Indian J. Hort. 68(4), December 2011: 516-521

Identification of resistant sources against early blight disease of tomato

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ABSTRACT

One hundred forty two tomato genotypes including wild and cultivated lines were screened for resistance against early blight disease caused by *Alternaria solani*. Evaluations were conducted *in vivo* and *in vitro* for disease severity and host resistance of the plants. Eight lines (EC-520057, EC-520058, EC-520059, EC-520061, EC-508765, EC-538394, H-88-78-1 and EC-501583) showed highly resistant reaction against the fungus; three lines were found resistant, 5 lines moderately resistant whereas 33 lines showed moderately susceptible besides 57 susceptible and 36 highly susceptible lines against the disease under natural epiphytotic condition. Screening under *in vitro*, revealed that eight genotypes were highly resistant, 3 resistant and 7 as moderately resistant. It was found that the accessions of wild relatives of tomato were highly resistant which may be utilized for the development of pre-bred lines or recombinant inbred lines or in other molecular research activities for the improvement of tomato. Some of the cultivated genotypes as resistant/moderately resistant under *in-vivo* and *in-vitro* like H-86, VRT-2, NC EBR-4 and RCMT-1 may directly be promoted for growing in disease prone areas.

Key words: *Alternaria solani*, Early blight resistance, tomato genotypes.

INTRODUCTION

Early blight is the major disease of tomato [*Solanum lycopersicum* L. (Peralta *et al*., 13) syn. *Lycopersicon esculentum* Mill.)] caused by the fungus *Alternaria solani* (Ellis & Martin) Sorauer. The disease in severe cases can lead to complete defoliation and is most damaging on tomato in regions with heavy dew, rainfall, high humidity, and fairly high temperatures (24-29°C). Epidemics can also take place in semi-arid climates where frequent and prolonged nocturnal dews occur (Rotem and Reichert, 16). Early blight causes considerable yield loss to the tomato crop especially in northern plains and peninsular parts of India. It is increasingly becoming a limiting factor for successful cultivation of tomato in these regions. Apart from the leaf symptoms of circular concentric rings with yellow halo, known as early blight (EB), *A. solani* can also cause symptoms as collar rot (basal stem lesions at the seedling stage), stem lesions on the adult plant, and fruit rot (Walker, 20). Yield losses up to 79% from early blight damage have been reported from India, Canada, United States and Nigeria (Basu *et al*., 2). Collar rot can cause seedling losses of 20 to 40 per cent in the field (Sherf and MacNab, 17). *A. solani* has the capability to grow over a wide range of temperatures, i.e. 4 – 36°C (Pound, 14).

Application of several fungicides has been recommended to control the disease, however, non-judicial use of the fungicides adds up to the human and environmental hazards. As the disease severity is more during fruiting stage, the toxic effects of fungicides also restrict the applicability of these chemicals. Thus the availability of resistant to moderately resistant genotypes may reduce the dependency on fungicides and can also be an effective component of integrated disease management strategy. The available sources of resistance are mostly confined to the weedy relatives, like *Lycopersicon hirsutum* (Barksdale and Stoner, 1), *L. pimpinellifolium* (Kalloo and Banerjee, 10), *L. esculentum* var. *cerasiforme* (Fageria, 6), which are not in practical or commercial use due to several unacceptable linked traits. However, cultivars with moderate degree of resistance have been evolved, *e.g.* Meltive and Nemato (Vakalounakis, 19), NC EBR-1, 2, and NC EBR-4 (Gardner, 7,8). The genetics of resistance to early blight has been reported as both, monogenic dominant (Datar and Lonkar, 5) as well as polygenic recessive at both seedling and adult plant stage (Thirthammallappa and Lohithaswa, 18). This manuscript reports the results of an experiment planned to identify resistant sources in tomato through screening under natural as well as artificial condition with the objective to identify genotype which may be used for commercial cultivation in disease prone areas and/or could be utilized in development of population for genetical/molecular studies.

MATERIALS AND METHODS

A total of 142 genotypes including cultivars, pre-bred lines and weedy relatives of tomato were selected for this study; twenty plants of each genotype were planted on the raised beds at the 60 cm × 45 cm spacing

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after 25 days of seed sowing in three replications. The investigation was carried out at the experimental farm of Indian Institute of Vegetable Research (IIVR), Varanasi during the main cropping seasons of (Rabi) 2007-08 & 2008-09. All the recommended package of practices for cultivation of tomato was followed in order to raise a good crop, except fungicide application. The plant materials and facilities used herein were obtained from tomato breeding unit of the Vegetable Improvement Division.

