INHERITANCE ANALYSIS AND IDENTIFICATION OF SSR MOLECULAR MARKER LINKED TO LATE BLIGHT RESISTANT GENE IN TOMATO (*Lycopersicon esculentum* Mill.)

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INTRODUCTION

Late blight caused by the fungal pathogen *Phytophthora infestans* de Bary is one of the most serious diseases of tomato cultivation regions in Vietnam. This oomycete pathogen attacks on leaves, stems, fruits and seeds of tomato. Spraying fungicides is the most effective method to control this disease. However, this strategy increases production costs and badly affects to the environment. Introduction of resistant varieties is therefore the most effective measure to control this disease. Two types of resistance to late blight on tomato have been reported. First, race-specific or vertical resistance is controlled by a dominant gene and is very effective against a specific race (strain) of the fungus. Plants with race-specific resistance react to infection by forming small, dead, non-spreading sport. Three dominant genes have been identified; *Ph-1* on chromosome 7 (Clayberg et al. 1965; Perirce 1971), *ph-2* on chromosome 10 (Morear et al, 1998), *Ph-3* on chromosome 9 (Chunwongse et al, 1998). The second type of resistance, horizontal resistance, is controlled by several genes.

The use of molecular markers can facilitate tomato breeding through marker assisted selection (MAS) for improvement of agronomic traits such as disease resistance, yield and fruit quality. Simple sequence repeats (SSR) or microsatellites are not only very common but also hypervariable among the types of tandem repetitive DNA in the eukaryotic genome (He et al, 2003). SSR markers are becoming the preferred molecular markers in crop breeding and genetic studies.

The research aims at using the SSR markers to analyze the inheritance of late blight resistance and identify SSR molecular marker associated with late blight resistant gene in tomato.

Figure 1. Tomato plant

Figure 2. Field affected by late blight susceptible tomato cultivar

Figure 3. Symptom of late blight disease in leaf, stem and fruit of tomato

MATERIALS AND METHODS

Plant materials

The tomato population was developed by crossing a susceptible cultivar Ta sie inbred line and the resistant genotype of L3708 to generate F_1, then back crossing to get BC_1F_1- BC_2F_1-BC_2F_2. Leaves collected from each plant were divided into two parts, one for evaluation of late blight resistance by detached-leaflet assay and the other for DNA extraction.

DNA extraction and SSR analysis

Genomic DNA was extracted from young leaves following the method modified from He et al. (2003). A total of 41 SSR primers previously mapped on tomato (Suliman Pollatsche et al. 2002) and SOL Genomics Network (SGN) of Cornell University were assayed for polymorphism between Ta sie inbred line and L3708.

The PCR reaction mixture (25µL total volume) consisted of 30ng template DNA, 1µM primer, 1.5 mM MgCL2, 400µM dNTP, 2.5 L 10 x PCR buffer containing, 10 mM Tris-HCL (pH 8.3), 50 mM KCL, 0.01% gelatin, and 1.5 unit of Taq DNA polymerase. PCR cycles started at 96^0C (2 min) in an eppendorf mastercyler AG, followed by 30 cycles of 94^0C (10s), 45-49^0C (1 min), 72^0C (1 min), and ended with 10 min 72^0C. PCR products were side-separated on a 2.5% agarose gel in 1 x

TBE buffer, stained with ethidium bromide. Bands were scored as “1” for homozygote from resistant parent L3708, “2” for homozygote from susceptible parent Ta Sie, “3” heterozygote , and “-” for missing data.

Data analysis

The data of late blight reaction of detached-leaflet and natural infection assays were tested for significant deviation from the expected Mendelian ration of 1:1 using chi-square (χ^2) test. Linkage analysis was undertaken using Mapmaker/EXP, and genetic distances were measured in Cent Morgan (cM).

RESULT

Inheritance analysis

The plant of Ta sie inbred line shriveled and dead at the final period of natural infection. The detached-leaflet of Ta sie inbred line became brown fig 4.

