Variation in Aggressiveness of some Isolates of *Ralstonia solanacearum* on Tomato

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ABSTRACT

Bacterial wilt caused by *Ralstonia* solanacearum E.F. Smith is one of the destructive diseases of tomato. Aggressiveness of seven different bacterial wilt isolates collected from Panchkhal, Kavre; Fosre, Myagdi; Balazu and Koteswor, Kathmandu; Nawalpur, Sarlahi; Kumfur, Dhading and Rampur, Chitwan were evaluated under screen house conditions in 1999. Isolate from Myagdi was found the most aggressive whereas Chitwan isolate was the least aggressive. Myagdi isolate overcame the resistance of moderately resistant (L 180) and resistant varieties (L 986). Other tested isolates did not produce any wilt disease in these two varieties. Reactions of the tested isolates in the susceptible variety (L 390) were statistically different in their virulence or aggressiveness. Myagdi isolate resulted in a rapid disease progress in this variety. Number of other management tactics might be necessary in those locations where highly aggressive strain of bacterial wilt is prevalent, as resistant variety alone may not provide adequate protection against the disease.

Keywords: Bacterial wilt, *Ralstonia solanacearum,* Aggressiveness, Strain, Virulence

INTRODUCTION

Bacterial wilt caused by *Ralstonia solanacearum* E.F. Smith is widely distributed disease in the world. It is one of the limiting factors to the successful cultivation of solnaceous vegetables throughout the world, where warm and humid climate is prevalent (Kelman, 1953; Vawdrey and Gounder, 1993). In Nepal, tomato (*Lycopersicon esculentum* L.) is one of the fruit vegetables grown extensively in terai, foothills and valleys of Nepal. Bacterial wilt is the second most important disease limiting its cultivation (Timila and Shrestha, 2001). In Nepal, after late blight, bacterial wilt is considered to be the second most important disease in tomato. This disease was first recorded in tomatoes in 1978 (Shrestha, 1990).

The initial symptom in mature plants is wilting of upper leaves without yellowing the foliage. Under hot and humid conditions, complete whole plant wilts suddenly leading to plant death. The vascular tissues of the lower stem of the wilted plants usually show brown discoloration. Infested soil, plant debris and weeds serve as the primary source of inoculum of the pathogen (Kelman, 1953). Bacterial wilt is a difficult disease to control. Once established in a field, because of wide host range (covering more than 44 plant families), ability to survive in the soil for long time and vast genetic variability of the pathogen makes it more challenging to control this pathogen (Hayward, 1991).

Wide use of pesticides has favoured the development of pathogen resistance towards pesticides (Melhotra et al., 1996). Concerns for human health and the environment have pushed the crop production to reduced use of pesticides. Planting resistant varieties is the most effective and simplest methods for controlling bacterial wilt disease and also

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environmentally sound. However, a major problem using resistant lines is due to its variability in degree of resistance offered to different strains of pathogen (Wang et al., 1997). Among the races of *R. solanacearum*, race 1 is the most diverse group (Hayward, 1991). Large variation, both in genotypes and aggressiveness, has been observed in several race 1 populations (Prior et al, 1990 and Hayward, 1991). Virulence differences among pathogen strains can cause resistance instability although other biotic and abiotic factors also affect host resistance (Hayward, 1991). The virulence of strain is related to its aggressiveness on the susceptible cultivar. The least aggressive strain affects least on the resistant cultivar (AVRDC, 1991). Based on the virulence of the strain the isolates were grouped into a number of profiles in different geographic regions (Prior et al., 1990).

For breeding and epidemiological purposes it is very important to analyze the variability of aggressiveness (Darrasse, et al., 1997). Understanding in aggressiveness of the pathogen has the implications in the development of management strategies of bacterial wilt. In Nepal, biovar II, III and IV (or races 1, and 3) have been reported but their aggressiveness have not been determined (Timila and Shrestha, 2001)). So, the experiment was conducted to analyze the variability in aggressiveness of isolates of *R. solanacearum* collected from different locations. Variation was evaluated using three different tomato cultivars rated as susceptible, moderately resistant and resistant to *R. solanacearum*.

MATERIALS AND METHODS

The experiment was conducted under screenhouse conditions at Khumaltar Lalitpur Nepal in 1999. The study was conducted in randomised complete block design with two replications. Fifteen plants of each variety were used against each isolate in each replication. Seedlings of three tomato entries, susceptible (L390), moderately resistant (L180-1) and highly resistant (L986) received from Asian Vegetable Research and Development Center were raised by direct sowing in plastic pots using steam sterilized soil. Only one seedling per pot was maintained, which had 4-5 true leaves at the time of inoculation.

