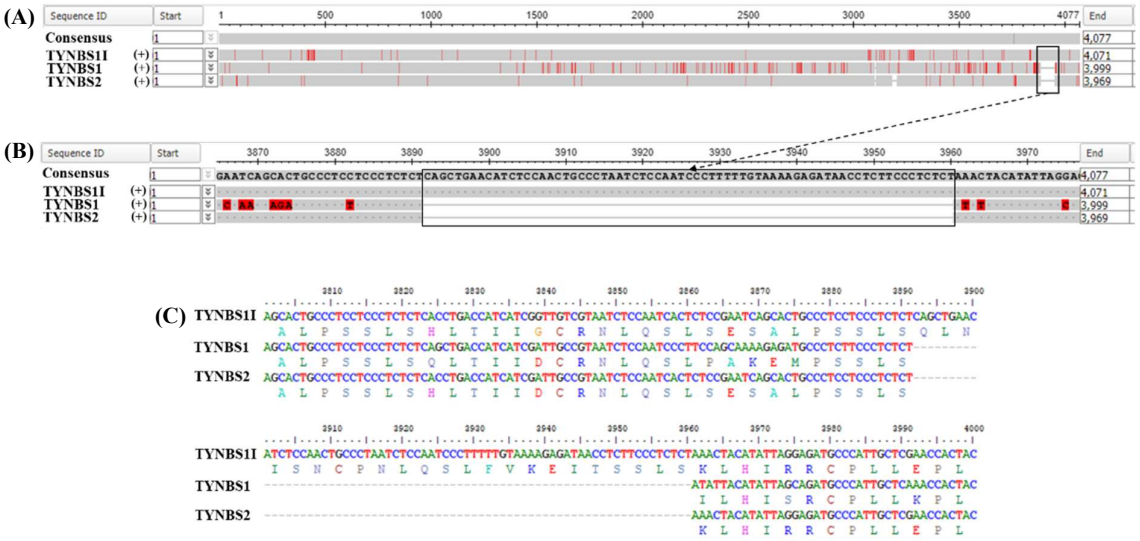

Figure 2 DNA sequence variation among *TYNBS1I;* TOMAC647, *TYNBS1*; SL3.0, and *TYNBS2*: SL3.0 (A); overview illustration of DNA sequence alignment at 69 bp insertion of *TYNBS1I* compared to *TYNBS1* and *TYNBS2* (B); and aligned nucleotide sequence at 69 bp insertion region (C).

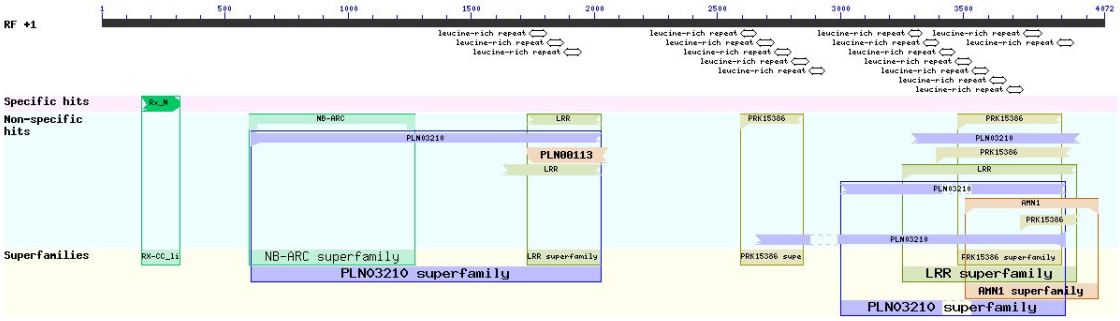

Figure 3 *TYNBS1I* DNA sequence conserved domain structure analysis using NCBI domain search tool.

Table 2 *TYNBS1I* conserved domain prediction using NCBI domain search tool [22].

