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# Screening of tomato genotypes against early blight pathogen *Alternaria solani* using detached leaf assay method

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**ABSTRACT:** Early blight (EB) caused by *Alternaria solani* is a destructive fungal disease of tomato (*Solanum lycopersicum* L.). Leaf blight, stem blight and apical fruit rot are the most prominent symptoms of the disease. It can cause complete loss of the crop when disease is severe. Thirty genotypes including wild relatives and hybrids of tomato were screened for early blight resistance under artificial inoculation by detached leaf assay method at ICAR-IIHR, Bengaluru. The genotypes screened under artificial conditions were grouped into six distinct categories based on percent disease index (PDI) recorded 7 days after inoculation (DAI) and disease was scored using 0-4 scale. Genotype LA-1777 was highly resistant. Six genotypes *viz*., NCEBR-1, NCEBR-4, Arka Alok, Arka Rakshak, Arka Saurabh and 8-3-3 were resistant. Seven genotypes were moderately resistant and rest of the genotypes were susceptible to early blight disease. The resistant genotype LA-1777 was very poor in its horticultural traits like size of the fruit and yield and it cannot be directly exploited, but it can be used in resistance breeding programme for developing superior cultivars and hybrids having resistance to early blight.

**Keywords**: *Alternaria solani,* Early blight, percent disease index

## INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the most important vegetable crops grown worldwide for its high nutritive value. India ranks second among top producers with the annual production of 207 lakh Tonnes (FAOSTAT, 2016). Tomato plants are highly prone to diseases caused by bacteria, viruses, fungi and nematodes. One of the major constraints in tomato cultivation is early blight (EB) caused by *Alternaria solani.* It is a destructive fungal disease of tomato which affects the yield and reduces the fruit quality (Kumar *et al*., 2013). Early blight is common in tropical, sub-tropical and temperate humid climates and it occurs on plants at all the stages of development. The yield loses were reported to be up-to 79% from India, United states and Canada and Nigeria (Prabhash *et al*., 2011). In the most severe cases the disease leads to complete defoliation (Peralta *et al.,* 2005). The symptoms of disease in leaf were found to be circular in shape initially with 0.5 inches in diameter and turn yellow, light brown and then to brown and finally the leaf sheds off. Spots appear on ripe or green fruits. On fruits it is usually affected at the stem end and in most cases appears as depressed rot. The source of resistance is very important and hence, both field evaluation and artificial evaluation by detached leaf method are used to screen. In order to develop a stable resistance source, management with chemicals has not been found effective as the weather conditions are favourable towards epidemics. Moreover, control with fungicide spraying is not attainable as the disease appears always at the fruit maturity. However genetic resistance provides more economically sound and environmentally safe approach (Pandey *et al*., 2003). Therefore, an alternative method of artificial screening by detached leaf method was found more reliable as it overcomes the physiology of plant disease and also reduces the space, time and labour requirement. Based on the above facts, the present study was carried out with the main objective to screen genotypes including wild relatives and hybrids of tomato for early blight disease resistance under artificial inoculation by detached leaf method.

## MATERIALS AND METHODS

Thirty genotypes including wild relatives, hybrids and released varieties were collected from Division of Vegetable Crops, ICAR-IIHR, Bengaluru and the genotypes were sown during the month of September 2016 in the Block-8 field, ICAR-IIHR, Hessaraghatta, Bengaluru. Seeds were sown in pro-trays and seedlings were raised in the shade net for 25 days. Twenty five day-old healthy seedlings were transplanted in main

field with two replications each. Six plants from each accession were transplanted.

**Isolation of pathogen *Alternaria solani***

*Alternaria solani* was isolated from infected tomato leaves collected at ICAR-IIHR, Bengaluru, using the tissue segment method (Aneja, 2001) under aseptic conditions. The pathogenicity was confirmed by inoculation and re-isolation. Further the pathogen was maintained on PDA slant.

**Artificial inoculation (Detached leaf assay):** The leaf samples were collected from the field which were free from pests and diseases. Artificial inoculation was made on detached leaves. Two replicates from each of the mature leaf samples (fourth leaf from tip of the plant) were collected for the study. In order to maintain the moisture content the moist blotting sheet was placed inside the Petri plate (18 cm in diameter). Two ml of double distilled water was added daily to maintain the relative humidity of about 90-95% to facilitate the disease development. Each leaf was placed separately in a Petri plate and a sterile glass slide was placed below the leaf to avoid direct contact with the moist surface and rotting.

