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Screening tomato genotypes for resistance against collar rot disease caused by *Alternaria solani* Sorauer

Suresh Reddy Yerasu*, Loganathan Murugan[1], Prasanna HC[2] and Arpita Singh

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Abstract

Alternaria solani is a soil borne pathogen causing heavy losses to tomato (*Solanum lycopersicum* L) crop worldwide. It causes early blight symptoms on foliage, collar rot symptom at basal stem region of seedlings, stem lesions on stem of adult plants and fruit rot symptoms on fruits. In comparison to early blight, collar rot screening and differentiating genotype into different resistance classes is relatively easy. To identify collar rot resistant genotypes thirty tomato accessions of the ICAR-Indian Institute of Vegetable Research, Varanasi, gene bank was screened by inoculating the pathogen under screen house conditions. Three genotypes including two tomato wild species LA 2325(*S. neorickii*) and WIR 3928 (yellow fruited wild species), and an advanced breeding line H-88-78-1 showed immune reaction to collar rot with zero mean disease severity index. The identified lines are being used as a source of resistance to *A. solani* to develop disease resistant cultivars.

Keywords: *Alternaria solani*, early blight, collar rot, biotic stress, resistance

Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most important vegetable crops of the world. Due to its culinary, nutritional, industrial, economic etc, importance it is grown in a wide range of climates and when open field cultivation is not possible due to extreme temperatures, the crop is being grown under different kind of protected structures. In India along with potato and onion, it is one of the most important vegetable. India ranks in second position in the total world production of tomatoes after China (FAO STAT 2017). In India, in the year 2017-18, tomato was grown in 0.79 million hectares with 19.7 million metric tons production (DAC database). Tomatoes are consumed in several ways like salad, mixed in other food items while cooking, different processed products like sauce, ketchup, puree, paste, soup, pickled etc. The diverse use and importance of the crop creates a demand for regular production and supply of tomatoes. However, the commercial production of tomato has been hindered by biotic stresses like viral, fungal diseases, insects, nematodes etc.

Early blight disease is a highly destructive fungal disease of tomato caused by fungus *Alternaria solani* Sorauer. in many tomato producing areas worldwide (Sherf and MacNab 1986). It caused fruit yield loss up to 79% in major tomato growing countries like Canada (Basu 1974), India (Datar and Mayee 1982), USA (Sherf and MacNab 1986), Nigeria (Gwary and Nahunnaro 1998), Australia, Israel and UK (Vloutoglou and Kalogerakis 2000).

A. solani produces air-borne spores (conidia) and can cause poly phyletic infection. Depending on the weather conditions, the pathogen can affect all the aerial parts of tomato plant in all different stages of the crop growth. Walker (1952), referred symptoms on foliage as early blight, symptoms on seedlings basal region as collar rot and symptoms on adult plant stem as stem lesions and symptoms on fruit as fruit rot. Early blight is characterized by dark, small, necrotic, coalescing lesions and target board like characteristic concentric lesions on the leaf surface. The lesions are surrounded by yellow rings (Sherf and MacNab 1986). Collar rot consists of initial dark, sunken lesions or cankers and proceeding to girdling the stem at ground level and disintegration of the vascular system (Foolad et al. 2008). When the stem is girdled by the lesion, seedlings become weak leading to a break in that region and subsequent death of the seedling. On the main stem and side branches of adult plants, the disease causes small, dark, slightly sunken

ICAR-Indian Institute of Vegetable Research, Varanasi, Uttar Pradesh
[1]ICAR-National Research Centre for Banana, Tiruchirappalli, Tamil Nadu
[2]ICAR-Indian Institute of Horticulture, Hessaraghatta, Karnataka
*Corresponding author; E-mail: yerasusureshreddy@gmail.com

areas that enlarge to form dark brown elongated spots which may have concentric rings. These spots are scattered along the stem and branches (Walker 1952). On fruits, the fungus causes dark, sunken, leathery and purple lesions on the stem-end. These lesions may expand in size and extend deep into the flesh of the fruit and makes the fruit unmarketable (Chaerani and Voorrips 2006).

Extensive work with respective to screening methods, identification of resistance sources, efforts to transfer the disease resistance etc. were done for early blight symptoms caused by the fungus. Relatively fewer reports are available on collar rot (Andrus et al. 1942; Gardner 1988, 2000; Gardner and Shoemaker 1999; Gardner and Panthee 2012) as it is less familiar among growers. Infected seedlings act as carrier of inoculum to main filed. Seedlings with collar rot may die or fail to grow vigorously after transplanting. Screening for leaf blight resistance under green house conditions has been a difficult task as distinguishing symptoms for different resistant classes was not enough to classify plants for levels of resistance (Barksdale 1969; Nash and Gardner 1988). This makes it very difficult to study the genetic resistance and transfer of the resistance to susceptible cultivars. Gardner (1990) reported close association between collar rot and leaf blight. This association made it easy to screen for collar rot resistance under green house conditions to identify plants with foliar resistance. In view of this importance for collar rot, thirty genotypes were screened for resistance against the disease.

