World Applied Sciences Journal 32 (11): 2259-2263, 2014
ISSN 1818-4952

DOI: 10.5829/idosi.wasj.2014.32.11.1312

Field, Greenhouse and Detached-Leaf Evaluation of Tomato (*Lycopersicon esculentum* Mill.) Genotypes for Late Blight Resistance

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Abstract: Twenty one tomato (Lycopersicon esculentum Mill.) genotypes were evaluated during September 2011 to August 2012 to estimate the level of their resistance to late blight infection so as to identify better resistant varieties. The experiment was conducted at Mizan-Tepi University trial field using Randomized Complete Block Design (RCBD) with three replications. The genotypes CLN-2037I, Roma VF, CLN-2037H, Melkashola, CLN-2037H and CLN-2037E showed relatively better resistance to late blight infection under field condition, green house and detached-leaf evaluation, revealing their better resistance performance to late blight infection. So that, these genotypes could be selected as resistant varieties to late blight and also could be considered in hybridization program as a source of resistant gene to late blight in developing resistant varieties.

Key words: Late blight · Tomato · Disease resistance · Detached leaf assessment

INTRODUCTION

Tomato is one the most important vegetable crops cultivated by commercial and small holder farms. However, its productivity potential severely threatened by different abiotic and biotic factors such as diseases including late blight, insect pests, salinity, heat complexes and low yield and sometimes total crop failure. Late blight which is caused by the oomycete pathogen (*Phytophthora infestans* (Mont.) de Bary) is the most destructive disease causing severe economic losses in tomato production all over the world including tropical region. According to [1], the use of fungicides to control late blight problem may lead to the emergency of resistant *Phytophthora infestans strains* which could result even the worst economic and environmental problem. In addition, in field conditions, because of its very short life cycle it is detectable only after the crop is severely damaged which makes difficult to control the disease through fungicides application [2]. As reports by many workers indicate late blight resistance in tomato is controlled by few genes [3, 4] and this makes its control through developing resistant varieties is more feasible. Therefore, the best and sustainable way to control the problem of this disease is the development of resistant varieties. Hence, identification of sources of resistance to late blight and combining the resistance to other traits of economic importance is of prime objective for the tomato improvement. Therefore, the objective of this study was to assess the relative resistance of the genotypes to late blight infection under field, greenhouse and detached-leaf assessment conditions so as to identify the better resistant variety which could be considered in breeding programme for farther improvement of the crop.

MATERIAL AND METHODS

Testing Location and Season: The study for field late blight infection evaluation has been conducted under irrigation condition during main production season from September 2011 to May 2012 under Mizan agro-ecology at trial field (farm field) of Mizan-Tepi University, which is located between 6°09'N latitude and 35°E longitude at an altitude of 1400m above sea level, in sub humid tropic South Western part of Ethiopia. The area receives annual rain fall of 2000mm and average mean annual minimum and maximum temperature is 20°C and 28°C respectively.

Experimental Materials: The study was conducted using 21 tomato genotypes of different origin. The seeds of the germplasms were obtained from Melkasa Agricultural Research Center where they were collected from different part of the world and maintained.

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Table 1: The Scales for assessment of leaves, whole plants and fruits damaged by late blight infection

Rating	Leaf infections (detached leaf assay)	Leaf infections (whole plant assay)	Fruit infections	Level of Resistance category	% Disease Index
1	No infections	No infections	No infections	immune	0
2	1-10% leaf area infected	First symptoms as grey-green to brown lesion observed on leaves)	Up to 12.5% of the fruits with grey-green to brown spots	Very highly resistant	1-10
3	11-20% leaf area infected)	Symptoms obvious. Yellowing or browning of some leaves or small lesions 50% of plant height	Up to 25% of the fruits with typical dark spots	Highly resistant	11-20
4	21-30% leaf area infected	Increased yellowing or browning, or small lesions to 75% of plant height	Up to 37.5% of the fruits with typical dark spots	Resistant	21-30
5	31-40% leaf area infected	Plant severely affected, about 50% of the leaves dead	Up to 50% of the fruits with typical dark spots	Less resistant	31-40
6	41-50% leaf area infected	Yellowing or browning to 50% of plant height	Up to 62.5% of the fruits with typical dark spots	Least resistant	41-50
7	51-60% leaf area infected	Yellowing or browning to 75% of plant height	Up to 75% of the fruits with typical dark spots	susceptible	51-60
8	61-70% leaf area infected	Entire plant yellow to brown, all leaves infected	More than 75% of the fruits with typical dark spots	Highly susceptible	61-70
9	71-100% leaf area infected	All leaves dead	All fruits infected	Very highly susceptible	>71

Experimental Design and Trial Management: The experiment was conducted using Randomized Complete Block Design (RCBD) with three replications and with the plot size of 2.10 m x 5.0 m each having five rows. Inter-row spacing of 1m and intera-row spacing of 0.3m was maintained during the layout. Fertilizer 200Kg/ha DAP was broadcasted at transplant & 100Kg/ha urea was side dressed at early flowering stage [5]. And other agronomic practices were performed as required.

