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Susceptibility of *Capsicum* Species and Cultivars to *Ralstonia solanacearum*: Anatomical Differences and Bacterial Multiplication in Resistant and Susceptible Cultivars

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ABSTRAK

Penilian rumah hijau terhadap 42 aksesi/kultivar Capsicum *spp. kepada penyakit layu bakteria yang disebabkan oleh* Ralstonia solanacearum, *memperlihatkan bahawa kultivar tempatan Malaysia "Kulai" menunjukkan paras keresistanan yang tinggi. Tiga aksesi, iaitu CO 1969, CO 1970 dan CO 1553, menunjukkan keresistanan sederhana sementara aksesi yang lain adalah rentan kepada sangat rentan. Kajian anatomi terhadap kultivar resistan dan rentan menunjukkan perbezaan bermakna bagi ciri-ciri anatomi yang tertentu. Bilangan urat vaskular per akar lebih banyak, korteks lebih tebal dan kepanjangan unsur xilem lebih pendek dalam akar kultivar resistan berbanding kultivar rentan. Bilangan purata berkas vaskular per pokok dan bilangan unsur xilem per berkas vaskular juga lebih banyak dan kepanjangan diameter unsur xilem lebih pendek di bahagian batang tengah kultivar resistan. Ciri-ciri anatomi pokok resistan ini mungkin menjadi faktor penyumbang yang menghad atau melambatkan proses penjangkitan, pergerakan dan penggandaan patogen. Penggandaan* R. solanacearum *pada bahagian batang tengah, kolar dan akar bagi kedua-dua kultivar yang diinokulatkan menerusi batang menunjukkan bahawa dalam kultivar rentan populasi bakteria tidak berbeza pada setiap bahagian yang dikaji, tetapi terdapat perbezaan yang bermakna antara kultivar pada bahagian penyempalan yang sama. Penemuan ini menunjukkan bahawa mekanisma resistan kepada penyakit adalah, sebahagian besarnya, bergantung kepada ketidakbolehan patogen untuk membahagi di dalam perumah yang resistan.*

ABSTRACT

Greenhouse evaluation of 42 accessions/cultivars of Capsicum *spp. to bacterial wilt caused by* Ralstonia solanacearum *revealed that the Malaysian local cultivar 'Kulai' was highly resistant. Three accessions, namely CO 1969, CO 1970 and CO 1553, were moderately resistant; all other accessions were susceptible to highly susceptible. Anatomical study of resistant and susceptible cultivars indicated significant differences in certain anatomical characters. The number of vascular strands per root was fewer, the cortex was thicker, and the length of xylem elements was shorter in the roots of resistant cultivars than the susceptible cultivar. The average number of vascular bundles per plant and the number of xylem elements per vascular bundle were fewer and the length and diameter of xylem elements were shorter in the mid-stem of resistant cultivars. These anatomical characters of resistant plants may contribute to limit or slow down the infection process, movement and multiplication of the pathogen. Multiplication of* R. solanacearum *at mid-stem, collar and tap root in stem-inoculated susceptible and resistant chilli cultivars revealed that in susceptible cultivars the bacterial population did not differ at all sites tested, but it differed significantly between cultivars at similar sampling sites. These findings show that the mechanism of resistance to the disease is mainly due to the reduced rate of multiplication of the pathogen within the resistant host.*

INTRODUCTION

Bacterial wilt of capsicums and chillis (*Capsicum* spp.) caused by *Ralstonia solanacearum* (previously called *Burkholderia solanacearum*) is one of the major constraints for chilli/capsicum production in the humid tropics of Malaysia (Abdullah and Kamaruzaman 1992; Syed and Loke 1995). The bacterium is a soil-inhabitant and rhizosphere survivor and enters the roots from artificial or natural openings (Kelman and Sequeira 1965). So far, limited work has been done on bacterial wilt-resistance of chilli pepper (Peter *et al.* 1984; Jyothi *et al.* 1993; Perera *et al.* 1993).

Various attempts to control bacterial wilt have been reported (He 1990; Hartman *et al.* 1993) but the success was limited. Breeding for resistant varieties, therefore, remains the best control strategy.

