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Studies on variations among prevailing races of *Xanthomonas vesicatoria* causing Bacterial spot of tomato in Karnataka

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Tomato, a highly profitable vegetable crop is severely affected by Bacterial spot pathogen and is rapidly spreading across Karnataka state (India). The pathogen *Xanthomonas vesicatoria* is difficult to manage due to its genetic variation and development of resistance to bactericides. The diversity study within the prevalent population of pathogen (25 isolates) indicates the occurrence of race T1 predominantly based on host differentials. The 20 isolates that infected Bonny Best and H7981 were designated as race T1, two isolates (*I*9 and *I*21) infecting Bonny Best and H7998 as race T2 and isolates *I*17 and *I*24 that infected Bonny Best, H7998 and H7981 was recorded as race T3.The study recorded three races infecting tomato prevailing in the state of Karnataka. The antibody developed for race T1 on interaction against the LPS of all 25 isolates revealed the presence of homology and heterology within the races serologically by ELISA and Western Blot techniques, differentiating the isolates into serovars. The 20 isolates that had four bands were included under serovar 1 whereas, strains of race T2 (serovar 2) and T3 (serovar 3) had 2 bands, also race P1 (serovar 4) had one band. The band found in all serovar groups were commonly found under serovar 1 (90-70 kDa range). The expression of bands correlated the presence of epitopes in correspondence within the races on antigen-antibody interaction.

Key words: *Xanthomonas vesicatoria,* host differentials, LPS, serovars

INTRODUCTION

Successful cultivation of tomato (*Solanum lycopersicum* L.) is hindered by several factors. Among these the Bacterial spot caused by *Xanthomonas vesicatoria* (Doidge) Dye is assuming greater proportions in recent years, thereby affecting the quality and yield of fruits resulting in severe monetary loss to farmers. Another major impediment is that the pathogen is transmitted through seeds. In India the loss due to this disease ranges from 10-80 per cent (Reddy *et al.* 2012) and in Karnataka it appeared to the range of 20-50 per cent (Kavita and Umesha, 2007). Under severe infection, even dropping off of the flowers and tender fruits are seen. This pathogen also affects bell pepper (*Capsicum annum* L.). The pathogen is pathogenically, physiologically, serologically and genetically diverse. Since chemical measures for the management is a limitation due to non-availability of effective antibiotics/bactericides and the pathogen is known to develop resistance to the antibiotics very fast. Hence, the key component of management relies on development of resistant variety for which studies on biology of the pathogen and prevalence of races in different agro climatic regions is important. Thus the present classification and distribution studies on the strains/ races of pathogen have predictive value in epide-

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miology and management of the disease especially through gene deployment..

X. vesicatoria is grouped into different races on the basis of the reaction with hosts *viz*., tomato (XCVT- *Xanthomonas campestris* pv. *vesicatoria*- Tomato group), pepper group (XCVP) and pepper tomato group (XCVPT). Again based on host differentials XCVT group has three races T1, T2 and T3, whereas pepper has races P0 to P8. The strains reacting with both hosts tomato and pepper (XCVPT) are designated as T1P1 and so on (Jensen 2006; Chet *et al.* 2010 and Ganeva *et al.* 2014).

The distribution of these races is increasing wherein initially only race T1 was present worldwide. In India, the race prevalent was reported to be T1P3 during 1994 and it is also reported about of the prevalence of tomato race T1, T3 and pepper race 2 in Karnataka. The present study is in relevance to the prevalence of races, nearly after a decade based on host differentials and serological variations for their homology/heterology. LPS an important virulence/immunogenic factor of plant pathogenic bacteria considered to be a vital entity in differentiating the races/groups within the population was exploited here for serological studies to obtain a refined differentiation of races. The study is very essential for development of management strategies with a main aim for gene deployment programmes.

MATERIALS AND METHODS

Collection, isolation, identification of pathogen and pathogenicity studies

The symptoms of bacterial spot appeared as small pale greenish yellow to dark brown spots with a halo on leaves and, on fruits it appeared as sunken brown spots with irregular lobed margins with water soaked or yellow halo. The pathogen was isolated from diseased tomato and pepper fruits and leaves exhibiting typical symptoms. The samples were obtained from different agro climatic zones of Karnataka (eastern, central, southern and northern dry zone, southern transition zone and hilly zone) and Low hills sub-tropical zone of Palampur, Himachal Pradesh.

