

## Screening of Chilli germplasms for Resistance Against Soil Borne Pathogen *Ralstonia solanacearum* causing Bacterial Wilt

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(Received: 07 July 2023; Revised: 04 August 2023; Accepted: 05 September 2023; Published: 15 September 2023)  
(Published by Research Trend)

**ABSTRACT:** Bacterial wilt caused by *Ralstonia solanacearum* is one of the most serious soil borne disease causing yield loss upto 30-100% in Solanaceous vegetables. Several methods have been tried to control bacterial wilt but they are ineffective and not economically feasible. This study was aimed to develop resistant lines against bacterial wilt. Three week old chilli seedlings of 50 accessions each, were root dipped with bacterial suspension and screened against *Ralstonia solanacearum* by transplanting seedlings into sick plot at Horticultural Research and Extension Centre (HREC), Devihosur, Haveri, Karnataka, during summer 2019-20. Out of which, none of the varieties/accessions found immune or highly susceptible to bacterial wilt incited by *Ralstonia solanacearum*. Only 4 varieties viz., Ujwala, Anugraha, Khandari and Utkal Ava showed 15% wilt incidence and were categorized as resistant. Utkal Rasmi, KDC-1, DCA-21, 20 indigenous collections and 2 exotic collections revealed 21-40% wilt incidence were regarded as moderately resistant. Byadgi Kaddi, Byadgi Dabbi and Pusa Jwala were grouped under susceptible lines (61-80%) and rest of the varieties which showed percent wilt incidence of 41-60% were found to be moderately susceptible. On the basis of present investigation, it is concluded that chilli accessions showing resistant to moderately resistance reaction to bacterial wilt can be further utilized for hybridization and crop improvement programme to develop resistance hybrids against bacterial wilt.

**Keywords:** Bacterial wilt resistance, *Ralstonia solanacearum*, Chilli, Screening.

### INTRODUCTION

Bacterial wilt caused by *Ralstonia solanacearum*, is one of the most economically important diseases of tomato (*Solanum lycopersicum* L.), brinjal (*Solanum melongena* L.), chilli (*Capsicum annum* L.), potato (*Solanum tuberosum* L.) and other solanaceae plants in the tropics and subtropics (Hayward, 1994; Genin and Denny 2012). This bacterium invades the plant body through wounded skin layers of the roots, immigrates into the vascular system and spreads into the wood tissue system. Due to pathogenicity related factors, such as viscous polysaccharides, bacterial cells themselves, and various enzymes, the conduit part is occluded to

reduce the water-passing ability, leading to yellowing of foliage, causing wilting symptoms and eventually plant death (Huet, 2014; Rahman *et al.*, 2002; Wang *et al.*, 1998). *R. solanacearum* occurs around the world mainly in tropical, subtropical, and temperate regions, with more than 200 plant species recognized as hosts (Denny 2006; Hayward 1991, 1994).

*R. solanacearum* is characterized by diverse phenotypic and genetic variations. According to the host range and physiological traits, it has been classified into five races and six biovars (Buddenhagen *et al.*, 1962; Hayward 1964). The phylogenetic analysis mainly classified into four phylotypes, depending on the geographical origin:

phylotype I (Asia), II (Americas), III (Africa), and IV (Indonesia) (Fegan and Prior 2005; Horita *et al.*, 2014). Several methods have been used to control bacterial wilt, including soil disinfection, soil amendment, biological and chemical controls, and resistant cultivars or rootstocks for grafting (Pradhanang *et al.*, 2011; Fujiwara *et al.*, 2011; Hu *et al.*, 2012; Keatinge *et al.*, 2014). Chemical control is not economically practical, especially in the field, due to the localization of the pathogen inside the xylem and its ability to survive at high depths in the soil (Mansfield *et al.*, 2012). To date, there are no bactericides available to efficiently control bacterial wilt (Grimault *et al.*, 1994; Hartman *et al.* 1993). Antibiotics such as penicillin, ampicillin, tetracycline and streptomycin have been reported as having little efficacy in repressing *R. solanacearum* growth (Murakoshi and Takahashi 1984), particularly in open fields. Previous studies have reported that biological control using different strains of *Pseudomonas fluorescens*, *Bacillus subtilis*, *B. amyloliquefaciens*, and rhizobacteria could suppress soil-borne diseases, including bacterial wilt, but validation on a larger scale is still needed (Weller, 1988; Lemessa and Zeller 2007; Singh *et al.*, 2012). Breeding for resistance to bacterial wilt is still the most appropriate, economical, and environmentally promising strategy for controlling this pathogen (Huet *et al.*, 2014; Boshou *et al.*, 2005). However, the development of resistant cultivars has been hampered by polygenic inheritance, and sometimes the association of resistance with horticulturally detrimental traits associated with the wild species.

