INTERNATIONAL JOURNAL OF PLANT PROTECTION / VOLUME 5 | ISSUE 2 | OCTOBER, 2012 | 324-328

IJPP

RESEARCH ARTICLE

Genetic diversity of *Ralstonia solanacaerum* from major tomato growing areas of Karnataka

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ARITCLE INFO

Received : 12.05.2012
Revised : 15.06.2012
Accepted : 30.08.2012

Key Words :
Ralstonia solanacearum, RAPD, Genetic variability

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ABSTRACT

Ralstonia solanacearum isolates from Karnataka (India) were analyzed by random amplified polymorphic DNA technique,the data distinguished the isolates into seven major clusters.High level of polymorphism (73.93%) indicated diverse genetic base. Maximum genetic diversity of 0.61 per cent was observed between Hosalli (Rs-7) and Doddaballapur (Rs-9) isolates, Distribution of strains into genetic clusters did not relate to geographic origin.

How to view point the article : Sumangala, K., Lingaraju, S., Hegde, Yashoda R. and Byadagi, A.S. (2012). Genetic diversity of *Ralstonia solanacaerum* from major tomato growing areas of Karnataka. *Internat. J. Plant Protec*., **5**(2) : 324-328.

INTRODUCTION

Ralstonia solanacearum(Yabucchi *et al.,* 1995), a causal agent of bacterial wilt of several crops like potato, tomato, pepper, tobacco, etc. is one of the important disease causing organisms in tropical, subtropical and warm temperate regions of the world (Hayward, 1991). *R. solanacearum* embraces a diverse array of populations that differ in host range, geographical distribution, pathogenicity, genetic and physiological properties. To describe this intra-specific variability, binary classification systems are used. There is considerable genetic variation among strains within each race or biovar (Cook *et al.,* 1989). In recent years, research has been directed towards developing rapid, sensitive and specific diagnostic assays to detect the *R. solanacearum* in plant and soil samples (Baker *et al.* 1984; Hendrick and Sequiera, 1984). Random amplified polymorphic DNA (RAPD) analysis (Williams *et al.,* 1990) has many advantages such as speed, low cost, minimal requirement of DNA, and lack of radioactivity, as a means of characterizing genetic variability. Major polymorphisms in RAPD pattern indicate genetic distinctness which can be used to distinguish unrelated groups. Minor polymorphisms may indicate genetic distinctness within groups or may occur because of experimental variability and, therefore, must be verified by repetition. RAPD analysis has been used effectively to distinguish between *R. solanacearum* strains.

MATERIALS AND METHODS

Laboratory experiments were carried out at the Department of Plant Pathology and Institute of Agri - Biotechnology (IABT), College of Agriculture, University of Agricultural Sciences, Dharwad. Karnataka (India), during 2008-2010. *Ralstonia* affected samples were collected from twenty four locations from major tomato growing areas of Karnataka. The details of location and designation given for each isolates are furnished in Table A. The pathogen was isolated on tetrazolium chloride (TZC) medium. Typical mucoid, creamy white colonies with pink centre was observed on medium ofter 48 h incubation and such single colony of each isolate was inoculated to 25 ml of Nutrient broth taken in 100 ml flasks. The flasks were kept for incubation at 32°C for 24 h. Pure cultures of the isolates were subjected to RAPD analysis.

Table A : *Ralstonia solanacearum* isolates from major tomato growing areas of Karnataka

Districts	Location	Isolate number
Dharwad	UAS Dharwad	Rs-1
	Garag	Rs-2
	Sapthapur	Rs-3
Bangalore	UAS Bangalore	Rs-4
	Doddaballapur	Rs-5
Tumkur	Gubbi	Rs-6
	Hosalli	Rs-7
Gadag	Bannikoppa	Rs-8
	Lakkundi	Rs-9
Haveri	Ranebennur	Rs-10
	Chalageri	Rs-11
Belgaum	Arabhavi	Rs-12
	Gokak	Rs-13
	Kanapur	Rs-14
Mysore	Mysore	Rs-15
	Hunsur	Rs-16
Chikkballapur	Chikkballapur	Rs-17
	Chintamani	Rs-18
Shimoga	Shimoga	Rs-19
	Sagar	Rs-20
Ramanagar	Ramanagar	Rs-21
Kolar	Kolar	Rs-22
Chikkmangalur	Chikkmangalur	Rs-23
Davanagere	Davanagere	Rs-24

