Assessment of resistance to bacterial wilt incited by Ralstonia solanacearum in tomato germplasm

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ORIGINAL ARTICLE

Assessment of resistance to bacterial wilt incited by *Ralstonia solanacearum* in tomato germplasm

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Abstract Bacterial wilt incited by *Ralstonia solanacearum* has been found the most damaging and widespread diseases of tomato throughout the world and causes heavy yield losses. Management of the disease is mainly relied on chemicals, and their use is fraught with health risks. The hazardous effects of pesticides can be dispensed with using non-chemical strategies, and resistant cultivars can prove a promising alternative. In the present study, 30 tomato cultivars were assessed for their resistance to bacterial wilt. None of the cultivars was immune or highly resistant to *R. solanacearum*. Two cultivars Early King and Lerica were found resistant (R), and four viz. Red Hero, Giant Cluster, Red Ruby and Red Stone showed moderately resistant (MR) reaction. Eleven cultivars each appeared as moderately susceptible and susceptible, while two cultivars (Bonny Best and Roma VF) were assessed as highly susceptible (HS) to the bacterium. In HS cultivars, symptoms appeared 4 days after inoculation. The symptoms were first observed on leaves and then progressed toward other parts of plants resulting in complete wilting in susceptible cultivars within 14 days. Brown discoloration in vascular systems of transversely cut parts of HS plants was also observed. On the other hand, in R and MR cultivars, symptoms appeared on leaves followed by chlorosis and no wilting was observed even after 14 days of inoculation. The R and MR cultivars are therefore recommended for cultivation under integrated production systems and in developing new resistant tomato cultivars.

Keywords Bacterial wilt · Resistance · Susceptibility · *Solanum lycopersicum*

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Introduction

Tomato (*Solanum lycopersicum* L.) is an extensively grown solanaceous vegetable throughout the world. It is a cheap source of vitamins A, C and E and contains large quantity of water, calcium and niacin (Olaniyi et al. 2010). Its regular consumption can reduce the risks of cancer, cardiovascular diseases and osteoporosis (Bhowmik et al. 2012). Short duration and low input costs attract the farmers to cultivate tomato and gain great production throughout the year especially in warmer countries (Naika et al. 2005). A large number of promising varieties and cultivars of tomato are cultivated throughout the world, which vary not only in size, shape, quality and firmness of fruit, but also in yield (Georgiev et al. 1988; Suwwan and Abu-Baker 1986). It can easily adapt to a variety of environmental conditions (Tiwari et al. 2012). Diversified climatic conditions in Pakistan favor the production of good quality tomatoes throughout the year (Chohan et al. 2016). Pakistan stands at 33rd rank globally in tomato production. The harvested area of tomato in Pakistan is 58,196 ha, and the total production is 574,052 t. Per acre yield of tomato in Pakistan is quite low as compared to many developed countries of the world. This happens due to the attack of several diseases caused by fungi (Iqbal and Mukhtar 2014; Iqbal et al. 2014), viruses (Ashfaq et al. 2014, 2015), bacteria (Tiwari et al. 2012) and nematodes (Babaali et al.

2017; Hussain et al. 2014; Kayani et al. 2017; Mukhtar et al. 2017a) during its growth.

Wilt incited by the bacterium *Ralstonia solanacearum* has been found the most disturbing and widespread diseases throughout the world damaging tomato, eggplant, chili and various solanaceous crops (Poussier et al. 1999; Yabuuchi et al. 1995). The pathogen has over 450 hosts resulting in heavy yield losses (Wicker et al. 2007). The bacterium has been divided into five races and five biovars on the basis of host range variations and metabolic properties, respectively (Alghuthaymi et al. 2016; Hayward 1994; He et al. 1983). The disease has been reported to cause 26% loss of fresh fruit production of hybrid tomatoes, and yield losses can go up to 90.62% under severe disease incidence (Artal et al. 2012). The pathogen has affected crop productivity of more than eighty countries throughout the globe causing annual losses to the tune of over US $ 1 billion every year (Champoiseau et al. 2009; Hong et al. 2012; Yuliar et al. 2015). In Pakistan, the prevalence of the disease is widespread in all agro-ecological zones with varying intensity (Aslam et al. 2015; Shahbaz et al. 2015). Furthermore, the incidence and severity of bacterial wilt increased when root-knot nematodes were found present conjointly with *R. solanacearum* in many vegetables (Hussain et al. 2016; Mukhtar et al. 2017b, 2014; Tariq-Khan et al. 2016).

