Mycopath (2015) 13(1): 37-41 **Research Article**

Incidence and prevalence of bacterial wilt of chili in Punjab, Pakistan

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Abstract

Chili (*Capsicum annuum* L.) is among the major vegetables of Punjab. Bacterial wilt caused by *Ralstonia solanacearum* (Smith) Yabuuchi is responsible for significant reduction in its production. In the present study, incidence and prevalence of this disease on chili were determined and biovars were identified in five major chili growing districts of Punjab, Pakistan namely Multan, Kasur, Pakpattan, Bahawalpur and Attock. The study revealed an overall 11% incidence and 85% prevalence of bacterial wilt in Punjab. The highest disease incidence of 16% was found in Pakpattan district followed by Kasur (12%) and Attock (12%). Disease incidence was found to be minimum in Multan and Bahawalpur districts (9% each). Prevalence of bacterial wilt was 90% in Pakpattan and Kasur and 80% in rest of the districts. Out of 42 *R. solanacearum* strains isolated from various localities, 82% were identified as Biovar 3, while the remaining 18% were recognized as Biovar 4. Biovar 3 was recorded from all the districts while Biovar 4 was present in all districts except Attock. As bacterial wilt is widely prevalent throughout the province with varying intensities; therefore, stringent surveillance and control measures are needed to preclude the spread and severity of the disease.

Key worlds: Bacterial wilt, biovar, disease incidence, disease prevalence, *Ralstonia solanacearum*.

Introduction

Chili (*Capsicum annuum* L.) is one of the most important and commonly cultivated crops all over the world. It belongs to Solanaceae family and requires warm, humid and dry weather for its growth and maturity. Chili is consumed on large scale in different forms throughout the world. It contains different chemicals like proteins, fatty oils, capsaicinoids, vitamins, fibers and mineral elements. It is used in pharmaceutical and cosmetic industry (Bosland and Votava, 2003). Pakistan is among the top 10 chili producing countries of the world, ranked 5th in cultivation and 10th in production (FAOSTAT, 2013). The area under chili cultivation in the country is 6.5×10^4 ha with total production of 1.5×10^5 ton. In Punjab, chili is cultivated on an area of 5.1×10^3 ha and contributes 7.4×10^3 ton of production. Multan, Kasur, Pakpattan, Bahawalpur and Attock are major chili producing districts of Punjab with 1800, 1200, 1120, 760 and 615 acres under chili cultivation, respectively (FAOSTAT, 2013). The average yield of chili is very low in the country (2.53 ton ha^{-1}) as compared to many developed countries which can be attributed to many biotic factors; bacterial wilt being the major constraint. Bacterial wilt incited by *Ralstonia solanacearum* is ubiquitous in distribution and is considered as a serious constraint to the cultivation of solanaceous crops in tropical, sub tropical and temperate regions of the world (Hayward, 1991; Zubeda and Hamid, 2011; Begum *et al*., 2012; Shahbaz *et al*., 2015). The bacterium has been divided into five races and five biovars on the basis of host range variations and metabolic properties respectively (Buddenhagen *et al*., 1962; He *et al.*, 1983; Hayward, 1994). Biovar 3 is prevalent in different agro ecological and climatic zones of India (James *et al.*, 2003). More than 450 plant species from 54 different botanical families are invaded by *R. solanacearum* resulting in heavy yield losses (Wicker *et al*., 2007). Over 80 countries all over the world are affected by this pathogen which causes annual damage of more than $1billion each year (Champoiseau *et al.*, 2009; Hong *et al*., 2012).

As the information regarding distribution of bacterial wilt on chili is lacking in the major chili

growing districts of the Punjab province of Pakistan, therefore, in the present study a survey was conducted in five major chili producing districts of Punjab to determine its incidence, prevalence and distribution of different biovars of *R. solanacearum*.