Little is known about the mechanism of resistance of chilli to *R. solanacearum*. In cultivars of alfalfa which have been cultivated for a long time in the USA and Canada, the mechanism of resistance to *Clavibacter michiganensis* subsp. *insidiosum* appears to be stable due to morphological and/or physio-chemical barriers to the pathogen (Cho *et al.* 1973).

Vascular anatomy of plants is known to play an important role in susceptibility to vascular pathogens (Elgersma 1970; McNabb *et al.* 1970; Cho *et al.* 1973). Restricted upward or downward movement of *Erwinia amylovora* in resistant apple shoots appears to be due to immobilization of the pathogen as well as anatomical features such as reduced vessel diameter and vessel branching (Tomasik and Goodman 1986). Therefore, anatomical characters may be important for an understanding of the mechanism of resistance during host-pathogen interactions.

Grimault and Prior (1993) detected *Pseudomonas solanacearum* in wild resistant tomato, but the infection was found only in selected progeny, indicating that resistant plants may become symptomless carriers. The information on the growth and multiplication of the pathogen in resistant and susceptible hosts is important in understanding the mechanism of infection.

Literature on anatomical differences and bacterial multiplication in chilli plants resistant and susceptible to bacterial wilt is lacking. The present study was, therefore, undertaken to evaluate the susceptibility of some chilli accessions/ cultivars to *R. solanacearum;* whether anatomical differences between resistant and susceptible cultivars of *Capsicum* spp. are related to bacterial wilt-resistance; and to investigate the multiplication of the pathogen in chilli with different levels of susceptibility.

MATERIALS AND METHODS

Propagation of Test Plants

Seeds were germinated in the greenhouse in trays with sterilized soil mixture (soil : sand : compost) in the ratio of 3 : 2 : 1 for a period of 10 days. They were then transferred to individual pots containing approximately 1.5 kg of sterilized soil mixture

Evaluation of the Susceptibility of 42 Accessions/ cultivars of Chilli to R. solanacearum

Seedlings of 42 accessions and cultivars (Table 1) of *Capsicum* spp., 40 from AVRDC, Taiwan; and one each from Taiwan and Malaysia were tested. Plants were inoculated when they were approximately 35 days old. The *R. solanacearum* strain used in this study was isolated from wilted chilli (cv. Chili Merah), *C. annuum* L. plants collected from UPM research field. On isolation on triphenyl tetrazolium chloride (TZC) agar medium (Kelman 1954), the pathogen yielded greyish white, fluidal colonies with light pink centres. After purification, the isolate was characterized as race 1 and biovar III according to pathogenicity and host range (Buddenhagen *et al.* 1962), and biochemical test (Hayward 1964). The isolate was kept as stock culture in lyophilized form and in 5 ml sterilized tap water at 15°C. Before each preparation of inoculum, the bacteria was streaked on TZC from the stock culture. The inoculum was prepared by culturing the virulent, wild type and fluidal colony of *R. solanacearum* on casamino acid-peptone-glucose (CPG) agar medium incubated at 30°C for 48 h. A micropipette tip carrying 20 µl of the bacterial suspension, containing approximately 2.0×10^8 cfu/ml was inserted at the axil of 3rd fully expanded leaf from the top of the plant. The initiation of wilt symptoms after inoculation and the number of wilted plants for each accession were recorded at 7-day intervals up to 63 days after inoculation. The reactions of species/ cultivars were graded on a 0-5 rating scale based on percentage of wilting obtained at 63 days. The rating scale was as follows: 0 = HR (highly resistant, plants did not show any symptoms of wilt); 1= R (resistant, 1- 20% plants wilted); 2 =

MR (moderately resistant, 21-40% plants wilted); 3 = MS (moderately susceptible, 41-60% plants wilted); 4 = S (susceptible, 61-80% plants wilted); and 5 = HS (highly susceptible, more than 80% plants wilted). The incubation period (time taken to produce 50% wilt at days after inoculation, DAI) and temperature and relative humidity using hygro-thermograph were also recorded. Plants were arranged on greenhouse benches following the complete randomized design with three replicates. One replication consisted of 25 plants.

