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IDENTIFICATION OF HOST RESISTANCE TO BACTERIAL CANKER DISEASE ON APRICOT, PLUM AND PEACH GROWN IN PUNJAB AND KPK, PAKISTAN

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ABSTRACT

Bacterial canker disease caused by *Pseudomonas syringae* pv. *syringae* (Pss) has expanded rapidly in Pakistan, notably on stone fruits. The present research aimed to determine peach, plum and apricot cultivars' resistance to Pss bacterial canker. During the 2014–2015 growing season, diseased samples exhibiting symptoms of bacterial canker were collected from peach, plum, and apricot trees in Punjab and KPK provinces, and 48 *P. syringae* isolates were recovered. In a pathogenicity test, three Pss isolates (PS3, PS9, and PS17) were found to be highly virulent on peach, plum, and apricot, and their resistance to Pss was evaluated. Leaves and shoots of five peach varieties; Early grand, Florida king, 4 ½, 5 ½, 6 ½, four plum varieties; Red beauty, Fazal-e-man ani, Stanley, Producer, and two apricot varieties; Chinese apricot and golden amber, were foliar sprayed with a mixed culture of Pss at a concentration of 10⁻⁸ cfu ml⁻¹. Sprayed cultivars were covered with plastic bags for three days to retain moisture and kept in a glass house, where they were closely monitored for the appearance of symptoms. 5 ½ peach, Fazal-e-Manani plum, and Chinese apricot were found resistant to Pss, 6 ½ peach, Stanley plum were susceptible while Golden amber apricot was found moderately susceptible to Pss. This is the first report of apricot, peach, and plum host resistance to Pss in Pakistan.

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INTRODUCTION

Bacterial canker of stone fruits is caused by *Pseudomonas syringae* pv. *syringae*, a widespread organism that generally causes disease on stressed trees. All stone fruit can be affected by this disease, but cherries, peaches, and apricots are the most susceptible (Ong and Rhodes, 2022).

Bacterial canker of stone fruits has emerged as a serious threat in many parts of the world (Mohammadi *et al.*, 2001; Ali *et al.*, 2021). It causes localized sunken lesions which are known as cankers or the death of an entire tree. Symptoms of bacterial canker are developed on plant parts like buds, fruits, twigs, and branches. Most

noticeable symptoms include; sunken lesions known as cankers that secrete or ooze out the gummy secretions during the late summer and spring (Hetherington, 2005).

This disease infects all the *Prunus* species including peach (*Prunus persica*), Plum (*Prunus domestica*), apricot (*Prunus armeniaca*), and cherry (*Prunus avium*). Two distinct pathovars of bacterial canker of stone fruits; *P. syringae* pv. *syringae* and *P. syringae* pv. *morsprunorum* are known and associated with different host range (Ivanova, 2007; Ahmed *et al.*, 2018; Ahmed *et al.*, 2016). *Pseudomonas* spp. are difficult to control because to a lack of proper control techniques, plant resistance, and

endophytic behaviour of the pathogen during various disease phases (Kennelly *et al.*, 2007; Bibi *et al.*, 2017).

The control of bacterial canker of stone fruits is unattainable, especially under optimal environmental conditions for infection and disease development (Giovanardi *et al.*, 2016).

The use of stone fruit cultivars resistant to Pss is economically and technically the most practical method for effective management of bacterial canker (Bassi, 1997). Use of resistant cultivars against Pss is technically and economically most effective procedure for the management of many diseases of stone fruits (Bassi, 1997; Donmez *et al.*, 2010). The objective of this study was to evaluate stone fruit cultivars commonly grown in Pakistan for their response to Pss.

MATERIALS AND METHODS

Germplasm Collection

Germplasm of Peach; Early grand, Florida king, locally cultivated peach numbers; 4 ½, 5 ½ and 6 ½, Plum; Red beauty, Fazal-e-manani, Stanley and Producer, two varieties of Apricot; Chinese apricot and golden amber were collected from Swat Green and Ali nursery farm Mingora Swat, Pakistan. The germplasm was used to determine the host resistance response against *Pseudomonas syringae*.

Isolation of Pathogen

Infected fruits, stems and leaves of stone fruits were collected from Punjab and KPK province of Pakistan during 2014-15 (Table 1). Pathovars of pathogen were recovered on artificial nutritional media; nutrient agar followed by purification on King's B media. Incubation was done at 28 ± 2 °C for 48 hrs followed by preservation of pure cultures in 30% sterile glycerol at -80 °C (King *et al.*, 1954; Klement *et al.*, 1990).

Table 1. Representation of surveyed locations of Punjab and Khyber Pakhtoon Khwa, Pakistan during 2014-15 and 2015-16.

