

Evaluation of chili germplasm for resistance to bacterial wilt caused by *Ralstonia solanacearum*

M. N. Aslam^{1,2} · T. Mukhtar² · M. Ashfaq² · M. A. Hussain³

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Abstract Bacterial wilt incited by *Ralstonia solanacearum* is a serious threat to economically important crops throughout the world and warrants strict control strategies. Use of resistant cultivars is one of the most important strategies to dispense with the pernicious consequences of chemicals. In the present study, twenty eight chili cultivars were assessed for their relative resistance or susceptibility to a highly virulent strain of *R. solanacearum* biovar 3 (RsBd6). Two cultivars namely Skyline-II and Hifly appeared highly resistant. Sanam was the only cultivar found to be resistant while twelve cultivars were categorized as moderately resistant to the bacterium. On the other hand, seven cultivars were rated as moderately susceptible and three showed susceptible reaction. Two cultivars, Talhari and Maxi, behaved as highly susceptible and California Wonder was found to be extremely susceptible. In susceptible cultivars, symptoms appeared 4 days after inoculation resulting in complete wilting within 14 days while in resistant and moderately resistant cultivars, no wilting was observed even after 14 days of inoculation. Brown discoloration in vascular systems of highly susceptible plants was also observed. The resistant and moderately resistant cultivars are therefore recommended for cultivation and in developing new resistant chili cultivars.

Keywords *Capsicum annuum* · Bacterial wilt · Resistance · Susceptibility

Chili pepper (*Capsicum annuum* L.) is one of the world's most popular vegetables from solanaceae family after potato and tomato and is being used mainly as spices and condiments (Bosland and Votava 2000). In Pakistan, it is cultivated on an area of 65 thousand hectares and the total annual production of the country is 150 thousand tons. In terms of yield, Morocco stands first with 22.04 tons/ha while Pakistan ranks at 28th position with 2.54 tons/ha yield (FAO 2012). The low yield is attributable to a number of biotic factors including fungi (Iqbal et al. 2014), viruses (Ashfaq et al. 2015), nematodes (Kayani et al. 2017; Mukhtar et al. 2017a) and particularly the bacteria. *Ralstonia solanacearum*, the incitant of bacterial wilt of chilies, is one of the most economically important plant parasitic bacteria due to its large host range, wide geographic distribution and high destructive potential (Elphinstone 2005; Wicker et al. 2007). The bacterium is soil borne and usually survives in the rhizospheres of the plants and penetrates the roots through natural openings and wounds (Kelman and Sequeira 1965). It has resulted in serious economic losses worldwide particularly in the tropics and is ranked as one of the most important pathogen causing heavy losses worldwide (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 one billion USD every year (Hong et al. 2012). In Pakistan, the pathogen is a serious threat to economically important solanaceous crops and its prevalence is widespread in all the agro-ecological zones with varying intensities (Aslam et al. 2015, 2017; Shahbaz et al. 2015). The incidence and severity of bacterial wilt increased when root-knot nematodes were found present conjointly with *R. solanacearum* in many vegetables

✉ M. N. Aslam
naveed.aslam@iub.edu.pk

¹ University College of Agriculture and Environmental Sciences, The Islamia University of Bahawalpur, Bahawalpur, Pakistan

² Department of Plant Pathology, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan

³ Plant Pathology Section, Regional Agricultural Research Institute, Bahawalpur, Pakistan

(Hussain et al. 2016; Khan et al. 2017; Mukhtar et al. 2014, 2017b; Tariq-Khan et al. 2016).

Huge losses caused by this bacterium in Pakistan warrants strict control strategies. The management of the disease is mainly relied on chemicals which are being discouraged due to health hazards coupled with their use. Resistant cultivars are, therefore, one of the most important alternative strategies to dispense with the pernicious consequences of chemicals. Breeding for resistance requires suitable sources of resistance. For this process, the suitable sources of resistance are necessary and there is no information about the resistance to this disease in the available chili germplasm in Pakistan. Therefore, in the present study, 28 chili cultivars were assessed for their relative resistance or susceptibility to *R. solanacearum* biovar 3.

Chili germplasm consisted of 28 commercially available cultivars collected from different sources (Table 1). A highly virulent strain of *R. solanacearum* biovar 3 designated as RsBd6 commonly prevalent in District Badin of Sindh Province of the country (Aslam 2015) was used in the studies. The bacterium was isolated from diseased chili plants showing typical bacterial wilt symptoms, confirmed immunologically (Opina and Miller 2005) and 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). After molecular confirmation (Opina et al. 1997), the purified culture of the highly virulent strain of *R. solanacearum* biovar 3 (RsBd6) was prepared and adjusted to 10^7 cfu/ml (Aslam 2015).