## MATERIALS AND METHODS

The present investigation entitled “Screening of chilli germplasm for resistance against soil borne pathogen *Ralstonia solanacearum* causing bacterial wilt” was carried out at the Horticultural Research and Extension Centre (HREC) Devihosur, Haveri, Karnataka, during summer 2019-20. Fifty diverse chilli germplasm seeds collected from different sources (Table 1) were sown in nursery beds during summer 2019-20. Three week old seedlings were transplanted into sick plot at a distance of 15 × 15 cm with two replications along with one control and methodology for isolation of plant pathogenic bacteria *Ralstonia solanacearum* and artificial inoculation method is as follows

**Table 1: List of chilli genotypes from different sources used for the study.**

Sr. No.	Genotype	Source
1.	Ujwala	KAU, Thrissur, Kerala
2.	Anugraha	KAU, Thrissur, Kerala
3.	Khandari	KAU, Thrissur, Kerala
4.	UtkalRashmi	OUAT, Odisha
5.	Utkal Ava	OUAT, Odisha
6.	PusaJwala	IARI, New Delhi
7.	Pant C-1	GBPUAT, Uttarakhand
8.	PhuleJyothi	MPKV, Rahuri, Maharashtra
9.	KDC-1	HREC, Devihosur
10.	DCA-21	HREC, Devihosur
11.	GCS-94/68	HREC, Devihosur
12.	ByadgiKaddi	HREC, Devihosur

13.	ByadgiDabbi	HREC, Devihosur
14.	IC-264468	NBPGR, New Delhi
15.	IC-265198	NBPGR, New Delhi
16.	IC-265199	NBPGR, New Delhi
17.	IC-275953	NBPGR, New Delhi
18.	IC-278306	NBPGR, New Delhi
19.	IC-283328	NBPGR, New Delhi
20.	IC-343788	NBPGR, New Delhi
21.	IC-347044	NBPGR, New Delhi
22.	IC-362007	NBPGR, New Delhi
23.	IC-362010	NBPGR, New Delhi
24.	IC-362012	NBPGR, New Delhi
25.	IC-362020	NBPGR, New Delhi
26.	IC-362023	NBPGR, New Delhi
27.	IC-362025	NBPGR, New Delhi
28.	IC-369588	NBPGR, New Delhi
29.	IC-369592	NBPGR, New Delhi
30.	IC-413048	NBPGR, New Delhi
31.	IC-505241	NBPGR, New Delhi
32.	IC-505305	NBPGR, New Delhi
33.	IC-505476	NBPGR, New Delhi
34.	IC-537588	NBPGR, New Delhi
35.	IC-537595	NBPGR, New Delhi
36.	IC-537650	NBPGR, New Delhi
37.	IC-565066	NBPGR, New Delhi
38.	IC-565072	NBPGR, New Delhi
39.	IC-572456	NBPGR, New Delhi
40.	IC-572468	NBPGR, New Delhi
41.	IC-572470	NBPGR, New Delhi
42.	IC-572475	NBPGR, New Delhi
43.	IC-572487	NBPGR, New Delhi
44.	IC-572491	NBPGR, New Delhi
45.	IC-572493	NBPGR, New Delhi
46.	IC-572495	NBPGR, New Delhi
47.	EC-399562	NBPGR, New Delhi
48.	EC-399565	NBPGR, New Delhi
49.	EC-399572	NBPGR, New Delhi
50.	EC-402101	NBPGR, New Delhi

IC – Indigenous Collection, EC- Exotic Collection, NBPGR- National Bureau of Plant Genetic Resources, KAU – Kerala Agricultural University, OUAT- Odisha University of Agriculture and Technology, IARI- Indian Agricultural Research Institute, GBPUAT- Govind Ballabh Pant University of Agriculture and Technology, MPKV- Mahatma Phule Krishi Vidyapeeth, HREC– Horticultural Research and Extension Centre.