Field Late Blight Resistance Assessment: Six weeks old seedlings of each genotype were transplanted. Three weeks after transplanting, following the occurrence of late blight in the field, 500 lts/ha of the fungicide Ridomil® Gold MZ 68 WG (Syngenta, Crop protection AG, Basle/Switzerland) was uniformly sprayed. Evaluation of disease severity was started six weeks after transplanting. The assessment was undertaken every 7 days and total 6 records were taken for every plots of each genotype. The estimation of leaves and fruits damaged by late blight was carried out following the scale used [6] by making some modifications. The description of the scale for assessment is given below in Table 1.

The area under the disease progress curve (AUDPC) was estimated according to the formula suggested by [7] as follows:

$$AUDPC = \sum_{i=1}^{n-1}\left(\frac{x_{i+1}+x_i}{2}\right)\left(t_{i+1}-t_i\right)$$

where: x_i = Score (rating) at time i, t = the day of ith observation and n = total number of observations. The level of resistance of the genotypes against the late blight infection was estimated based on the number of AUDPC score, i.e. is the genotype scored maximum *AUDCP* value considered to be relatively less resistant to the late blight infection. In addition, starting 50% flowering 15 plants which look severely affected from each plot were tagged and total number of infected fruits and the total number of leaves dead or 75% of their leaf areas infected from each plant were recorded. Then the score was expressed as a percentage as follows:

$$\%\text{of number of fruits infected} = \frac{\text{Number of infected fruits}}{\text{Total number of fruits}} \text{ x } 100$$

$$\%\text{of number of leaves infected} = \frac{\text{Number of infected leaves}}{\text{Total number of leaves}} \text{ x } 100$$

Greenhouse (Whole Plant Assay) and Detached-leaves assay Evaluation: The seedlings of the genotypes were grown under greenhouse condition. The average temperature of the greenhouse during the evaluation period was 20 to 26°C.

Isolation of the Pathogen and Inoculums Preparation: The infected leaves were collected from tomato field and transferred to prepared rye agar culture media for sporangial development. To check the development of sporangia, the samples were taken from each petri dish and the well developments of sporangia were observed under microscopes. The sporangia were collected from all petridishes and mixed together uniformly in sterilized distilled water to get sporangial suspension. Then the sporangial suspension was put in the dark for 90 to 100 minutes at 11 to 12°C before inoculation [8].

Inoculation: Six weeks old plant was inoculated using hand sprayer by spraying to run-off with sporangial suspension. For each genotype (Fifteen plants per growing media/container) 125ml of sporangial suspension was used to inoculate the plants. The inoculation was done early in the evening starting at 8:00 pm. The temperature inside the greenhouse during the inoculation was 19°C and the relative humidity (RH) was 83%. Fifteen days after inoculation, visual observation of the plants, estimation of diseased proportion of the plants and estimation of level of resistance was made based on the scale and the descriptions mentioned in table (Table 1). The leaves were also detached from each genotype and were put in petri dishes on moistened filter paper. The leaves (Four leaves of each genotype) were inoculated with 5ml of sporangial suspension and incubated at 18°C for 24hrs. Seven days after inoculation, the leaves were evaluated for the proportion of leaf area blighted and the level of resistance was assessed using the scale described below (Table1).

Data Analysis: The level of infections for detached leaves assay and whole plant assay were computed and expressed in average percentages. The collected AUDPC values were calculated for each genotype and subjected to analysis of variance for Randomized Complete Block Design as per [9]. SAS statistical software package [10] was employed for analysis of variance. The statistical significance was determined by using F-test. List significance difference (LSD) was used to separate the mean performance of the genotypes which were significantly different [9].

RRESULTS AND DISCUSSION

Result from analysis of variance showed highly significant differences among the genotypes ($P<0.001$) for the characters evaluated (Table 2). This indicated the existence of sufficient genetic variability among evaluated genotypes. The genotypes CLN-2037I (129.50, 186.76), CLN-2037F (135.33, 147.00), Melkashola (136.50, 222.83), Roma VF (141.17, 166.83) and CLN-2037H (165.637, 171.33) recorded relatively lower infected leaves and fruits AUDPC values respectively for late blight infection assessment under field condition, revealing their better resistance to late blight infection (Table 2). Lower percentage of infected leaves and fruits were recorded by the genotypes CLN-2037F (16.47%, 24.51%), CLN-2037I (17.19%, 54.52%), CLN-2037H (18.82%, 39.92%) and Roma VF (20.13%, 78.17%) (Table 2), indicating their better resistance to late blight infection under field condition.

