Evaluation of tomato inbred lines for resistance to the tomato yellow leaf curl disease complex in Oman

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\textbf{A B S T R A C T}

Tomato yellow leaf curl disease (TYLCD), caused by viruses of the genus \textit{Begomovirus} (family \textit{Geminiviridae}), is a major constraint to tomato production in Oman. The development of tomato lines with resistance to the viruses that cause TYLCD is an important target for tomato breeding programs worldwide. The study assessed fourteen tomato inbred lines harbouring one or more of five TYLCD resistance genes (Ty-1/Ty-3, Ty-2, ty-5) for resistance against the TYLCD complex in Oman by screening in the field for TYLCD incidence, severity and viral load over two consecutive years. All Ty gene-containing lines performed better than the susceptible controls, with lower disease incidence, severity and viral DNA load. Lines harbouring Ty-2 alone or in combination with other Ty genes performed poorly. A line harbouring only ty-5 performed the best, displaying no symptoms and having the lowest viral load. The presence of a disease enhancing betasatellite in lines harbouring Ty-2 suggests that this may compromise Ty-2 resistance. The results indicate that the Ty genes are useful in Oman as well as other Gulf Cooperation Council countries and should be incorporated into local varieties.

1. Introduction

The genus \textit{Begomovirus} is the largest genus of the family \textit{Geminiviridae} of single-stranded DNA viruses and contains many of the most economically important geminiviruses (Zerbini et al., 2017). Begomoviruses are transmitted by whiteflies of the \textit{Bemisia tabaci} species complex and have genomes that are either bipartite (components known as DNA A and DNA B) or monopartite (with a genome that is a homolog of the DNA A component of bipartite begomoviruses). Collectively the begomoviruses are widespread geographically, occurring throughout the tropics and subtropics, and infecting a wide host range of plant within the eudicots, including crops in the family \textit{Solanaceae}. Begomoviruses cause tomato yellow leaf curl disease (TYLCD), a very destructive disease of tomato globally. The major cause of TYLCD is the monopartite begomovirus \textit{Tomato yellow leaf curl virus} (TYLCV; Moriones and Navas-Castillo, 2000). This virus has its origins in the Middle East but has since spread to almost all tomato producing areas of the world (Mabvakure et al., 2016) including, most recently, into Pakistan (Zaidi et al., 2017).

Tomato cultivation in Oman has recently been seriously affected by begomoviruses (Khan et al., 2014a,b). Analysis implicated at least five distinct begomovirus species; TYLCV (Idris et al., 2011), \textit{Chili leaf curl virus} (ChLCV; Khan et al., 2013b), \textit{Tomato leaf curl Sudan virus} (ToLCSVD; Khan et al., 2013a), as well as begomoviruses found only in Oman, including \textit{Tomato leaf curl Liwa virus} (ToLCLwV, previously known as Tomato leaf curl Al-Batinah virus; Khan et al., 2014a,b) and \textit{Tomato leaf curl Barka virus} (ToLCBrV; Al-Shihi et al., 2014). Furthermore, most begomoviruses that infect tomato in Oman may also be associated with a betasatellite, small ssDNA molecules that require a helper begomovirus for replication in and spread between plants. Betasatellites, in many cases, enhance the symptoms induced by the helper virus as well as increasing viral DNA levels in plants by overcoming host plant resistance based on RNA interference (Zhou, 2013).

Traditional measures to reduce losses due to begomovirus infection are based on management of the vector, including the use of insecticides, physical barriers and reflective mulches (Hilje et al., 2001; Lapidot et al., 2014). Other cultural practices include virus-free transplants, crop-free periods, weed control and removal of infected plants (Navas-Castillo et al., 2011). However, these tactics have not been effective in controlling whitefly populations or begomovirus infection. The wide host range, high virus evolution rates, and the presence of a large population of efficient vectors (whitefly) make it difficult to...
develop effective long-term management strategies. For this reason conventional breeding and selection has been the mainstay for obtaining resistance against begomoviruses in tomato crops.

