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Screening of tomato (*Solanum lycopersicum* L.) hybrids for bacterial wilt resistance in pot culture

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Abstract
The project entitled "Screening of tomato (*Solanum lycopersicum* L.*)* Hybrids for yield and bacterial wilt resistance in pot culture" was carried out at the department of Olericulture, College of Agriculture, Vellayani, during 2016-2017. 10 hybrids were evaluated by completely randomized design. Screening in bacterial sick soil under field condition does not provide satisfactory results. An efficient artificial inoculation screening gives better results with respect to bacterial wilt resistance. Method followed for the artificial inoculation was, soil drenching along with root injury that increased the intensity of wilt compared to uninjured root. The studies revealed significant difference among treatments for percent disease incidence at a bacterial concentration of 10^5 cfu ml^{-1} as inoculum. It was lowest in LE 16 × Vellayani Vijai and resistant check (26.67%) whereas incidence was 100 per cent in the susceptible check. LE 20 × Manulekshmi. For bacterial wilt resistance, LE 16 × Vellayani Vijai was the best based inoculated trial.

Keywords: Tomato, bacterial wilt, pot culture, artificial innoculation

Introduction
Tomato (*Solanum lycopersicum* L.), belonging to the family Solanaceae, is the world's largest grown vegetable crop after potato. Tomato is universally treated as 'protective food'. It forms an essential part of human diet as an important source of vitamin A and C as well as minerals and carotenoids. Lycopene is a powerful antioxidant synthesized in tomato (Sangrit *et al.* 2011) [5] it is a good appetizer and good remedy for patients suffering from constipation and an important ingredient in the cocktail known as "Bloody Mary". Based on its importance, tomato is known as "Golden apple" or "Love apple" in England (Sharma *et al.* 2015) [6]. India is the second largest producer of tomato in the world. Tomato is grown in 7.67 lakh ha with a production of 163.84 lakh MT and productivity of 22.00t ha^{-1} in 2014-2015 (NHB, 2016) [3].
The tomato production scenario in India has changed tremendously over the past decade with the increase in popularity of hybrids in commercial cultivation. At the same time crop suffers from several biotic and abiotic stresses during its growing season. Among these stresses, bacterial wilt caused by *Ralstonia solanacearum* is one of the most devastating and wide-spread disease worldwide (Poussier *et al.* 1999) [4]. Bacterial wilt has become a serious problem in tomato grown in humid tropical regions of the world (Hayward, 1991) [1] and Kerala in particular. This disease has also become the limiting factor for raising tomato crop in Kerala. Warm humid tropical climate and acidic soil condition in Kerala favour the incidence of the disease. It is widely distributed and highly destructive. Use of resistant varieties is the most effective means for control, as the application of chemicals is practically ineffective
Even though there are many hybrids, still there is need to develop new hybrids having excellent yield and quality performance. Keeping in view the pace with which the hybrids of tomato are gaining popularity, it is imperative to obtain such hybrids having a complex of valuable attributes, *viz.* earliness, uniformity, high yield, resistance to diseases and adaptability to different environmental conditions. Yield is a complex character and selection for yield and yield components deserves considerable attention. A crop breeding programme, aimed at increasing the plant productivity requires consideration not only of yield, but also of its components that have direct or indirect bearing on yield. Hence it is the present day need to breed varieties/hybrids for bacterial wilt resistance combined with high yield and quality.

Materials and method
Bacterial wilt pathogen (*Ralstonia solanacearum*) was isolated from already infected tomato

plant and grown in pure culture using SM selective agar medium. Isolate was tested for pathogenicity and used for inoculation.

A single colony of the inoculants pathogen was transferred to CPG (Casamino acid-peptone-glucose) broth and incubated overnight. The OD of the suspension was made to 1.0 at 600 nm (approximate population of 10^7 cfu ml^{-1}). The bacterial growth was further diluted 100 fold for getting suspension with a population of 10^5 cfu ml^{-1}. Eight superior hybrids along with two standard checks one resistant (Jessica) and another susceptible (Naveen) were transplanted in pots. 5 ml of the bacterial suspension was poured to the base of the transplanted seedling. Two sets of experiments, one with higher bacterial population and the other with lower bacterial population, were carried out independently. Periodical observations on the appearance of wilt symptom were done.

The experiment was carried out in completely randomized design. For the experiment 10 hybrids ((8 + 2 (1 resistant + 1 susceptible)) were evaluated in 3 replications during October 2016 to January 2017. Observations like number of plants wilted, percentage disease incidence and days taken for wilting from inoculation were noted, Statistical analysis - Analysis of variance was computed based on Completely randomized design. The significance was tested by referring to the values of 'F' table.

$$Y_{ij} = u + t_i + e_{ij}$$

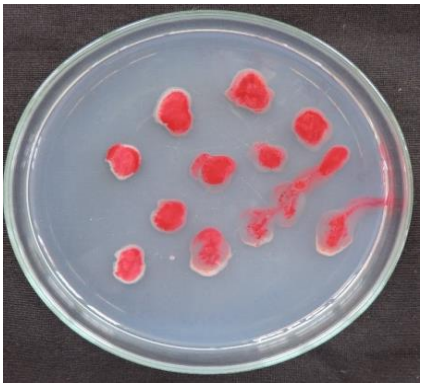
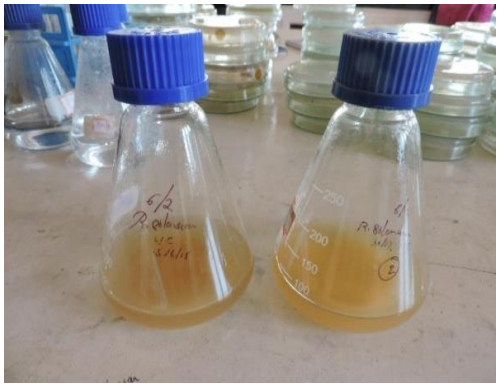
Where, Y_{ij} = phenotypic observation of i^{th} treatment and j^{th} replication; μ = general men; t_i = effect of i^{th} treatment; e_{ij} = random error associated with i^{th} treatment and j^{th} replication