The bacterium was isolated and purified on CKTM medium. The identification of the 25 isolates from tomato and pepper on the basis of morphology (shape, size, capsulation, sporulation, Gram reaction), cultural (colony-colour, shape, appearance), physiological and biochemical (oxidase reaction, indole production, urease reaction, nitrate reduction, methyl-red reaction, utilization of asparagines and different sugars, starch and pectin hydrolysis, nitrate reduction, oxidase and catalase reaction, indole production, esculin hydrolysis, curdling of skimmed milk, catalase reaction, gelatin hydrolysis, casein hydrolysis and hydrogen sulphide production) and pathogenic characteristics (host and non-hosts) (Anon, 2013). Susceptible variety Arka Vikas (tomato) and California Wonder (pepper) were used as hosts to establish the pathogenicity.

Identification of the races of X. vesicatoria on the basis of pathogenicity on host differentials in tomato and pepper differential cultigens

The 25 isolates were separately multiplied on NBYGA (Nutrient broth yeast glucose agar) in Roux flasks for 72 hrs at 28^0C and the cells were harvested, concentrated to $2X10^5$ cfu/ml in spectronic 20D (Milton & Roy, USA) at 460 nm. The plants were pre-incubated for 24 hrs in humid chamber and the bacterial cells were swabbed with a pinch of abrasive celite into the fully expanded leaves at 4-5 leaf stage of tomato cultigens *viz*., Bonny Best, Hawaii 7998 and Hawaii 7981 and pepper cultigens Early Calwonder (ECW) and its isogenic lines ECW 10R, ECW 20R and ECW 30R. The inoculated plants were kept for 24 hrs in humid tent then shifted to green house. The reactions were recorded on the differentials once in every 24 hrs based on the development of necrotic spot for resistance or susceptibility.

Immunogenic variation among X. vesicatoria isolates infecting tomato and pepper

Lipopolysaccharide (LPS), an important virulence factor of plant pathogenic bacteria, a major constituent of the gram-negative bacterial cell wall complex used to study variations among the isolates. Antibodies developed for *X. vesicatoria* cells are reported to be more specific and sensitive against LPS. LPS is also known to induce expression of plant defense system and it exhibits variation within different strains of the bacteria (Casabuono *et al.* 2011; Petrocelli *et al.* 2012).

Extraction of LPS was done by two methods to note

the highest efficiency of extraction in plant pathogenic bacteria - Hot phenol water extraction. The precipitated LPS fraction was vacuum dried and stored under 4^{0}C. The concentration of the extracted lipopolysaccharide was estimated by Lowry's method. The LPS from 25 isolates was used as antigen in the immunological studies.

Antiserum were developed in rabbits immunized with the purified bacterial cells of *I*14 (*X. vesicatoria)* as antigen for producing the antibodies. The IgG from the antiserum was separated by ammonium sulphate precipitate method. The immunoglobulin (IgG) content was determined by recording OD at 280 nm in the spectrophotometer (ELICOSL159, UV VIS) for 3 ml of 10% solution and calculated as OD of 1.0 ml at 280 nm = 0.74 mg/ml of protein. Antibodies in the prepared antiserum was standardised by tube agglutination test for ELISA and Western Blot.

Enzyme Linked Immunosorbent Assay (ELISA)

The LPS of each isolate lyophilized in the vial and their concentration was determined by Lowry-Folin Ciocalteau Reagent method was diluted in one ml of sterile distilled water for ELISA. The optimum concentration of antigen and antiserum dilution for ELISA was determined by running the test assay at different antiserum dilutions of 1:10, 1:20, 1:40, 1:80, 1:160, 1:1320, 1:680 and 1:1280, while the antigen was used at a concentrations of 800, 400, 200, 100 and 50 ng/ml for 25 isolates. Direct ELISA determined the optimum concentration for maximum antigen-antibody reaction recorded in the OD value of 492 nm (ELISA scanner, Microtek, USA).

The enzyme conjugate, goat anti-rabbit (Bangalore Genei), was used at the dilution of 1:5000. Pure culture cells of the isolates of *X. vesicatoria* – Race T1 was used as positive control and negative control was coating buffer containing FCS; no antigens, no serum and no enzyme linked antibody were added to have antigen, serum and conjugate control. The immediate preceding values from the highest OD value (peak) was considered as the optimum dilution of antisera and antigen concentration for ELISA studies.

Indirect ELISA was carried out with 200 ng and 100ng of antigen suspension for 25 isolates diluted in coating buffer at 100 µl for each well in 96 wells microtitre plate and 100 µl of Homologous antiserum (1:80), 100 µl antirabbit goat enzyme conjugate (1:5000) was added to each well and lastly 100 µl substrate chromogen solution was added per well. The intensity of colour in the wells was read at 492 nm.

Western blot technique (WB)

The LPS of isolates were prepared by mixing 10 µg of the sample in 8 µl of 4X Sample Buffer and boiled at 100^{0}C for 10 min and cooled on ice, loaded into the wells for Sodium Dodecyl Sulphate – Polyacrylamide gel electrophoresis (SDS PAGE) and electrophoresed at 75-90 mA until the band reached the end of resolving gel.

Transfer of the LPS from the gel onto the membrane: The Poly Vinelydene Di Fluoride (PVDF) substrate membrane was initially soaked for few seconds in methanol and then transferred into Transfer Buffer Solution (TBS). Soaked filter papers of membrane size were placed in the TBS before laying them on the Transfer Apparatus (Bio rad Transblot SD, semidry transfer cell). The soaked filter papers were transferred onto the apparatus one after the other and trapped air bubbles were removed after each time of transfer. Soaked PVDF membrane were placed over the filter papers and gel was placed over the membrane, next two more filter papers were placed and the apparatus was set to 15 V for 30 min.

Immunodetection of LPS: After the transfer, the membrane was removed and placed in the Blocking Buffer whereas the gel was transferred to Staining Solution to check the transfer success. The membrane was placed in 20 ml of prepared Blocking Solution on shaker at 30^{0}C for one hour. The membrane was washed with TBS-T and transferred into the antiserum solution diluted with Blocking Buffer (1:80) and placed on shaker at 30^{0}C for an hour. Again it was washed and transferred into conjugate solution-anti-rabbit goat antiserum conjugated with HRPO diluted with blocking buffer (1:5000) and placed on shaker at 30^{0}C for half an hour. After conjugation membrane was washed and incubated in substrate buffer for 10 min. under dark conditions and observations were made for the presence of protein bands on the PVDF membrane.

Based on the antigenic binding to the antibody raised against isolate *I*14 (race T1) by the 25 isolates, they were categorised into different serovars.

RESULTS AND DISCUSSION

Identification and confirmation of pathogen

Cell morphology: The 25 isolates observed were small rods, present in singles, Gram negative, capsulated, non-sporulating, non-acid fast staining and monotrichously flagellated (transmission electron microscope). Colonies utilized glucose, trehalose, cellobiose, arbinose, mannose and produced acid, but failed to produce acids in case of sucrose, lactose, rhamnose and maltose sugars. The isolates

Table 1: Isolate characterisation based on host differentials

Isolates	Place of collection	Hosts	Tomato host differentials			Group	Race
			Bonny Best	H7998	H7981		
*I*1	GKVK, Bangalore	Tomato	+	HR	+	XCVT	T1
*I*2	Hoskote	Tomato	+	HR	+	XCVT	T1
*I*3	Chitradurga	Tomato	+	HR	+	XCVT	T1
*I*4	Davangere	Tomato	+	HR	+	XCVT	T1
*I*5	Hassan	Tomato	+	HR	+	XCVT	T1
*I*6	Kolar	Tomato	+	HR	+	XCVT	T1
*I*7	Mudigere	Tomato	+	HR	+	XCVT	T1
*I*8	Mysore	Tomato	+	HR	+	XCVT	T1
*I*9	Shimoga	Tomato	+	+	HR	XCVT	T3
*I*10	Chikkaballapur	Tomato	+	HR	+	XCVT	T1
*I*11	Madanpalli	Tomato	+	HR	+	XCVT	T1
*I*12	Burgoor, Tumkur	Tomato	+	HR	+	XCVT	T1
*I*13	Magadi	Tomato	+	HR	+	XCVT	T1
*I*14	Agrahara, Mandya	Tomato	+	HR	+	XCVT	T1
*I*15	Maddur	Tomato	+	HR	+	XCVT	T1
*I*16	Mulbagul	Tomato	+	HR	+	XCVT	T1
*I*17	Chitanahalli, Kolar	Tomato	+	+	+	XCVT	T2
*I*18	Ankatahalli, Kolar	Tomato	+	HR	+	XCVT	T1
*I*19	Kaiwara	Tomato	+	HR	+	XCVT	T1
*I*20	KR Nagar	Tomato	+	HR	+	XCVT	T1
*I*21	Hebbal, Bangalore	Tomato	+	+	HR	XCVT	T3
*I*22	Bijapur	Tomato	+	HR	-	XCVT	T1
*I*23*	Palampur, HP	Pepper	-	-	-	XCVP	P1
*I*24	Arasikere	Tomato	+	+	+	XCVT	T2
*I*25	Kanakapura	Tomato	+	HR	+	XCVT	T1

+ Positive reaction / Virulence
- Negative reaction / Avirulence
HR-Hypersensitive reaction
* Except *I*23 from bell pepper, rest all the isolates are from tomato

Table 2A : Reaction of ELISA for the detection of variations among the strains of *X. vesicatoria*

Antigen / Isolate (200ng)	OD Value												
	*I*1	*I*2	*I*3	*I*4	*I*5	*I*6	*I*7	*I*8	**I**9	*I*10	*I*11	*I*12	*I*13
R1	1.043	1.121	1.006	0.937	1.731	1.148	1.114	1.632	0.218	1.731	1.630	1.411	1.169
R2	1.066	1.121	1.011	0.934	1.748	1.145	1.119	1.670	0.218	1.738	1.788	1.312	1.166
Avr.	1.055	1.121	1.009	0.936	1.74	1.147	1.116	1.651	0.218	1.734	1.709	1.362	1.168
	*I*14	*I*15	*I*16	*I*17	*I*18	*I*19	*I*20	*I*21	*I*22	*I*23	*I*24	*I*25	*I*26 (Serum check)
R1	1.643	1.557	1.015	0.330	0.936	1.462	1.040	0.227	0.551	0.173	0.208	0.925	0.346
R2	1.666	1.571	1.021	0.328	0.891	1.793	1.059	0.238	0.565	0.183	0.222	0.948	0.373
Avr.	1.655	1.564	1.018	0.329	0.913	1.628	1.050	0.233	0.558	0.178	0.215	0.937	0.360
Mean	1.023												
CD (0.01)	0.147												
S.Em	0.037												
CV%	5.14												

Table 2B : Reaction of ELISA for the detection of variations among the strains of *X. vesicatoria*

Antigen / Isolate (100ng)	OD Values												
	I 1	*I*2	*I*3	*I*4	*I*5	*I*6	*I*7	*I*8	*I*9	*I*10	*I*11	*I*12	*I*13
R1	1.362	1.015	0.978	0.897	1.241	1.010	1.106	1.243	0.175	1.243	1.397	1.213	1.510
R2	1.368	1.017	0.997	0.796	1.236	1.119	1.098	1.221	0.169	1.253	1.274	1.122	1.145
Avr.	1.365	1.016	0.987	0.847	1.239	1.065	1.102	1.232	0.172	1.248	1.336	1.168	1.328
	*I*14	*I*15	*I*16	*I*17	*I*18	*I*19	*I*20	*I*21	*I*22	*I*23	*I*24	*I*25	*I*26 (Serum check)
R1	1.342	1.306	1.119	0.178	0.575	1.499	1.025	0.187	0.471	0.109	0.164	0.918	0.342
R2	1.380	1.343	1.109	0.174	0.602	1.521	1.048	0.189	0.473	0.106	0.162	0.909	0.371
Avr.	1.361	1.325	1.114	0.176	0.589	1.510	1.037	0.188	0.472	0.108	0.163	0.913	0.357
Mean	0.901												
CD (0.01)	0.167												
S.Em	0.042												
CV%	6.64												

tested were positive for catalase reaction, gelatin hydrolysis, casein hydrolysis and hydrogen sulphide production which confirmed that the pathogen is *X. vesicatoria* (Anon, 2013).

