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Conventional and molecular marker-assisted selection and pyramiding of genes for multiple disease resistance in tomato

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ABSTRACT

Tomato (*Solanum lycopersicum* L.) is widely grown in the tropics but production is subject to high losses from diseases. AVRDC—The World Vegetable Center initiated a program to develop fresh market tomato lines resistant to begomoviruses causing tomato yellow leaf curl disease, *Phytophthora infestans* causing late blight, *Ralstonia solanacearum* causing bacterial wilt, *Stemphyllium* spp. causing gray leaf spot, *Fusarium oxysporum* f. sp. *lycopersici* race 2, and *Tobacco mosaic virus*. This work provides greenhouse, field, molecular marker, and laboratory protocols used in the screening and selection process that were applied to segregating populations during generation advance over three years to develop five multiple disease resistant F_7 fresh market tomato lines. Resistance of the five lines to the abovementioned diseases was confirmed in subsequent evaluations. Average yields of the five lines exceeded 100 t/ha under optimal temperatures in a dry season trial, but yields were reduced in a second trial under higher temperatures and rainfall. Seed of three multiple disease resistant $F_{7:8}$ lines is available from AVRDC (http://avrdc.org/seed/improved-lines/); these lines have potential for release as inbred line cultivars, hybrid parental lines, or breeding stock.

1. Introduction

Tomato (*Solanum lycopersicum* L.) is a widely grown vegetable throughout the tropics and subtropics and is an important source of vitamins A and C. Production of high value fruit and vegetables such as tomato offer some smallholders the opportunity to change from subsistence to commercial farming and substantially increase their incomes (Weinberger and Lumpkin, 2005; Fan et al., 2013). However, tomato crops can be infected by disease-causing bacterial, fungal, and viral pathogens that reduce yields, fruit quality, shelf-life, and nutritional content. In extreme cases, these diseases force farmers to abandon tomato production altogether. In the absence of resistant cultivars, farmers often depend on pesticides to control diseases. High reliance on pesticides poses health hazards to farmers and their families, the environment, and consumers; intensive pesticide use also can substantially increase production costs, which increase farmer financial risks and pass the accrued higher costs to consumers (Wilson and Tisdell, 2001). Resistant cultivars are among the cheapest, simplest, and most environmentally safe ways to manage disease.

Many diseases affect tomato in the tropics and subtropics, but three of the most important in terms of widespread incidence and potential to cause high yield losses include tomato yellow leaf curl disease caused by whitefly-vectored begomoviruses (Hanssen et al., 2010; Navas-Castillo et al., 2011), bacterial wilt caused by *Ralstonia solanacearum* (Hayward 1991; Mansfield et al., 2012), and late blight caused by *Phytophthora infestans* (Mont.) De Bary (Fry, 2008; Nowicki et al., 2012). The pathogens causing these diseases are genetically diverse with vast potential to generate new forms (Hayward, 1991; Fry, 2008). Most disease resistance in commercial tomato cultivars is conditioned by single genes, each conferring resistance to a specific pathogen or pathogen race, strain, or phylotype (Yang and Francis, 2007; Scott and Gardner, 2007; Scott, 2007). Six tomato yellow leaf curl disease resistance genes (*Ty-1/Ty-3*, *Ty-2*, *Ty-4*, *Ty-5*, *Ty-6*) are available in cultivated tomato (Ji et al., 2007a,b,c; Verlaan et al., 2013; Hutton and Scott, 2015). Five late blight resistance genes were introgressed from *S. pimpinellifolium* into cultivated tomato (Nowicki et al., 2012) and *Ph-2* and *Ph-3* have been used in commercial cultivars (Zhang et al., 2014). Two major

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bacterial wilt resistance quantitative trait loci (QTLs), *Bwr-12* and *Bwr-6*, were identified in tomato cultivar 'Hawaii 7996' (H7996) (Thoquet et al., 1996; Carmeille et al., 2006; Wang et al., 2013) and *Bwr-12* is important for resistance to Phylotype 1 (Asia) bacterial wilt strains (Wang et al., 2013). Three race-specific genes (*I*, *I–2*, *I–3*) condition resistance to the fusarium wilt pathogen (*Fusarium oxysporum* f. sp. *lycopersici*) (Scott and Gardner, 2007). The incompletely dominant gene *Sm* offers resistance to four species of the gray leaf spot pathogen (*Stemphyllium* spp.) (Scott and Gardner, 2007). Several genes condition resistance to *Tobacco mosaic virus* (TMV) in tomato and the $Tm2^2$ allele conditions resistance to multiple strains (Scott, 2007).

Effective selection for disease resistance in segregating populations requires accurate, cost-effective screening methods that permit rapid testing of thousands of plants. Common disease screening techniques include field testing under natural disease pressure, and greenhouse/growth room screening procedures in which plants are inoculated with specific pathogen strains. Field screening is appropriate when the breeding is conducted in the region where the cultivars will be released and high disease pressure can be expected. Greenhouse seedling inoculation can assess disease reactions quickly, reduce some sources of environmental variation by use of characterized pathogen strains and defined inoculum concentrations, and avoid confounding effects from other pests or diseases. Many disease resistance genes have been mapped in tomato, and molecular markers linked to these genes are available for marker-assisted selection (MAS). The choice of screening method depends upon effectiveness, availability, cost, and convenience. Disease resistance alone is insufficient to ensure farmer adoption; commercial cultivars also must possess high yield potential, early maturity, and other horticultural traits, as well as fruit quality and nutrient content. Consequently, disease resistance breeding must be conducted with selection for important horticultural and fruit characters.

