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ANALYSIS OF AUBERGINE GERMPLASM FOR RESISTANCE SOURCES TO BACTERIAL WILT INCITED BY *Ralstonia solanacearum*

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Bacterial wilt caused by *Ralstonia solanacearum* has become a serious threat to the profitable cultivation of aubergine in Pakistan. Management of the disease is mainly relied on chemicals and their use is often 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, twenty four aubergine cultivars were assessed for their resistance to bacterial wilt. Assessment of cultivars on the basis of disease index showed that none of the cultivar was immune, highly resistant or resistant to *R. solanacearum*. Thirteen genotypes viz. Neeli, Saravan, Purple Round, Black Round, Neelum, Kenha 91, Karishna, Violet Prince, Black Beauty, Shamli, Black Nagina, Twinkle Star and Kalash F_1 were found moderately resistant. Six genotypes viz. Dilnasheen, Black Long, Nirala, Purple Long, Pusa Purple and Black Beauty Long were observed as moderately susceptible while three genotypes namely Purple Queen, Purple Rani and Luffa were found susceptible. Two cultivars viz. Bemissal and Eggplant Round were detected as highly susceptible. The resistant and moderately resistant cultivars are therefore, recommended for cultivation under integrated production systems and in developing new resistant aubergine cultivars.
Keywords: Bacterial wilt, resistance, susceptibility, brinjal, disease index

INTRODUCTION

Aubergine, an important solanaceous vegetable, is widely cultivated in Pakistan and the area under its cultivation was 8325 hectares with 82999 tons production during 2014-15. In Punjab, the area under aubergine cultivation was 4452 hectares which produced 54159 tons of aubergine. Many biotic factors have been found to affect the successful production of aubergine including viruses (Ashfaq *et al*., 2015, 2017; Riaz *et al*., 2015), Fungi (Fateh *et al*., 2017), nematodes (Kayani *et al*., 2017, 2018; Khan *et al*., 2017; Mukhtar *et al*., 2017a,b, 2018), insect pests (Javed *et al*., 2017a,b; Kassi *et al*., 2018; Nabeel *et al*., 2018) and particularly bacteria (Shahbaz *et al*., 2015; Aslam *et al*., 2017a).

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). The pathogen has over 450 hosts resulting in heavy yield losses (Wicker *et al*., 2007). The disease has been reported to cause 26 percent loss of fresh fruit production of hybrid tomatoes and yield losses can go up to 90.6 percent 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 one billion USD every year (Hong *et al*., 2012). In Pakistan, the prevalence of the disease is widespread in all the agro-ecological zones with varying intensity (Aslam *et al*., 2017b). 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; Tariq-Khan *et al*., 2017).

The disease is commonly controlled by using chemicals which are being discouraged due to health hazards coupled with its use. The resistant cultivars can be employed as an important component in integrated disease management programs (Shahzaman *et al*., 2015; Rahoo *et al*., 2017, 2018a, b). Development of resistant varieties is the only possible and feasible way of managing this disease. Breeding for resistance requires suitable sources of resistance. For this process, the suitable sources of resistance are necessary and there is scanty information about the resistance to this disease in available aubergine germplasm in Pakistan. Therefore, the objective of the present study was to assess the degree of resistance among the available aubergine germplasm against this quarantine pathogen.

MATERIALS AND METHODS

***Germplasm collection*:** Aubergine germplasm comprising 24 cultivars and genotypes (Table 1) was collected from Federal Seed Certification and Registration Department, Islamabad; National Agricultural Research Center, Islamabad; Punjab Seed Cooperation, Ayub Agricultural Research Institute, Faisalabad and local markets.

Table 1. Response of each genotype to *R. solanacearum* and disease index on each genotype.

