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Screening of Tomato Cultivars through Qualitative and Quantitative Evaluation for Bacterial Canker (*Clavibacter michiganensis* subsp *michiganensis)* in Open and Protected Conditions

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Authors' contributions

This work was carried out in collaboration between all authors. Author KV designed and coordinated the study. Author RT executed the experiments, performed the statistical analysis and wrote the first draft of the manuscript. Author RT managed the literature searches, designed the protocol and managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Twenty-five tomato cultivars were evaluated in two growing season and two growth conditions against bacterial canker (*Clavibacter michiganensis* subsp *michiganensis*) at the Vegetable Research Centre (V.R.C.) Pantnagar. The experiment was completely randomized in controlled conditions (glass house) and randomized block design in open field conditions. The experiment was done by two methods, first, through alteration in the enzymatic concentrations of the ROS-scavenging enzymes estimation (quantitative method) and second, through symptom expression (qualitative method) in open and protected conditions. Out of the 25 cultivars screened between September 2015–January, 2016 for the first growing season (5-35ºC), disease appearance was observed in cultivar Pusa Ruby (21.60%) followed by Rohini (20.60%) and Arka Vikash (19.56%) in open field conditions. In the protected crop conditions of glasshouse the cultivar with maximum disease appearance was Arka Vikash with a disease severity of 24.1% followed by Rohini (19.50%)

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and Pusa Ruby (18.00%). During the second growing season (24-45°C), Feb 16–June 16, all the cultivars exhibited susceptible disease reaction in both the open field and glass house. The cultivar screening resulted in Phule Raja, a potential cultivar with resistance by delayed expression of the pathogen presence at the end of the growth period (May), while the remaining 24 varieties exhibited susceptible disease reaction early in the growth period.

Keywords: Tomatoes; Clavibacter michiganensis subsp michiganensis (Cmm); resistant cultivars; disease reaction; ROS scavenging enzymes.

1. INTRODUCTION

Bacterial canker of tomato [*Solanum lycopersicum* L.] is one of the dreadful bacterial diseases affecting the tomato crop [1,2]. It is caused by *Clavibacter michiganensis* subsp. *michiganensis,* first identified by (Smith) Davis and was described in 1910 in Michigan, USA [3] for the first time. The plant debris served as the primary sources of inoculums and the infected seed serves as the primary source of inoculum for the long distance dissemination of the pathogen [4]. The transmission rates of pathogen from seed to seedling can vary from 0.25 to 85% [5], and even a very low bacterial population density may result in infected seedlings [6]. The management of *Cmm* became complicated, primarily due to unavailability of resistant cultivars, and due to the seed-borne nature of the pathogen together with the absence of effective chemical control measures.

Plants when exposed to the biotic or abiotic stresses exhibit an increase in the concentration of Reactive Oxygen Species (ROS) causing significant plant cell damage. However, the plants possess the antioxidant defenses required for detoxifying the ROS. The ROS-scavenging enzymes in plant system have been extensively studied and the results demonstrate an increase in the enzymatic activity in response to environmental stress [7]. Studies describing correlations of high PPO levels, (ROS-scavenging enzymes) in cultivars or lines showing high pathogen resistance provides an evidence for the role of these enzymes in pathogen defense [8].

The present investigation was therefore undertaken to evaluate the 25 different cultivars for their disease reaction on the basis of enzymatic activities and the ambient environment condition (first growth season: 5-35°C and second growth season: 24-45°C) and thereby to understand the role of the enzymes and environment in the disease reaction of the host towards the pathogen.

2. MATERIALS AND METHODS

The experiments were performed in the protected conditions in the Dept. of Plant Pathology, GBPUA&T (Pantnagar) and the field trials were conducted at VRC, Pantnagar. The planting soil was of clay-loam texture in field. The maximum temperature ranges between 35-45°C and the minimum temperature ranges between 2-4°C during the experimental period.

2.1 Bacterial Culture and Its Growth Conditions

The bacterium *C. michiganensis* subsp. *michiganensis* was routinely sub-cultured in Nutrient broth – glucose-yeast medium (NGY: Nutrient Broth: 8.0 g, Yeast extract: 2.0 g, K_2HPO_4: 2.0 g, KH_2PO_4: 0.5 g, Glucose: 2.5 g, Agar: 15.0 g, in 1L of distilled water followed by sterilization at 121°C, at 15 psi for 15-20 min). The culture was stored and maintained on NGY slants at 4°C for short time duration, and in glycerol stock in -80°C for long time storage.