Name	Accession	Description	Location	E-value
Rx_N	pfam18052	Rx N-terminal domain;	160-315	5.09E-08
NB-ARC	pfam00931	NB-ARC domain;	598-1269	6.36E-46
PLN03210	PLN03210	Resistant to *P. syringae* 6; Provisional	607-2028	4.10E-07
LRR	COG4886	Leucine-rich repeat (LRR) protein [Transcription];	1630-2028	9.35E-03
PLN00113	PLN00113	LRR receptor-like protein kinase; Provisional	1723-2055	2.27E-03
LRR	COG4886	LRR protein [Transcription];	1726-2028	3.33E-04
PRK15386	PRK15386	type III secretion effector GogB;	2593-2847	3.60E-03
PLN03210	PLN03210	Resistant to *P. syringae* 6; Provisional	2656-3915	7.99E-04
PLN03210	PLN03210	Resistant to *P. syringae* 6; Provisional	3001-3915	2.37E-05
LRR	COG4886	LRR protein [Transcription];	3253-3960	1.08E-05
PLN03210	PLN03210	Resistant to *P. syringae* 6; Provisional	3289-3972	2.10E-07
PRK15386	PRK15386	type III secretion effector GogB;	3388-3936	1.09E-06
PRK15386	PRK15386	type III secretion effector GogB;	3475-3900	1.13E-11
AMN1	cd09293	Antagonist of mitotic exit network protein 1	3505-4047	4.40E-05
PRK15386	PRK15386	type III secretion effector GogB;	3730-3969	1.34E-04

Table 3 Identity of *TYNBS1I* compared to 14 other resistance proteins in the R gene family.

Gene	%Identity	Protein domain	Disease	Accession no.
N	23	TNL	Tobacco mosaic virus	AAA50763
Bs4	23	TNL	Bacterial spot disease	AAR21295
R1	24	CNL	Late blight (potato)	AAL39063
Rx	30	CNL	Potato virus X	CAB50786
Sw5-a	24	CNL	Tospovirus	AAG31013
Sw5-b	24	CNL	Tospovirus	AAG31014
Tm-2	28	CNL	Tomato mosaic virus	NP_001318061
RCY1	28	CNL	Cucumber mosaic	BAC67706
CYR1	34	CNL	Mungbean yellow mosaic virus	ADU57957
Ph-3	25	CNL	Tomato late blight	AAL39063
R3a	67	CNL	Potato late blight	AAW48299
I2	67	CNL	Fusarium wilt	AAD27815
TYNBS1	94	CNL	Tomato yellow leave curl virus	BBC83283
TYNBS2	96	CNL	Putative R gene	BBC83286

TYNBS1 and *TYNBS1I* are resistance alleles introgressed from different genetic sources. *TYNBS1* was inherited from *S. habrochaites* accession 'B6013' [6, 7], while *TYNBS1I* is a resistance allele derived from *S. habrochaites* accession 'L06112' [15]. The linked co-segregating marker (T0302) to the *Ty-2* gene, located at 89 cM, 51.878 Mb of chromosome 11 [13], can separate *Ty-2*, *Ty-2a*, and *ty-2* alleles [14]. The marker is located close to the *Ty-2a* allele with significant confidence values of the LOD score of 3 [23]. Other studies have used 17,532 SNPs indicating levels of similarity between *Ty-2*, *Ty-2a,* and *ty-2*, with nucleotide base differences at 51,359,485 bp and 53,374,347 bp on chromosome 11, which is mapped in the *TYNBS1* (*Ty-2*) gene in three tomato lines: TOMAC643 (*Ty-2/ Ty-2*), TOMAC463 (*ty-2/ ty-2*), and TOMAC647 (*Ty-2a/Ty-2a*) [24]. However, TOMAC647 did not differ from other resistance lines in another study of a Thailand virus isolate from Nakhon Pathom [24]. Nonetheless, the 69 bp insertion in the *TYNBS1I* gene region may be specific to certain environmental conditions or other TYLCV isolates. *TYNBS1I* was compared with not only *TYNBS1* but also *TYNBS2*, *I2*, *R3a*, and other resistance genes. The results showed that the 69 bp insertion in *TYNBS1I* was unique compared to the other resistance genes, as illustrated in Figure 4. This exceptional 69 insertion sequence in *TYNBS1I* (*Ty-2a*) contains an interval leucine-rich repeat (LRR) motif, as identified through the NCBI domain search tool, which is an important domain in the family of disease-resistance genes in plants, as shown in Figure 3 and Table2. Another paper reviewed LRR domain mutations, such as duplications and deletions of entire repeats, and discussed the derived diversification and evolving new interaction specificities [25]. Notably, the correlation between resistance to TYLCV and the insertion and deletion was established in this gene family. Verlaan MG et al. (2013) [26] reported a deletion of 4 amino acids in the first amino-terminal part of the protein in the ty-1 allele compared to *Ty-1* and *Ty-3*. Noris E et al. (1996) [27] demonstrated that the deletion of 420 nt from the 3' end of the TYLCSV Rep gene gains resistance to the monopartite tomato yellow leaf curl Sardinia virus (TYLCSV). Pramanik, D et al. (2021) [28] reported a 73 bp deletion in *Ty-5* (SlPelo) trigger resistance in tomatoes. The deletion TYLCV resistance *Ty-1* and *Ty-3* genes establish resistance in tomato through an siRNAs mechanism [29].