**Disease assessment:** The mycelial disc of 5 mm in diameter from the growing edge of the seven days old colony maintained and multiplied on the PDA media was used for the study. The leaves were pricked slightly at the centre using a fine needle to facilitate the entry of the mycelium of the pathogen which was inoculated on the upper surface of the leaf. Pathogen inoculation was done only on the middle of the leaf. The inoculated leaves were incubated under at 22$^0$C and exposed to a photoperiod of 12 h under a cool white fluorescent light and 12 h dark. The lesion development was checked daily on the inoculated leaf and the disease severity was calculated on the seventh day by measuring the area covered by the pathogen by using the 0-4 scale developed by (Devananthan and Ramanujam, 1995): 0= infection free or healthy; 1= 1-25% leaf area blighted; 2= 26-50% leaf area blighted; 3= 51-75% leaf area blighted; 4= 76-100% leaf area blighted. Thus the individual leaf ratings were recorded and per cent disease index (PDI) was calculated by using the formula:

$$PDI = \frac{\text{Sum of numerical values}}{\text{Number of leaves graded} \times \text{Maximum rating}} \times 100$$

Tomato genotypes including wild relatives and hybrids were then grouped into five categories based on the PDI value (Mckinney, 1923) as: <1%=immune; 1-10%=highly resistant; 10.1-25%=resistant; 25.1-40 % =moderately resistant; 40.1-50%=susceptible; >50 %=highly susceptible.

**Apparent infection rate (r):** The apparent infection rate is the measure of disease development at a speed which an epidemic develops. Disease incidence were recorded from three days to seven days and the apparent infection rate were calculated with the formula sated by (Van der Plank, 1968)

$$r = 2.3/t_2\text{-}t_1 \{\log(x_2(1\text{-}x_1)/x1(1\text{-}x_2))\}$$

where, r = apparent infection rate in non-logarithmic phase, $X_1$= disease index at the time $t_1$, $X_2$= disease index at subsequent week time $t_2$.

**Area under disease progress curve (AUDPC):**

AUDPC is another criteria for recording the speed of pathogen progression and which differentiates between the resistant and susceptible genotypes. Disease incidences were recorded from three days to seven days after inoculation and AUDPC were recorded as a measure of quantitative disease resistance involving repeated disease assessments. The disease scoring for AUDPC was calculated by the formula (Jeger and Rollinson, 2001)

$$\text{Ak} = \sum_{i=1}^{Ni-1} \frac{\text{yi} + \text{yi} + 1}{2} (ti + 1 - ti)$$

Where, yi = proportion of disease on the i$^{th}$ observation, $t_i$ = time (days) of observation expressed as days after sowing (DAS) and N = total number of disease severity readings (PDI) taken throughout the experiment.

**RESULTS AND DISCUSSION**

**Screening by artificial detached leaf method**

Thirty tomato accessions including wild relatives and hybrids were screened for early blight resistance. The screening results indicated that LA1777 was highly resistant with PDI of 4.17. NCEBR-1, Arka Alok, Arka Saurabh, 8-3-3, NCEBR-4 and Arka Rakshak showed resistance with average PDI of 25 and line number 4-3-3 (25.42%), Arka Vikas (17.08%), Arka Samrat (24.58%), Arka Abha (28.75%), Vaibhav (22.92%), H-335 (15%) and 5-3-7 (14.17%) genotypes showed moderately resistant response; Arka Meghali (40.42%), H-329 (75%), H-331 (31.67%) and H-369 (17.50%) were susceptible and Punjab Chhuhara,CO-3, PKM-1, Pusa Ruby, PED, LA-1670, LA-2656, LA-2093, 917, Kashi Anupama, 2809 and 2805 were highly susceptible to