2.2 Inoculum Preparation

Inoculum was prepared from early log-phase cells which were obtained by growing the bacterial strain in nutrient yeast extract broth in 25 mL sterile tubes, incubated at 27±1° C on an orbital shaker at 200 rpm for 24 h. Bacterial culture was subsequently pelleted by centrifugation (twice, each at 3500 ***g*** for 5 min) and washed in sterile distilled water (SDW). The pellet thus, obtained were rinsed twice by sterilized water and adjusted to the value of 0.06 at OD 660nm that corresponds to 10^8 cfu / ml for inoculations [9]. The bacterial suspension was inoculated into the plants by a hypodermic syringe at the node of the first true leaf emergence [10].

2.3 Weather and Climatic Condition

Pantnagar in Uttarakhand state is situated in the Tarai region, in the foothills of Kumaon Himalayas with the geographical coordinates of 29°3´0" N, 79°31´0" E at an altitude of 243.84 MSL. The maximum temperature ranges between 35-45°C and the minimum temperature ranges between 2-4°C. The average rainfall is 1350 mm, most of which is received during southwest monsoons during months from July to September. The meteorological data was compiled during the crop growing season and is represented in the Table No. 5 and 6.

2.4 Recording of Weather Parameter, Relationship with Disease Reaction and Cultivar Screening

The data on the weather parameter viz., the temperature range ($T_{max.}$ and $T_{min.}$) and relative humidity (R.H. $_{max.}$ and R.H. $_{min.}$) were obtained from the Department of Agro-Meteorology, GBPUA&T, Pantnagar for both the growth seasons of the crop and the influence of the weather parameter on the disease reaction was analyzed on qualitative basis (symptom expression) as Highly Susceptible (HS), Susceptible (S), Moderately Susceptible (MS), Moderately Resistant (MR), Resistant (R) and Highly Resistant (HR) in the open field and Protected conditions (Table No. 5 and 6).

2.5 Glasshouse Experiment (Protected Conditions)

A soil mixture of sandy soil, vermicompost, and farmyard manure (2:1:1) was placed in polypropylene bags and sterilized in an autoclave for three consecutive days at 15 lbs. of pressure for 30 min, then 1.5 kg of the mixture was placed in plastic pots (15 x 10 cm). Seeds of the 25 tomato cultivars were surface sterilized with 1% sodium hypochlorite for 30 s, rinsed twice with sterile distilled water, and dried under a sterile air stream. Two pots with two plants per pot were used for each cultivar. The pots (14 inches dia.) were placed in the *glasshouse* and irrigation was provided as needed or at 2 day intervals until partial saturation. A cycle of 10:14 h dark: light and a temperature of 28±2°C were maintained in the glasshouse. The plants were inoculated with pathogen at 5th week stage. Disease reduction was monitored 20 days after infection by recording the disease incidence in infected plants in untreated challenged control plants and defense inducers-treated challenged plants.

2.6 Sample Collection and Biochemical Analysis

The leaf tissues were taken from 25 cultivars screened at two different durations i.e. before the pathogen inoculation and after the pathogen inoculation, to assess the change in the enzymatic activities. The samples were taken for biochemical analysis after 48hrs of pathogen inoculation in both the set of experiments to assess the change in enzymatic activity. The experiments were replicated thrice. For each treatment, leaf tissues were taken from each set of the treatment and stored in a deep freezer (-80 ºC) until used for biochemical analysis.

2.7 Biochemical Analysis

2.7.1 Peroxidase (POD) activity EC 1.11.1.7 assessment

Leaf samples (0.1 g) were homogenized in 2 ml of ice cold phosphate buffer (0.1 M/l), (pH 7.0), at 4°C, centrifuged at 16,000 g at 4°C for 15 min and the supernatant was used as enzyme source. The reaction mixture consisted of 1.5 ml pyrogallol (0.05 M/l), 0.05 ml enzyme extract and 0.5 ml H_2O_2 (1% v/v). Reaction mixture without enzyme served as control. The changes in the absorbance at 420 nm were recorded after 30 s intervals for 3 min. The enzyme activity was expressed as change in the unit / min / g FW [11].