**Isolation of *Ralstonia solanacearum*.** The pathogen *Ralstonia solanacearum* was isolated from bacterial wilt infected plants from the field. The culture was prepared from the ooze of infected plants which was streaked on to the petriplates containing TTC (2, 3, 5 Triphenyl Tetrazolium Chloride) medium (Fig. 1). Petriplates were incubated at room temperature (26±2°C) and examined daily for the growth of the pathogen. Within 24-48 hours the virulent strain bacterium was observed as creamy white growth with pointed pink colour at the centre of the colony (Chandrashekar *et al.*, 2006; Sridhar, 2012; Raghu, 2014). Sub culturing was done with the single colony of the prepared culture plate on TTC medium. The petriplates with multiplied cultures were maintained in the refrigerator at 4°C for further studies.

The inoculum was prepared by scrapping off colonies from the medium using a sterile glass slide and suspending in 100 ml sterile distilled water. The

concentration of the inoculum was then adjusted to 0.3 OD at 600nm ( $1.0 \times 10^6$  cfu/ml) using spectrophotometer following serial dilution method.

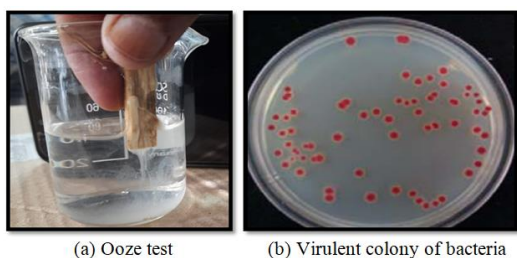


Fig. 1.

**Artificial inoculation on plants. Sick plot experiment.** Sick plots were prepared at Horticultural Research and Extension Centre, Devihosur, Haveri. Three week old seedlings were root dipped with bacterial suspension culture and transplanted to sick plot (Fig. 2). Plants without root dip with bacterial suspension culture were served as the control. Disease incidence in plants were monitored daily and the severity of the disease incidence in selected varieties were scored according to the scoring system reported by Winstead and Kelman 1952 and followed by Hussian *et al.* (2005).



Fig. 2. General view of sick plot screening against bacterial wilt at HREC, Devihosur, Haveri.

**Scoring schedule:**

**Percent wilt Incidence (PWI):** The incidence of bacterial wilt was recorded at two week interval after inoculation by using the following formula

$$\text{Wilt incidence (\%)} = \frac{\text{Number of wilted plants}}{\text{Number of total tested plants}} \times 100$$

Sr. No.	Disease Reaction	PWI
1.	Highly Resistant (HR)	0
2.	Resistant (R)	1-20
3.	Moderately Resistant (MR)	21-40
4.	Moderately Susceptible (MS)	41-60
5.	Susceptible (S)	61-80
6.	Highly Susceptible (HS)	>80

Table 2: Analysis of variance for bacterial wilt incidence among the accessions in chilli

Sr. No.	Source of variation/Characters	Replications	Treatments (Genotypes)	Error	S.Em ±	CD (5%)	CD (1%)
	Degrees of freedom		49	49			
1.	Bacterial wilt (PWI)	121.00	455.59**	51.61	5.08	14.44	19.25

\*\*Significant at 1%

**Screening of chilli germplasm lines for bacterial wilt resistance.** Artificial inoculation was carried out by root dipping of three week old seedlings at concentration of  $1.0 \times 10^6$  cfu/ml and then transplanted into sick plot (Fig. 2). Within 5-6 days the infected plants started showing symptoms of bacteria caused by *Ralstonia solanacearum* which was confirmed with the ooze test. One month after inoculation with the pathogen the disease severity were scored in accordance to the scale and genotypes were categorized into 6 groups, based on the percent wilt incidence. Out

The bacterial wilt disease severity was scored by using an arbitrary scale as described by Winstead and Kelman (1952) and followed by Hussian *et al.* (2005) and each plant was scored in accordance to the scale and the genotypes were categorized as resistant and susceptible based on the reaction to wilt incidence.