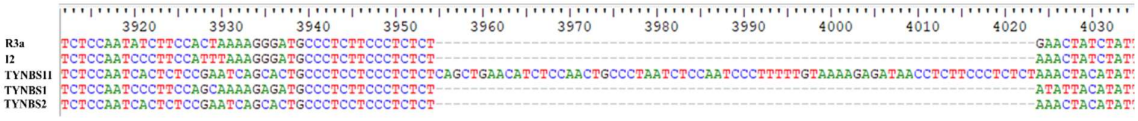

Figure 4 DNA sequence variation among *TYNBS1I*, *TYNBS1*, *TYNBS2*, *I2*, and *R3a.*

Our DNA marker effectively distinguishes between *Ty-2 (TYNBS1)* and *Ty-2a (TYNBS1I)* alleles at the 3' downstream region, making it a reliable tool for identifying plants carrying the new allele. The essential advantage of this marker set is that it targets the gene region, unlike the previous marker, T0302. This newly developed marker detects *TYNBS1I* based on sequence variations between *TYNBS1* and *TYNBS1I* at the 3' downstream region. To validate the marker, the previously reported 20IY10 marker specific to the *TYNBS1* resistance gene was employed. This marker produces PCR amplicons of 738 bp and 600 bp for the Ty-2 resistance and susceptible line, respectively (Figure 5(A)). Additionally, a specific primer, TYNBS1I2, was developed, with its first base located 294 bp downstream of the *TYNBS1I* stop codon. This primer, illustrated with a purple arrow in Figure 5(B), cannot bind to *TYNBS1*, ensuring its specificity to *TYNBS1I*. For validation, the 20IY10 marker was used as a combination primer in this study (Figure 5(C)), confirming the precise presence of the *Ty-2a* allele. The TYNBS1I2 marker produced a 164 bp PCR amplicon in TOMAC647 (*Ty-2a/Ty-2a*) but failed to amplify in TOMAC648 (*Ty-2/Ty-2*) and TOMAC643 (*Ty-2/ty-2*), as shown in Figure 5 (D). Meanwhile, the 20IY10 marker produced PCR amplicons corresponding to *Ty-2* resistance and susceptibility, as depicted in Figure 5(E). Finally, the combined use of 20IY10 and TYNBS1I2 markers in PCR assays demonstrated the specificity of TYNBS1I2 for detecting the *TYNBS1I* or *Ty-2a* allele, while excluding other alleles, such as *Ty-2* and *ty-2* (Figure 5(F)). This

marker exhibited dominance, generating PCR amplicons exclusively in tomato lines harboring the *Ty-2a* allele. However, it was not possible to discern homozygosity or heterozygosity solely based on this marker. To ascertain the genotype accurately in our breeding program, we found it necessary to apply a combination of this specific marker with others for comprehensive genotype confirmation.

We developed TYNBS1I2F, the forward primer of TYNBS1I2, to be specific to *TYNBS1I* in the unique region. Therefore, it was not possible to amplify this on DNA samples of TOMAC648, Seedathip3, TOMAC646, and hybrids between these tomato lines because of a primer mismatch in other genotypes. DNA amplicons with a size over 1 kb (1,188 bp as analyzed by in-silico PCR) were produced from primer 20IY10F and TYNBS1I2R amplification. The monomorphic band that occurs in the combination marker set at product size of around 300 bp may amplify from other homolog genes in the same family that are located in different genome regions. This result confirmed that TYNBS1I2 is a specific primer to *TYNBS1I* or *Ty-2a* allele. The result of the combination marker identified different alleles in the tested lines. As an intragenic marker, the DNA marker developed in this study might help more efficient and accurate selection of plants containing the Ty-2 gene than the linked T0302 marker. Including the variation identified in this region, it might also be an alternate source of resistance to virus variation according to the function of this NB-LRR family. Therefore, it will be helpful for plant selection in future breeding programs.