2.7.2 Total Phenol Content (TPC) estimation

Total phenol content estimation was done in accordance to the procedure described [12]. Leaf tissues (0.1 g) were placed in 5 ml ethanol (95%) and were placed at 0°C for 48 h. Individual samples were homogenized followed by centrifugation at 10,000 rpm for 10 min. 1 ml of 95% ethanol, 5 ml of autoclaved distilled water and 0.5 ml of 50% Folin–Ciocalteau reagent was added to 1 ml of the supernatant, and the reaction mixture was shaken vigorously for proper mixing of the constituents. 1 ml of 5% sodium carbonate was added after 5 min, the reaction mixture was incubated at room temperature for an hour and the absorbance of the color developed was recorded at 725 nm. Standard curves were prepared for each assay using different gallic acid concentrations in 95% ethanol. Absorbance

values were converted to mg GA equivalents (GAE) per g FW.

2.7.3 Pathogenesis Related Protein 2 (β- 1, 3- glucanase) activity assay

The activity of β- 1, 3- glucanase activity was assayed by the laminarin - dinirosalicyclic acid method [13]. The reaction mixture consisted of 62.5µL of 4% laminarin and 62.5 µL of enzyme extract. The reaction mixture was carried out at 40°C for 10 min. The reaction was stopped by adding 375 µL of dinirosalicyclic acid and was heated for 5 min in boiling water, vortexed and the absorbance was measured at 500nm. The enzyme activity was measured as µg glucose released min^{-1} mg^{-1} protein.

2.7.4 Polyphenol Oxidase (PPO) activity assay (EC 1.14.18.1)

Leaf samples (0.1 g) were homogenized in 2 ml of ice cold phosphate buffer (0.1 M/l), at pH 6.5. The homogenate was centrifuged at 16000 rpm for 30 min at 4ºC, and the supernatant, thus, obtained was used directly in the enzyme assay. The reaction mixture consists of 0.4 ml catechol (1 mM/l) in 3 ml of (0.05 M/l) sodium phosphate buffer pH 6.5 and 0.4 ml enzyme extract. Only substrate containing reaction mixture served as control. The substrate for PPO estimation was catechol, and the change in absorbance was recorded at 405 nm [14]. The PPO enzyme activity was expressed as change in OD min/mg/ Fresh Weight (FW).

2.8 Qualitative Screening of the Germplasm in Response to Ambient Temperature Conditions in two Growth Seasons and Locations

The qualitative screening of the cultivars in the two growth season and two locations was conducted by evaluating the cultivars for symptom production one week after pathogen inoculation till the end of the growth period. The rating was done on the basis of symptom expression on plants ranging from HS- Highly susceptible, S-Susceptible, MS- Moderately susceptible, MR- Moderately resistant, R- Resistant and HR- Highly resistant.

2.9 Statistical Analysis

Analysis of variance (ANOVA) was performed using STPR software package versions 2 and 3, where significance level of 0.05 was used for all statistical interpretation.

3. RESULTS AND DISCUSSION

The enzymatic activity assay of the 25 cultivars evaluated in the protected condition revealed following results.

3.1 Biochemical Analysis

3.1.1 Peroxidase (POD) activity assay (EC 1.11.1.7)

The glasshouse screening of 25 cultivars /lines was done for the purpose of observing the difference in the peroxidase activities before and after the inoculation of the pathogen in different cultivars. An average range of temperature between 5 - 32°C during Sep 2015 – Jan 2016 and 24-45°C, during Feb – June, 2016 was observed under protected conditions of cultivation.

The maximum decline in the enzymatic activity was observed in the cultivar Ankit, which was recorded 3.92 before inoculation and declined to 1.17 after inoculation however, the minimum decline in the enzymatic activity was observed in the variety Phule Raja which was 1.23 before inoculation and declined to only 1.13 after inoculation (Table 1).

These findings indicate that the more is the decline in enzymatic activity, higher is the susceptibility of the cultivar towards the pathogen infection.

It has also been reported by many workers that phytohormones induce plant defense against many biotic and abiotic stresses and plant defense in addition to their impact on plant growth and development [15,16]. Simultaneous inclusion of phenolic compounds in the cell wall during incompatible plant–microbe/elicitor interactions can be associated with increase in POD activity [17].