**Table 1. Screening of tomato genotypes using detached leaf assay method**

| Genotype | IV day | V day | VI day | VII day | Mean PDI | Disease reaction |
|---|---|---|---|---|---|---|
| Punjab Chhauraha | 31.67 (5.712) | 58.33 (7.701) | 73.33 (8.617) | 80 (9.71) | 60.83 | HS |
| CO-3 | 40 (6.403) | 73.33 (8.617) | 100 (10.05) | 100 (10.05) | 78.33 | HS |
| Pusa Ruby | 25 (5.099) | 56.67 (7.587) | 80 (9) | 100 (10.05) | 65.42 | HS |
| PKM-1 | 18.33 (4.388) | 46.67 (6.895) | 73.33 (8.617) | 80 (9) | 54.58 | HS |
| 8.3.3 | 8.33 (3.05) | 10 (3.32) | 11.67 (3.56) | 15 (4) | 11.25 | R |
| 4.3.3 | 10 (3.317) | 18.33 (4.40) | 26.67 (5.26) | 46.67 (6.9) | 25.42 | MR |
| PED | 10 (3.32) | 20 (4.58) | 43.33 (6.66) | 73.33 (8.617) | 36.67 | MR |
| Arka Vikas | 5 (2.449) | 13.33 (3.79) | 23.33 (4.911) | 26.67 (5.26) | 17.08 | R |
| LA-1670 | 36.67 (6.092) | 66.67 (8.207) | 80 (9) | 93.33 (9.71) | 69.17 | HS |
| LA-2656 | 28.33 (5.383) | 40 (6.403) | 76.67 (8.809) | 83.33 (9.18) | 57.08 | HS |
| LA-2093 | 13..33 (3.739) | 40 (6.403) | 63.33 (7.998) | 83.33 (9.18) | 50.00 | HS |
| L1-1777 | 0 (1) | 5 (2.45) | 5 (2.449) | 6.67 (2.739) | 4.17 | HR |
| H-369 | 8.33 (3.028) | 10 (3.255) | 25 (5.045) | 26.67 (5.26) | 17.50 | R |
| 917 | 15 (4) | 26.67 (5.255) | 50 (7.118) | 83.33 (9.18) | 43.75 | S |
| H-331 | 5 (2.449) | 18.33 (4.388) | 50 (7.118) | 53.33 (7.341) | 31.67 | MR |
| H-329 | 18.33 (4.388) | 25 (5.083) | 33.33 (5.846) | 60 (7.81) | 34.17 | MR |
| Kashi Anupama | 30 (5.568) | 70 (8.412) | 73.33 (8.617) | 83.33 (9.18) | 64.17 | HS |
| Arka Rakshak | 3.33 (2.08) | 10 (3.32) | 13.33 (3.79) | 18.33 (4.4) | 11.25 | R |
| Arka Samrat | 8.33 (3.028) | 20 (4.561) | 26.67 (5.239) | 43.33 (6.649) | 24.58 | MR |
| Arka Meghali | 25 (5.045) | 26.67 (5.239) | 53.33 (7.268) | 56.67 (7.547) | 40.42 | S |
| Arka Abha | 15 (3.966) | 23.33 (4.911) | 33.33 (5.846) | 43.33 (6.649) | 28.75 | MR |
| Arka Saurabh | 5 (2.449) | 10 (3.32) | 13.33 (3.79) | 20 (4.583) | 12.08 | R |
| Vaibhav | 10 (3.32) | 10( 3.255) | 25 (5.045) | 46.67 (6.895) | 22.92 | MR |

| | | | | | | |
|---|---|---|---|---|---|---|
| 2809 | 33.33 (5.846) | 70 (8.426) | 93.33 (9.71) | 100 (10.05) | 74.17 | HS |
| NCEBR-4 | 5 (2.45) | 10 (3.32) | 13.33 (3.79) | 20 (4.58) | 12.08 | R |
| NCEBR-1 | 3.33 (2.08) | 10 (3.317) | 16.67 (4.20) | 20 (4.58) | 12.50 | R |
| Arka alok | 5 (2.449) | 10 (3.317) | 11.67 (3.544) | 16.67 (4.194) | 10.83 | R |
| H-335 | 8.33 (3.028) | 13.33 (3.772) | 15 (3.966) | 23.33 (4.911) | 15.00 | MR |
| 5.3.7 | 5 (2.45) | 8.33 (3.028) | 13.33 (3.772) | 30 (5.568) | 14.17 | MR |
| 2805 | 8.33 (3.06) | 33.33 (5.796) | 60 (7.752) | 73.33 (8.603) | 43.75 | S |
| **C.D.** | 0.09 | 0.10 | 0.14 | 0.14 | | |
| **SE(m)** | 0.03 | 0.04 | 0.05 | 0.05 | | |
| **SE(d)** | 0.04 | 0.05 | 0.07 | 0.07 | | |
| **C.V.** | 1.41 | 1.22 | 1.37 | 1.24 | | |

Alternaria blight (Table -1 and Fig-1).