For RAPD analysis, the genomic DNA was isolated from the isolates following the protocol given by Sambrook and Rausell (2001). To test the quality, DNA samples were run on 0.8 per cent agarose gel in 1X TAE buffered and stained with ethidium bromide and checked for contamination by RNA (which usually runs ahead) and the DNA was evaluated by comparing it with a standard undigested DNA sample. Serial dilutions were carried out to get desired quantity of DNA for polymerase chain reaction (PCR). Thirty decamer primers under OPA, OPB and OPF series procured from M/s Bangalore Genei, Pvt. Ltd., Bangalore were tested for DNA amplification by RAPD, for producing polymorphism among the strains. Reaction mixture was prepared in 0.2 ml thin walled PCR tubes containing the following components. The total volume of each reaction mixture was 20 µl. The following reaction mixture was found to be optimum for PCR amplification. 10x assay buffer with 15 mM$MgCl_2$:2.5 µl, dNTPs mix (2.5 mM each):1.0 µl, Primer (5pM/ µl):1.0µl, Template DNA (25ng/ µl): 1.0 µl, Sterile distilled water:14.30µl, Taq DNA polymerase (3.0U/ µl):0.2 µl. DNA amplification consisted of 40 cycles of denaturation at 94°C for 1 min, annealing at 37°C for 1 min and extension at 72°C for 2 min in a Eppendorf Master cycler gradient supplied by Eppendorf Gradient, 2231, Hamburg Germany was used for cyclic amplification of DNA. The amplified products were separated on 1.5 per cent agarose gel in 1x TAE buffer at 120V and visualized on a UV transilluminator.

RESULTS AND DISCUSSION

RAPD was used to detect the variation among the isolates of *R. solanacearum* collected from different districts of Karnataka. The profile of amplicons of different primers for *R. solanacearum* isolates is given in Table 1 and Fig. 1. A total of 241 DNA bands were detected using 30 primers, total of the 139 bands were polymorphic. Out of 30 primers OPA 10, OPB 3, OPF 7 and OPF 8 showed 100 per cent polymorphism. The banding profile per primer also varied from minimum of 4 bands