Bacterial culture and inoculum preparation

A total of 7 isolates of *Ralstonia solanacearum* were collected from Panchkhal, Kavre; Fosre, Myagdi; Balazu and Koteswor, Kathmandu; Nawalpur, Sarlahi; Kumfur, Dhading and Rampur, Chitwan infecting tomato plants were used in the study. The bacteria was isolated from infected tomato plant on Tetrazolium chloride medium (TTC medium) (Kelman, 1954). The bacteria was multiplied or cultured on 523- medium ($MgSO_4$. 7H2O 0.3 g, K_2HPO_4 2 g, Yeast extract 4 g, Casein hydrolysate 8 g, Sucrose 10 g, Agar 18 g and Distilled water 1 liter) at 28^oC for 24 hours as described by Asian Vegetable Development and Research Center and stored at room temperature in sterile distilled water. A loopful of bacterial suspension of each isolates was streaked in TTC medium and incubated at 28^o C for 48 hours for single colony development. Then a single colony was multiplied in 523 medium. After 24 hours, bacterial mass of each isolate was suspended in sterile distilled water and the concentration was estimated at OD_{600}=0.300 (1 x 10^8 colony forming units per ml).

Inoculation and evaluation:

Water stress was created on the test plants by skipping irrigation one day prior to inoculation. Inoculation of each strain in each entry was done by pouring 30 ml of bacterial

suspension at the base of each test seedling. Normal irrigation schedule was resumed from the next day of inoculation.

Observations were recorded on the number of wilted plants at 5, 10, 15, 20 and 25 days after inoculation. Wilt incidence was determined from the number of infected plants against total number of inoculated plants for each entry and bacterial strain and expressed as percentage. Temperature profile was collected from Agronomy Division, NARC at Khumaltar.

Statistical analysis:

Because L 180-1 and L 986 showed resistant reaction to strains except Myagdi strain, no any statistical analysis was performed. The disease incidence on susceptible variety (L 390) at 25 days after inoculation were submitted to ANOVA procedures after Arcsine square root transformation using MSTAT C statistical package and means were separated by (Duncan's Multiple Range Test)

RESULTS AND DISCUSSION

Variations in wilt incidence were found in different isolates in different varieties with different level of resistance. The data presented in Table 1 also shows variable reaction to wilt by different isolates in the susceptible variety, L390. Isolate from Fosre of Myagdi district was found to be the most aggressive one causing highest rate of incidence (73%), which was significantly different from the incidence caused by rest of the tested isolates. The isolate from Chitwan was the least aggressive one with the lowest wilt incidence (<3%), which was significantly less than the incidence from rest of the six isolates. The isolate from panchkhal (Kavre) produced significantly higher wilting when compared with that of Balazu (kathmandu) isolate but did not differ from the isolates from Koteshwor (Kathmandu), Nawalpur (Sarlahi) and Kumfur (Dhading). Similarly, the rate of wilting produced by stains from Koteshwor, Balazu, Nawalpur and Kumfur also dod not differ from each other.

Time taken to appearance of wilt symptom was also different for different isolates varieties. In the susceptible variety (L 390), Wilting started in just 5 days after inoculation with Myagdi isolate. In the same variety, it took 10 days for the development of wilt symptoms when inoculated with the panchkhal and Koteshwor the isolates. Similarly, the Dhading and balazu isolates produced wilting in 15 days whereas the Sarlahi and Chitwan isolates took 20 and 25 days respectively for the appearance of the symptoms. The Myagdi isolate showed wilting symptoms in 17 days in the moderately resistant variety (L180) and 20 days in the resistant variety (L 986) after inoculation.

The rate of disease progress in the susceptible variety was highest for Myagdi isolate followed by Panchkhal, Koteshwor, Sarlahi, Dhading, Balazu and then Chitwan isolates (Figure 1).

The moderately resistant (L 180) and resistant (L 986) varieties evaluated in this study did not show any wilting symptoms when inoculated with six different isolates of *R. solanacearum*, but succumbed to wilting against isolate Myagdi isolate (Table 1). The rate of disease development was highest in the susceptible variety followed by the moderately resistant and resistant variety (Figure 2). Myagdi isolate, thus, appears to be most aggressive when compared to other isolates used in the study.

Wilt incidence percent and degree of severity of disease could be affected by temperature as mentioned by Mew and Ho (1976). However, during experimental period, the maximum and minimum temperature at the experimental site during the study period ranged from 28º–29º C and 18º -19º C which is ideal for the growth of the given pathogen and disease development. It was observed that the most aggressive isolate overcame the resistance of L 986 while the least aggressive isolate caused low wilting (2.77 %) even in the susceptible variety, L 390. Low aggressiveness of *R. solanacearum* strains on tomato is more related to slower multiplication rather than retarded upward movement of bacteria (Wang et al., 2005).