Differences in Anatomical Characters of Resistant and Susceptible Chilli Cultivars

Two cultivars of chilli *viz.* highly resistant cv. Kulai and highly susceptible cv. Long Chilli 455 (LC 455) were selected from the previous study. One hundred plants of each cultivar were raised in trays and carefully removed from the soil when they were 30 days old. Two 5-mm sections were taken 1.5 cm below the ground for root samples and two stem sections of the same length were taken from the upper part of the first internode of the plants. All the samples were fixed and preserved in formalin-acetic acid-alcohol solution. One pair of root and stem segments was processed for microtomy and another sample was used for measuring the length of the xylem elements.

Samples for microtomy were dehydrated through an ethanol series and embedded in paraffin blocks. Sections 7-8 µm thick were prepared using a rotary microtome and stained with either safranin-fast green or 0.5% toluidine blue O with 1% borax, and observed under the light microscope. The thickness of the cortex, diameter of xylem vessels and number of vessel elements were measured or counted.

For measurement of the length of vascular elements, tissues were macerated in 10% chromic acid and 10% nitric acid in 1 : 1 ratio for 16 h. The length of 10 complete vascular elements was taken randomly for each specimen. Means of the above characters were subjected to statistical analyses using the SAS package.

Population Dynamics of R. solanacearum *in Resistant and Susceptible Chilli Cultivars*

Fifty plants each of resistant cv. Kulai and susceptible cv. LC 455 were propagated as above, then transferred into pots containing sterilized soil mixture. Thirty-five day old plants were stem-inoculated as above with a bacterial suspension containing approximately 2. 2 x 10^8 cfu/ml.

Bacterial population was assessed by randomly harvesting five plants for each cultivar at 4-day intervals. Samples from the tap root, mid-stem and collar region were cut and macerated with phosphate buffer in a stomacher laboratory blender for 2 min. The tissues were incubated for 20 min at 15°C, after which serial dilutions were made and 0.1 ml of the bacterial suspension was plated on tetrazolium chloride (TZC) agar medium (Kelman 1954) in five replications. Plates were incubated at 30°C for 48 h. The bacterial population was expressed in log unit per g fresh matter (FM). Sampling of the susceptible plants was carried out for 16 days when plants showed complete wilting. Assessment of bacterial population in the resistant cultivar was assessed 32 days after inoculation. The experimental design was a complete randomized design of 5 replications with 20 plants per replicate. All bacterial population data were analysed statistically using the SAS package.

RESULTS

The reaction of the chilli accessions and cultivars is shown in Table 1. Analyses of variance of the percentage of wilted chilli pepper accessions revealed significant differences among the accessions ($p= 0.01$). The cultivar "Kulai" showed a high level of resistance with complete absence of wilting caused by *R. solanacearum* under the present test conditions. Three accessions, CO1969, CO1970 and CO1553, were moderately resistant with wilt incidence of 21-40%, whereas all other accessions were moderately to highly susceptible.

The moderately resistant accessions and some of the moderately susceptible accessions did not show 50% wilting even after 35 days after inoculation (DAI), whereas most of the susceptible accessions were more than 80% wilted within 10 DAI. The moderately resistant accessions had longer incubation periods (8 - 10 days) than the susceptible accessions (3 - 9 days).

Mean difference of thickness of root cortex in resistant and susceptible varieties was significantly different (Table 2), but not in the case of stems. There were significantly fewer vascular bundles in the stem and roots of plants of the resistant cultivar than the plants from susceptible cultivar.

