Molecular screening for *Ty-1, Ty-2, Ty-3, Ty-4* and *Ty-5* genes in two tomato breeding lines

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Abstract

Tomato yellow leaf curl virus (TYLCV) is a major tomato virus disease in tropical and subtropical regions. *Ty-1, Ty-2, Ty-3, Ty-4* and *Ty-5* are genes conditioning resistance to TYLCV disease in tomato. Molecular screening for these genes in two tomato breeding lines resistant to TYLCV disease (Multichiltylc-95-J0-C2 and Pimpertylc-J-13) was done using five sequence tagged site (STS) markers included (TG178/Taq1), (T0302), (FLUW25), (P3-81/Mas1) and (SINAC1/Taq1) which linked to *Ty-1, Ty-2, Ty-3, Ty-4* and *Ty-5* respectively. Five TYLCV resistant lines in addition to a susceptible one were used as checks. The experiment was conducted at Biotechnology and Molecular Breeding Unit of the Asian Vegetable Research Development Center (AVRDC), the World Vegetable Center in Taiwan. Results indicated that Multichiltylc-95-J0-C2 did not carry *Ty-1, Ty-2*, *Ty3, Ty-4* or *Ty-5* genes. Pimpertylc-J-13 did not carry *Ty-1, Ty-2*, *Ty3, Ty-4* but it appeared that it may carry *Ty-5*, a resistance gene from *Solanum peruvianum.*

Keywords:

Introduction

DNA marker technology has been used in commercial plant breeding programs since the early 1990s, and has proven helpful for the rapid and efficient transfer of useful traits into agronomical desirable varieties and hybrids [1]. Markers linked to disease resistance loci can now be used for marker-assisted selection (MAS) programs and thus allowing several resistance genes to be accumulated in a single genotype ("pyramiding" resistance genes). In addition, markers linked to resistance genes may be also useful for cloning and sequencing the genes. The use of DNA markers allows identification of desirable genotypes in the laboratory instead of the field, thus enhancing plant breeding efficiency. Since 1980s molecular markers are being widely used as a major tool for breeding of tomato [2]. More than 40 genes (including many single genes and quantitative trait loci, QTL) that confer resistance to all major classes of pathogens have been mapped on the tomato molecular map [3]. Moreover, other resistance genes were added to the map [4]. Currently, over 285 morphological, physiological and disease resistance markers, including 36 isozymes and over 1000 restriction fragment length polymorphism (RFLP) markers have been mapped to the 12 individual chromosomes of tomato [5].

Zamir and his team were the first to report the mapping of a begomovirus resistance

gene using lines derived from *Solanum chilense* accession LA 1969 and they revealed that a major incompletely dominant gene (*Ty-1*) on chromosome 6 accounted mostly for the resistance [6]. Considerable progress has been made to identify resistance sources by introgressing genes from the wild species of tomato especially *Lycopersicon chilense, Solanum peruvianum* and *S. habrochaites* (previously known as *L. hirsutum*) [7]. Five genes conferring resistance to TYLCV have been reported, which included *Ty-1* from *S. chilense* LA 1969 [6], *Ty-2* from *S. habrochaites* [8], *Ty-3* from *S. chilense* LA 2779 [9], *Ty-4* from *S. chilense* LA1932, LA2779 and LA1938 [10] and *Ty-5* from *S. peruvianum* (Kadirvel, AVRDC, Personal communication, 2009).

The purpose of this study is to test the presence of *Ty-1, Ty2, Ty3, Ty4* and *Ty5* genes in two tomato breeding lines bred by INRA (France) for resistance to TYLCV disease named Multichiltylc-95-J0-C2 and Pimpertylc -J-13.

Materials and methods

Experiment was conducted at Biotechnology and Molecular Breeding Unit of the Asian Vegetable Research and Development Center (AVRDC), the World Vegetable Center at Taiwan in 2009. Eight tomato genotypes (Table 1) were grown in October 2009 in a greenhouse. Five sequence tagged site (STS) markers included (TG178/Taq1), (T0302), (FLUW25), (P3-81/Mas1) and (SINAC1/Taq1) which linked to *Ty-1, Ty-2, Ty-3, Ty-4* and *Ty-5* genes respectively were used to detect the presence of these genes in two tomato breeding lines resistant to TYLCV disease named Multichiltylc-95-J0-C2 and Pimpertylc -J-13. DNA was extracted according to the protocol of Dellaporta with few modifications [11].

Two apex leaves were collected from each plant at seedling stage and kept in a separate well of the 96-well plate. The samples were collected randomly from five plants on each of the eight tested lines. The plates were placed in Freeze-Dryer, which was pre-cooled until -50˚C, for 24 hours. Then the plates were placed in a Minibeadbeater and after grinding for few seconds the samples looked like very fine powder. The homogenate was incubated in 600 µl of extraction buffer (100 mM Tris-HCl (pH 8), 50 mM EDTA, 500 mM NaCl, 10 mM 2-β-mercapto ethanol and 1% (w/v) SDS) at 65 °C for 10 minutes and mixed with a half volume of Chloroform: Isoamyl alcohol (24:1v/v). The mixture was centrifuged at 11269 ×*g* for 15 minutes and the supernatant (500 µl) was transferred to a 1.5 ml micro centrifuge tube and the DNA was precipitated by adding 150 µl of sodium acetate (5 M, pH 5.2) and 600 µl of isopropanol. The pellet was washed with 70

% (v/v) ethanol, air dried and dissolved in 100 µl of sterile double distilled water. The polymerase chain reaction (PCR) procedure was carried out following the standard protocol described by (Kadrevil, AVRDC, Personal communication, 2009) to identify the STS markers linked to *Ty-1, Ty-2, Ty-3, Ty-4* and *Ty-5* locus in the eight genotypes. The preparation of the cocktail mixture was done as follow: 1056 µl ddH2O, 160 µl 10xbuffer, 128 µl dNTP, 80 µl primer and 16 µl hot start 4 x Taq enzyme were added, respectively in an 0.5 ml tube. Then 1.0 µl of the DNA template was added to each well in the two 96-well plates followed by 9.0 µl of the cocktail mixture and the plates were spin at 2000 *rpm* for one minute. After that the plates were placed in the PCR engine, the PCR reaction (35 cycles) was run. 6% Acrylamide gel was used to assess *Ty-1* and *Ty-5* genes markers. 2 µl of 1x red dye was added to each well already loaded with DNA template in the 96-well plates. 2 µl of each DNA sample and the marker were loaded into the wells using a micropipette equipped with a drawn out plastic tip. Then the electrophoresis was run for 50 minutes. After that the glass plates were detached and laid on the bench. The gel remained attached to the lower plate. Upper plate was pulled smoothly away and the spacers were removed. The gel was stained with Ethidium Bromide and visualized under UV and a photograph was saved in the computer. 1.5% and 2% Agrose gels were used to assess *Ty-2, Ty-3* and *Ty*-4 genes markers. 2 µl of 1x blue dye was added to each well already loaded with DNA template in the 96-well plates. Then 2 µl of each sample was loaded in addition to the marker to the agrose gel which was run at 160 V for 80 minutes. After that the gel was removed from the casting tray to a deep tray containing 1 µl of 10 mg/ml Ethidium Bromide solution in 150 ml of 1 x TBE and stained for 10 minutes and the gel was rinsed briefly with water. The gel was visualized under UV and a photograph was saved in the computer.

Results and discussion

DNA of TY52 (carried *Ty-1* gene) amplified at 650 bp, 230 bp and 210 bp, whereas DNA of both of Multichiltylc-95-J0-C2 and Pimpertylc-J-13 amplified at 180 bp. DNA of CLN2498E (carried *Ty-2* gene) amplified at 900 bp whereas DNA of both of Multichiltylc-95-J0-C2 and Pimpertylc-J-13 amplified at 800 bp. DNA of CA4 (carried *Ty-3*) amplified at 650 bp whereas DNA of Multichiltylc-95-J0-C2 and Pimpertylc-J-13 amplified at 450 bp. DNA of Lianero (carried *Ty-4*) amplified at 811 bp, 106 bp, 65 bp and 3 bp whereas DNA of Multichiltylc-95-J0-C2 and Pimpertylc-J-13 amplified at 913 bp and 64.3 bp. DNA of Puntero (carried *Ty-5* gene) amplified at 450 bp whereas Pimpertylc-J-13 DNA amplified