The disease is commonly controlled by using different management strategies such as crop sanitation, chemicals, disease-free planting material, crop rotation. These methods have one or the other drawbacks and as alone strategy are not successful. The use of chemicals is being discouraged due to health hazards coupled with its use. Development of resistant varieties is the only possible and feasible way of managing this disease. Breeding for resistance requires desired resistant sources and continuous screening against the pathogen. As there is scanty information about the resistance in available tomato germplasm to this disease in Pakistan, therefore, the objective of the present study was to assess the degree of resistance among the available tomato germplasm against this quarantine pathogen. The resistant cultivars can be employed as an important component in integrated disease management programs.

Materials and methods

The bacterium *Ralstonia solanacearum*

The bacterium, *R. solanacearum*, was isolated from diseased tomato plants showing typical bacterial wilt symptoms confirmed immunologically (Opina and Miller 2005) and from infested soil using semi-selective medium, South Africa (SMSA) (Englerbrecht 1994) and triphenyltetrazolium chloride (TTC) medium (Hugh and Leifson 1953). The bacterium was purified from a single colony. The hypersensitive reaction was performed on tobacco and tomato plants, and pathogenicity was confirmed on susceptible cultivars of tomato and tobacco (Elphinstone et al. 1996).

The DNA of the bacterium was isolated and amplified using *egl* gene primers in PCR (Opina et al. 1997). The PCR-amplified product was run on 1% agarose gel by using GeneRuler™ 1 kb DNA Ladder. The bacterium gave positive results by giving fragments at 750 bp with *egl* gene and was confirmed as *R. solanacearum*.

Germplasm collection

Tomato germplasm comprising 30 cultivars (Table 4) was collected from National Agricultural Research Center (NARC), Federal Seed Certification and Registration Department, Punjab Seed Cooperation and local markets.

Evaluation of tomato cultivars for resistance to *R. solanacearum*

The nurseries of all the 30 tomato cultivars were raised separately in sterilized potting mixture in germination trays in the greenhouse. The daily temperature of the greenhouse ranged 25–27 °C. The trays were watered when required.

The screening of tomato cultivars was done in polythene bags measuring 12.75 × 10.15 cm. The bags were filled with sterilized soil containing sand, silt and compost at the ratio 3:1:1, respectively. Three-week-old seedlings were transferred individually to polythene bags. There were ten replications for each treatment. The bags were arranged randomly in a glasshouse at a temperature of 25 °C and were properly moistened in alternative days. One week after transplantation, the plants of each cultivar were inoculated with 30 ml of bacterial culture containing 1×10^7 cfu/ml through soil drenching. One-third root system of each cultivar was slightly injured by inserting a sharp sterilized knife about 2 cm away from the stem prior to drenching to facilitate penetration of the bacterium. After inoculation, the plants were watered at alternative days and symptoms of bacterial wilt were observed. The wilt symptoms and wilted plants were recorded and graded with disease rating scale of 0–5 (Winstead and Kelman 1952) given in Table 1. The association of bacterium with symptom development was confirmed by immunostrips (Opina and Miller 2005). The disease index of bacterial wilt of each cultivar was calculated by the following formula.