3.1.2 Total Phenol Content (TPC) estimation

The glasshouse screening of 25 cultivars/lines was done in the protected condition (glasshouse) for the purpose of observing the difference in the TPC before and after inoculation of pathogen.

Table 1. Peroxidase activities in different tomato cultivars before and after the pathogen inoculation

S.No	Variety	Before inoculation	After inoculation	S.No	Variety	Before inoculation	After inoculation
1	Phule Raja	1.23	1.13	14	Ankit	3.92	1.17
2	Dhanshree	1.19	1.02	15	Lakshmi	1.16	0.58
3	Bhagyashree	0.86	0.63	16	Syngenta (To-1458)	0.83	0.89
4	Amrutha	1.17	0.61	17	PPT-1	1.21	1.03
5	Trisha	0.90	0.79	18	PPT-2	1.17	0.82
6	Calyx-248	1.52	1.31	19	Arka Vikash	1.38	0.91
7	Suricha	1.53	0.91	20	US 2853	1.35	0.92
8	Pradhan	1.06	0.86	21	Pusa Ruby	1.38	0.95
9	Shivam	0.84	0.81	22	Rohini	1.05	0.74
10	NTH2350	1.12	0.76	23	Noble	0.62	0.58
11	Vaishnavi 2082	0.91	0.85	24	CLN	0.59	0.37
12	Lyco	1.14	1.02	25	Siroji	0.58	0.48
13	Himgiri	0.63	0.51				
CD at 5%; a 0 .11; b 0.031; axb 0.15; CV 9.42							

a= interaction within the varieties
b= interaction within the time of inoculation
axb= interaction between varieties and time of inoculation

Table 2. The total phenolic content in different cultivars of tomato plants before and after the pathogen inoculation

S.No	Variety	Before inoculation	After inoculation	S.No	Variety	Before inoculation	After inoculation
1	Phule Raja	2981.70	2509.10	14	Ankit	3444.29	3609.23
2	Dhanshree	2700.89	1914.66	15	Lakshmi	2861.28	2289.15
3	Bhagyashree	3272.40	2805.60	16	Syngenta (To-1458)	2420.70	2530.83
4	Amrutha	3274.10	3383.20	17	PPT-1	7084.50	3471.26
5	Trisha	4009.80	2354.80	18	PPT-2	2392.79	3910.63
6	Calyx-248	2883.20	2750.78	19	Arka Vikash	2355.20	1716.27
7	Suricha	3180.20	3300.72	20	US 2853	2381.86	1868.63
8	Pradhan	3906.20	3019.72	21	Pusa Ruby	3173.70	3003.69
9	Shivam	2843.70	2481.19	22	Rohini	5016.84	3521.40
10	NTH2350	3174.20	3256.83	23	Noble	3840.20	4982.37
11	Vaishnavi 2082	2931.90	2817.34	24	CLN	2931.80	3067.83
12	Lyco	4114.71	2234.20	25	Siroji	2299.60	2385.61
13	Himgiri	1321.20	1519.25				
CD at 5%; a 2.30; b 0.65; axb 3.25; CV 0.16							

During screening of 25 different tomato cultivars/lines for the TPC (Table 2), the maximum phenolic content was observed in the line PPT-1 7084 µg/g FW which suddenly declined to 3471 µg/g FW after inoculation of the pathogen. The minimum decline in the phenolic content was observed in the cultivar Phule Raja, where, the phenolic content declined to 2509.10 µg/g FW after inoculation from the initial content of 2981.70 µg/g FW before inoculation. These findings indicate that more is the decline in the phenolic content; the higher is the susceptibility of the tomato cultivars.

The physiology and the plant metabolism can be altered by oxidation of phenols which may

produce many defensive compounds that helps the plant in surviving against different stresses either directly or through diverse plant signaling pathways [18].

3.1.3 Pathogenesis Related Protein 2 (β- 1, 3-glucanase) activity assay

The twenty-five tomato cultivars were screened in protected condition (glasshouse), for the purpose of observing the differences in the PR-2 protein activity before and after pathogen inoculation.