Kumar and Srivastava (2013) screened tomato genotypes for early blight under natural field condition for two seasons and they reported that disease reaction ranging from highly susceptible to highly resistant. In an another study, Leyva- Mir *et al.* (2013) screened advanced lines of tomato for tolerance to early blight and observed that lines 60 and 10 were tolerant to early blight with less AUDPC and the severity of 33% and 35% respectively suggesting that there may be variability in resistance to early blight depending on the environmental conditions and the genetic makeup of the cultivar. Earlier, screening genotypes for early blight resistance was carried out by Choulwar *et al.* (1992), Fageria *et al.* (1997), Thirthamallappa *et al.* (2000) and Suryavanshi *et al.* (2000). The resistant genotype identified in the present study was very poor in their fruit weight and slow plant growth. These resistance sources cannot be exploited directly but can be used in a resistance breeding programme for developing superior cultivars and hybrids having resistance to early blight.

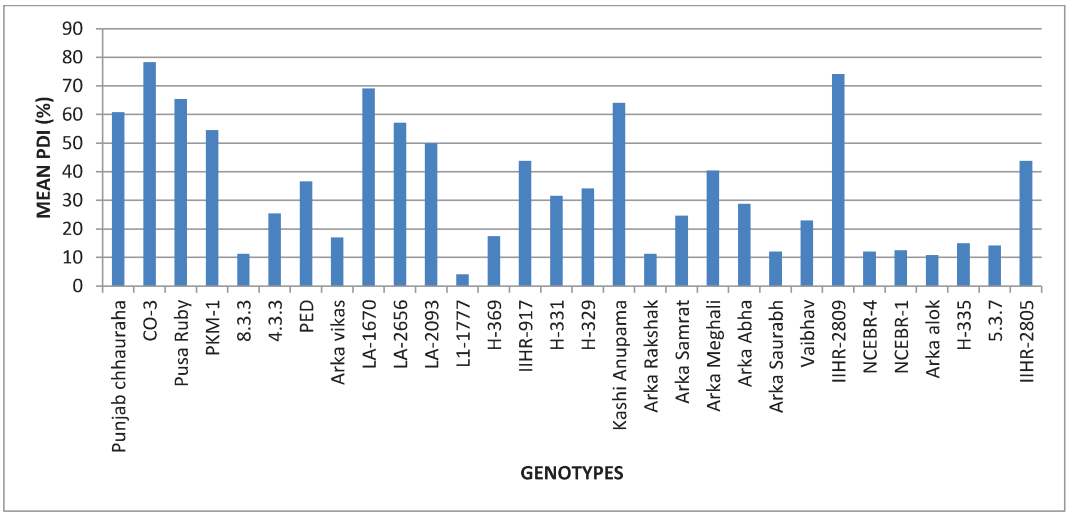



**Fig-1 Screening of tomato genotypes by artificial inoculation for early blight resistance using detached leaf assay**

**Table 2. Apparent infection rate (r) per unit per day of with progression of early blight disease of tomato**

| Genotype | Between 4th to 5th day | Between 5th to 6th day | Between 6th to 7th day | Avg. AIR |
|---|---|---|---|---|
| Punjab chhauraha | 2.53 | 0.67 | 0.37 | 1.19 |
| CO-3 | 1.42 | 2.88 | 0.70 | 1.66 |
| Pusa Ruby | 1.37 | 1.12 | 3.21 | 1.90 |
| PKM-1 | 1.36 | 1.14 | 0.37 | 0.96 |
| 8.3.3 | 0.00 | 0.75 | 0.17 | 0.31 |
| 4.3.3 | 0.17 | 0.68 | 0.80 | 0.55 |
| PED | 0.81 | 1.12 | 1.28 | 1.07 |
| Arka vikas | 1.07 | 0.68 | 0.00 | 0.58 |
| LA-1670 | 1.24 | 0.69 | 1.25 | 1.06 |
| LA-2656 | 0.52 | 1.59 | 0.42 | 0.84 |
| LA-2093 | 1.46 | 0.95 | 1.06 | 1.16 |
| L1-1777 | 0.00 | 0.00 | 0.31 | 0.10 |
| H-369 | 0.20 | 1.10 | 0.09 | 0.46 |
| H-917 | 0.72 | 1.01 | 1.61 | 1.11 |
| H-331 | 1.45 | 1.49 | 0.13 | 1.02 |
| H-329 | 0.39 | 0.41 | 1.10 | 0.63 |
| