Gene-Based Markers for the *Tomato Yellow Leaf Curl Virus* Resistance Gene Ty-3

Panpan Dong1,3, Koeun Han1, Muhammad Irfan Siddique1, Jin-Kyung Kwon1, Meiai Zhao2, Fu Wang3, Byoung-Cheorl Kang1*

1Department of Plant Science, Plant Genomics and Breeding Institute, and Vegetable Breeding Research Center, College of Agriculture and Life Sciences, Seoul National University, Seoul 08826, Korea
2College of Life Science, Qingdao Agricultural University, Qingdao 266-109, China
3College of Horticulture, Qingdao Agricultural University, Qingdao 266-109, China

**ABSTRACT** The viral disease induced by *Tomato yellow leaf curl virus* (TYLCV) reduces tomato (*Solanum lycopersicum*) yield significantly in tropical and subtropical regions. A number of loci, including Ty-1 to Ty-5, conferring resistance to TYLCV have been described and introgressed into modern tomato cultivars. The availability of molecular markers linked to these genes would expedite the introgression of TYLCV resistance into commercial cultivars. In the present study, we developed gene-based markers linked to the Ty-3 gene using a segregating population derived from a cross between the TYLCV-resistant line *S. lycopersicum* 'A45' and the susceptible line *S. lycopersicum* 'A39'. *Agrobacterium*-mediated screening was used to test TYLCV resistance of plants in the segregating population, and the resistance was evaluated by a visual scoring method and polymerase chain reaction analysis. By comparing sequences of the Ty-3 genes of the resistant and susceptible lines, two high-resolution melting (HRM) markers (Ty3-HRM1 and Ty3-HRM2) and one sequence characterized amplified region (SCAR) marker (Ty3-SCAR1) were developed. The HRM markers were based on single nucleotide polymorphisms at the 13th exon and the 15th intron, whereas the SCAR marker was based on a 246-bp deletion in the 16th intron. These gene-based markers will be useful tools for marker-assisted selection in breeding programs to improve TYLCV resistance of tomato.

**Keywords** TYLCV, Gene-specific makers, SCAR, High-resolution melting

**INTRODUCTION**

Tomato (*Solanum lycopersicum*) belongs to the Solanaceae family and is one of the most important cultivated vegetables worldwide. Since it has high nutritional value, tomato has become a popular vegetable grown in large scale (Naika et al. 2005). *Tomato yellow leaf curl virus* (TYLCV) disease transmitted by the whitefly (*Bemisia tabaci*) is one of the most devastating diseases causing quality and yield reduction in tomato (Liu et al. 2013). According to recent reports, TYLCV disease is spreading due to global warming, with current outbreaks in the United States and China (Rojas et al. 2007; Zhang et al. 2009; Melzer et al. 2010). Recently, TYLCV incidence was also reported in Korea (Lee et al. 2010). TYLCV is a pathogen with broad host range, including tomato (*S. lycopersicum*), potato (*S. tuberosum*), pepper (*Capsicum annuum*), tobacco (*Nicotiana tabacum*), and several other dicot species (Polston and Anderson 1997). Leaf curling, yellowing and plant stunting are typical disease symptoms in infected plants (Ji et al. 2009a). If plants are attacked by TYLCV at the juvenile stage, up to 100% yield losses can occur (Varma and Malathi 2003). Disease management for TYLCV has focused on whitefly control, and these practices are uneconomical and laborious as well as including periodical application of insecticide (Hilje et al. 2001; Palumbo et al. 2001). Furthermore, insecticide resistance in whitefly has been described (Horowitz et al. 2007), highlighting the urgent need for alternative approaches to TYLCV disease management, such as the...
development of resistant cultivars (Verlaan et al. 2013). To speed up the process of introgression of disease resistance genes to elite breeding materials, molecular markers have been developed and utilized in major crop plants. Accordingly, molecular markers have become essential tools for marker-assisted selection in breeding programs, including those aimed to improve resistance to TYLCV, and also for map-based cloning to isolate the genes controlling traits (Gonzalez-Cabezuelo and Lozano 2012). Most tomato cultivars are susceptible to TYLCV; however, several resistance sources have been identified in wild tomato species, such as S. pimpinellifolium, S. chilense, S. peruvianum, S. habrochaites, and S. cheesmaniae (Ji et al. 2007; Verlaan et al. 2013). TYLCV resistance sources include Ty-1, Ty-2, Ty-3, Ty-4, and Ty-5, and the genes underlying Ty-1 and Ty-3 have been identified (Verlaan et al. 2013). The Ty-1 locus in S. chilense LA1969 is linked with the Ty-3 locus on tomato chromosome 6 (Ji et al. 2007). Ty-1 was first mapped to the interval between markers T0774 and SL_2.40ch06_30.891, and Ty-3 was mapped between the markers UF_TY3_P1 and UF_TY3_P23 in a recombinant inbred line carrying S. chilense LA2779 introgression; ultimately, it was found that Ty-1 and Ty-3 are alleles of the same gene (Verlaan et al. 2013). The Ty-1/Ty-3 gene encodes a DFDGD-class RNA-dependent RNA polymerase.