at 450 bp and Multichiltylc-95-J0-C2 DNA amplified at 400 bp (Fig.1). Results indicated that Multichiltylc-95-J0-C2 did not carry *Ty-1, Ty-2*, *Ty3, Ty-4* or *Ty-5* genes and Pimpertylc-J-13 did not carry *Ty-1, Ty-2*, *Ty3,* or *Ty-4* genes. It appeared that Pimpertylc-J-13 may carry *Ty-5*, a resistance gene from *S. peruvianum*. Multichiltylc-95-J0-C2 was previously reported as tolerant to TYLCV [12, 13]. On the other hand, Pimpertylc-J-13 was previously determined as a good source of tolerance to TYLCV [14]. So these findings suggest verifying these results through marker-trait association in a segregating population by screening with virus and genotyping with the marker. Also the results confirmed the presence of the above mentioned marker genes *Ty-1* in TY52, *Ty-2* in CLN2498E, *Ty-3* in CA4, *Ty-4* in Lianero (F1) and *Ty-5* in Puntero. These different sources of resistance to TYLCV will help in constructing a program aimed at pyramiding these genes in one genotype that can express high resistance to this disease.

Table 1. Tomato genotypes used for genotyping for marker-assisted selection of *Ty-1, Ty-2,Ty-3, Ty-4* and *Ty-5* genes.

Variety	Reaction to TYLCV	Resistance source	Source
CLN1621L	Susceptible (Check)	-	AVRDC
Ty52	Resistant (Check)	*Ty*-1	AVRDC
CLN2498E	Resistant (Check)	*Ty*-2	AVRDC
CA4	Resistant (Check)	*Ty*-3	AVRDC
Llanero(F1)	Resistant (Check)	*Ty*-4	AVRDC
Puntero	Resistant (Check)	*Ty*-5	AVRDC
Pimpertylc-J-13	Resistant	Unknown	INRA-France
Multichiltylc-95-J0-C2	Resistant	unknown	INRA-France

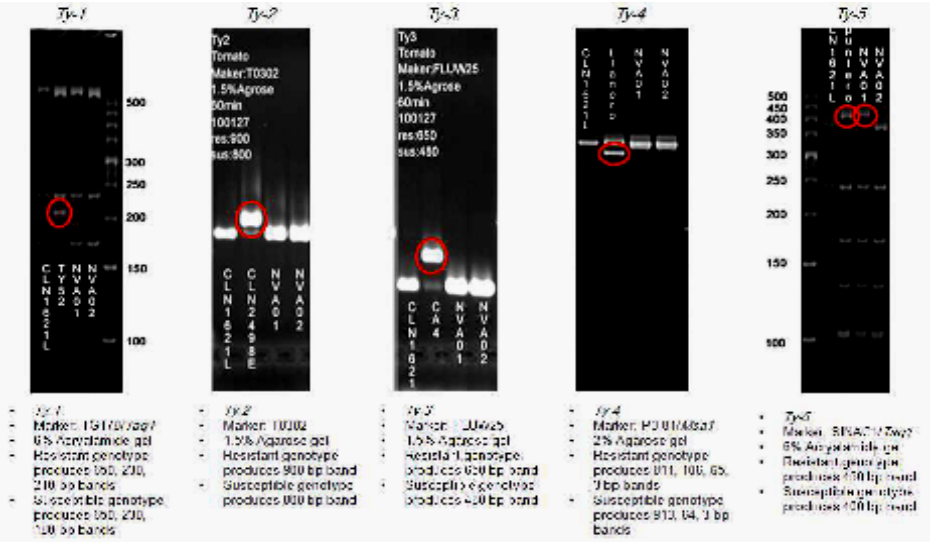

Fig. 1. Genotyping for marker assisted selection of five TYLCV resistance genes markers *Ty-1, Ty-2, Ty-3, Ty-4* and *Ty-5;* NVA01= Pimpertylc-J-13, NVA02= Multichiltylc-95-J0-C2.

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