TABLE 1
Reaction of 42 chilli accessions/cultivars to *Ralstonia solanacearum*

Accession no.	Name of accession	Source	% of wilt*	Reaction**	Incubation period (day)	50% wilt DAI***
	Capsicum annuum					
FI hybrid	Long chilli 455	Taiwan	100	HS	4	6
CO 1436	P3/78	Mexico	96	HS	5	7
COO 801	Nose Gay	USA	96	HS	7	10
COO 751	Chilli Tepine	USA	96	HS	7	10
CO 2800	PI 271322	Mexico	92	HS	5	7
CO 2489	Chile Dulce Num-135 RJE	Mexico	92	HS	5	6
COO 800	P 204/76	USA	92	HS	6	8
COO 222-2	PI 163201	India	88	HS	5	7
COO 788	Little Tomato	USA	88	HS	5	7
CO 3865	W-C 2068 Habanero	Mexico	84	HS	5	7
COO 829	Fordhook	USA	84	HS	7	10
COO 832	Red Cherry Hot	USA	80	S	6	10
COO 767	Thick Wall World Beater	USA	80	S	7	10
COO 722	VP 46A	India	76	S	6	8
CO 1966	PI 165588	India	68	S	7	12
CO 3982-A	PI 439274	USA	68	S	6	9
COO 707	Pimento	USA	64	S	7	9
COO 721	VP 34	India	52	MS	7	11
CO 1964	PI 165510	India	44	MS	9	-
CO 1970	PI 166368	India	40	MR	10	-
CO 1969	PI 65691	India	32	MR	10	-
Local	Kulai	Malaysia	0.0	HR	-	-
	Capsicum spp.					
CO 1175	Crillo de Marelos 334	France	96	HS	4	7
COO 268	Paul Smith's A Serano 1534	USA	96	HS	3	5
COO 758	CAP 291/96	Guatemala	92	HS	4	7
CO 1826	PI 138560	Iran	88	HS	5	8
COO 679	Num 598	Guatemala	88	HS	5	9
COO 835	Orias Kossarvu	Hungary	72	S	7	11
CO 1664	P 493/80	Denmark	48	MS	7	-
	Capsicum chacoense					
CO 4394	PI 260435	Bolivia	96	HS	6	9
CO 1553	P498/78	England	36	MR	8	-
	Capsicum frutescens					
COO 776-A	Ose Utoro	Nigeria	100	Hs	5	6
COO 776	Ose Utoro	Nigeria	92	HS	6	7
CO 4166	136 BGH 412 Pimenta Wild	Brazil	92	HS	7	10
CO 1379	P8/85	Mexico	60	MS	7	11
CO 4171	146 BGH 823 Pimenta	Brazil	60	MS	7	9

Table 1 (contd.)

Accession no.	Name of accession	Source	% of wilt*	Reaction**	Incubation period (day)	50% wilt DAI***
	Capsicum chinense					
CO 4675	PI 441598	Brazil	100	HS	5	7
CO 4681	PI 441604	Brazil	100	HS	5	9
CO 4685	PI 441608	Brazil	96	HS	6	7
CO 4690	PI 441613	Brazil	92	HS	7	7
CO 4682	PI 441605	Brazil	88	HS	5	8
CO 4678	PI 441601	Brazil	80	S	6	10
		LSD = 14.47				

*Means derived from angular transformed data.
**0 = HR (highly resistant, plants did not show any symptoms of wilt); 1 = R (resistant, 1-20% plants wilted) ; 2= MR (moderately resistant, 21-40% plants wilted); 3 = MS (moderately susceptible, 41-60% plants wilted); 4 = S (susceptible, 61-80% plants wilted); and 5 = HS (highly susceptible, more than 80% plants wilted).
***DAI= Days after inoculation

The numbers of vessel elements in the stem were fewer and the lengths of vessel elements in roots and stems of resistant plants were shorter than in susceptible cultivars with the differences statistically significant. Xylem elements in roots and stems of cv. LC 455 were 83.03 and 61.00 µm longer respectively than those of cv. "Kulai". The mean diameter of xylem elements in roots did not differ statistically but was significantly different for the stem.

Both cultivars tested were infected by the pathogen, which then multiplied regardless of their susceptibility. Bacterial population density differed significantly at similar sites of detection i.e. mid-stem, collar and tap root at different times for susceptible cv. LC 455 and resistant cv. "Kulai" cultivars (Table 3, 4, 5). However, bacterial population density among the mid-stem, collar and tap root regions did not differ significantly in the susceptible cultivar (Table 6). Some susceptible plants showed symptoms of wilt after 5 days' inoculation, with complete wilting by 16 days. Accordingly, the test plants were no longer suitable for further investigation.