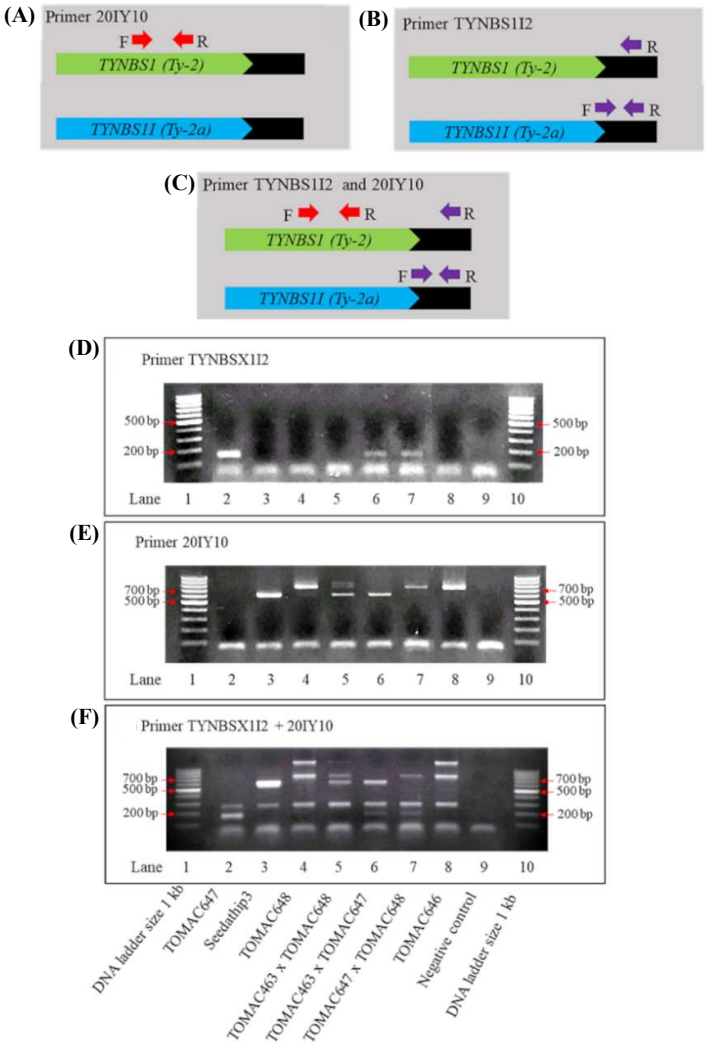

Figure 5 Primer detection procedures: Primer 20IY10 detect intragenic region of *TYNBS1* and *TYNBS1*I (A); primer TYNBS1I2 detected at 3' downstream of *TYNBS1* and *TYNBS1I* (B); combined two-primer detection in *TYNBS1* and *TYNBS1I* (C); and agarose gel electrophoresis of TYNBS1I2 (D); 20IY10 (E); and 2 primer combination (F), where Lane1, DNA ladder size 1 kb; Lane2, TOMAC647; Lane3, Seedathip3; Lane4, TOMAC648; Lane5, hybrid between TOMAC463 and TOMAC648; Lane6, hybrid between TOMAC463 and TOMAC647; Lane7. hybrid between TOMAC647 and TOMAC648; Lane8, TOMAC646; Lane9, negative control, and Lane10, DNA ladder size 1kb.

4. Conclusion

Ty-2a or the *TYNBS1I* allele differ in various tomato genotypes. There were 69 nucleotides identified in *Ty-2a*, which encoded CC-NBS-LRR domains such as the *TYNBS1*, *I2*, and *R3a* resistance genes. The specific marker developed from the unique *Ty-2a* region confirmed *Ty-2a* was a novel *Tomato Yellow Leaf Curl Virus* resistance allele that could be used in plant improvement.

5. Acknowledgments

This research is funded by the Graduate School Fellowship Program in Agriculture and Agro-industry from the Agricultural Research Development Agency (Public Organization) as of fiscal year 2022, Kasetsart University Research and Development Institute (KURDI), and the Center of Excellence on Agricultural Biotechnology, Office of the Permanent Secretary, Ministry of Higher Education, Science, Research and Innovation. (AG-BIO/MHESI). We acknowledge Professor Hans de Jong, who guided us in the writing of this manuscript. Special appreciation goes to Dr Andrew Warner and KURDI for kindly making English suggestions.