Even though Ty-3 and Ty-1 are allelic, Ty-3 behaves differently than other TYLCV resistance genes. Ty-3 is effective against TYLCV and the bipartite begomovirus ToMoV (Agrama and Scott 2006). Ty-1 and Ty-2 genes express incomplete or nearly complete dominance pattern (Ji et al. 2009b). By contrast, Ty-3 has been reported to have an additive effect, and it is a major locus explaining a high proportion of resistance (Ji et al. 2009b). Gene-based molecular markers of Ty-3 would be useful to predict the phenotypic variations. There have been gene-based markers developed for Ty-1/Ty-3 by comparative analysis of tomato accessions (Jung et al. 2015; Lee et al. 2015). In the present study, we developed Ty-3 gene-based co-dominant markers in a bi-parental population to improve the efficiency of genotyping and to increase the diversity of molecular markers.

MATERIALS AND METHODS

Plant materials

The accessions used in this genetic analysis were kindly provided by Prof. Wang Fu (College of Horticulture, Qingdao Agricultural University, China). The F1 progeny were produced by crossing a susceptible line S. lycopersicum ‘A39’ and a resistant line S. lycopersicum ‘A45’ containing Ty-3, and F1 plants were self-pollinated to construct a segregating population of 150 F2 plants. In addition, F1 was backcrossed to the susceptible line S. lycopersicum ‘A39’, and 75 BC1F1 plants were produced for genetic analysis. The segregating populations were tested to validate the developed molecular markers.

Virus inoculation and phenotype evaluation

Agrobacterium cells containing the TYLCV genome (GV3101 pCAMBIA0390_Ty-1.9mer) were provided by Professor Young-Su Seo (Pusan University, Korea). A 5-ml culture of the Agrobacterium cell stock was grown overnight at 30°C in LB medium containing kanamycin (50 mg/ml) and gentamycin (50 mg/ml). The culture was transferred to a 2-ml tube and centrifuged at 35,000g for 2 minutes. The pelleted cells were re-suspended in infiltration buffer (200 μM acetosyringone, 10 mM MgCl2, 10 mM 2-(N-morpholino) ethanesulfonic acid) to a final optical density at 600 nm (OD600) of 0.3. The suspension was incubated on a rocker for 3 hours at room temperature. Agro-infiltration using a 1-ml needleless syringe was performed on cotyledons of tomato seedlings at the 1-2 true leaf stage. The inoculated plants were placed in a growth chamber at 25°C to 28°C. Disease response was scored at 21 and 28 days post inoculation (dpi) according to the Lapidot scale (Lapidot et al. 2006). Plants with phenotypes scored 0 to 1 were marked as resistant, whereas those scored 2 to 4 were considered susceptible. To confirm the phenotype, DNA was extracted from infected leaves and polymerase chain reaction (PCR) was performed to detect TYLCV (Deng et al. 1994).

DNA extraction

Fresh leaves of plants were sampled before and after infiltration with TYLCV. DNA was extracted from young