In the resistant chilli cv. "Kulai", the *R. solanacearum* population differed significantly (p=0.01) between mid-stem and tap root regions (Table 6). The highest population obtained from all sites tested was c. 10^7 cfu/g FM.

The bacterial population increased initially, remained stable for a few days and then appeared to decline in the resistant cv. "Kulai" (Table 7) during the period of investigation. The population differed significantly among the sites, i.e. tap root, collar and mid-stem regions, and at various sampling times. The resistant plants did not produce any symptom of wilt during the whole study period.

In the susceptible cultivars, bacterial population did not differ significantly at 12-16 sampling dates among mid-stem, collar and tap root. The population obtained was c. 10^9 cfu/g FM from 8-16 days after inoculation (Table 6 & 7).

DISCUSSION

Forty-two accessions/cultivars of *Capsicum* species were evaluated for resistance to *R. solanacearum* under greenhouse conditions. Results revealed that cv. "Kulai" showed a high level of resistance to the pathogen without any visible signs of wilting. Three accessions (CO1969, CO1970 and CO1553) were moderately resistant whereas all the other accessions were moderately to highly susceptible.

For tomato, expression of resistance under high temperature may be lacking (Krausz and Thurston 1975) or may break down (Mew and Ho 1977). Resistance in potato was also found to be temperature sensitive and strain specific (French and De Lindo 1982). Difference in resistance may also be due to the occurrence of different races. Buddenhagen and Kelman (1964) reported that three races can occur in

TABLE 2
Anatomical characters of susceptible chilli cv. LC 455 and resistant cv. Kulai to *Ralstonia solanacearum*

Characters	Variety					
	Long chilli 455		Kulai			
	Range	Mean ± S.E.	Range	Mean ± S.E.	Significance	CV(%)
Root						
Thickness of cortex (μm)	61.3 - 127.2	88.2 ± 1.65	80.0 - 187.5	117.9 ± 3.87	**	8.48
Number of vascular bundles	4.0 - 5.0	4.54± 0.07	4.0 - 5.0	4.18± 0.06	*	4.56
Length of xylem element (μm)	149.0 - 460.0	303.8 ± 11.9	103.6 - 334.5	220.5 ± 7.08	**	11.49
Diameter of xylem element (μm)	22.0 - 55.0	28.5 ± 0.61	17.0 - 50.0	28.0 ± 0.55	ns	6.52
Stem						
Thickness of cortex (μm)	125.0 - 396.0	222.0 ± 9.9	165.0 - 372.0	238.0 ± 5.74	ns	10.75
Number of vascular bundles	4.0 - 5.0	4.5 ± 0.07	4.0 - 5.0	4.18± 0.06	*	4.61
Length of xylem element (μm)	208.1 - 588.1	438.5 ± 12.02	138.0 - 334.5	377.5 ±17.3	**	8.78
Diameter of xylem element (μm)	20.0 - 63.2	32.8 ± 0.83	18.7 - 60.0	28.5 ± 0.44	**	6.56
Xylem elements per vascular bundle	13.0 - 23.0	17.1 ± 0.94	11.0 - 17.0	14.0 ± 0.58	**	15.86

S.E. = standard error, ns= not significant; * and **= significant at (p = 0.05) and (p = 0.01) respectively; CV = coefficient of variation

TABLE 3
Bacterial population at the mid-stem region of chilli for the susceptible cv. LC 455 and resistant cv. Kulai after stem inoculation

Days after inoculation	Bacterial population ± S.E. (log cfu/g FM)		Significance
	LC 455	Kulai	
4	8.88 ± 0.03	6.09 ± 0.05	**
8	9.28 ± 0.2	7.69 ± 0.2	**
12	9.29 ± 0.06	8.29 ± 0.3	**
16	9.22 ± 0.1	8.01 ± 0.02	**

** = significant at p = 0.01 (t-test); S.E. = standard error; FM = fresh matter

TABLE 4
Bacterial population at the collar region of chilli for the susceptible cv. LC 455 and resistant Kulai after stem inoculation