Analysis of variance

Source	Degrees of freedom	Sum of squares	Mean sum of squares	F-ratio
Treatment	(t-1)	Tr. SS	Tr.MS = Tr. SS/(t-1)	F= Tr.MS/EMS
Error	(n-t)	ESS	EMS= ESS/(n-t)	
Total	(n-1)	TSS		

(t = number of treatments; r = number of replications (equal replications for all treatments); n = t × r = total number of observations; Tr. MS = mean sum of squares due to treatments; EMS = mean sum of squares due to error)

Preparation of bacterial inoculums

Result and discussion

An efficient artificial inoculation screening gives better results with respect to bacterial wilt resistance. Soil drenching is the best method of inoculation which is reliable, effective and exactly simulates the natural infection as the bacterium is soil borne and enters the plant through roots under field condition. Singh *et al.* (2014a) [7] used soil drenching along with root injury that increased the intensity of wilt compared to uninjured root. Hence in the present investigation, soil drenching along with root injury method was used.

Hybrids and checks were inoculated with higher concentration of inoculum (10^7 cfu ml^{-1}) and lower concentration of 10^5 cfu ml^{-1}. The results showed non-significance among the treatments with higher concentration of inoculum (10^7 cfu ml^{-1}) and significant difference with lower concentration of inoculum (10^5 cfu ml^{-1}). The results are presented in Tables 1, 2and 3.

The disease incidence ranged from 26.67 % to 100 %. Naveen (susceptible check) exhibited 100 % wilting in 9.33 days. Both LE 16 × Vellayani Vijai and Jessica (resistant check) exhibited 26.67 % disease incidence, in 8.50 and 8.00 days respectively. According to Kishan and Chand (1990) [2], as concentration of inoculum increases, mortality of plants also increase and takes less number of days for wilting.

Among the hybrids and checks, 4 plants out of 15 of LE 16 × Vellayani Vijai and resistant check (Jessica) wilted which is lower compared to other hybrids (Plate 1). With respect to percentage Disease Incidence (PDI) (%) among the hybrids and checks, LE 16 × Vellayani Vijai and resistant check (Jessica) (26.67%) showed lowest incidence followed by LE 16 × Anagha (40.00%), LE 13 × Manulekshmi, LE 20 × Manulekshmi and LE 13 × Vellayani Vijai (46.67%). 100% bacterial wilt incidence was exhibited by the susceptible check, Naveen. The LE 16 × Vellayani Vijai took 8.50 days and resistant check (Jessica) 8.00 days for 26.67% disease incidence where as susceptible check, Naveen took 9.33 days for 100% wilting (Plate 10).

The studies revealed significant difference among treatments for percent disease incidence at a bacterial concentration of 10^5 cfu ml^{-1} as inoculum. It was lowest in LE 16 × Vellayani Vijai and resistant check (26.67%) whereas incidence was 100 per cent in the susceptible check. LE 20 × Manulekshmi. For bacterial wilt resistance, LE 16 × Vellayani Vijai was the best based inoculated trial.

Table 1: Number of plants wilted at a concentration of 10^5 cfu ml^{-1} (Artificial inoculation)

Hybrids	Number of plants wilted (out of 15)
LE13 × Manulekshmi	7
LE 16 × Manulekshmi	8
LE 20 × Manulekshmi	7
LE 19 × Manulekshmi	7
LE 16 × Anagha	6
LE 19 × Anagha	8
LE 16 × Vellayani Vijai	4
LE 13 × Vellayani Vijai	7
Jessica (Check)	4
Naveen (Check)	15

Table 2: Performance of hybrids and checks for bacterial wilt incidence under pot culture

Hybrids	% disease incidence (10^7 cfu ml^{-1})	% disease incidence (10^5 cfu ml^{-1})
LE13 × Manulekshmi	73.33	46.67
LE 16 × Manulekshmi	66.67	53.33
LE 20 × Manulekshmi	73.33	46.67
LE 19 × Manulekshmi	80.00	46.67
LE 16 × Anagha	73.33	40.00
LE 19 × Anagha	73.33	53.33
LE 16 × Vellayani Vijai	46.67	26.67
LE 13 × Vellayani Vijai	73.33	46.67
Jessica (Check)	46.67	26.67
Naveen (Check)	100.00	100.00
CD (0.05)	NS	0.250

Table 3: Days taken for wilting at a concentration of 10^5 cfu ml^{-1}(Artificial inoculation)

Hybrids	% disease incidence (10^5 cfu ml^{-1})	Days taken for wilting
LE13 × Manulekshmi	46.67	7.78
LE 16 × Manulekshmi	53.33	8.78
LE 20 × Manulekshmi	46.67	7.89
LE 19 × Manulekshmi	46.67	6.78
LE 16 × Anagha	40.00	6.17
LE 19 × Anagha	53.33	7.28
LE 16 × Vellayani Vijai	26.67	8.50
LE 13 × Vellayani Vijai	46.67	8.33
Jessica (Check)	26.67	8.0
Naveen (Check)	100.00	9.33

Susceptible seedlings

Resistance seedlings

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