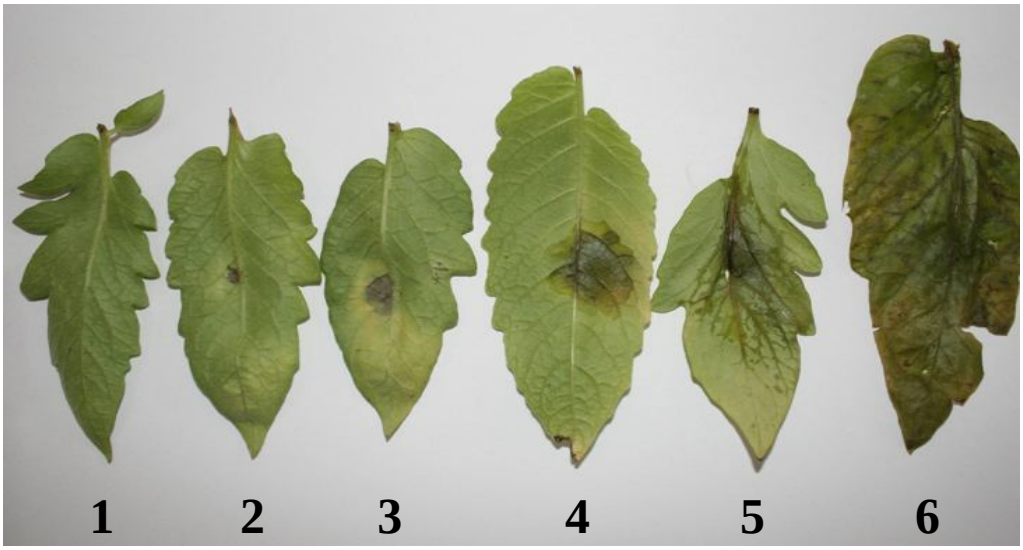

Figure 4. Compatible inheritance after inoculation detached-leaflets

The response of BC_2F_1 population in infection and deteched-leaflet assays showed that the segregation fits a 1:1 ratio follow (χ^2) test, indicating the resistance is dominant and suggested that it is inherited as monogenic dominant trait in L3708 resistant accession.

Detection of the marker

Of 41 SSR markers used in this study, 16 SSRs were able to detect polymorphism between Ta sie inbred line and L3708 donor. Among five markers as SSR112, SSR559, SSR333, SSR110 and TOM236, located on chromosome 9, only TOM236 was significantly linked to the resistant gene.

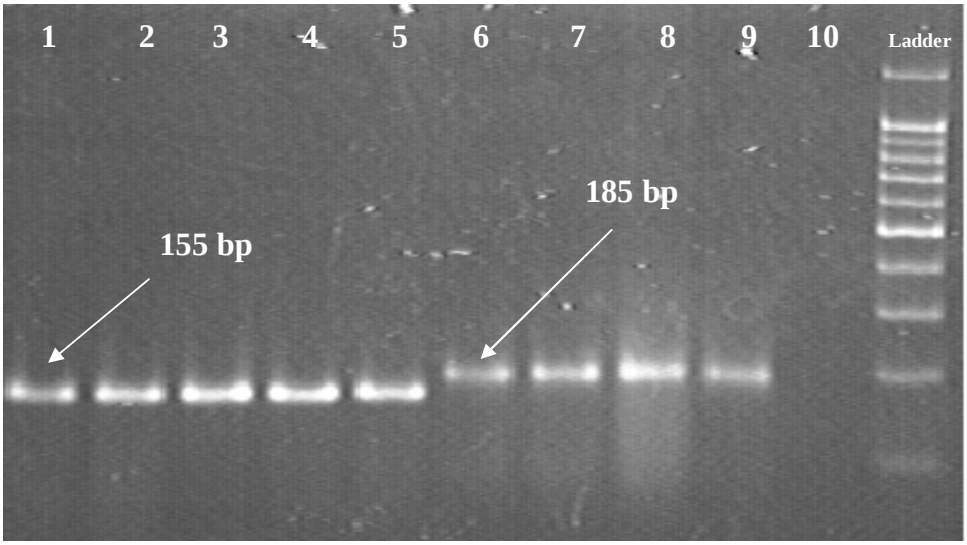

Figure 5. PCR products at the locus TOM236 on chromosome 9

Note: 1: L3708, 6: Ta sie, 2,3,4,5,7,8,9 and 10 BC_2F_1 populations

DISCUSSION

The disease test on the BC_2F_1 population demonstrated that resistance to late blight of tomato L3708 is the dominant monogenic trait located on chromosome 9, which is similar to the result of Ph3 (Chunwongse et al. 1998), but it is not known with certainty whether the resistance gene is *Ph3*. Sine no polymorphism between Ta sie and L3708 was detected for other SSR markers on chromosome 9.

CONCLUSION

L3708 tomato shown resistance of late blight in isolates collected in tomato cultivation region of Viet Nam.

Resistance gene conveyed in L3708 tomato genotype is dominant.

It is inherited as monogenic dominant trait linkage with TOM236 marker located on chromosome 9.