Field screening of 142 tomato genotypes was done against early blight resistance during the cropping season of 2007-08 and 2008-09 in the month of February-March under natural epiphytotic conditions. Average disease severity of early blight was recorded at 90 and 120 days after transplanting. Ten plants from each genotype were randomly selected and scored individually using 0-5 rating scale (Table 1) based on leaf area, stem and fruit covered by blight symptoms following the rating scale described by Pandey *et al.* (12). Disease incidence was calculated on the basis of per cent of infected leaves and stem.

Percentage disease index (PDI) was calculated as follows:

$$PDI = \frac{\text{Sum of all rating} \times 100}{\text{Total no. of observations} \times \text{Maximum rating grade}}$$

Table 1. Scale for rating of early blight disease in tomato.

Rating	Reaction description
0	Free from infection
1	< 10% surface area covering leaf, stem and fruit infected by early blight
2	11-25% foliage of plant covered with a few isolated spot
3	Many spot coalesced on the leaves, covering 26-50% surface area of plant
4	51-75% area of the plants infected, fruits also infected at peduncle end defoliation and blightening started. Sunken lesions with prominent concentric ring on stem, petioles and fruits
5	< 75% area of plant part blighted, severe lesion on stem and fruit rotting on peduncle end

The mean value of the PDI from ten individual plants was calculated for each of the observations at 90 and 120 days after transplanting and averaged. Host plant reaction was classified based on the mean PDI value as highly resistant (0-5) resistant (5.1-12), moderately resistant (12.1-25), moderately susceptible (25.1-50), susceptible (50.1-75) highly susceptible (> 75).

Early blight of tomato is more prevalent during the month of January to April in northern India when it receives congenial condition for its perpetuation. Diseased samples were collected from tomato plots at IIVR, Varanasi. Pathogen was isolated from leaves, twigs, and fruit of tomato, and was purified by hyphal tip method. Circular margin, dark brown and smooth velvety zonation was recorded in the Varanasi isolate (Va). The isolate were maintained in the media containing potato dextrose agar (PDA). The entire culture slant were sealed and preserved at 4°C.

Artificial screening was done in order to confirm the field screening during 2007-08. Genotypes were tested against the most virulent isolate of *A. solani*, *Va-6* (Kumar *et al*., 11). under artificial condition on healthy stem pieces (bits) of tomato. Thirty six genotypes were selected for artificial screening on the basis of their reaction under field condition ranging from highly resistant to susceptible. An inoculation technique developed by Pandey *et al*. (11) was followed to test the genotypes with pure mycelial culture of *A. solani*. Ten-day-old cultures of Varanasi (Va-6) isolate was taken for inoculation. Culture bits of 20 mm size was ground in 50 ml of sterilized distilled water with sterilized pestle and mortar and filtered with sterilized muslin cloth in a clean test tube aseptically. The culture suspension was maintained up to 150 colonies per ml and was transferred in conical flask. Young and healthy stem pieces of tomato were washed thoroughly with sterilized distilled water and then surface sterilized with 0.1% $HgCl_2$ solution followed by rinsing with sterilized distilled water for three times. The surface-sterilized stems were then placed over sterilized moist blotting sheets in a plastic tray. The stems were place in two rows, one row of stem pieces were inoculated with sterilized needle, and the other was used as control. Each stem was inoculated with 5 µl of freshly prepared fungal suspension. Plastic trays were incubated at 25 ± 2°C and 95 per cent humidity for 9 days and observations were recorded twice after 6th and 9th day of incubation. The lesion size was measured for each observation as length of the lesion multiplied by its width and the mean was calculated. The genotypes were categorized on the basis of the average size of the lesions (mm^2) as 0 or no lesion development (highly resistant), 1-15 mm^2 (resistant), 16-30 (moderately resistant), 31-40 (moderately susceptible), 41-50 (susceptible) and > 51 mm^2 (highly susceptible) following Chaerani *et al*. (4) with slight modifications.

RESULTS AND DISCUSSION

A total of 142 genotypes/lines of diverse origin were transplanted and screened against early blight disease under natural epiphytotic condition. Out of 142 lines, eight (EC-520057, EC-520058, EC-520059,

Table 1. Screening of tomato genotypes against early blight disease under natural epiphytotic condition.