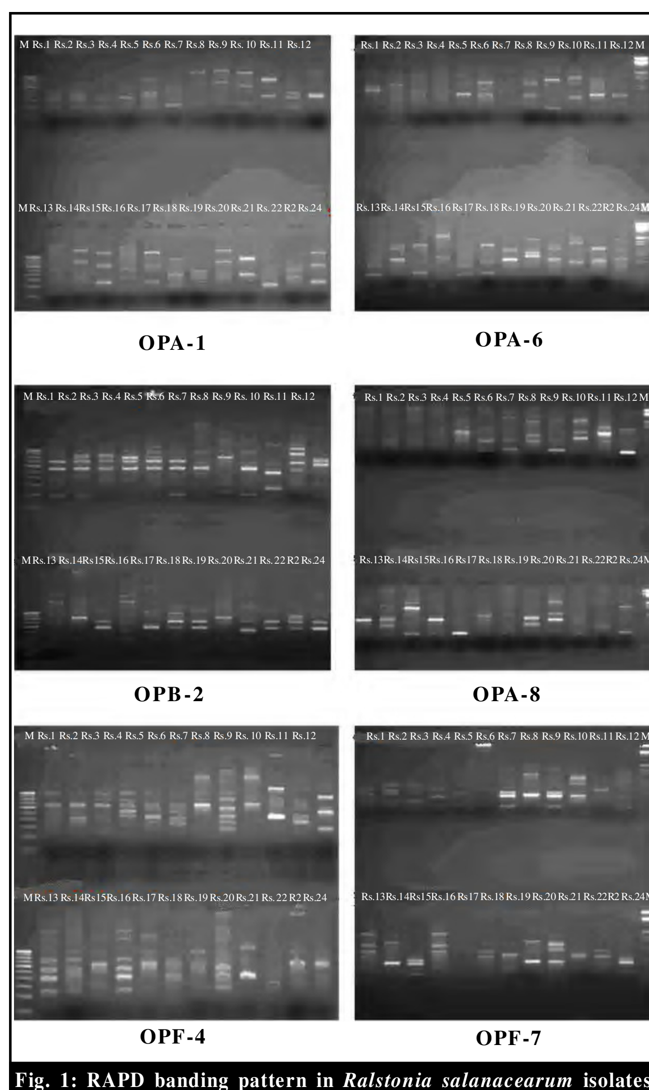

Fig. 1: RAPD banding pattern in *Ralstonia salanacearum* isolates

Table 1 : Banding profile of different primers for different isolates of *Ralstonia solanacearum*

Primers	Total bands	Polymorphic bands	% Polymorphism
OPA 1	8	7	87.5
OPA2	9	7	77.77
OPA3	4	3	75.00
OPA4	6	5	83.33
OPA5	8	5	62.50
OPA6	9	7	77.77
OPA7	7	6	85.71
OPA8	11	9	81.81
OPA9	5	4	80.00
OPA10	9	9	100.0
OPB1	6	4	66.66
OPB2	8	7	87.5
OPB3	6	6	100.0
OPB4	12	11	91.66
OPB5	6	5	83.33
OPB6	7	5	71.42
OPB7	9	5	55.55
OPB8	9	6	66.66
OPB9	8	5	62.50
OPB10	4	2	50.00
OPF1	12	12	100.0
OPF2	13	7	53.84
OPF3	16	6	37.50
OPF4	12	8	66.66
OPF5	5	4	80.00
OPF 6	6	3	50.00
OPF7	6	6	100
OPF8	8	8	100
OPF9	5	3	60.00
OPF10	7	4	57.14
Total	241	139	73.93

(OPA3) to maximum of 16 bands (OPF3). From the RAPD analysis, the results revealed that a total of 73.93 per cent polymorphism was found between the isolates, indicating that there was a high molecular variability among the isolates. Based on the simple matching coefficient a genetic similarity matrix was constructed to access the genetic relatedness among the isolates. The similarity co-efficient ranged from 0.19 to 0.61 (Table 2). The maximum genetic diversity of 0.61 per cent was observed between Hosalli (Rs-7) and Doddaballapur (Rs-9) isolates, whereas least similarity (0.19 %) was observed between Kolar (Rs-22) and Garag (Rs-2) isolates.

Information on the banding pattern for all the primers was used to determine the genetic distance between the isolates and to construct a dendrogram by using unweighted pair group arithmetic mean method (UPGMA). The dendrogram for pooled data showed seven major clusters (Fig 2). The isolates Rs-1, Rs -3, Rs-2, Rs-4, Rs-5 and Rs-6 (UAS Dharwad, Sapthapur, Garag, UAS Bangalore, Doddaballapur and Gubbi) were found in one cluster, isolates Rs-18, Rs-19, Rs-22 and Rs-23(Chintamani, Shimoga, Kolar and Chikkmangalur) formed second cluster, Rs-11 and Rs-12 of (Chalageri and Arabhavi) isolates were found in third cluster, isolates Rs-7 and Rs-9 (Hosalli and Doddaballapur) were found in fourth cluster with high genetic similarity. Rs-15, Rs-17 and Rs-24 of Mysore, Chikkaballapur and Davangere isolates were found in fifth cluster. Isolates Rs-8, Rs-14, Rs-20, Rs-13 and Rs-16 isolates of Bannikoppa, Khanapur, Shimoga, Gokak and Hunsur were found in sixth cluster. Isolates of Rs-10 and Rs-21 (Ranebennur and Ramanagar) isolates were found in seventh cluster. The genetic relation between Rs-1, Rs-3 and Rs-2 (Dharwad Garag and Sapthapur) and isolates Rs-4 and Rs-5 (UAS Bangalore and Doddaballapur) may by correlated to their geographical affiliations as they grouped into same clusters. However,