Sr.	Genotype	Disease Index	Reaction
1	Neeli	0.33	MR
2	Saravan	0.32	MR
3	Purple Round	0.35	MR
4	Black Round	0.37	MR
5	Neelum	0.40	MR
6	Kenha 91	0.34	MR
7	Karishna	0.36	MR
8	Violet Prince	0.38	MR
9	Black Beauty	0.39	MR
10	Shamli	0.34	MR
11	Black Nagina	0.35	MR
12	Twinkle Star	0.38	MR
13	Kalash F_1	0.33	MR
14	Pusa Purple	0.44	MS
15	Black Beauty Long	0.45	MS
16	Dilnasheen	0.45	MS
17	Black Long	0.46	MS
18	Nirala	0.47	MS
19	Purple Long	0.42	MS
20	Purple Queen	0.57	S
21	Purple Rani	0.55	S
22	Luffa	0.59	S
23	Bemissal	0.85	HS
24	Eggplant Round	0.88	HS

***Bacterial culture*:** A highly virulent strain of *R. solanacearum* commonly prevalent in Pakistan was used in the studies. The bacterium was isolated from diseased plants showing typical bacterial wilt symptoms, confirmed immunologically 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. After molecular confirmation, the purified culture of the highly virulent strain was prepared and adjusted to 10^7 cfu/ml (Aslam *et al*., 2017a, b).

***Preparation of nursery*:** The nurseries of 24 aubergine 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.

***Assessment of resistance to R. solanacearum in aubergine germplasm*:** The screening of aubergine cultivars was done in polythene bags measuring 12.75×10.15 cm. The bags were filled with sterilized soil containing 3:1:1 sand, silt and compost respectively. Three week old seedlings were transferred individually to polythene bags. There were five 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 bacterial culture containing 1×10^7 cfu/ml through soil drenching as described by Aslam *et al*. (2017a). After inoculation, the plants were watered at alternative days and the wilt symptoms and wilted plants were recorded and graded with disease rating scale of 0-5 (Winstead and Kelman, 1952). The disease index of bacterial wilt of each cultivar was calculated and the germplasm were then categorized following the scale described by Aslam *et al*. (2017b).

RESULTS

Reaction of aubergine germplasm to *Ralstonia solanacearum*: The results regarding the response of aubergine germplasm to *R. solanacearum* has been summarized in Table 2. Assessment of cultivars on the basis of disease index (Table 1) showed that none of the cultivar was immune, highly resistant or resistant to *R. solanacearum*. Thirteen genotypes viz. Neeli, Saravan, Purple Round, Black Round, Neelum, Kenha 91, Karishna, Violet Prince, Black Beauty, Shamli, Black Nagina, Twinkle Star and Kalash F_1 were found moderately resistant. Six genotypes viz. Dilnasheen, Black Long, Nirala, Purple Long, Pusa Purple and Black Beauty Long were observed as moderately susceptible while three genotypes namely Purple Queen, Purple Rani and Luffa were found susceptible. Two cultivars viz. Bemissal and Eggplant Round were detected as highly susceptible with disease index of 0.85 and 0.88 respectively.

Table 2. Summary of responses of different genotypes of aubergine to *R. solanacearum*.

Disease Index	Genotypes	Reaction
0.00-0.2	-	HR
0.21-0.3	-	R
0.31-0.4	Neeli, Saravan, Purple Round, Black Round, Neelum, Kenha 91, Karishna, Voilet Prince, Black Beauty, Shamli, Black Nagina, Twinkle Star, Kalash F_1	MR
0.41-0.5	Dilnasheen, Black Long, Nirala, Purple Long, Pusa Purple, Black Beauty Long	MS
0.51-0.6	Purple Queen, Purple Rani, Luffa	S
0.61-0.9	Bemissal, Egg Plant Round	HS
0.91-1.0	-	ES

Resistant (R), Moderately Resistant (MR), Highly Resistant (HR), Susceptible (S), Moderately Susceptible (MS), Highly Susceptible (HS), Extremely Susceptible (ES).

DISCUSSION

Among different management strategies for bacterial wilt in

aubergine, use of resistant cultivars is a cheap, eco-friendly and durable. In many countries, the researchers have tried to identify resistant sources in aubergine against *R. solanacearum* (Rahman *et al*., 2011; Gopalakrishnan *et al*., 2014; Bhavana and Singh, 2016). In the present studies, some of the tested aubergine cultivars showed 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 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.

***Conclusion*:** It is concluded from the present assessment that aubergine cultivars showed variations in their resistance response to *R. solanacearum*. Thirteen genotypes viz. Neeli, Saravan, Purple Round, Black Round, Neelum, Kenha 91, Karishna, Violet Prince, Black Beauty, Shamli, Black Nagina, Twinkle Star and Kalash F_1 were found moderately resistant and therefore, are recommended for cultivation under integrated production systems and in developing new resistant aubergine cultivars.

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