Materials and Methods

Survey of chili growing areas

To determine the incidence and prevalence of bacterial wilt, an extensive survey of chili was conducted during 2013-14 in five major chili growing districts of Punjab namely, Multan, Kasur, Pakpattan, Bahawalpur and Attock. From each district ten sites were randomly selected making a total of fifty. One field of chili (~ 1 acre) was randomly selected from each site for recording bacterial wilt incidence. From each field fifty plants, each after every 5 steps following zigzag pattern, were randomly observed and by applying the following formula, incidence of bacterial wilt of each site was calculated.

$$\text{Incidence (\%)} = \frac{\text{No. of wilted plants}}{\text{Total No. of plants}} \times 100$$

The incidence from all districts and the whole province was calculated.

Similarly, the disease prevalence of each district and the province was calculated by following formula.

$$\text{Prevalence (\%)} = \frac{\text{No. of infected fields}}{\text{Total No. of fields observed}} \times 100$$

Collection of samples

A total of 42 *R. solanacearum* strains associated with chili plants were collected from the fields from 5 major chili growing districts of Punjab. Wilting was observed symptomatologically (Osborn, 1995; Lemay *et al*. 2003) and the association of *R. solanacearum* with the wilted plants in the field was confirmed serologically by using immuostrip (Opina and Miller, 2005). The infected plants showing typical wilt symptoms were uprooted along with soil from the rhizosphere, packed in polythene bags, labeled and taken to the lab in a cold container for further analyses.

Isolation and purification of *R. solanacearum*

The bacterium was isolated from soil by taking 1 gm of soil and making serial dilution after homogenizing in 9 mL of sterilized distilled water. By adding requisite amount distilled water dilution series of 10^6 and 10^7 cfu/ml were made and streaked on SMSA (specific media, South Africa) plates and incubated at 30 ºC for bacterial growth for 48 h (Englerbrecht, 1994). Partially infected stem segments were cut into small pieces of 10 cm length, surface sterilezed with 70% ethanol and streaked on TTC (triphenyle tetrazolium chloride) media and incubated at 30 ºC for bacterial growth (Hugh and Leifson, 1953). The pure culture was obtained by restreaking the single colonies on media plates and keeping the fresh pure culture in sterilized distilled water at room temperature for further analyses

Hypersensitive reaction (HR)

A bacterial suspension of 10^7 cfu mL^{-1} from fresh pure culture was made and infiltrated in the epidermis of tobacco leaves by using a sterilized syringe while distilled water was used as control. This practice was repeated twice on the same leaf and incubated at 28 ºC for 24-48 h for necrosis development in inoculated areas.

Biochemical characterization of *R. solanacearum* strains

After positive HR, the strains were further tested through different biochemical tests like Gram staining, Levan production, catalase activity (Schaad, 1980), oxidase activity (Kovacs, 1956), KOH loop test (Suslow *et al.*, 1982), Pigment Production (King *et al.*, 1954), Gas production (Van den Mooter, 1987), and bacterium growth at 37 °C and 41 ºC

Molecular confirmation of *R. solanacearum*

The DNA from the 42 purified strains was extracted, quantified and amplified in PCR by using primer pair JHFegl: 5'GACGATGCATGCCGCTGGTCGC 3' and JHRegl:5'CACGAACACCACGTTGCTCGCATTGG 3'. The amplified PCR products were run on 1% agarose gel containing ethidium bromide and visualized in Gel Doc System. All strains gave a 750-bp band that corresponded to *R. solanacearum.*

Identification of biovars

The *R. solanacearum* strains were characterized into biovars on the basis of utilization of different disaccharides and hexose alcohols (Hayward, 1964; He *et al*., 1983).

Results and Discussion

The overall incidence of bacterial wilt caused by *R. solanacearum* in Punjab was 11%

while the disease was found to be prevalent in 85% areas of the province.