Province	District/ Area	Sub Areas
Punjab	Rawalpindi	Murree, Gujar Khan, Chak Shehzad, National Agricultural Research Center, Islamabad
	Attock	Dheri Kot, Shakar Dara, Qaziabad, Madrotha, Thekrian
	Chakwal	Barani Agricultural Research Institute, Chakwal
Khyber Pakhtoon Khwa	Peshawar	Peshawar
	Haripur	Jatti Pind, Nara Amaz, Tofkian, Pind Hashim Khan, Khan Pur
	Abbotabad	Singi Mera, Bagnotar, Balakot, Malkot, Nambal, Sajikot, Nara
	Mansehra	Baffa, Battal, Hilkot, Jaloo, Shinkiari, Dhodal, Bandi, Malik Pura
	Swat	Sher Garh, Sakha Kot, Thanra, Barikot, Mingora, Takht, Sher Palam, Bagh Deri, Matta

Hypersensitive Response and Pathogenicity Test

Hypersensitive reaction was performed to check the virulence of recovered bacterial isolates on tobacco, peach, plum and apricot seedlings (Johansson *et al.*, 2015; Doolotkeldieva and Bobusheva, 2020). Suspension was prepared using 24-48 hours grown bacterial cultures in sterilized distilled water to make a final concentration of 10^8 cfu ml⁻¹. Inoculation of healthy leaves of pre-maintained peach, plum and apricot nursery was done followed by the covering of leaves with polythene bags in order to maintain 25 ± 2 °C temperature and 65-70 % relative humidity. The response was then recorded after 3-7 days with an interval of 2 days post inoculation (DPI).

Host Resistance Response against Bacterial Canker Pathovars

Preparation of Inoculum

The inoculum of *P. syringae* was prepared in nutrient broth media incubated for two days at 28 °C in a shaking

incubator at 200 rpm. Fresh bacterial colonies from nutrient broth were collected by centrifugation at 10000 rpm for 10-15 minutes. Final concentration of bacterial population 10^8 cfu/ml was made at OD6000 using spectrophotometer in sterilized distilled water as solvent.

Inoculation and Host Response

Bacterial inoculation was done onto the germplasm of 10-12 week old seedlings of peach, plum, and apricot grown in pots on sterile soil under controlled conditions (Temperature (d/n) = 28/24 °C) by applying 30 ml of bacterial suspension (10^8 cfu/ml) as foliar application. Plants inoculated with distilled water were used as control (Lelliott and Stead, 1987). After inoculation, plants were covered with plastic bags to maintain 25 ± 2 °C temperature and 65-70 % relative humidity in controlled conditions, and plants were then examined for disease development. After 2 to 3 weeks, average disease severity rating (ADSR) was assessed by using rating scale; 1 = symptomless; 2 = a

few necrotic spots; 3 = more spots, some coalescing; 4 = severe spot and leaf defoliation; and 5 = plant dead (Ahmed *et al.*, 2016). Cultivars were arranged separately in glass house by using CRD design with three replicates.

RESULTS AND DISCUSSION

Recovered isolations were identified comparing the findings of Crosse (1959) who revealed *P. syringae* pv. *syringae* and *P. syringae* pv. *morsprunorum* can be readily isolated from leaf surfaces of peach and apricot during the growing season. A total of 48 bacterial isolates were recovered from the presumed infected tissues and leaf samples and kept in 20% glycerol for further investigations.

Hypersensitive Response and Pathogenicity Test

A total of 48 isolates were examined for hypersensitive reaction (HR) on tobacco which revealed 13 isolates showed negative HR response, while the remaining 35 isolates showed positive HR response. Pathogenicity test revealed only 3 isolates were highly virulent on apricot, peach and plum plants in the initial 10 days (Table 2). These findings are in line with the findings of Kotan and Şahin (2002), where they identified *P. syringae* pv. *syringae* from bacterial canker symptoms on apricot trees in Turkey and confirmed its pathogenicity. Kennelly *et al.* (2007) also reported the strains of *P. syringae* pv. *syringae* are highly susceptible to stone fruits except to sour cherry.

Host resistance response against *P. syringae* isolates

All the test cultivars of cultivars of peach, plum and apricot showed a susceptible to resistant response following disease severity scale against *P. syringae* pv. *syringae* (Table 3).

Change in leave color, mostly in susceptible cultivars including 6 ½ in peach, Stanley in plum and Golden Amber in apricot was observed 5 days after inoculation followed by characteristic symptoms of *P. syringae*

appeared on leaves after 10 days. Water-soaked spots of 1 to 3 mm of diameter appeared as first which later on, turned to brownish, dryer and brittle, and eventually fell out giving a shot-hole appearance confirming the symptoms of bacterial canker disease on stone fruits (Agiros, 2005; Doolotkeldieva and Bobusheva, 2020).

Average disease severity rating (ADSR)

Average disease severity rating (ADSR) was determined between 1.87 (5 ½) and 3.87 (6 ½) in peach, 1.73 (Fazal-e-manani) and 3.93 (Producer), 1.10 (Chinese apricot) and 2.63 (Golden amber) (Table 3).