Resistance to begomoviruses has been successfully introgressed from resistant accessions of the wild tomato species *Solanum pimpinellifolium*, *Solanum peruvianum*, *Solanum chilense* and *Solanum habrochaites* into cultivated tomato (Ji et al., 2007b). Five major begomovirus resistance genes originating from wild species were identified and mapped through molecular markers. Ty-1, a partially dominant major resistance gene, was introgressed from *S. chilense* accession LA1969 and mapped to the short arm of chromosome 6 (Zamir et al., 1994). Ty-2, a dominant resistance gene introgressed from *S. habrochaites* accession into tomato line H24, was mapped to the short arm of chromosome 11 (Hanson et al., 2000). Ty-3, a partially dominant gene introgressed from *S. chilense* accessions LA2779 and LA1932, was mapped to chromosome 6 (Ji et al., 2007a). Ty-3 introgressed from LA2779 was reported to be longer and linked to Ty-1. However, recent studies by Verlaan et al. (2013) indicate that Ty-1 and Ty-3 are allelic and encode an RNA dependent RNA polymerase; this resistance is likely part of the host RNA interference-based defense against foreign nucleic acids. Consistent with this idea Butterbach et al. (2014) showed increased cytosine methylation of viral genomes in Ty-1 plants. Ty-4, introgressed from *S. chilense*, was mapped to the long arm of chromosome 3, and it was shown to condition a lower level of resistance compared to Ty-3 (Ji et al., 2009). A recessive resistance gene ty-5 was mapped to chromosome 4 (Anbinder et al., 2009) and also found in the tomato cultivar ‘Tyking’ (Hutton et al., 2012). Recent evidence suggests that ty-5 resistance is due to a RNA surveillance factor ‘Pelo’ (Lapidot et al., 2015). A major TYLCD resistance gene mapped to chromosome 10 in tomato line FLA456 (Kadirvel et al., 2013) was later assigned the name FLA456 (Kadirvel et al., 2013) was later assigned the name

2. Materials and methods

2.1. Experimental site and lay out of trials

The experiment was carried out at Tawoos Agricultural Systems, in Al-Batinah South Governorate, Barka from September to February 2012–2013 and 2013–2014. Fourteen tomato inbred lines with differing combinations of Ty begomovirus resistant genes, developed by the World Vegetable Center, were used in the study. A local susceptible variety, ‘GS 12’, was used as control (Table 1). Lines and variety were replicated three times and arranged in a randomized complete block design. Each experimental unit (plot) included one row of 10 plants. Within and between row spacings were 0.5 m and 1.5 m, respectively. Border rows consisted of the susceptible line GS 12 to increase whitely and TYLCD pressure in the trials.

2.2. Screening and disease scoring

Individual tomato plants were evaluated for disease incidence and severity for four consecutive weeks, starting from the first appearance of symptoms in the field, using a modified disease assessment scale (DAS) (Friedmann et al., 1998; Shahid et al., 2013) where 0 = no discernible symptom, 1 = slight leaf curling of the margins and yellowing, 2 = mild leaf curling, yellowing and vein swelling, 3 = severe leaf curling, yellowing and vein swelling, 4 = overall severe leaf curling, vein yellowing, crumpling and stunting of the whole plant. This modified scale was used for all the tomato plants under observation. The experiment was repeated twice over consecutive growing seasons to validate the results.

Disease incidence and severity were assessed for each plant in each plot as described earlier by Elbaz et al. (2016). Disease incidence was calculated using the formula

\[ I(\%) = \frac{DP}{TP} \times 100 \]

where I = disease incidence, DP = number of symptomatic plants and TP = total number of plants.

The disease severity was estimated for each individual tomato seedling/plant and then the average disease severity was calculated for 10 plants in each treatment using the following formula:

\[ S = \sum_{i=1}^{10} \frac{DP_i}{TP} \]

where “i” is plant number (from 1 to 10), DP i is the disease severity for scale for plant “I” and TP is the total number of plants in each replicate (10).

2.3. Statistical analysis

The data were subjected to statistical analysis to detect possible effects of the cultivar and gene group (GN) on disease incidence, severity and viral load as described earlier by Elbaz et al. (2016). The differences in disease incidence, severity scales and viral load between tomato cultivars and GN were analyzed using ANOVA in SAS. Tukey’s Studentized range test (SAS, version 8) was used to determine whether the cultivars or gene groups differed significantly from each other in the incidence and severity of the disease as well as in the viral load. The fixed effects at each time were the cultivars or groups, where values representing the incidence, severity or viral load varied.

2.4. Sampling, DNA extraction and virus amplification

Leaves from each line/variety were collected from the field. Total genomic DNA was extracted from all tomato leaves using a modified CTAB method (Permingeat et al., 1998). Samples were tested for the presence of begomoviruses and begomovirus-associated satellites by polymerase chain reaction (PCR) with degenerate primers FDCP382/RDCP1038, for begomoviruses (or begomovirus DNA A components; Khan et al., 2013a), degenerate primers BV1855/BC2751, for begomovirus DNA B components (Idris and Brown, 1998), beta01/beta02, for betasatellites (Bridgon et al., 2002), and DNA101/DNA102, for alphasatellites (Bull et al., 2003). PCR products were visualized in 1% agarose gels, stained with ethidium bromide, under ultraviolet light.

2.5. Identification of viruses and satellites

PCR positive and selected PCR negative tomato plants were selected to amplify circular DNA molecules by rolling-circle amplification (RCA) using an IllustraTM TempliPhi 100 Amplification Kit (Amersham Biosciences, Piscataway, NJ, USA). RCA products were digested with a