The 24 tomato isolates of *X. vesicatoria* artificially inoculated to 25 days Arka Vikas seedlings exhibited small greenish yellow to dark brown spots on the leaves which turned necrotic leading to blighting after 15 days with halo. Similarly the pepper isolate (XCVP) produced lesions on pepper leaves of California Wonder, later turned to dark brown in colour. Re-isolation of the pathogen from the lesions on tomato and pepper confirmed to be *X. vesicatoria* by biochemical analysis as mentioned above. Also cross infection of all tomato isolates did not infect pepper or *vice versa.* The identified isolates were designated as *I*1 to *I*25 and stored in -80^{0}C for long term preservation and served as stock culture for further studies (Table 1).

Identification of the races of X. vesicatoria on the basis of host differentials

The results of the reaction between the host differentials and the pathogen are presented (Table 1). The 20 tomato isolates out of 24 except *I*9, *I*17, *I*21 and *I*24 were avirulent on tomato genotype Hawaii 7998 and produced hypersensitive reaction within 24hrs of inoculation. The isolates *I*1 to *I*25 except *I*23 pepper isolate were

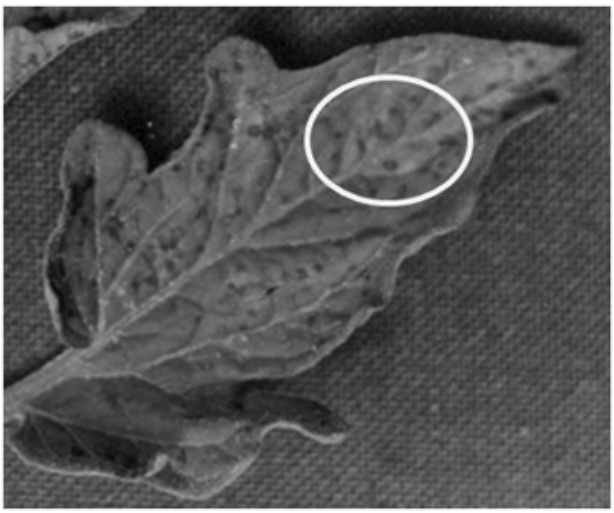

Fig. 1 : Pathogenic reaction of isolate I14 on host H7981

pathogenic on Bonny Best and recorded typical necrotic leaf spot symptom (Fig. 1). The strains *I*9 and *I*21 were pathogenic on H7998 but, induced a HR reaction in H7981 whereas, strains *I*17 and *I*24 were pathogenic to all three genotypes. None of these tomato isolates were infective on the pepper cultivar *viz*., Early Calwonder. The inoculation

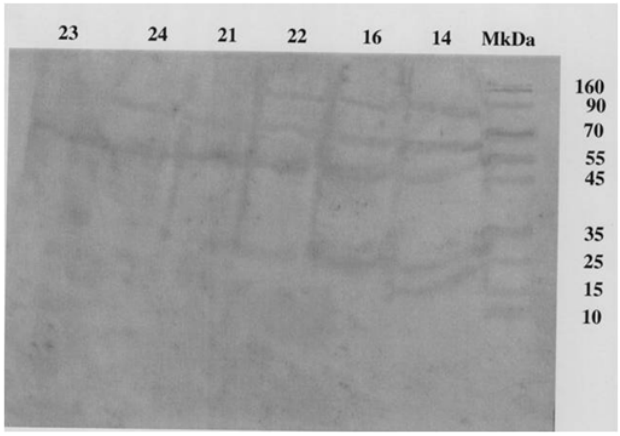

Fig. 2 : WB assay for isolates (representive six isolates)

of the 25 isolates of *X. vesicatoria* obtained from different agroclimatic zones, revealed the prevalence XCVT group in Karnataka (Table 1), since the 24 isolates from tomato infected only tomato cultivars and none were pathogenic to pepper plants.