A maximum decline of 0.20 concentrations in the PR-2 protein activity was observed in the line PPT-1, in which the protein concentration before inoculation of the pathogen was 0.85 which was dropped to 0.65 after inoculation. The similar trend was observed in other cultivar Pusa Rubi, Rohini, Arka Vikash and US2853 showing a decline in PR 2 protein activity after inoculation of the pathogen.

The Pathogenesis Related proteins such as β-1, 3-glucanases (PR-2) and chitinases (PR-3) have been recognized to possess the enzymatic activities that lead bacterial cell wall hydrolysis [19]. These PR proteins breakdown the plant cell wall components which act as elicitors to plant defense responses [20]. These proteins have often been considered as the biochemical basis for induced resistance in plants [21], which serve as bimolecular markers for breeding purposes in crop improvement for disease resistance.

3.1.4 Polyphenol Oxidase (PPO) activity assay (EC 1.14.18.1)

The glasshouse screening of 25 cultivars / lines was done in the protected condition to assess the PPO activity before and after inoculation of pathogen in plants.

Amongst the 25 different varieties screened for the PPO activity (Table 4), the highest decline in PPO activity was observed in the cultivars Arka Vikash in which the PPO conc. before inoculation of the pathogen was 24.57 µg/g FW which declined to 9.81 µg/g FW after inoculation of the pathogen. The same cultivar also exhibited the highest susceptibility indicating that more is the decline in the PPO activity; the higher is the susceptibility of the cultivar toward the disease. The role of PPOs as one of the most important enzymes involved in plant defense against many biotic and abiotic stresses was also reported previously [22].

Table 3. The PR-2 protein (µg glucose released min^{-1} mg^{-1} protein) activity in different tomato cultivars before and after the pathogen inoculation

S.No	Variety	Before inoculation	After inoculation	S.No	Variety	Before inoculation	After inoculation
1	Phule Raja	0.21	0.17	14	Ankit	0.52	0.48
2	Dhanshree	0.24	0.21	15	Lakshmi	0.43	0.37
3	Bhagyashree	0.28	0.39	16	Syngenta (To-1458)	0.29	0.23
4	Amrutha	0.46	0.42	17	PPT-1	0.85	0.65
5	Trisha	0.41	0.38	18	PPT-2	0.53	0.38
6	Calyx-248	0.48	0.59	19	Arka Vikash	0.22	0.18
7	Suricha	0.80	0.72	20	US 2853	0.71	0.61
8	Pradhan	0.27	0.18	21	Pusa Ruby	0.26	0.17
9	Shivam	0.42	0.34	22	Rohini	0.29	0.18
10	NTH2350	0.69	0.62	23	Noble	0.27	0.21
11	Vaishnavi 2082	0.82	0.78	24	CLN	0.71	0.48
12	Lyco	0.49	0.42	25	Siroji	0.54	0.32
13	Himgiri	0.17	0.18				
CD at 5%; a		**0.021; b**	**0.0057; axb**	**0.028; CV**		**4.231**	

Table 4. The PPO activity (OD min/mg/ FW) in different tomato germplasm before and after the pathogen inoculation

S. No	Variety	Before inoculation	After inoculation	S. No	Variety	Before inoculation	After inoculation
1	Phule Raja	17.95	9.87	14	Ankit	9.19	7.62
2	Dhanshree	9.90	7.36	15	Lakshmi	8.65	7.75
3	Bhagyashree	22.8	5.44	16	Syngenta (To-1458)	8.28	7.62
4	Amrutha	8.49	8.26	17	PPT-1	6.75	6.83
5	Trisha	11.17	6.40	18	PPT-2	8.95	6.15
6	Calyx-248	10.95	7.50	19	Arka Vikash	24.57	9.81
7	Suricha	8.16	7.82	20	US 2853	28.60	12.50
8	Pradhan	6.65	6.59	21	Pusa Ruby	10.85	8.62
9	Shivam	8.23	7.81	22	Rohini	6.71	6.52
10	NTH2350	7.4	10.08	23	Noble	5.86	5.26
11	Vaishnavi 2082	10.64	8.59	24	CLN	13.19	12.35
12	Lyco	9.86	7.84	25	Siroji	16.25	14.88
13	Himgiri	11.20	8.71				
CD at 5%; a		**0.341; b**	**0.096; axb**		**0.482; CV**	**2.980**	

3.2 Qualitative Screening of the Tomato Germplasm in Response to Ambient Temperature Conditions in Two Growth Seasons and Locations

In contrast to Gram-negative plant-pathogenic bacteria, an incompatible reaction between *Cmm* and a tomato cultivar has not yet been found and all efforts to obtain resistant tomato cultivars by breeding so far have not been satisfactory.

