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Screening of F_6 Generation Superior Recombinant Inbreed Lines of Tomato for Bacterial wilt Disease Resistance under Sick Pot Condition

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Abstract: Sixteen superior recombinant inbred lines (RILs) of F6 generation, bacterial wilt resistant check Anagha and susceptible check ArkaVikas were screened for bacterial wilt disease resistance under sick pot condition. The experiment was laid out in completely randomized design. The results of screening showed that 6 superior RILs including resistant check Anagha are resistant, 4 moderately resistant, 3 moderately susceptible and 3 superior RILs including susceptible check ArkaVikas are susceptible to bacterial wilt disease.

Keywords: Bacterial wilt, resistance, recombinant inbred lines

INTRODUCTION

Tomato (*Solanumlycopersicum* L.) is the most economically important and widely grown vegetable crop in the world. It belongs to the Solanaceae family with its diploid chromosome number 2n =24 and believed to have its origin in the mountainous regions of the Andes comprising Peru, Ecuador and Chile. It is universally treated as a protective food because of its rich lycopene content- a powerful antioxidant, valued for anti-cancerous property worldwide (Bose *et al*., 2002) and generally eulogised as poor man's orange because of its nutritive value and attractive appearance. It is also a treasure of vitamin A, C and minerals. Tomatoes are used directly as raw vegetable in sandwiches and salad. It is regarded as No. 1 processing vegetable in the world. Various processed products *viz*., paste, puree, syrup, juice, ketchup, sauce, whole peeled tomato, *etc*. are prepared from tomato. Tomato puree and tomato paste have great export demand.

World-over, tomato cultivation spans over an area of 5.02 million hectare, with a production of 170.75 million tonnes. India is the second largest producer (11.5 %) after China (30.7 %) followed by U.S.A. (8.1 %). In India, an area of 0.81 million hectare is under tomato cultivation with an annual production of 19.67 million tonnes. Average productivity of tomato in India remains low at 24.36 t/ha as against 33.99 t/ha, the world average. Madhya Pradesh, Karnataka, Andhra Pradesh, Telangana, Gujarat, Odisha, West Bengal, Bihar and Maharashtra are the leading states in the production of tomato in India (Anon., 2024).

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Worldwide, tomato crop is affected by more than 50 biotic stresses. Among them, bacterial wilt caused by *Ralstoniasolanacearum* (Smith) a vascular pathogen, is a major constraint for production of tomatoes in tropics and subtropics (Yabuuchi*et al*., 1995). It causes yield loss up to 90.62 per cent (Ramakishun, 1987). Sometimes it destroys the entire crop (Rao *et al*., 1975).

The bacterial wilt disease in tomato was first reported from Italy in 1882 (Walker, 1952). In India, the bacterial wilt of tomato wasfirst reported from West Bengal (Hedayatullah and Saha, 1941). Presently the disease has been reported in all major tomato growing states like Madhya Pradesh, Karnataka, Andhra Pradesh, Telangana, Gujarat, Odisha, West Bengal, Bihar and Maharashtra owing to major yield loss. *Ralstoniasolanacearum* is an aerobic, non-sporing, gram-negative, soil borne plant pathogenic bacterium. It colonises in the xylem, causing bacterial wilt in a very wide range of potential host plants. Because of its devastating lethality, *R. solanacearum* is more intensively studied phytopathogenic bacteria and bacterial wilt of tomato is a model system for investigating mechanisms of pathogenesis.

The symptoms of the disease includes drooping of lower leaves of the infected plants. The whole plant may appear stunted before wilting occurs. Affected plants will rapidly wilt and die without yellowing of leaves. If cut stem sections are suspended in water, a milky bacterial ooze will flow from the cut surface. The decay of the pith indicates the advanced stage of the disease. These symptoms differentiates the bacterial wilt disease from other fungal wilts (Fusarium and Verticilliumwilt).

Ralstoniasolanacearum(Smith) can survive upto three years in the soil, even in the non-appearance of any hosts. With the progression of the disease, the pathogernquintessence near the root zone and found upto 45 cm depth. The disease spreads throughsoil, irrigation water, infected plant materials, farm implements *etc*. The pathogen survives insoil layersplant debris, and the rhizosphere of the weed hosts. *R. solanacearum* is known to have several races and infects more than 200 species in 50 families (Hayward, 1991), including tomato, potato, eggplant, pepper, tobacco, banana, chilli and peanut (French and Sequeira, 1970). Therefore, it is difficult to eradicate the disease. Though a few effective chemicals are available to manage this disease, their practical use under field conditions is limited due to soil borne nature of the pathogen and ecological concerns associated with the pesticides. Thus, host plant resistance is the most effective and economical means to manage this disease (Denny, 2006).

MATERIAL AND METHODS

The experiment has been carried in a completely randomized design. Recombinant inbred lines population were developed involving the cross combination of Vaibhav x Anagha. Further, these recombinant inbred line populations were evaluated for growth, yield and quality components with resistance to bacterial wilt by which superior recombinant inbred lines were identified. These superior recombinant inbred lines of F_6 generation were further screened for bacterial bacterial wilt disease resistance under sick pot condition in polyhouse along with resistant check Anagha and susceptible check ArkaVikas.