The screening of chili germplasm for resistance or susceptibility to *R. solanacearum* was carried out in the glasshouse. The seeds of 28 chili cultivars were soaked in water for 24 h for their proper germination. The soaked seeds were sown in germination trays containing sterilized peat mass. The trays were put in the glasshouse at a temperature of 25 °C. Three weeks after emergence, the seedlings at 3–4 leaf stage were

transferred to polythene bags measuring 12.75×10.15 cm containing sterilized soil (sand, silt and compost at the ratio of 3:1:1). There were 40 plants of each cultivar which were divided into 5 batches of 8 plants and placed on a plastic tray. Each batch served as a replication, thus making 5 replications for each cultivar. The trays were arranged in completely randomized design in the glasshouse at a temperature of 25 °C and bags were properly moistened at alternative days. One week after transplantation, the plants of each cultivar were inoculated with 50 ml of bacterial culture containing 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 symptoms were recorded till the complete wilting of susceptible cultivars and graded with 0–5 disease rating scale (Winstead and Kelman 1952). The association of the bacterium with the symptom development was confirmed immunologically (Opina and Miller 2005). The disease index of bacterial wilt of each cultivar was then calculated (Aslam et al. 2017).

On the basis of mean disease index, two cultivars namely Skyline-II and Hifly appeared as highly resistant. Sanam was the only cultivar found to be resistant while twelve cultivars were categorized as moderately resistant to the bacterium. On the other hand, seven cultivars were rated as moderately susceptible and three viz. NARC 16/9, Loungi and Neelum showed susceptible reaction. Two cultivars, Talhari and Maxi appeared as highly susceptible and California Wonder was the only cultivar found to be extremely susceptible (Table 1).

It was also observed that in highly susceptible cultivars, characteristic symptoms of bacterial wilt appeared 4 days after inoculation. The symptoms were first observed on leaves and then progressed towards other parts of plants resulting in complete wilting in highly susceptible cultivars. The complete

Table 1 Reaction of chili cultivars to *Ralstonia solanacearum*

Sr. No.	Mean Disease Index	Names of cultivars	Response
1	0.00–0.2	Hifly ¹ , Skyline II ¹	Highly Resistant
2	0.21–0.3	Sanam ¹	Resistant
3	0.31–0.4	Ghotki ² , Yolo Wonder ² , Sangri ² , Baby Hot Chili ³ , Jalapeno ³ , Hot Shot ³ , Red Giant ¹ , Hot Queen ¹ , Black Master ³ , Bahawalpur Desi ³ , Long Green ⁴ , Gola Peshawari ³	Moderately Resistant
4	0.41–0.5	Tata Puri ³ , NARC 16/7 ⁴ , NARC 16/4 ⁴ , Sky Red ¹ , Nagina ² , Elpaso ³ , Red Fire ²	Moderately Susceptible
5	0.51–0.6	NARC 16/9 ⁴ , Loungi ² , Neelum ²	Susceptible
6	0.61–0.9	Talhari ² , Maxi ²	Highly Susceptible
7	0.91–1.0	California Wonder ²	Extremely Susceptible

Sources of seed ¹ = Federal Seed Certification, Islamabad; ² = Chili Research Station, Kunri; ³ = Authorized Dealer; ⁴ = National Agricultural Research Center, Islamabad

wilting occurred in 14 days within susceptible cultivars. Brown discoloration in vascular system of transversely cut parts of highly susceptible plants was also observed. Re-isolation of bacterium from susceptible plants confirmed the disease. On the other hand, in resistant cultivars, symptoms appeared on leaves followed by chlorosis and no wilting was observed even after 14 days of inoculation. The plants of the extremely susceptible cultivar (California Wonder) wilted entirely and died.

Resistance against *R. solanacearum* has been reported to be due to certain genes. In some cases a single dominant gene is involved while in other cases few genes are responsible for resistance to the bacterium (Grimault et al. 1995; Monma et al. 1997). A number of quantitative trait loci are also known to control resistance in tomato to bacterial wilt (Ishihara et al. 2012). The resistance and susceptibility have also been associated with symptom development. In susceptible cultivars 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. This recommends that the germplasm should be evaluated at its local conditions against particular isolates of the pathogen of that area.

In the present study, chili cultivars showed wide variations in their response to *R. solanacearum* from extremely susceptibility to highly resistance. Two cultivars, Skyline-II and Hifly, appeared as highly resistant, Sanam was resistant and twelve other cultivars were found moderately resistant to the bacterium. The resistant and moderately resistant cultivars are therefore recommended for cultivation under integrated production systems and in developing new resistant chili cultivars.

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