**RESULTS AND DISCUSSION**

**Analysis of variance.** The results of analysis of variance among the 50 genotypes of chilli revealed that the mean sum of squares due to genotypes were highly significant for bacterial wilt incidence This suggested the presence of substantial amount of genetic variation among the genotypes that could be exploited in identification of lines resistance to bacterial wilt. The result of analysis of variance for bacterial wilt incidence is presented in Table 2.

of 50 accessions, none of the germplasms found highly susceptible (>80%), highly resistant or immune (0%) to bacterial wilt (Table 3). Only four varieties Ujwala, Anugraha, Khandari and Utkal Ava showed less than 20% wilt incidence and were categorized as resistant. Utkal Rasmi, KDC-1, DCA-21, 20 indigenous collections and 2 exotic collections (Table 4) revealed percent wilt incidence between 21-40%. So, they were grouped as moderately resistant. Byadgi Kaddi, Byadgi Dabbi and Pusa Jwala were grouped as susceptible lines (61-80%) and rest of the varieties which showed PWI

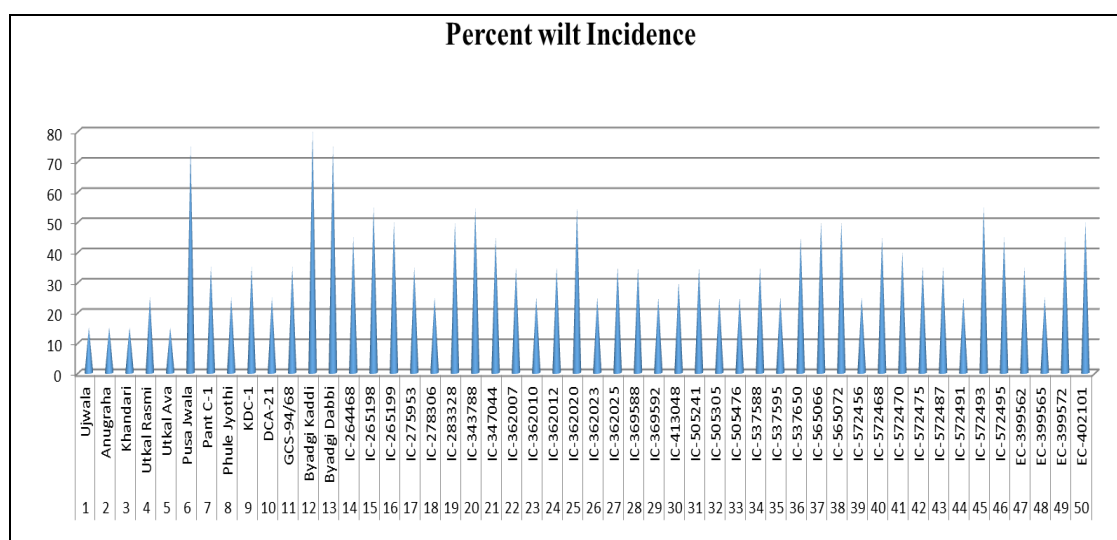
of 41-60% were found to be moderately susceptible (Table 4). Four chilli varieties Ujwala, Anugraha, Khandari and Utkal Ava released from Kerala Agriculture University (KAU), Kerala and Odisha University of Agriculture and Technology (OUAT), Odisha, respectively, showed 15% of resistance to bacterial wilt which confirms resistance to bacterial wilt (Fig. 3). Similar findings as reported by Sridhar (2012); Gopalakrishnan and Peter (1991); James *et al.* (2017); Thakur *et al.* (2014); Mathew, (2020).

**Table 3: Screening of chilli germplasm against bacterial wilt incidence.**

Sr. No.	Genotypes	Bacterial wilt (PWI)	Disease reaction
1.	Ujwala	15.00	R
2.	Anugraha	15.00	R
3.	Khandari	15.00	R
4.	UtkalRasmi	25.00	MR
5.	Utkal Ava	15.00	R
6.	PusaJwala	75.00	S
7.	Pant C-1	35.00	MR
8.	PhuleJyothi	25.00	MR
9.	KDC-1	35.00	MR
10.	DCA-21	25.00	MR
11.	GCS-94/68	35.00	MR
12.	ByadgiKaddi	80.00	S
13.	ByadgiDabbi	75.00	S
14.	IC-264468	45.00	MS
15.	IC-265198	55.00	MS
16.	IC-265199	50.00	MS
17.	IC-275953	35.00	MR
18.	IC-278306	25.00	MR
19.	IC-283328	50.00	MS
20.	IC-343788	55.00	MS
21.	IC-347044	45.00	MS
22.	IC-362007	35.00	MR
23.	IC-362010	25.00	MR