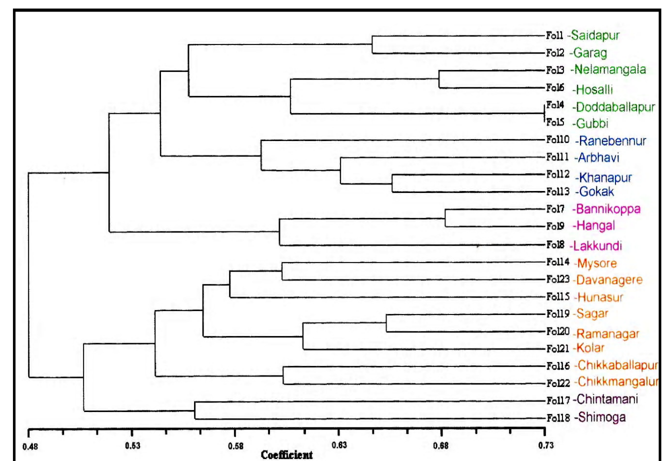

Fig. 2 : Dendrogram for twenty three isolates of *Ralstonia salanacearum* based on RAPD analysis

Table 2 : Similarity coefficient of twenty four isolates of *Ralstoniasolanacearum*

Isolates	Rs 1	Rs 2	Rs 3	Rs 4	Rs 5	Rs 6	Rs 7	Rs 8	Rs 9	Rs 10	Rs 11	Rs 12	Rs 13	Rs 14	Rs 15	Rs 16	Rs 17	Rs 18	Rs 19	Rs 20	Rs 21	Rs 22	Rs 23	Rs 24
Rs 1	1.00																							
Rs 2	0.52	1.00																						
Rs 3	0.53	0.51	1.00																					
Rs 4	0.47	0.47	0.49	1.00																				
Rs 5	0.44	0.44	0.49	0.46	1.00																			
Rs 6	0.43	0.40	0.43	0.36	0.50	1.00																		
Rs 7	0.24	0.21	0.23	0.22	0.30	0.31	1.00																	
Rs 8	0.20	0.23	0.31	0.28	0.38	0.35	0.37	1.00																
Rs 9	0.29	0.21	0.27	0.25	0.23	0.30	0.61	0.31	1.00															
Rs 10	0.26	0.19	0.29	0.24	0.35	0.26	0.23	0.32	0.27	1.00														
Rs 11	0.25	0.24	0.28	0.26	0.43	0.35	0.25	0.25	0.29	0.29	1.00													
Rs 12	0.28	0.28	0.35	0.29	0.32	0.38	0.27	0.32	0.26	0.28	0.36	1.00												
Rs 13	0.29	0.28	0.32	0.33	0.37	0.36	0.32	0.42	0.29	0.28	0.23	0.30	1.00											
Rs 14	0.29	0.21	0.23	0.24	0.38	0.42	0.38	0.46	0.29	0.33	0.27	0.31	0.44	1.00										
Rs 15	0.25	0.26	0.28	0.21	0.34	0.22	0.33	0.29	0.33	0.29	0.31	0.27	0.31	0.25	1.00									
Rs 16	0.29	0.27	0.32	0.24	0.37	0.35	0.30	0.42	0.32	0.29	0.26	0.30	0.59	0.47	0.35	1.00								
Rs 17	0.22	0.23	0.27	0.28	0.36	0.36	0.35	0.29	0.35	0.29	0.29	0.27	0.32	0.31	0.51	0.36	1.00							
Rs 18	0.27	0.26	0.30	0.26	0.33	0.36	0.25	0.36	0.28	0.29	0.35	0.31	0.39	0.34	0.27	0.41	0.24	1.00						
Rs 19	0.39	0.32	0.36	0.33	0.38	0.37	0.25	0.25	0.25	0.28	0.34	0.33	0.30	0.31	0.29	0.32	0.26	0.42	1.00					
Rs 20	0.32	0.29	0.29	0.24	0.40	0.36	0.44	0.47	0.41	0.39	0.28	0.34	0.44	0.49	0.35	0.50	0.37	0.37	0.29	1.00				
Rs 21	0.29	0.20	0.26	0.23	0.34	0.38	0.36	0.43	0.32	0.38	0.30	0.32	0.33	0.37	0.28	0.32	0.20	0.39	0.39	0.38	1.00			
Rs 22	0.34	0.19	0.33	0.25	0.33	0.36	0.26	0.26	0.20	0.31	0.30	0.30	0.29	0.29	0.26	0.35	0.27	0.39	0.45	0.35	0.32	1.00		
Rs 23	0.38	0.21	0.39	0.33	0.37	0.39	0.30	0.32	0.22	0.26	0.25	0.35	0.25	0.32	0.22	0.26	0.23	0.33	0.36	0.34	0.31	0.43	1.00	
Rs 24	0.33	0.33	0.39	0.28	0.41	0.39	0.35	0.33	0.33	0.28	0.31	0.31	0.32	0.33	0.42	0.39	0.43	0.36	0.37	0.43	0.35	0.42	0.34	1.00