Breeding inbred lines with resistance to multiple diseases is a worthy but often difficult goal. Selecting screening protocols and the sequence of trait screening, and managing segregating populations to achieve the desired outcome, can be challenging. This paper describes a three-year selection process and the sequence of field, lab, greenhouse and molecular marker protocols applied by AVRDC-The World Vegetable Center (AVRDC) to a segregating population, which led to the development of fresh market tomato lines resistant to late blight, tomato yellow leaf curl disease, bacterial wilt, fusarium wilt, gray leaf spot, and *Tobacco mosaic virus* (TMV).

2. Materials and methods

2.1. Parents and cross

A three-parent cross, [(CLN2777G × G2-6-20-15B) × LBR-11], coded CLN3241 was created at AVRDC in 2006–2007 to develop tropically adapted, multiple disease resistant lines. CLN2777G is homozygous for resistance genes *Bwr-12* (bacterial wilt), *Ty-2* (tomato yellow leaf curl disease), and $Tm2^2$ (TMV). G2-6-20-15B is homozygous for *Ty-3* (tomato yellow leaf curl disease resistance). LBR-11 is an AVRDC F_6 selection from North Carolina State University F_2 population NC3220x-20 and is homozygous for resistance genes *Ph-2* and *Ph-3*, *I2* (resistance to race 2 of the fusarium wilt pathogen), and *Sm* (resistance to the gray leaf spot pathogen).

2.2. Line development

Segregating populations were managed by pedigree selection appropriate for self-pollinating crops (Fehr, 1987). Sixteen protocols to assess disease resistance, horticultural and fruit traits (Tables 1 and 2) were used to screen populations and lines. Selection and generation advance began in 2008 with a segregating triple-cross F_1 population (Table 3) and continued until completion of F_7 lines in June 2012. Selection was based on individual plant performance in the F_1 and F_2 generations and single plot progeny rows (30 plants per plot) with individual plant selection practiced within rows from the F_3 to F_7 generations. Two tomato crops were produced in southern Taiwan during the dry season (October–February and March–June) allowing two cycles of generation advance per year. Seed was sown in trays and seedlings were maintained in a plastic house for about 30 days before field transplanting. Before transplanting, seedlings were screened for resistance to one or more diseases, either by MAS or by greenhouse inoculation. Resistant plants were transplanted to the field for evaluation of fruit and horticultural traits. Intensive selection for tomato yellow leaf curl disease and late blight resistance was practiced during the F_1–F_4 generations. Tomato yellow leaf curl disease in southern Taiwan is caused by *Tomato yellow leaf curl Thailand virus* (TYLCTHV) and *Tomato leaf curl Taiwan virus* (ToLCTWV) (Tsai et al., 2011) with the highest pressure occurring from March to June and coinciding with high whitefly populations. Plants with tomato yellow leaf curl disease resistance genes identified by MAS were assessed in the field for tomato yellow leaf curl disease severity. Late blight screening relied on seedling inoculation with selected pathogen isolates, and also by MAS after gene markers for *Ph-2* and *Ph-3* became available in 2009. Greenhouse seedlings were screened for bacterial wilt resistance using drench inoculation, and by MAS after markers for *Bwr-12* became available in 2011. Fusarium wilt, TMV, and *Stemplyllium* screenings were performed with seedlings of $F_{7:8}$ lines. Selection for plant vigor, vine cover (extent to which foliage cover protects the fruit), early maturity, visual fruit load, fruit size and shape, fruit size uniformity within the fruit cluster, fruit firmness, fruit color development, and absence of fruit defects such as cracking was carried out during generation advance.

2.3. Disease resistance evaluations and confirmation

2.3.1. Late blight

Details of the protocols for inoculum preparation and inoculation are given in Chen et al. (2008). Briefly, 35-day-old seedlings were spray-inoculated with zoospore/sporangia suspensions of 5×10^4/mL of selected pathogen isolates. Inoculated seedlings were incubated in a growth room at 100% relative humidity and 20 ± 2 °C without light for the first 24 h. Afterwards, growth room conditions were maintained at 60–95% RH, a daily 14-h light period (70 μE m^2 s^{-1}) and 20 ± 2 °C. Each plant was visually scored 10 days after inoculation according to the following scale, where 0 = no symptoms; 1 = 1–5% leaf area affected, small lesions <2 mm and no stem lesions; 2 = 6–15% leaf area affected, necrosis-restricted leaf lesions and no stem lesions; 3 = 16–30% leaf area affected, coalescing leaf lesions or tiny water-soaked stem lesions; 4 = 31–60% leaf area affected, edge-expanding leaf lesions or a few small stem lesions (< 5 mm); 5 = 61-90% leaf area affected, drying leaf lesions or edge-expanding stem lesions; 6 = 91–100% leaf area affected, leaves blighting, extensive stem damage, or death. Resistant checks included WV700 (homozygous for *Ph-2*) and CLN2037B (homozygous for *Ph-3*). Entries and checks were tested for reactions to isolates Pi39Aand Pi237 in separate trials. WV700 is susceptible and resistant, respectively, to Pi39A and Pi237; conversely, CLN2037B is resistant to Pi39A and susceptible to Pi237. Plots included 12 plants and entries were arranged in a randomized complete block design (RCBD) with three replications.

2.3.2. Bacterial wilt

A detailed description of the bacterial wilt drench method is given in Hai et al. (2008). Inoculations were conducted with virulent