24.	IC-362012	35.00	MR
25.	IC-362020	55.00	MS
26.	IC-362023	25.00	MR
27.	IC-362025	35.00	MR
28.	IC-369588	35.00	MR
29.	IC-369592	25.00	MR
30.	IC-413048	30.00	MR
31.	IC-505241	35.00	MR
32.	IC-505305	25.00	MR
33.	IC-505476	25.00	MR
34.	IC-537588	35.00	MR
35.	IC-537595	25.00	MR
36.	IC-537650	45.00	MS
37.	IC-565066	50.00	MS
38.	IC-565072	50.00	MS
39.	IC-572456	25.00	MR
40.	IC-572468	45.00	MS
41.	IC-572470	40.00	MR
42.	IC-572475	35.00	MR
43.	IC-572487	35.00	MR
44.	IC-572491	25.00	MR
45.	IC-572493	55.00	MS
46.	IC-572495	45.00	MS
47.	EC-399562	35.00	MR
48.	EC-399565	25.00	MR
49.	EC-399572	45.00	MS
50.	EC-402101	50.00	MS
	<b>Mean</b>	<b>37.60</b>	
	<b>SEm±</b>	<b>5.08</b>	
	<b>CD @5%</b>	<b>14.44</b>	
	<b>CV (%)</b>	<b>19.11</b>	

R-Resistant; S-Susceptible; MR-Moderately Resistant; MS-Moderately Susceptible



**Fig. 3.** Reaction of chilli genotypes against *Ralstonia solanacearum* by artificial inoculation.



**Table 4: Grouping of chilli germplasm against Bacterial wilt reaction.**

PWI	Disease reaction	Germplasm lines
0 %	Highly Resistant (HR)	Nil
1-20%	Resistant (R)	Ujwala, Anugraha, Khandari and Utkal Ava.
21-40%	Moderately Resistant (MR)	UtkalRasmi, KDC-1, DCA-21, IC-275953, IC-278306, IC-362007, IC-362010, IC-362012, IC-362023, IC-362025, IC-369588, IC-369592, IC-413048, IC-505241, IC-505305, IC-505476, IC-537588, IC-537595, IC-572456, IC-572470, IC-572475, IC-572487, IC-572491, EC-399562, EC-399565.
41-60%	Moderately Susceptible (MS)	Pant C-1, PhuleJyothi, GCS 94/68, IC-264468, IC-265198, IC-265199, IC-283328, IC-343788, IC-347044, IC-362020, IC-537650, IC-565066, IC-565072, IC-572468, IC-572493, IC-572495, EC-399572, EC-402101
61-80%	Susceptible (S)	ByadgiKaddi, ByadgiDabbi, PusaJwala
>80%	Highly Susceptible (HS)	Nil

PWI- Percent Wilt Incidence

## CONCLUSIONS

On the basis of present investigation, it is concluded that chilli accessions showing resistant to moderately resistance reaction to bacterial wilt can be further utilized for hybridization and crop improvement programme to develop resistance hybrids against bacterial wilt.

**Acknowledgement.** We are grateful to acknowledge the financial support received from Department of Science and Technology, Ministry of Science and Technology, New Delhi, for conducting this work under the research titled “Genetic mapping for bacterial wilt resistance and heritability studies in chilli (*Capsicum annuum* L.). Authors are sincerely thankful to National Bureau of Plant Genetic Resources (NBPGR), New Delhi, for providing the seed materials of different chilli accessions to the first author.

**Conflict of Interest.** None.

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**How to cite this article:** Thilak J.C., Ajjappalavara P.S., Ganiger V.M., Sandhyarani N., Ramangouda S.H., Abdul Kareem M. and Vinutha D.B. (2023). Screening of Chilli germplasm for Resistance Against Soil Borne Pathogen *Ralstonia solanacearum* causing Bacterial Wilt. *Biological Forum – An International Journal*, *15*(9): 913–918.