The disease incidence was found to be maximum (16%) in Pakpattan district followed by Kasur and Attock with 12% each. The incidence was found to be minimum in Multan (9%) followed by Bahawalpur (9%) as shown in Fig. 1. As regards prevalence, it was maximum (90%) in the districts of Pakpattan and Kasur followed by 80% in the rest of the districts as shown in Fig. 2.

Identification of biovars

Out of 42 *R. solanacearum* strains, 82% were identified as biovar 3, while the remaining (18%) were recognized as biovar 4. Biovar 3 was recorded from all the districts while biovar 4 was present in four districts barring Attock as shown in Fig. 3.

Bacterial wilt caused by *R. solanacearum* is widely distributed throughout the world. Earlier a number of surveys has confirmed the prevalence of bacterial wilt in different parts of the world. Bekele *et al*. (2011) reported an incidence of 55% and 25% on chili and potato crops respectively from the major chili and potato producing regions of Ethiopia. Ahmed *et al.* (2013) reported an incidence of 23% of bacterial wilt disease in Munshigonj followed by 20% in Nilphamari and the lowest disease incidence of 9% in Jamalpur. In Bangladesh up to 31% disease incidence has been reported on egg plant (Hussain *et al.*, 2005).

Variations in the incidence and prevalence of bacterial wilt are attributable to the diversity of *R. solanacearum* strains, variations in soil types. The pathogen has been reported to survive in the soil for longer periods of time and disease development is favored by warm and humid soil conditions (Ahmed *et al*., 2013). The other major sources of dispersal of *R. solanacearum* are infected planting material (infected potato tubers and seedlings raised in infested soils) (Olsson, 1976), use of uncertified seed and contaminated farm implements. The inoculum of the bacterium builds up in the soil due to repeated and continuous growing of same crops and intercropping with susceptible hosts results in severity of the disease.

Contaminated mechanical implements and weeds are another major means of dispersal of *R. solanacearum* (Buddenhagen and Kelman, 1964). In developing countries like Pakistan the implements are not disinfected prior to use in other healthy fields. Similarly, the bacterium develops on different weeds which provide a continuous source of its survival and infection.

It is concluded from the present study that bacterial wilt is widely prevalent in the province of Punjab, Pakistan as the environmental conditions and other factors mentioned above are favourable for its spread. Therefore, control strategies should be adopted accordingly to minimize losses caused by this disease.

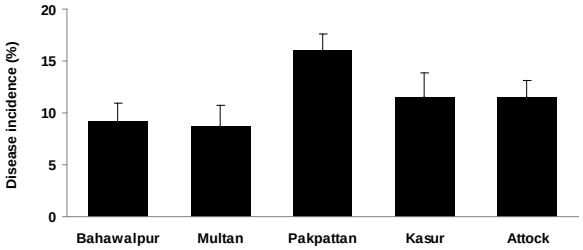

Fig. 1: Disease incidence (%) in five major chili growing districts of Punjab.

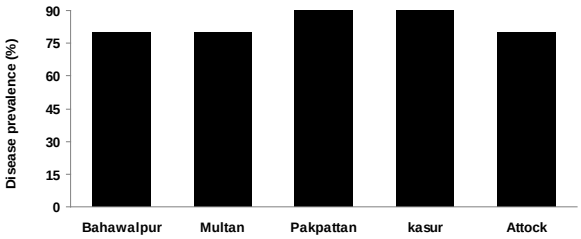

Fig. 2: Disease prevalence (%) in five major chili growing districts of Punjab.

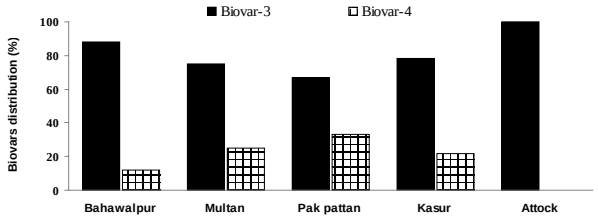

Fig. 3: Biovar distribution (%) of *R. solanacearum* in five major chili growing districts of Punjab.

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