Material and Methods

Plant material: Thirty tomato genotypes comprising four wild species, eight released varieties, advanced breeding lines and exotic collections (lines with a prefix EC; Table 1) from vegetable gene bank, ICAR-Indian Institute of Vegetable Research, Varanasi, India were screened for collar rot symptom caused by *A. solani.*

Screening for resistance to collar rot under screen house conditions: Seeds of all the tomato accessions were sown in pots filled with sterile potting mixture (Soil: Sand: well decomposed farm yard manure at 2:1:1) and were allowed for 4 weeks to grow. After four weeks, the seedlings were uprooted gently, inoculated and transplanted in big five kg pots filled with the potting mixture. Each pot contained three seedlings of different genotypes chosen randomly. In this way three replications with a seedling per replication of all the genotypes were accommodated in thirty pots.

For inoculum preparation, virulent *Alternaria solani* isolate UP-7 (Murugan et al. 2016) was established in Petri plates containing readymade V-8 agar medium (Himedia Pvt Limited, India) amended with asparagine (1.2 g/l) and the plates were incubated at 25 ±2ºC under alternate light/darkness (12 h each). After 10 days, culture mat was harvested by applying 10-15 ml of sterile water per plate, scraping the mycelial mat followed by its maceration (Yerasu *et al*, 2019). Macerated culture was diluted four times with distilled water. For inoculation, except roots all parts of the seedling were dipped in the inoculum and transplanted in pots. Seedlings were watered regularly in such a way that water should not come in direct contact with above ground parts of the seedlings. It was done to avoid wash away of the inoculum. Collar rot readings were taken on 14th and 21st day after inoculation. Final disease severity was recorded by following Andrus *et al*, (1942) rating method with modifications. The Disease severity Index (DSI) was 0 – No collar lesions; 1 – Collar lesions very shallow or with a definite tendency to heal; 2 - Plants with well developed collar lesions but still erect; 3 – Plants alive but broken over at collar region; 4 – Plants killed. Based on mean DSI plant were described as immune - 0 mean DSI, resistant 0.1-1mean DSI, moderately susceptible 1.1-2, susceptible 2.1-3 mean DSI and highly susceptible 3.1-4mean DSI.

Statistical Analysis: Analyses of variance (ANOVA) and multiple comparison test based on Tukey's honestly significant difference (HSD) were calculated in computing environment R v 30102 (R Core Team, 2012) with the Agricolae package.

Results and Discussion

The study was carried out with an aim to identify resistance sources for collar rot symptom caused by *A. solani*. For this, thirty genotypes including wild species, advanced breeding lines and released tomato verities were inoculated with virulent *A. solani* isolate UP-7. The pathogen produces relatively less conidia on agar media. *In vitro* sporulation needs creating unfavorable conditions for vegetative growth by manipulating nutrition, light spectrum and temperature (Rotem and Bashi 1969, Shahin and Shepard 1979,

Table: 1 ANOVA (one way) for disease severity index of collar rot caused by *A solani*

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F-value	Pr(>F)
Genotype	29	105.43	3.636	8.18	4.95 e-12***
Residual	60	26.67	0.444		

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Walker 1980). Further, periodic sub culturing of the pathogen leads to reduction in conidial production (Rotem 1994). To avoid these difficulties/limitations associated with conidial production of *A. solani*, water suspension of macerated culture of *A. solani* was used as inoculum. Infectivity of macerated mycelia growth of *A.solani* in tomato was reported by many authors (Andrus et al. 1942; Gardner et al. 1990; Yerasu et al. 2019).

Genotypes gave significantly different reaction for collar rot symptoms after inoculated with *A. solani* (Table 1). ANOVA and Tukey's HSD analysis based on mean DSI classified genotypes into significantly different groups (Table 2). In highly susceptible genotypes collar rot symptoms clearly developed within 14 days after inoculation. Necrotic leaf blight symptoms on these genotypes were also observed within 14 days. By 21st day all plants of EC620540 and Hawaii – 3998 died with a break at collar region. Within 14 days, very small necrotic leaf blight symptoms appeared in medium susceptible and resistance genotypes. Progress of leaf blight symptoms varied among medium susceptible and resistance genotypes, and the plants did not collapse due to leaf blight. DSI was used to evaluate collar rot severity and to differentiate the genotypes into different susceptibility groups. The ratings of the genotypes used in the work took different aspects of collar rot symptom development like survival, freedom from collar rot, the extent of development or severity of collar rot lesions and it considered the apparent ability of some genotypes to recover or heal after infection (Andrus et al. 1942).