Table 1 Disease rating scale for bacterial wilt of tomato caused by *R. solanacearum* (Winstead and Kelman 1952)

Rating	Reaction observed
0	No wilting
1	Less than 10% wilted plants
2	11–25% wilted plants
3	26–50% plants wilted
4	51–75% plants wilted
5	>75% plants wilted

Table 2 Scale based on disease index for the categorization of tomato germplasm

Disease index	Reaction
0.00–0.2	Highly resistant
0.21–0.3	Resistant
0.31–0.4	Moderately resistant
0.41–0.5	Moderately susceptible
0.51–0.6	Susceptible
0.61–0.9	Highly susceptible
0.91–1.0	Extremely susceptible

Disease Index = {(No. of plants at disease rating score "1" × 1) + (No. of plants at disease rating score "2" × 2) + (No. of plants at disease rating score "3" × 3) + (No. of plants at disease rating score "4" × 4) + (No. of plants at disease rating score "5" × 5}/(Total number of plants observed *5).

The cultivars were then categorized following the scale given in Table 2.

Results

Response of tomato germplasm to *Ralstonia solanacearum*

The reaction of tomato germplasm to *R. solanacearum* is summarized in Table 3. Assessment of cultivars on the basis of disease index (DI) (Table 4) showed that none of the cultivar was immune or highly resistant to *R. solanacearum*. Two cultivars Early King and Lerica were found resistant (R), and four viz. Red Hero, Giant Cluster, Red Ruby and Red Stone were moderately resistant (MR). Eleven cultivars each appeared as moderately susceptible (MS) and susceptible (S) with DI ranging 26–50 and 51–75, respectively, while tow cultivars (Bonny Best and Roma VF) were highly susceptible (HS) to the bacterium (Tables 3, 4).

It was also observed that in HS cultivars, symptoms appeared 4 days after inoculation. The symptoms were first observed on leaves and then progressed toward other parts of plants resulting in complete wilting in HS cultivars. The complete wilting occurred in 14 days within susceptible cultivars. Brown discoloration in vascular system of transversely cut parts of HS plants was also observed. Re-isolation of bacterium from susceptible plants confirmed the disease. On the other hand, in R cultivars, symptoms appeared on leaves followed by chlorosis and no wilting was observed even after 14 days of inoculation.

Discussion

Use of resistant cultivars is a cheap, ecofriendly and durable disease management strategy. The search for a source of genetic resistance to bacterial wilt of various vegetables has been pursued in several countries. Resistance to bacterial wilt in tomato genotypes Acc 99, Acc 151, Hy 5 and Sweet 72 has been reported by Tewari (1986), in tomato cultivar Sonali by Patil et al. (1990), in BWR-1 and BWR-5 by Bora et al. (1993) and in BT-18 by Mishra et al. (1995). Similarly, In et al. (1996) screened 31 tomato varieties to *R. solanacearum* and assessed only three as moderately resistant, while the rest were susceptible. Sharma and Kumar (1997) and Sharma et al. (1997) tested new bred lines of tomato for resistance to *R. solanacearum* and recommended three of them which were moderately resistant and high yielding. Sharma et al. (2006) found

Table 3 Host status of tomato germplasm against *R. solanacearum*

DI	Genotypes	Reaction
0–0.2	–	HR
0.21–0.3	Early King, Lerica	R
0.31–0.4	Red Hero, Giant cluster, Red Ruby, Red Stone	MR
0.41–0.5	Prince, Avinash, Gala, BSS-173, Sultan, BSS-30, Red Boy, T-55, Super Blockey, T-60, Red Diamond	MS
0.51–0.6	Yaqii, Roma Super, BSS-39, Kalam, Rio Fuego, Rio Grande, Galia, Roma VW, Marjan, Sahel, Roma King	S
0.61–0.9	Bonny best, Roma VF,	HS
0.91–1.0	–	ES

HR highly resistant, *R* resistant, *MR* moderately resistant, *MS* moderately susceptible, *S* susceptible, *HS* highly susceptible, *ES* extremely susceptible, *DI* disease index

Table 4 Individual disease index of different tomato cultivars and their reaction to bacterial wilt