Use of resistant varieties is one of the best methods of bacterial wilt control, but resistance of a cultivar can vary among different geographic locations depending on the strains of *R. solanacearum* present (Kelman and Person, 1961). Therefore, a single method will not be effective in controlling bacterial wilt in tomato (Hartman, 1992). Tomato varieties provide location specific nature of resistance in to bacterial wilt due to difference in the agressiveness of the pathogen strains, climate or soil characteristics present in the location (Wang et al, 1997). Loss of resistance at higher temperature has been reported for other cultivars (Prior et al., 1990). In association with the use of resistant variety, number of other management tactics might be needed in those locations where highly aggressive strain of bacterial wilt is prevalent, because resistant variety may not be stable in such locations. Thus resistace of a cultivar can vary among different geographic locations depending on the strains of *R. solanacearum* present (Kelman and Person, 1961). It also indicated that the programs for improvement of tomatoes for resistance to bacterial wilt should include all groups of strains prevalent in the given area (Prior et al., 1990).

CONCLUSION

The present study indicated that there is existence of variation in aggressiveness among the strains which belongs to race 1, biovar 3 (Timila and Shrestha, 2001) of *R. solanacearum* on tomato prevalent in Nepal. More studies are required in this area as only a few isolates were included in the present study. Such studies can help develop tomato wilt management strategy for different egroecological region of the country

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Table 1. Variation in wilt incidence percent induced by 7 different isolates of *R. solanacearum* in three differetial varieties of tomato, 4 weeks after inoculation.

S. No.	Isolates	Locations	Wilt incidence percent in different varieties		
			L 390**	L 180 *	L 986*
1.	Panch	Panchkhal, Kavre	42.25 b	0	0.0
2.	Kath	Koteswor, Kathmandu	34.53 bc	0	0.0
3.	Bala	Balazu, Kathmandu	17.78 c	0	0.0
4.	Chit	Rampur, Chitwan	02.77 d	0	0.0
5.	Sarl	Nawalpur, Sarlahi	25.83 bc	0	0.0
6.	Myag	Fosre, Myagdi	73.33 a	40.97	23.33
7.	Dhad	Kumfur, Dhading	26.15 bc	0	0.0
		CV (%)	14.95	-	-

* Not analysed. ** in the column means with the same letter are not significantly different according to Duncan's multiple range test (P= 0.05).

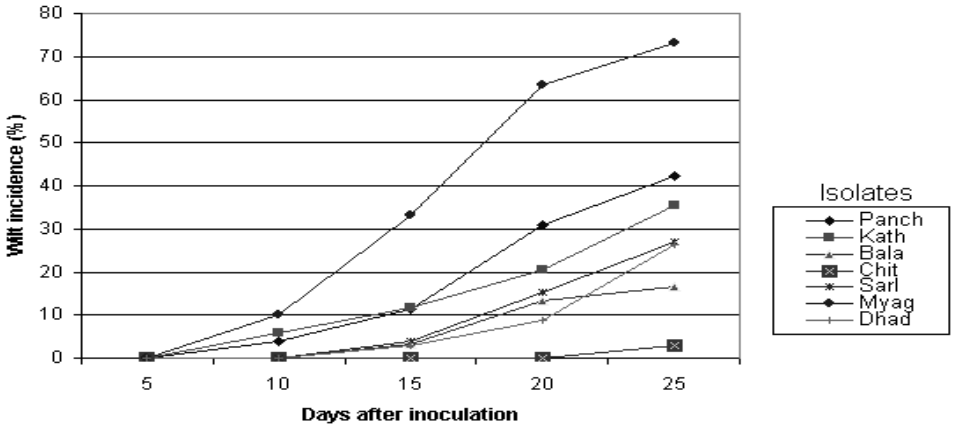

Figure 1. Disease progress cruves of wilt caused by different isolates of *R. solanacearum* in susceptible tomato variety, L 390

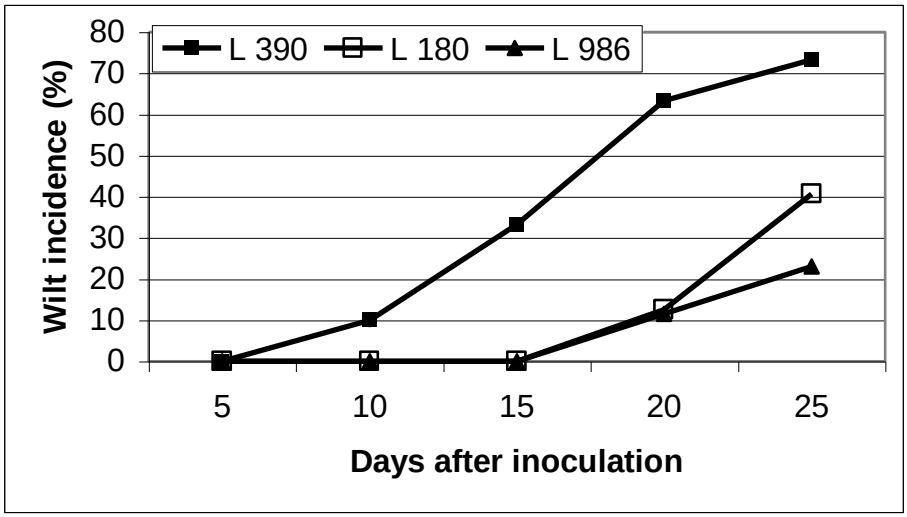

Figure 2.