The pepper isolate *I*23 from Palampur, Himachal Pradesh used as check in the study was pathogenic on ECW and ECW 10R but produced hypersensitive reaction on ECW 20R and 30R. This isolate was not pathogenic to any of the tomato cultigens infecting only the pepper plant indicating that it belonged to XCVP group and Pepper race 1 (P1) based on its reaction with isogenic lines (Stall *et al*., 2009).

Three groups of *X. vesicatoria*- XCVT, XCVP and XCVPT are usually associated with the disease. Strains belonging to XCVT and XCVP groups were further divided into races on the basis of virulence on tomato cultigens and pepper near isogenic lines. Accordingly on the basis of race nomenclature, the 20 isolates in the XCVT group could be designated as belonging to tomato Race 1 (T1), whereas *I*9 and *I*21 isolates belonged to tomato Race 3 (T3) as they induced hypersensitive reaction on H7981 but, infected Bonny Best and H7998 cultigens. *I*17 and *I*24 isolates we categorized into tomato Race 2 (T2), as these isolates were pathogenic to all three cultigens. The study confirmed that 20 tomato isolates from Karnataka belonged to tomato Race T1 and T3 and in addition T2 has been added to the group as it could infect all three tomato cultigens (Bonny Best, H7998 and H7981). Pepper isolate from Himachal Pradesh belonged to pepper Race P1. None of the tomato and pepper isolates tested were pathogenic to pepper and tomato on cross inoculation, hence the possibility of prevalence of XCVPT group in Karnataka was ruled out. This was also confirmed by earlier workers. The prevalence of all the three races (T14, T2 and T3) is recorded here on tomato in Karnataka for the first time. Previously T1 and T2 were reported in the state. Identification of new races is very important to understand the change in biology of the pathogen. The reason for the prevalence of new races could be the seeds which are being imported regularly for various purposes by public and private sectors.

Antigenic variation among the isolates of X. vesicatoria using serological techniques

The extraction of LPS by Hot Phenol Water Extraction was efficient, it yielded highest amount of

LPS (quantity). The purified cells was immunized to rabbit and purified immunoglobulins was quantified at OD 280nm was estimated to be 1.275 mg/ml. The titre of the antiserum on the basis of agglutination reaction was 1:160.

ELISA: At 200-100 ng/100 µl and 1:160 antigen concentration and antiserum dilution high OD values of 1.042 and 1.041 were recorded. Without compromising the efficiency of ELISA the optimum antiserum dilution was fixed at immediate preceding OD values to the highest, hence 200 and 100 ng at 1:80 was used for determining serological relationship among the isolates.

The reaction was recorded between the LPS of 25 isolates and the antiserum (1:80 dilution) developed against *I*14 by Indirect ELISA at OD value of 492nm (Table 2A and 2B). As per standard procedures OD values double than the negative control (antiserum check) was taken as positive reaction to establish the antigenic relationship. Accordingly in the data presented all the isolates reacted positively with antiserum raised and the OD values ranged from 0.558 to 1.74 at an antigen concentration of 200 ng, those values double the check value was taken as positive reaction. Similarly, the reaction carried out between the antiserum and antigen at 100 ng concentration revealed that all the isolates reacted antigenically positive and OD values obtained were higher than antiserum check doubled except for *I*9, *I*17, *I*21, *I*23 and *I*24. OD values obtained in ELISA were significant as compared to negative control for 20 tomato isolates of race T1 and except for the five isolates under race T2 and T3. The observations were in accordance with host differential studies.

Western blot (WB) technique: WB determined the degree of antigenic relationship between the various isolates and the antibody developed on the basis of number of protein bands appearing in the PVDF membrane. WB was executed with all 24 tomato and one pepper isolates. The bands depicted in WB plate have been presented here for only six representative samples of the isolates for elaborative discussion (Fig. 2) including the homologous antigen *I*14. The isolate *I*14 showed presence of five bands, in range of 90-70 kDa (2bands) and single band in the range of 55-45, 35-25 and 25-15 kDa. Isolates *I*16 and I22 revealed the presence of four bands, 90-70 kDa (2 bands), 55-45 kDa (1 band) and 35-25 kDa (1band), *I*24 exhibited two close bands in the range, 90-70 and 55-45 kDa, whereas *I*21 revealed the presence of three bands in the range 90-70, 55-45 and 35-25 kDa. The pepper isolate *I*23 revealed only one band in range 55-45 kDa. The homologous antigen *I*14 exhibited more number of bands revealing strong affinity towards the antibody. Similarly WB studies were also done for the remaining 19 isolates which recorded the similar observations of banding patterns.