Table 2: Level of resistance of the genotypes to late blight infection under field evaluation

S.N	Genotype	Infected f leaves (AUDPC value)	Infected fruits (AUDPC value)	Infected leaves (%)	Infected fruits (%)
1	BL-1198	243.83^{bcd}	176.67^{bcde}	84.42	32.87
2	Metadel	249.67^{abcd}	186.67^{abcd}	44.15	35.79
3	Melkasalsa	222.83^{cde}	157.50^{defghi}	60.94	24.1
4	Beafsteak	224.00^{cde}	197.17^{abc}	92.43	41.8
5	CLN 2037F	147.00^{g}	135.33^{hi}	24.51	16.47
6	CLN 2037H	171.33^{fg}	165.67^{defgh}	39.92	18.82
7	Cochoro	232.17^{cde}	203.00^{ab}	85.43	32.91
8	Tomato1365/95	254.33^{abcd}	177.33^{bcde}	58.84	34.67
9	Chali	249.67^{abcd}	145.83^{fghi}	56.04	17.73
10	Unknown 13	224.00^{cde}	145.83^{fghi}	41.43	22.31
11	Bishola	268.33^{abc}	158.67^{defghi}	82.15	23.13
12	Eshet	290.17^{a}	166.83^{cdefg}	43.04	25.39
13	Melkashola	222.83^{cde}	136.50^{ghi}	41.1	20.52
14	Fetan	169.17^{fg}	156.33^{defghi}	69.47	35.28
15	H-1350	221.67^{de}	149.33^{efghi}	45.83	21.07
16	CLN 2037E	168.00^{fg}	159.83^{defgh}i	35.77	24.38
17	CLN-5915-4D-2-2-0	194.83^{ef}	175.00b^{cdef}	50.17	29.37
18	Pearson	247.33^{abcd}	201.83^{ab}	93.19	35.57
19	CLN 2037I	186.67^{efg}	129.50^{i}	54.52	17.19
20	Roma VF	166.83^{fg}	141.17^{ghi}	78.17	20.13
21	Marglobe	290.17^{ab}	213.50^{a}	93.82	41.63
	CV (%)	12.52	11.13		
	LSD (5%)	45.63	30.33		

Means with the same letter are not significantly different (at 5% level of significance)

AUDP= area under disease development progress curve

Table 3: Level of resistance of the genotypes to late blight infection under greenhouse and leaflet evaluation

S.N	Genotype	Level of infection (Detached-leaf assay)%	Level of infection (Whole plant assay)%	Level of Resistance category
1	BL-1198	84.65	80.32	9
2	Metadel	84.44	78.88	9
3	Melkasalsa	74.41	67.82	8
4	Beafsteak	85.3	85.37	9
5	CLN 2037F	61.91	59.01	7
6	CLN 2037H	48.7	45.49	6
7	Cochoro	89.53	87.27	9
8	Tomato1365/95	93.33	92.67	9
9	Chali	82.25	78.17	9
10	Unknown 13	72.91	70.66	9
11	Bishola	91.59	90.34	9
12	Eshet	92.25	88.48	9
13	Melkashola	72.45	71.6	9
14	Fetan	59.13	55.47	7
15	H-1350	90.72	88.15	9
16	CLN 2037E	50.31	47.52	6
17	CLN-5915-4D-2-2-0	48.17	46.45	6
18	Pirson	94.07	93.2	9
19	CLN 2037I	51.21	49.8	6
20	Roma VF	50.06	48.74	6
21	Marglobe	93.64	92.85	9

1 = Immune, 2 = Very highly resistant, 3 = Highly resistant, 4 = Resistant, 5 = Less resistant, 6 = Least resistant, 7 = Susceptible, 8 = Highly susceptible, 9 = Very highly susceptible

In case of green house and detached-leaves evaluation, only the genotypes CLN-2037H, CLN-2037E, Roma VF, CLN-2037I and CLN-5915-4D-2-2-0 categorized under least resistant category and all the rest genotypes categorized under susceptible, or very highly susceptible category (Table 3).

CONCLUSION

Analysis of variance revealed that there was highly significant differences among the genotypes for all characters evaluated. The genotypes CLN-2037I (129.50, 186.76), Roma VF (141.17, 166.83), CLN-2037H (165.637, 171.33), Melkashola (136.50, 222.83), CLN-2037H and CLN-2037E, showed relatively better resistance to late blight infection under field condition, green house and detached-leaves evaluation, revealing their better resistance performance to late blight infection. So that, these genotypes could be selected as resistant varieties to late blight and also could be considered in hybridization programme as a source of resistant genes to late blight in developing resistant varieties.

AKNOWLEGEMENTS

The author is grateful to Mizan-Tepi University for sponsoring this study.

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