S. no.	Genotypes	DI	Reaction
1	Early King	0.23	Resistant
2	Lerica	0.26	Resistant
3	Red Hero	0.32	Moderately resistant
4	Giant Cluster	0.34	Moderately resistant
5	Red Ruby	0.35	Moderately resistant
6	Red Stone	0.38	Moderately resistant
7	Prince	0.41	Moderately susceptible
8	Avinash	0.41	Moderately susceptible
9	Gala	0.43	Moderately susceptible
10	BSS-173	0.44	Moderately susceptible
11	Sultan	0.45	Moderately susceptible
12	BSS-30	0.45	Moderately susceptible
13	Red Boy	0.46	Moderately susceptible
14	T-55	0.47	Moderately susceptible
15	Super Blockey	0.47	Moderately susceptible
16	T-60	0.48	Moderately susceptible
17	Red Diamond	0.48	Moderately susceptible
18	Yaqii	0.51	Susceptible
19	Roma Super	0.54	Susceptible
20	BSS-39	0.54	Susceptible
21	Kalam	0.55	Susceptible
22	Rio Fuego	0.55	Susceptible
23	Rio Grande	0.56	Susceptible
24	Galia	0.57	Susceptible
25	Roma VW	0.58	Susceptible
26	Marjan	0.59	Susceptible
27	Sahel	0.59	Susceptible
28	Roma King	0.60	Susceptible
29	Bonny Best	0.83	Highly susceptible
30	Roma VF	0.90	Highly susceptible

resistance in tomato cultivars Swarna Lalima, Swarna Naveen and B-17. Scott et al. (2009) also demonstrated high level of resistance to *R. solanacearum* in large-fruited breeding lines from eight crosses at the F5 generation.

In the present studies, some of the tested tomato cultivars showed resistant and moderately resistant reactions against *R. solanacearum*. Resistance against *R. solanacearum* is due to certain genes. Grimault et al. (1995) found that a single dominant gene is responsible for resistance in tomato to bacterial wilt. Monma et al. (1997) observed that resistance to bacterial wilt is partially recessive, whereas Oliveira et al. (1999) reported additive effects of genes for resistance against the disease. Previously, Singh (1961) while working with the Hawaii and North Carolina germplasm resistant to bacterial wilt had indicated that resistance in tomato to the disease is due to recessive genes, while Acosta et al. (1964) reported the involvement of a relatively small number of genes with major effects in resistance. This shows that a simple genetic control may underlie the resistance to bacterial wilt in some resistant stocks originating from the tropical areas (Tikoo et al. 1990).

Plants frequently encounter many stress stimuli including pathogenic infection. As a result, the hosts respond to these cues by triggering a series of pathogen-inducible enzymes and other chemicals which have been found implicated in defending the host against the pathogens. The expressions of these defense enzymes and chemicals at initial stages and elevated levels are an important characteristic of host resistance to phytopathogens.

The resistance and susceptibility have also been associated with symptom development. In susceptible genotypes, symptoms appeared after 4 days, while resistant ones took 14 days to show symptoms and are in line with the findings of Anith et al. (2004). The resistance to bacterial wilt is strain specific and temperature dependent as has been observed in potato (French and De Lindo 1982). Environmental conditions and locations also influence resistance against bacterial wilt. Hanson et al. (1996) reported variable reaction of tomato lines to bacterial wilt evaluated at several locations in Southeast Asia. They found that tomato lines which were resistant to bacterial wilt in Malaysia and Taiwan showed susceptible reactions in Philippine and Indonesia. This recommends that the germplasm should be evaluated at its local conditions against particular isolates of the pathogen of that area.

It is concluded from the present assessment that tomato cultivars showed variations in their resistance response to *R. solanacearum*. Two cultivars Early King and Lerica were found resistant, and four viz. Red Hero, Giant Cluster, Red Ruby and Red Stone appeared as moderately resistant and therefore are recommended for cultivation under integrated production systems and in developing new resistant tomato cultivars.

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