The lowest ADSR was found in 5 ½ of peach, Fazal-e-Manani of plum and Chinese apricot of apricot. Response on these cultivars were statistically different from other cultivars. In contrast, the highest ADSR was found in 6 ½ of peach, Stanley of plum. These cultivars were statistically higher than other cultivars and were considered as susceptible, because they showed highest susceptibility to *P. syringae* infection. The cultivar 5 ½ of peach, Fazal-e-manani of plum and Chinese apricot of apricot showed small necrotic lesions and were considered as resistant cultivars to *P. syringae*. On the basis of ADSR, the remaining cultivars of peach, plum and apricot were considered as moderately susceptible. Considering the appearance of symptoms shown by the most virulent strains of *P. syringae*, 5 ½ of peach, Fazal-e-manani of plum and Chinese apricot of apricot were grouped as resistant cultivars and had an ADSR of 1.87, 1.73 and 1.10, respectively (Table 3). The response was significantly lower than ADSRs of moderately susceptible cultivars, Florida king and Early grand of Peach, Red beauty and Stanley of plum, and susceptible cultivars 6 ½ and 4 ½ of peach and Producer of plum, while Golden amber of apricot was found moderately susceptible. This is the first study that exhibits host resistance to peach, plum and apricot cultivars to bacterial canker disease in Pakistan.

Table 2. Hypersensitive reaction and virulence of *P. syringae* isolates on tobacco, peach, plum and apricot plants.

Isolate	Tobacco	Peach	Plum	Apricot
Ps1	+	+	-	+
Ps2	-	-	-	-
Ps3	+	+++	+++	+++
Ps4	-	-	-	-
Ps5	-	-	-	-
Ps6	+	-	++	++

Ps7	+	++	+	+
Ps8	+	++	+	++
Ps9	+	+++	++	+++
Ps10	+	++	++	+
Ps11	+	-	+	-
Ps12	+	-	+	-
Ps13	-	+	-	++
Ps14	+	-	+	-
Ps15	+	+	+	+
Ps16	-	-	-	-
Ps17	+	+++	++	+++
Ps18	+	+	++	+
Ps19	-	+	-	-
Ps20	+	+	-	+
Ps21	+	-	-	+
Ps22	+	-	-	-
Ps23	-	-	-	-
Ps24	+	+	+	+
Ps25	+	+	-	+
Ps26	-	-	-	-
Ps27	+	+	-	+
Ps28	+	+	++	-
Ps29	-	-	-	-
Ps30	-	-	-	-
Ps31	-	-	-	-
Ps32	+	++	+	+
Ps33	+	-	+	-
Ps34	+	+	-	+
Ps35	+	+	+	+
Ps36	+	+	+	+
Ps37	+	++	+	++
Ps38	+	-	-	-
Ps39	+	-	+	-
Ps40	+	-	-	-
Ps41	+	++	++	++
Ps42	+	-	-	-
Ps43	+	++	++	++
Ps44	+	-	-	-
Ps45	-	-	+	-
Ps46	-	-	-	-
Ps47	+	-	-	+
Ps48	+	-	+	-

Isolates marked '+++' are highly virulent; Isolates marked '++' are moderately virulent; Isolates marked '+' are weakly virulent.

Table 3. Host resistance response of different peach, plum and apricot varieties against *P. syringae* isolates.

Plant	cultivar	Mean	Response
Peach	Early grand	2.87	MS
	Florida king	2.10	MS
	4 1/2	3.12	S
	5 1/2	1.87	R
	6 1/2	3.87	S
Plum	Red Beauty	2.63	MS
	Fazal-e-manani	1.73	R
	Producer	3.93	S
	Stenley	2.89	MS
Apricot	Chinese Apricot	1.10	R
	Golden Amber	2.63	MS

Classes: R resistant, MS moderately susceptible, S susceptible

It is evident from the worldwide reports that *P. syringae* is undoubtedly critical bacterial pathogen and is still increasing its host range, infecting more crops, fruits and vegetable particularly posing serious threats to production of stone fruits.

Leaf spot can be found on cultivars of stone fruits susceptible to bacterial canker infection. These leaf spots are surrounded by chlorotic rings during early stages, and then the spots expand in diameter and finally falling out of leaf tissues resulting in shot-hole symptom (Kennelly *et al.*, 2007; Doolotkeldieva and Bobusheva, 2020). *P. syringae* isolates with virulence properties could produce compounds such as siderophore pyoverdine and phytotoxin, similar to syringomycin, which cause dark dry decay (Taguchi *et al.*, 2010; Jones *et al.*, 2007). In this aspect, cultivars which showed severe disease symptoms in leaves can produce critical symptoms on blossoms, shoots and trunks as well. Similarly, in cultivars showing lower leaf spot symptoms, pathogenic potential could be lower in other parts of the plant. Except reports of Kennelly *et al.* (2007) who suggested that mode of resistance to *P. syringae* *pv.* *syringae*, no reports are found on the area of investigations therefore, it is important that for further studies be conducted to find out the mechanism of resistance to *P. syringae* *pv.* *syringae*.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHORS CONTRIBUTIONS

All the authors contributed equally to this work.

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