Three genotypes including two tomato wild species LA 2325 and WIR 3928, and an advanced breeding line H-88-78-1 showed immune reaction to collar rot (Fig 1). Among the released varieties tested in the experiment Pusa Ruby and Kashi Anupam gave resistance reaction. LA 2325 (*S. neorickii*) is a green fruited wild relative to cultivated tomato. WIR-3928 is a yellow fruited wild relative of tomato. WIR-3928 has shown leaf blight resistance at 40 and 70 days after sowing. H-88-78-1 is an advanced breeding line and was susceptible to leaf blight. On the other hand, EC 520078 (*S. pimpinellifolium*) is a red fruited wild relative of tomato that has shown medium susceptible to collar rot and was resistance to leaf blight at 40 and 70 days after sowing (Yerasu et al. 2019). These results indicated that resistance to collar rot and leaf blight may have close association in WIR-3928 which may be used in

Table 2: Grouping of genotypes based on Tukey's HSD test for mean disease severity index of collar rot caused by *A solani*

S. No	Genotype	Mean Disease severity index (DSI)	Disease reaction
1	EC620540	4^{a}	HS
2	Hawaii-3998	4^{a}	HS
3	WIR3957 (yellow fruited wild species)	3.33^{ab}	HS
4	Arka Meghali*	3^{abc}	S
5	HisarLalit*	3^{abc}	S
6	VRT-1	2.67^{abcd}	S
7	EC538408	2.33^{abcd}	S
8	H-88-78-4	2.33^{abcd}	S
9	Indam-2103-6-1	2.33^{abcd}	S
10	Kashi Vishesh*	2.33^{abcd}	S
11	Punjab Chuhara*	2.33^{abcd}	S
12	Kashi Amrit*	2^{abcde}	MS
13	EC520078 (*S. pimpinellifolium*)	2^{abcde}	MS
14	H-88-78-2	2^{abcde}	MS
15	H-88-78-3	2^{abcde}	MS
16	Marutham(CO 3)*	2^{abcde}	MS
17	D-2-2-1	1.67^{bcde}	MS
18	EC620545	1.67^{bcde}	MS
19	Indam-2103-1-1	1.33^{bcde}	MS
20	Swarna Naveen*	1.33^{bcde}	MS
21	Kashi Anupam*	1^{cde}	R
22	H-88-78-5	1^{cde}	R
23	BL1208	0.67^{de}	R
24	EC620424	0.67^{de}	R
25	EC620444	0.67^{de}	R
26	EC625645	0.67^{de}	R
27	Pusa Ruby*	0.67^{de}	R
28	H-88-78-1	0^{e}	I
29	LA2325 (*S. neorickii*)	0^{e}	I
30	WIR3928 (yellow fruited wild species)	0^{e}	I

Means with different superscript letters are statistically different at $p < 0.05$ based on Tukey's HSD testLA 2325 was introduced from TGRC, UC Davis, USA*Released varieties*HS* highly susceptible, *S* susceptible, *MR* medium susceptible, *R* resistant and *I* immune

LA 2325 WIR 3928

Hisar Lalit VRT-1

Fig.1 Reaction of LA 2325, WIR3928, HisarLalit and VRT-1 for collar rot symptoms after 21 days of inoculation with *A solani*

breeding programmes to select leaf blight resistant genotypes through collar rot screening. Collar rot resistance in H-88-78-1 and leaf blight resistance in EC 520078 may be controlled by different genomic regions in respective genotypes. As a trait, early blight resistance is complex as it has quantitative inheritance and controlled by additive and no- additive interaction of many minor genes leading to more Genotype × Environmental interaction (Nash and Gardner 1988; Chaerani et al. 2007). In this backdrop study on inheritance of collar rot in the identified resistance genotypes has greater significance. If collar rot resistance has simple inheritance, pyramiding such genes in a single genotype may offer relatively good resistance to early blight. Identified collar rot immune genotypes can be utilized in early blight resistance breeding programmes to study collar rot genetics and to develop early blight resistant cultivars.

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टमाटर में *अल्टरनेरिया सोलेनाई* एक मृदा जनित रोग है, जिससे पूरे विश्व में अधिकतम नुकसान होता है। लक्षण के रूप में पत्तियों पर गहरे भूरे रंग के धब्बे बनते हैं और नवोद्भिद तनों के आधारीय क्षेत्र में सड़न, वयस्क तनों के पौधों एवं फलों पर सड़ने की प्रक्रिया देखी जा सकती है। अगेती झुलसा बीमारी की तुलना में कॉलर राट की पहचान और विभिन्न प्रतिरोधी प्रभेदों की छंटनी करना अपेक्षाकृत आसान होता है। भा.कृ.अनु.प.–भारतीय सब्जी अनुसंधान संस्थान, वाराणसी (उत्तर प्रदेश) के प्रक्षेत्र पर कुल 30 प्रभेदों की तना सड़न बीमारी की छंटनी स्किनहाउस में कृत्रिम रोगकारक दशा में परीक्षण किया गया। परीक्षण उपरान्त तीन प्रभेद जैसे एलए–2325 (*सोलेनम नियोरीकी*), जंगली प्रभेद, डब्ल्यूआईआर–3928 (पीले फल वाली जंगली प्रजाति) एवं एच–88–78–1 (उच्चीकृत प्रजनन वंशक्रम) को तना सड़न बीमारी की प्रति पूर्ण प्रतिरक्षित पाया गया जिसमें रोग की तीव्रता सूचकांक नगण्य था। इस प्रकार छंटनी की गयी पूर्ण प्रतिरक्षित प्रारूपों को *ए. सोलेनाई* रोग कारक के प्रति सुधरी किस्मों के विकास हेतु स्रोत के रूप में उपयोग किया जा सकता है।

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