Days after inoculation	Bacterial population ± S.E. (log cfu/g FM)		Significance
	LC 455	Kulai	
4	7.72 ± 0.03	5.15 ± 0.2	**
8	9.13 ± 0.05	7.56 ± 0.8	**
12	9.43 ± 0.04	8.00 ± 0.1	**
16	9.19 ± 0.1	8.17 ± 0.4	**

** = significant at p = 0.01 (t-test); S.E. = standard error; FM = fresh matter

TABLE 5
Bacterial population at the tap root region of chilli for the susceptible cv. LC 455 and resistant cv. Kulai after stem inoculation

Days after inoculation	Bacterial population ± S.E. (log cfu/g FM)		Significance
	LC 455	Kulai	
4	8.70 ± 0.1	4.76 ± 0.7	**
8	9.14 ± 0.05	7.21 ± 0.2	**
12	9.30 ± 0.4	7.62 ± 0.3	**
16	9.24 ± 0.1	8.01 ± 0.3	**

** = significant at p = 0.01 (t-test); S.E. = standard error; FM = fresh matter

Pseudomonas solanacearum. In the present study, under greenhouse conditions the air temperature and relative humidity were 20-33°C and 55-90%, respectively, and the bacterial strain used may be different. These factors, together with the influence of other environmental factors, such as soil moisture and temperature, may influence resistance. However, the results of the present study are in agreement with those obtained by Hanson *et al.* (1996), where the field reaction of tomato lines to different strains of *R. solanacearum* differed at different locations in South East Asia. They found that in Malaysia and Taiwan most tomato lines were resistant, but in the Philippines and Indonesia they were susceptible. This indicates that it is necessary to evaluate the germplasm in local conditions against a particular pathogen.

TABLE 6
Mean bacterial population in chilli susceptible cv. LC 455 and resistant cv. Kulai after stem inoculation upon 16 days of sampling

Sites of detection	Bacterial population*** (log cfu/g FM)	
	LC 455	Kulai
Mid-stem	9.15 a*	7.54 a**
Collar	9.11 a	7.23 ab
Tap root	8.90 a	6.76 b

* and ** any two means in a column followed by a common letter are not significantly different at p = 0.05 and p = 0.01 respectively, as determined by Duncan's multiple range test;
*** Mean bacterial population was obtained from 20 observations of 4, 8, 12 and 16 days sampling after inoculation of the plants

The longer incubation period of the resistant and moderately resistant accessions compared to the susceptible ones indicated that resistance could either delay the initial infection of the disease or slow down the rate of wilting. The incubation period for *R. solanacearum* in chilli appeared to be generally similar to that of tomato (Atabug and San Juan 1981); in 10% of the wilted plants assessed for the incubation period of the pathogen the resistant accessions had longer incubation periods compared to those of the susceptible accessions. The present study, based on the assessment of 50% wilted chilli plants, produced similar results, i.e. the resistant accessions had longer incubation periods and took a longer time to produce disease symptoms than the susceptible accessions.

The present study indicated that fewer vessels and a thicker cortex in roots, and a shorter length and smaller diameter of the xylem ele-

TABLE 7
Development of bacterial population in resistant cultivar Kulai and susceptible LC 455 after stem inoculation for 32-day period of investigation

Sites of detection	Cultivars	Mean bacterical population at day after inoculation (log cfu/g FM)							
		4	8	12	16	20	24	28	32
Mid-stem	Kulai	6.89 a	7.70 a	8.28 a	8.17 a	7.70 a	4.26 a	6.61 a	6.16 a
	LC 455	8.88 b	9.27 a	9.29 a	9.21 a	-	-	-	-
Collar	Kulai	6.10 b	7.26 a	8.00 b	8.08 a	7.68 a	7.02 b	6.62 b	5.59 b
	LC 455	7.73 c	9.13 b	9.43 a	9.19 b	-	-	-	-
Tap root	Kulai	5.26 c	7.21 b	7.62 c	7.41 b	6.15 b	5.41 c	5.11 c	4.66 c
	LC 455	8.70 c	9.14 b	9.30 a	9.24 a	-	-	-	-

Any two means in a row with the same letter are not significantly different at p = 0.01, as determined by the Duncan's multiple range test

ments in stems of resistant plants, may restrict the growth and movement of the pathogen. Since *R. solanacearum* is a vascular pathogen, these characters possibly hinder occurrence of infection and reduce bacterial movement post-entry. Plugging of vessels by the growth of tyloses (Beckman 1966; Wallis and Truter 1978), accumulation of gums (Elgersma 1967) and occlusion of vessels by vesicles (Shi *et al.* 1992) have been suggested as mechanisms for localizing or slowing the vertical distribution of vascular pathogen. Since large vessels will not be occluded as rapidly as small ones, the distribution of bacterial cells will not be hindered and the disease severity of susceptible cultivar will be higher.