majority of isolates with different geographical locations were found in same cluster. It may be sumrised that the population of *Ralstonia solanacearum* in Karnataka was genetically heterogeneous and the interrelationship among the different isolates can be reliably and precisely explained by RAPD marker. There are reports on the genetic diversity among the biovars of the pathogen (Jaunet and Wang, 1997; Gunathilake *et al.,* 2004).

REFERENCES

Baker, C. J., Neilson, M. N., Sequeria, L. and Keegstra, K.G. (1984). Chemical characterization of the lipopolysaccharide of *Pseudomonas solanacearum*. *Appl. & Env. Microbiol.*, **47**:1096-1100.

Cook, D., Barlow, E. and. Sequeira, L. (1989). Genetics diversity of *Pseudomonas solanacearum* detection of RFLP with DNA probes that specify virulence and the hypersensitive responce.*Mol. Plant Microb. Interact.,* **2**:1 13-121.

Gunathilake, P. M. P. C. K., Bandra, J. M. R. S. and Somarajeewa, P. K. (2004). Genetic diversity and pathogenecity of *Ralstonia solanacearum*in tomato.*Tropical Agric. Res.,***16**:51-60.

Hendrick, C.A. and Sequeira, L.(1984). Lipopolysaccharide defective mutants of the wilt pathogen, *Pseudomonas solanacearum*. *Appl.& Env. Microbiol***.**, **48**: 94-101.

Hayward, A.C.(1991). Biology and epidemiology of bacterial wilt caused by *Pseudomonas solanacearum.Annu. Rev. Phytopathol.,* **29**:65-87.

Jaunet, T. and Wang, J.F. (1997). Population structure of *Ralstonia solanacearum* from a diseased nursery and tomato production fields in Taiwan. Bacterial wilt disease: Molecular and ecological aspects. *Springer-Verlag, Berlin*, pp. 82-87.

Sambrook, J. and Rausell, B.W. (2001). *Molecular cloning- A laboratory manual,* (3rd Ed.), Cold Spring Harbor Laboratory Press, NEW YORK.

Williams, J.G.K., Kubelik, A.R., Livak, K. J., Rafalski, J. A. and Tingey, S.V. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res*., **18**: 6531-6535.

Yabuuchi, E., Kosako, Y., Yano, I., Hotta, H. and Nishiuchi, Y. (1995). Transfer of two *Burkholderia* and an alcaligenes species to *Ralstonia* gen. nov-proposal of *Ralstonia pickettii* (Ralston, Palleroni and Doudoroff 1973) comb.nov, *Ralstonia solanacearum*(Smith, 1896) comb. nov. and *Ralstonia eutropha* (Davis, 1969) comb. nov.*Microbiol.Immunol.*, **39**: 897-904.

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