Reaction	PDI	Genotype(s)
HR	0-5	EC-520057, EC-520058, EC-538394, EC-508765, EC-520059, EC-520061, EC-501583, H-88-78-1 (8)
R	5.1-12	EC-538404 (NC EBR-4), VRT-2, EC-538393 (3)
MR	12.1-25	KS-118, LA-4040-1, H-88-78-2, H-88-78-3, RCMT-1 (5)
MS	25.1-50	Arka Saurabh, IST-7, JTP-02-7, DT-2, F-6050-1, DARL-63, LA-4044-2, LA-4012-1, IIVR-SEL-1, Shalimar, BT-120, ATL-97-44, VLT-34, H-86-3, DARL-64, Pant-T-7, Punjab Chhuhara, DVRT -2, F-4036-1, F-5013-3, NDTS-2002-3, F-7001-1, F-7025-1, F-6102-1, LA-17-1, BT-136, VTG-87, LA-7421, Neptune, CHRT-4, F-7045-1, F-6012-1, SKAUT-2 (33)
S	50.1-75	F-6021-1, VRT-35-1, VRT-35-2, VRT-41-1, 126-PD-1, VRT-5-1, VRT-40-2, VRT-43-1, VRT-2-1, TLH-30-1, TMT-415, LA-3940-1, LA-3947-1, NDTS-2002-2, NDTVR-60-1, Punjab Upma, PS-1, PDT-3-1, PDT-3-1-1, Pant-T-3, RCMT-2, IIVR-Sel-3, Improved Shalimar-1, H-86, H-86-2, KTDS-171, KS-16, LA-3941-1, LA-3951-1, LA-3997-1, LA-4055-1, F-4002-1, F-5055-1, F-5025-1, F-6061-1, F-7028-1, F-6109-1, F-5010-1, Arka Vikas, Co-3, CH-3, DARL-62, DT-10, DVRT-1, DVRT-1-1, EC-519785-1, F-4036-2, F-5013-1, F-5013-2, F-5013-4, F-4049-1, F-5070-2, F-7011-1, F-6004-1, F-6010-1, F-6010-2, F-6016-1 (57)
HS	>75	DVRT-1-2, EC-519769-1, EC-519731-1, EC-519730-1, F-6024-1, F-5020-1, F-4012-1, F-6022-1, F-7012-1, F-4047-1, F-6059-1, EC-538401(NC EBR-1), FEB-2-1, FEB-2-2, FEB-4-1, FEB-4-2, FLB-4-1, H-88-1, H-86, HAT-118-1, HAT-122-1, LA-3971-1, LA-4059-1, LA-3772-1, LA-3772-2, LA-3959-1, VFN-8, TLH-17-1, VRT-32-1, SEL-7, TH-806, TLH-27-1, VRT-40-1, VRT-4-1, VRT-1-1, VRT-31-1 (36)

HR = Highly resistant, R = Resistant, MR = Moderately resistant, MS = Moderately susceptible, S = Susceptible, HS = Highly susceptible, PDI = Percent disease incidence

EC-520061, EC-508765, EC-538394, H-88-78-1 and EC-501583) were found as highly resistant, three lines (EC-538404 (NC EBR-4), VRT-2 and EC-538393) resistant, five lines (KS-118, LA-4040-1, H-88-78-2, H-88-78-3 and RCMT-1) moderately resistant, 33 lines showed moderately susceptible, 57 lines susceptible and 36 lines showed the highly susceptible reaction against early blight disease caused by *Alternaria solani* (Table 1). Among the highly resistant category, except EC-501583, all the four accessions belong to the wild relative (*L. hirsutum* syn. *Solanum habrochaites*) of tomato. All other lines were of *L. esculentum* species. It was observed that the genotypes with indeterminate growth habit showed either highly resistant, resistant or moderately resistant reaction as has been reported by Pandey *et al.* (12).

During screening, it was observed that symptoms of early blight appeared on all parts of the plant above ground. Leaf spot symptoms were scattered, brown to dark brown with concentric rings. As the natural inoculum's pressure increased, the spots coalesced and enlarged during the month of March every year. Chlorotic halo was also observed around the spot in most of the genotypes.. The stem lesions were usually restricted to one side of the stem and become elongated and sunken. Mature stem lesion clearly showed concentric rings. Fruit symptoms gradually progressed on apical portion of fruit as dark brown, depressed, firm with distinct continuous rings on fruits. The disease was more prevalent at fruit ripening stage and continued till the crop completely reached to senescence. Generally early blight of tomato was common during January to April (when average temperature varied from 15 to 30°C).

Confirmation of field screening was done through artificial screening on 36 selected genotypes. Although, the concentration of the inoculum was constant for all the genotypes during the inoculation process, the differential reactions of the genotypes against *A. solani* isolate suggests variable potential of genotypes against Va-6 isolate of *A. solani*. Among 36 genotypes, eight (EC-520057, EC-520059, EC-501583, EC-508765, EC-520058, EC-538394, EC-520061, H-88-78-1) were found highly resistant, three (NC EBR-4, VRT-2 and H-86) as resistant and seven (DVRT-2, EC-538393, DARL-63, H-88-78-2, LA-4040-1, RCMT-1 and KS-118) as moderately resistant while, eight lines were found moderately susceptible and seven lines as susceptible (Table 2). The earliest and most severe infection was observed in three genotypes; VRT-32-1, F-4012-1 and Co-3, indicating that these three genotypes are the most susceptible among the lot.