From the perusal of the data presented in Table 5 it is evident that the seedlings of the entire tomato germplasm (all the lines/cultivars) expressed restricted disease symptoms on artificial inoculation with *Cmm*. Most of the cultivars showed resistant (R) and moderately resistant (MR) reaction against the bacterial canker disease. However, five cultivars viz., Lyco, Syngenta (To-1458), Arka Vikash, Pusa Ruby and Rohini exhibited moderately susceptible reaction amongst all the tested germplasm. Maximum disease expression was observed in cultivar Pusa Ruby (21.60%) followed by Rohini (20.60%) and Arka Vikash (19.56%). The minimum disease severity was observed in cultivar Phule Raja (7.70%), followed by Dhanshree (8.40%) and Bhagyashree (7.60%). For the protected conditions, results indicate that among the 25 cultivars in the protected condition when screened only one cultivar Phule Raja exhibited resistant disease reaction with restricted symptom expression and least disease severity (8.59%) while most of the variety exhibited moderately resistant disease reaction (Table 5). The variety showing maximum disease expression was Arka Vikash with a disease severity of 24.1% followed by Rohini (19.50%) and Pusa Ruby (18.00%).

From the perusal of data, Table 6, it is observed that most of the germplasm exhibited susceptible and moderately susceptible reaction however five cultivars Viz., Trisha, NTH2350, Lakshmi, Arka Vikash and Pusa Ruby exhibited highly susceptible disease reaction. The cultivar showing maximum disease severity was Arka Vikash (66.10%) followed by Trisha (66.00%) and Pusa Ruby (64.80%). The cultivar showing least disease severity was Phule Raja(23.90%) followed by Suricha (32.53%) and Calyx (33.90%).Out of the twenty-five cultivars screened in protected conditions, six cultivars exhibited moderately susceptible (MS) disease reaction, thirteen exhibited susceptible (S) disease reaction and six of them exhibited highly susceptible (HS) disease reaction (Table 6). Highest disease susceptibility was observed in the cultivar Arka Vikash (67.80%), followed by Pusa Ruby (65.90%) and NTH 2350(65.70%). The variety exhibiting the minimum disease severity was Phule Raja (29.00%) followed by line PPT-1(38.30%) and variety Calyx-248(40.10%).

Season of planting1- September 2015 – January 2016

Table 5. Reaction of tomato germplasm to bacterial canker of tomato (*Clavibacter michiganensis* subsp. *michiganensis*) in open and protected condition in Rabi season