LPS is a ubiquitous component of Gram-negative bacteria can induce defense related response in plants, but in many cases the direct effect are weak. The structure of smooth LPS includes a hydrophilic polysaccharide and a hydrophobic Lipid A. The polysaccharide has two components O-specific polysaccharides and core oligosaccharides. The O-polysaccharide chains contain the most immune determinant structures which define the immunological O-specificity of LPS and is inherited from the parental bacterial strains while the core- and Lipid –A are less immune dominant (Petrocelli *et al*., 2012).

The LPS of 25 isolates analysed against the antibody of T1 (*I*14) revealed the homology and antigenic variation among the isolates. Out of 25 isolates, 20 isolates expressed positive reaction as evidenced by high significant OD values except five isolates for *I*9, *I*17, *I*21, *I*23 and *I*24. The LPS of isolates from different agroclimatic zones assessed by WB inferred that a single common band measuring 55-45 kDa detected in all the isolates belonging to the races T1 (serovar 1), T2 (serovar 2) and T3 (serovar 3) of XCVT group and P1 (seroar 4) of XCVP group also, this could possibly be an evidence of existence of a common epitope among the *X. vesicatoria* group. Among the strains belonging to group XCVT bands in range of 90-70 kDa is common whereas, T1 had two bands in this range but T2 and T3 had only one band which represents 2 and 1 epitope respectively for the races. Aggregatively T1 had four bands, T3 two bands and T2 three bands indication of having common closely located epitope as of T1 which means they are antigenically close and also distinct from each other.

The isolates were found to be heterologous on the basis of host differential studies and also antigen–antibody interaction revealed in ELISA and WB. The 20 isolates belonging to race 1 and four isolates

belonging to race T2 and T3 were antigenically distinct. The lone pepper isolate was also antigenically distinct from the tomato isolates. It is also reported that hypervariation at an *lps* locus between different strains of a plant pathogenic bacterium (*Xanthomonas oryzae* pv. *oryzae*) indicating multiple horizontal gene transfer events occurring at this locus in the Xanthomonad group of plant pathogens. Attributing the variations to be of advantage in evading the host immune system, can be accredited to the host differential studies. The races/ strains of *X. vesicatoria* are immunologically and antigenically distinct. Serological interaction was depicted in the protein bands expressed for each of the races which was specific and if standardised can play a vital role as a tool for identification. Based on host differential studies the 20 isolates (*I*1, *I*2, *I*3, *I*4, *I*5, *I*6, *I*7, *I*8, *I*10, *I*11, *I*12, *I*13, *I*14, *I*15, *I*16, *I*18, *I*19, *I*20, *I*22 and *I*25) belonged to race T1 while, *I*17 and *I*24 belonged to race T2, *I*9 and *I*21 falls under T3 and *I*23 the pepper isolate belongs to P1. The study also categorises the isolates under the same races in serological studies as Race 1-Serovar 1, Race 2 – Serovar 2, Race 3- Serovar 3 and Race P1-Serovar 4 respectively. Hence, we confirm that LPS can be used as a serological tool for race differentiation within a pathogen that can react to the differential host accordingly. Further the race grouping based on the host differentials and serogrouping are in consistent as per the revelation in the study. LPS also attributes the variation to be of advantage in evading the host immune system which also supports for differential host preference. Thus the findings in the investigation on immunological relationship using antibody developed, against LPS was found to be significant in differentiating races of *X.* v*esicatoria*serologically also, contributing for use as a tool to further know the prevalence of races under different agroclimatic zones for ease in gene deployment.The non-availability of differential host system can exploit the LPS based serological tool for race identification.

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