In addition, the small number of vascular bundles more deeply embedded in the cortical tissues of the resistant plants were less likely to become infected than numerous vascular bundles covered by a thin cortex, as seen in susceptible plants. The shorter elements of the resistant cultivar also probably hinder the movement of bacterial cells compared with the longer elements of the susceptible cultivar, due to the presence of more obstructions at the point of union of the elements.

Results of the present study confirm the earlier report of Cho *et al.* (1973) who found that in resistant alfalfa cultivars there were morphological barriers to *Corynebacterium insidiosum.* They indicated fewer vascular bundles, shorter vessel elements, and a thicker cortex in resistant alfalfa cultivars than susceptible cultivars.

Similarly, resistance in sugarcane cultivars to ratoon stunting disease (RSD) bacterium is attributed to the low number of large, continuous vessels, which pass through the nodes, which are effective in reducing pathogen movement (Teakle *et al.* 1975).

Thus, selection of anatomical characters such as thick root cortex, low numbers of vascular bundles and short vessel elements would be useful in breeding programmes designed to control bacterial wilt.

All chilli cultivars in this study were infected by *R. solanacearum* regardless of their degree of susceptibility to the disease. The resistant cultivar did not show any wilt symptoms throughout the period of study, even though the pathogen was detected in the plants. This shows that cv. "Kulai" could be latently infected. Difference in disease expression as well as the multiplication of the pathogen resulted from subsequent *in planta,* plant- *R. solanacearum.* interactions. The results of the occurrence of latent infection in resistant chilli corroborates those reported for resistant tomato cultivars (Prior *et al.* 1990) and resistant chilli peppers (Prior *et al.* 1994).

The susceptible cv. LC 455 showed initial symptoms of wilt within 5 days of inoculation, and high bacterial density was recovered from all sites. Complete wilting occurred within 16 days, suggesting that wilt susceptibility resulted from simultaneous rapid bacterial colonization and increase of bacterial population in vascular tissues.

Results revealed that the multiplication and distribution of the pathogen in resistant cultivars was slower than in the susceptible cultivars. Lower multiplication rates of *Xanthomonas phaseoli* on resistant bean leaves and *P. solanacearum* on resistant tomato plants were reported by Cafati and Saettler (1980) and Grimault *et al.* (1993), respectively. Morphological barriers that restrict pathogen spread have been reported in other bacteria-plant systems. For example, the population of *Erwinia amylovora* in susceptible apple cultivars was more than 10^4 times greater than in resistant cultivars (Tomasik and Goodman 1986). Besides immobilization of the pathogen, anatomical features such as vessel diameter and vessel branching in resistant plants appeared to be involved.

From these observations, it is hypothesized that the anatomy of resistant cultivars plays a significant role in restricting the spread of pathogen in the host. On the other hand, Grimault and Prior (1993) reported that conducting vessels of tomato plants resistant to bacterial wilt may have some tolerance capacity to bacterial mass because resistant chilli plants also showed an ability to tolerate the pathogen. Thus, the resistant cv. "Kulai" behaves as a symptomless carrier. Some other plant species, such as tobacco (Winstead and Kelman 1952), potato (Ciampi and Sequeira, (1980) and tomato lines (Prior *et al.* 1994), have also been reported to be symptomless carriers of *P. solanacearum.*

In conclusion, this study shows that cv. "Kulai", which bears locally acceptable fruits and has a high level of resistance to bacterial wilt, could be used in developing wilt-resistance cultivars and is recommended for cultivation in wilt-prone areas.

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