6. References

[1] Czosnek H, Ghanim M, Morin S, Rubinstein G, Fridman V, Zeidan M. Whiteflies: Vectors, and victims (?), of geminiviruses. Adv Virus Res. 2001;57:291-322.

[2] Prasad A, Sharma N, Gowthem G H, Muthamilarasan M, Prasad M. Tomato Yellow Leaf Curl Virus: Impact, Challenges, and Management. Trends Plant Sci. 2020;25(9):897-911.

[3] Zamir D, Ekstein-Michelson I, Zakay Y, Navot N, Zeidan M, Sarfatti M, et al. Mapping and introgression of a tomato yellow leaf curl virus tolerance gene, *TY-1*. Theor Appl Genet. 1994;88(2):141-146.

[4] Scott JW, Stevens MR, Barten JHM, Thome CR, Polston JE, Schuster DJ, et al. Introgression of resistance to whitefly-transmitted geminiviruses from lycopersicon chilense to tomato. Andover: Intercept Limited; 1996. p. 357-367

[5] Verlaan MG, Hutton SF, Ibrahem RM, Kormelink R, Visser RG, Scott JW, et al. The Tomato Yellow Leaf Curl Virus resistance genes *Ty-1* and *Ty-3* are allelic and code for DFDGD-class RNA-dependent RNA polymerases. PLoS Genet. 2013;9(3):e1003399.

[6] Hanson P, Bernacchi D, Tanksley S, Muniyappa V, Padmaja A, Chen H-M, et al. Mapping a wild tomato introgression associated with tomato yellow leaf curl virus resistance in a cultivated tomato line. J Am Soc Hortic Sci. 2000;125(1):15–20.

[7] Yamaguchi H, Ohnishi J, Saito A, Ohyama A, Nunome T, Miyatake K, et al. An NB-LRR gene, *TYNBS1*, is responsible for resistance mediated by the *Ty-2 Begomovirus* resistance locus of tomato. Theor Appl Genet. 2018;131(6):1345-1362.

[8] Ji Y, Scott J, Schuster D, Maxwell D. Molecular Mapping of *Ty-4*, a New Tomato Yellow Leaf Curl Virus Resistance Locus on Chromosome 3 of Tomato. J Am Soc Hortic Sci. 2009;134(2):281-288.

[9] Anbinder I, Reuveni M, Azari R, Paran I, Nahon S, Shlomo H, et al. Molecular dissection of Tomato leaf curl virus resistance in tomato line TY172 derived from *Solanum peruvianum*. Theor Appl Genet. 2009;119(3):519-530.

[10] Scott J, Hutton S, Freeman J. Fla. 8638B and Fla. 8624 Tomato Breeding Lines with *Begomovirus* Resistance Genes *ty-5 Plus Ty-6* and *Ty-6*, respectively. HortScience. 2015;50(9):1405-1407.

[11] Gill U, Scott J, Shekasteband R, Ogundiwin E, Schuit C, Francis D, et al. *Ty-6*, a major begomovirus resistance gene on chromosome 10, is effective against Tomato yellow leaf curl virus and Tomato mottle virus. Theor Appl Genet. 2019;132(5):1543-1554.

[12] El-Sappah A, Qi S, Soaud S, Qiulan H, Saleh A, Abourehab M, et al. Natural resistance of tomato plants to Tomato yellow leaf curl virus. Front Plant Sci. 2022;13:1081549.

[13] Yang X, Caro M, Hutton S, Scott J, Guo Y, Wang X, et al. Fine mapping of the tomato yellow leaf curl virus resistance gene *Ty-2* on chromosome 11 of tomato. Mol Breed. 2014;34:749-760.

[14] Chomdej O, Pongpayaklers U, Juejun N, Sinbunyama I, Chunwongse J. *Ty-2* resistance to Tomato yellow leaf curl virus in F1 and BC1F1 crosses between wild species tomato, *Solanum habrochaites* 'L06112' and a commercial cultivar, Seedathip3. Agric Sci J. 2016:47(2):189-200.