Sick pots harboring single isolate of bacterial wilt pathogen, *Ralstoniasolanacearum*culture were established. 16 superior recombinant inbred lines along with Anagha, a bacterial wilt resistant check and ArkaVikas, a bacterial wilt susceptible check were evaluated to assess their reaction to the bacterial wilt disease incidence under glasshouse condition with two replications. The lines that were resistant/ moderately resistant/ moderately susceptible/ susceptible to bacterial wilt disease were identified.

Inoculum material and its preparation

Collection of Sample

Ralstoniasolanacearum infected plants were collected from College of Horticulture, UHS Campus, GKVK, Bengaluru. When the longitudinal section of the collar portion of the disease infected plant was placed in a test tube containing clean water, fine white milky strands composed of masses of bacteria oozes out from the margin of the cut portion within a few minutes. This confirms the presence of pathogen.

Isolation of *Ralstoniasolanacearum*

The bacterial wilt affected plant was used for isolation. Using sterilized scalpel, the outer parts of infected material were removed. The cut pieces were placed in distilled water for 10 to 15 minutes. Through inoculation loop, ooze was streaked ontotriphenyltetrazolium chloride (TTC) media. The TTC media consists of peptone (10 g), dextrose (5 g), casein hydrolysate (1 g), agar (17 g)and 1 per cent TTC (5 ml) in one litre of distilled water.The streaked plates were incubated for 24 to 36 hoursat 31 ± 1^0 C. The virulent colonies were pink incolour at the centre with fluidal nature. They were isolated and then suspended in sterilized distilled water in screw capped vials and stored at room temperature. Colonies were regularly renewed and checked for virulence by plating on TTC medium.

Inoculation method

The *R. solanacearum* culture (OD_{600}= 0.3) was inoculated through soil drench method where, 5 ml of bacterial suspension is inoculated to each 30 days old seedlings around root zone with the help of micropipette. Before inoculation, the roots are slightly severed by sharp knife to ensure bacterial penetration through roots.

Phenotypic observation

The plants with bacterial wilt symptoms were counted and disease per cent was calculated. Accordingly, the lines were classified as resistant, moderately resistant and susceptible based on the percentage of disease incidence and grouped into following standard scale (Hussain*et al*., 2005).

Sl. No.	*Score*	*Disease reaction*	*Range*
1	0	Highly resistant (HR)	Plants did not show any bacterial wilt disease symptoms
2	1	Resistant (R)	1 - 20% plants wilted
3	2	Moderately resistant (MR)	21 – 40% plants wilted
4	3	Moderately susceptible (MS)	41 – 60% plants wilted
5	4	Susceptible (S)	61 – 80% plants wilted
6	5	Highly susceptible (HS)	More than 80% plants wilted

RESULTS AND DISCUSSION

Sixteen superior RILs, Anagha, a resistant check and ArkaVikas, a susceptible check were tested for bacterial wilt disease reaction in polyhouse. The per cent of wilted plants was estimated for all tomato genotypes. They were congregated into different groups based on the standard scale of Hussain*et al*., 2005.

Among 18 tomato genotypes, 8 genotypes were resistant, 4 moderately resistant, 3 moderately susceptible and 3 genotypes were susceptible to bacterial wilt disease (Table 1).

Superior RILs *viz*., COHBT-1 (5 %), COHBT-4 (15 %), COHBT-18 (15 %), COHBT-55 (10 %), COHBT-59 (5 %), COHBT-60 (10 %) and COHBT-69 (15 %) along with resistant check Anagha (5 %) were resistant to bacterial wilt.

Moderately resistant reaction to bacterial wilt was registered in COHBT-5 (25 %), COHBT-8 (25 %), COHBT-12 (30 %) and COHBT-52 (35 %). Whereas, COHBT-47 (45 %), COHBT-49 (60 %) and COHBT-50 (45 %) showed moderately susceptible reaction to bacterial wilt.

Susceptible check ArkaVikas (75 %) showed susceptible reaction to bacterial wilt along with COHBT-29 (75 %) and COHBT-65 (70 %).The findingsare in covenant with Gayathri (2004)

and Mane (2013) where, ArkaVikas showed susceptible reaction to bacterial wilt.

Seven superior RILs *viz*., COHBT-1, COHBT-4, COHBT-18, COHBT-55, COHBT-59, COHBT-60 and COHBT-69 along with resistant check Anagha were found to be bacterial wilt resistant and thus can be further used in breeding programs to develop bacterial wilt resistant cultivars.

Table 1: Reaction of superior RILs of tomato for bacterial wilt disease incidence in sick pot condition

Sl. No.	*Treatments*	*Per cent bacterial wilt incidence (%)*	*Disease reaction*
1	COHBT-1	5	R
2	COHBT-4	15	R
3	COHBT-5	25	MR
4	COHBT-8	25	MR
5	COHBT-12	30	MR
6	COHBT-18	15	R
7	COHBT-29	75	S
8	COHBT-47	45	MS
9	COHBT-49	60	MS
10	COHBT-50	45	MS
11	COHBT-52	35	MR
12	COHBT-55	10	R
13	COHBT-59	5	R
14	COHBT-60	10	R
15	COHBT-65	70	S
16	COHBT-69	15	R
17	ArkaVikas	75	S
18	Anagha	5	R

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

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