Table 2. Artificial screening of tomato genotypes using 'stem bits' against Va6 isolate of A. solani.

Sl. No.	Genotype	Source	Species	Lesion size (mm^2) a	Reaction
1	EC-520057	AVRDC, Taiwan	*L. hirsutum*	0	HR
2	EC-520059	AVRDC, Taiwan	*L. hirsutum*	0	HR
3	EC-501583	AVRDC, Taiwan	*L. esculentum*	0	HR
4	EC-508765	AVRDC, Taiwan	*L. esculentum*	0	HR
5	EC-520058	AVRDC, Taiwan	*L. hirsutum*	0	HR
6	EC-538394	AVRDC, Taiwan	*L. esculentum*	0	HR
7	EC-520061	AVRDC, Taiwan	*L. hirsutum*	0	HR
8	H-88-78-1	IIVR, Varanasi	*L. esculentum*	0	HR
9	NC EBR-4	NCSU, USA	*L. esculentum*	2	R
10	VRT-2	IIVR, Varanasi	*L. esculentum*	3	R
11	H-86	IIVR, Varanasi	*L. esculentum*	6	R
12	DVRT-2	IIVR, Varanasi	*L. esculentum*	12	MR
13	EC-538393	AVRDC, Taiwan	*L. esculentum*	12	MR
14	DARL-63	Pithoragarh	*L. esculentum*	12	MR
15	H-88-78-2	IIVR, Varanasi	*L. esculentum*	12	MR
16	LA-4041	TGRC, USA	*L. esculentum*	15	MR
17	RCMT-1	Shillong, Meghalaya	*L. esculentum*	24	MR
18	KS-118	Kalyanpur, UP	*L. esculentum*	24	MR
19	BT-136	OUAT, Orissa	*L. esculentum*	30	MS
20	FEB-2	IIVR, Varanasi	*L. esculentum*	35	MS
21	H-88-78-3	IIVR, Varanasi	*L. esculentum*	32	MS
22	EC-538401	AVRDC, Taiwan	*L. esculentum*	36	MS
23	F-5013-4	IIVR, Varanasi	*L. esculentum*	30	MS
24	Sel-7	HAU, Haryana	*L. esculentum*	30	MS
25	Arka Vikas	IIHR, Bangalore	*L. esculentum*	36	MS
26	VRT-43	IIVR, Varanasi	*L. esculentum*	36	MS
27	CH-3	HARP, Ranchi	*L. esculentum*	49	S
28	F-6050-1	IIVR, Varanasi	*L. esculentum*	48	S
29	EC-519769	AVRDC, Taiwan	*L. esculentum*	42	S
30	EC-519785	AVRDC, Taiwan	*L. esculentum*	49	S
31	FEB-4-1	IIVR, Varanasi	*L. esculentum*	48	S
32	VFN-8	USA	*L. esculentum*	42	S
33	H-88-1	IIVR, Varanasi	*L. esculentum*	49	S
34	VRT-32-1	IIVR, Varanasi	*L. esculentum*	50	HS
35	F-4012-1	IIVR, Varanasi	*L. esculentum*	60	HS
36	Co-3	TNAU, Coimbatore	*L. esculentum*	66	HS

[a] Av. of three stem bits of each genotype

In this study, eight genotypes were found as highly resistant under natural field screening as well as under artificially inoculated condition. The disease severity increased with growth of the plants. It has been observed that even on susceptible plants, the topmost younger leaves are usually free from early blight symptoms, whereas the older and lower leaves may be greatly affected and necrotized by the fungus (Johanson and Thurston, 9). Physiological mechanism controlling this apparent resistance in foliage has been clarified and Rotem (15) suggested that low sugar content as the cause of higher susceptibility in older or weakened leaves and plants. During later stages, leaves of maturing plant might be susceptible due to translocation of sugars to the ripening fruits. The genotypes exhibiting similar type of reaction under both natural and field conditions gives more reliable state for selecting the resistant or tolerant genotypes as there may be cases of disease escape under natural field condition due to environmental or some other reasons. The suggestion of Chaerani and Voorrips (3) is worth mentioning who opined that the carefully adapted laboratory assays on explants, like stem bits, however, show greater promise for studying particular aspects of resistance/susceptibility and for eliminating the confounding influences of whole-pant physiology under the natural conditions.

The results indicated that the accessions of wild species were highly resistant and may be utilized as sources for the development of pre-bred lines or recombinant inbred lines or in other molecular works for the improvement of tomato against early blight disease. Some of the cultivated genotypes as resistant/ moderately resistant like H-86, VRT-2, NC EBR-4 and RCMT-1 may also be promoted for growing in disease prone areas besides using them in development of resistant/tolerant varieties.

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Received: .December, 2009; Revised: July, 2011; Accepted : August, 2011