[15] Chomdej O, Whankaew S, Chatchawankanpanich O, Kositratana W, Chunwongse J. Resistance to tomato yellow leaf curl Thailand virus, TYLCTHV-[2] from *Solanum habrochaites* accession 'L06112' in F1 and BC 1F1 generations. Songklanakarin J Sci Technol. 2008;30:441-446.

[16] Sinbunyama I, Pongpayaklers U, Juejun N, Chomdej O, Chunwongse J. The Selection for Tomato Resistant Lines at BC6F1 Generation to Tomato Yellow Leaf Curl Virus (TYLCTHV-[2]) using Molecular Markers. Proceeding in the 10 th National Kasetsart University Kamphaeng Saen Conference. 2013 December 6-7, Kasetsart Univerity, Kamphaeng Saen Campus, Nakhon Pathom, Thailand; 2013.

[17] Sitadhani K. Seedathip 1, Seedathip 2, Seedathip 3, and Seedathip 4 Tomato variety. In: Innovative Agriculture: Collection of Innovative Research Works on the Occasion of the 72nd Anniversary of Kasetsart University. Book number. Bangkok: Kasetsart University; 2015. p.365. [In Thai]
[18] Fulton TM, Chunwongse J, Tanksley SD. Microprep protocol for extraction of DNA from tomato and other herbaceous plants. Plant Mol Biol Rep. 1995;13(3):207-209.
[19] Hall TA. BioEdit: A User-Friendly Biological Sequence Alignment Editor and Analysis Program for Windows 95/98/NT. Nucleic Acids Symp Ser (Oxf). 1999;41:95-98.
[20] Lu S, Wang J, Chitsaz F, Derbyshire MK, Geer RC, Gonzales NR, et al. CDD/SPARCLE: the conserved domain database in 2020. Nucleic Acids Res. 2020;48(D1):D265-D268.
[21] Ng A, Xavier RJ. Leucine-rich repeat (LRR) proteins: integrators of pattern recognition and signaling in immunity. Autophagy. 2011;7(9):1082-1084.
[22] Wang J, Chitsaz F, Derbyshire M, Gonzales N, Gwadz M, Lu S, et al. The conserved domain database in 2023. Nucleic Acids Res. 2023;51(D1):D384-D388.
[23] Pongpayaklers U. Molecular mapping of Tomato Yellow Leaf Curl Thailand Virus (TYLCTHV-[2]) resistance gene in wild tomato, *Solanum habrochaites* accession 'L06112' [thesis]. Bangkok, Thailand: Kasetsart University; 2011. [In Thai]
[24] Sinbunyama I. Evaluation of Ty-2 and Ty-2a allele for resistance to Tomato Yellow Leaf Curl Thailand Virus isolate Nakhon Pathom (TYLCTHV-[NP]) in 'Seedathip 3' tomato [thesis]. Bangkok, Thailand: Kasetsart University; 2018.
[25] Ellis J, Dodds P, Pryor T. The generation of plant disease resistance gene specificities. Trends Plant Sci. 2000;5(9):373-379.
[26] Verlaan MG, Hutton SF, Ibrahem RM, Kormelink R, Visser RGF, Scott JW, et al. The Tomato Yellow Leaf Curl Virus resistance genes *Ty-1* and *Ty-3* are allelic and code for DFDGD-class RNA-dependent RNA polymerases. PLoS Genet. 2013;9(3):e1003399.
[27] Noris E, Accotto GP, Tavazza R, Brunetti A, Crespi S, Tavazza M. Resistance to tomato yellow leaf curl geminivirus in *Nicotiana benthamiana* plants transformed with a truncated viral C1 gene. Virology. 1996;224(1): 130–138.
[28] Pramanik D, Shelake RM, Park J, Kim MJ, Hwang I, Park Y, et al. CRISPR/Cas9-mediated generation of pathogen-resistant tomato against tomato yellow leaf curl virus and powdery mildew. Int J Mol Sci. 2021;22(4):1878.
[29] Yang Y, Liu T, Shen D, Wang J, Ling X, Hu Z, et al. Tomato yellow leaf curl virus intergenic siRNAs target a host long noncoding RNA to modulate disease symptoms. PLoS Pathog. 2019;15(1):e1007534.
[30] Lee JH, Chung DJ, Lee JM, Yeam I. Development and application of gene-specific markers for tomato yellow leaf curl virus resistance in both field and artificial infections. Plants. 2020;10(1):9.