S. No.	Variety/lines	Open conditions				Protected conditions			
		Disease severity* (%)	Disease reaction	Temperature (ºC)	RH (%)	Disease severity* (%)	Disease reaction	Temperature (ºC)	RH (%)
1.	Phule Raja	7.70(16.1)	R	9.4-26.4	32-82	8.59 (17.05)	R	12.1-28.8	37-88
2.	Dhanshree	8.40(16.8)	R	9.4-26.4	32-82	11.90 (20.17)	MR	11.8-26.4	44-87
3.	Bhagyashree	7.600(16.01)	R	9.4-26.4	32-82	12.83(20.99)	MR	11.8-26.4	44-87
4.	Amrutha	13.50(21.55)	MR	9.4-26.4	32-82	13.10 (21.21)	MR	11.8-26.4	44-87
5.	Trisha	14.0(21.97)	MR	9.4-26.4	32-82	13.70(21.72)	MR	11.8-26.4	44-87
6.	Calyx-248	13.80 (21.81)	MR	9.4-26.4	32-82	15.20 (22.94)	MR	11.8-26.4	44-87
7.	Suricha	13.80(21.80)	MR	9.4-26.4	32-82	15.16 (22.91)	MR	11.8-26.4	44-87
8.	Pradhan	15.50(23.18)	MR	9.4-26.4	32-82	16.10 (23.65)	MR	11.8-26.4	44-87
9.	Shivam	16.40(23.88)	MR	9.4-26.4	32-82	15.80(23.41)	MR	11.8-26.4	44-87
10.	NTH2350	17.73(24.91)	MR	9.4-26.4	32-82	14.06(22.02)	MR	11.8-26.4	44-87
11.	Vaishnavi 2082	16.20(23.733)	MR	9.4-26.4	32-82	12.80(20.96)	MR	12.1-28.8	37.88
12.	Lyco	17.40(24.65)	MS	6.8-22.2	48-96	17.56(24.77)	MS	9.4-26.4	32-82
13.	Ankit	16.63(24.06)	MR	9.4-26.4	32-82	15.40(23.10)	MR	10.2-27.8	38.4-91
14.	Lakshmi	13.50(21.55)	R	9.4-26.4	32-82	18.70(25.61)	MR	11.8-26.4	44-87
15.	Syngenta (To-1458)	19.40(26.25)	MS	8.3-23.3	46-93	18.70(25.61)	MS	9.4-26.4	32-82
16.	PPT-1	15.33(23.05)	MR	9.4-26.4	32-82	12.900(21.04)	MR	11.8-26.4	44-87
17.	PPT-2	14.46(22.35)	MR	9.4-26.4	32-82	13.700(21.72)	MR	11.8-26.4	44-87
18.	Arka Vikash	19.56(26.13)	MS	9.4-26.4	32-82	24.100(29.40)	MS	11.3-29	36-90.6
19.	US 2853	16.70(24.12)	MR	9.4-26.4	32-82	13.36 (21.44)	MR	11.8-26.4	44-87
20.	Pusa Ruby	21.60(27.69)	MS	6.8-22.2	48-96	18.00(25.10)	MS	11.3-29	36-90.6
21.	Rohini	20.60(26.99)	MS	8.3-23.3	46-93	19.50(26.20)	MS	11.3-29	36-90.6
22.	Noble	13.70(21.72)	MR	9.4-26.4	32-82	13.10(21.21)	MR	11.8-26.4	44-87
23.	CLN	14.60(22.46)	MR	9.4-26.4	32-82	13.80(21.80)	MR	11.8-26.4	44-87
24.	Siroji	15.20(22.94)	MR	9.4-26.4	32-82	15.10(22.86)	MR	11.8-26.4	44-87
25.	Himgiri	16.73(24.14)	MR	9.4-26.4	32-82	17.40(24.65)	MS	11.3-29	36-90.6
	SEM	*0.147*				*0.25*			
	CD at 5%	*0.418*				*0.72*			

**Figures in parentheses are angular transformed values*

Season 2: February - June 2016

Table 6. Reaction of tomato germplasm to bacterial canker of tomato (*Clavibacter michiganensis* subsp. *michiganensis*) in open and protected condition in Feb-June growth season

S. No.	Variety/lines	Open conditions				Protected conditions			
		Disease severity* (%)	Disease reaction	Temperature (ºC)	R.H. (%)	Disease severity* (%)	Disease reaction	Temperature (ºC)	R.H. (%)
1.	Phule Raja	23.90 (29.26)	MS	22.6-34.1	44.6-71.7	29.90 (33.14)	MS	26-35.3	55.7-68.3
2.	Dhanshree	62.9 (52.47)	HS	14.0-31.3	28-80	65.20 (53.84)	HS	16.2-33.5	31-75
3.	Bhagyashree	54.06 (47.33)	S	16.2-33.5	31-75	56.00 (48.44)	S	26.-35.1	51.7-79.4
4.	Amrutha	49.50 (44.71)	S	16.2-33.5	31-75	46.80 (43.16)	S	21.3-36.3	29.6-66.6
5.	Trisha	66.0 (54.33)	HS	14.0-31.3	28-80	70.10(56.87)	HS	16.2-33.5	31-75
6.	Calyx-248	39.90 (39.17)	MS	24.5-33.8	52.9-72.7	40.10 (39.27)	MS	26.6-34.1	44.6-71.7
7.	Suricha	32.53 (34.77)	MS	24.5-33.8	52.9-72.7	45.90(42.64)	S	17.9-37.8	29-64
8.	Pradhan	46.10 (42.76)	S	22.6-34.1	44.6-71.7	53.70(47.12)	S	17.9-37.8	29-64
9.	Shivam	56.63 (48.81)	S	22.6-34.1	44.6-71.7	57.90(49.54)	S	26.-35.1	51.7-79.4
10.	NTH2350	65.30 (53.90)	HS	13.5-29.1	37-83	65.70(54.15)	HS	21.8-38.7	33-68
11.	Vaishnavi 2082	55.70 (48.27)	S	19.2-36.0	30-66.7	58.70(50.010)	S	17.9-37.8	29-64
12.	Lyco	47.10 (43.33)	S	19.2-36.0	30-66.7	58.70(50.01)	S	17.9-37.8	29-64
13.	Ankit	23.00 (28.65)	MS	22.6-34.1	44.6-71.7	48.80(44.31)	MS	26.6-34.1	44.6-71.7
14.	Lakshmi	62.70 (52.35)	HS	13.5-29.1	37-83	62.59(52.29)	HS	16.2-33.5	31-75
15.	Syngenta (To-1458)	33.90 (35.60)	MS	23.7-33.8	48.4-72.1	48.70(44.255)	S	26.-35.1	51.7-79.4
16.	PPT-1	39.60 (38.99)	MS	23.7-33.8	48.4-72.1	38.30(38.23)	MS	26-35.3	55.7-68.3
17.	PPT-2	28.80 (32.45)	MS	23.7-33.8	48.4-72.1	41.80(40.28)	MS	25.5-32.9	67.7-86.3
18.	Arka Vikash	66.10 (54.39)	HS	16.2-33.5	31-75	67.80(55.42)	HS	16.2-33.5	31-75
19.	US 2853	47.70 (43.68)	S	16.2-33.5	31-75	58.70(50.010)	S	17.9-37.8	29-64
20.	Pusa Ruby	64.80 (53.61)	HS	14.0-31.3	28-80	65.90(54.27)	HS	16.2-33.5	31-75
21.	Rohini	30.70 (33.64)	MS	25.5-32.9	67.7-86.3	60.30(50.95)	S	22.6-34.1	44.6-71.7
22.	Noble	34.90 (36.21)	MS	25.5-32.9	67.7-86.3	33.80(35.54)	MS	26-35.3	55.7-68.3
23.	CLN	44.09 (41.61)	S	16.2-33.5	31-75	48.60(44.19)	S	22.6-34.1	44.6-71.7
24.	Siroji	52.60 (46.49)	S	16.2-33.5	31-75	52.80(46.60)	S	22.6-34.1	44.6-71.7
25.	Himgiri	58.90 (50.12)	S	25.9-31.2	74.9-91.1	57.80(49.48)	S	26.-35.1	51.7-79.4
	SEM	*0.11*				*0.685*			
	CD at 5%	*0.33*				*1.948*			

**Figures in parentheses are angular transformed values*

The limited symptoms expression of the disease was observed, in the varieties grown between September 2015 –January 2016, both in open field and protected condition, where the temperature was observed ranging between 5 - 32ºC during the growth period. However the tomato germplasm grown between Feb - June of the year 2016 with an observed temperature range of 26 - 45ºC both in open field and protected condition, the cultivars which could not exhibit symptoms under lower temperature also expressed susceptible disease reaction. The optimum temperature for the maximum symptom expression has been reported to be in the range of 25-30ºC [23].Expression of symptoms in some of the cultivars may be due to relative humidity (87-97%) that enhanced the symptoms in 2-3 week-old tomato seedlings [24]. These finding indicate that the temperature is one of the important factors for the expression of the disease both in open and protected conditions.

4. CONCLUSION

The quantitative and qualitative methods of analysis for the assessment of disease reaction in tomato germplasm, it was observed that environmental factors like temperature and humidity play an important role in disease development, besides genetic factors of inheritance in various cultivars. It was observed that with the decline in the enzymatic concentration of the ROS scavenging enzymes, the cultivars tend to be more susceptible towards the pathogen. Out of the 25 cultivars, screened for two seasons between 2015-2016, disease appearance was observed in cultivar Pusa Ruby (21.60%) followed by Rohini (20.60%) and Arka Vikash (19.56%) in open field conditions and in ArkaVikash (24.1%) followed by Rohini (19.5%) in the protected crop cultivation conditions, The Cultivar screening resulted in Phule Raja variety with resistance by delayed expression of the pathogen presence at the end of the growth period (May), while the remaining 24 varieties exhibited susceptible disease reaction early in the growth period.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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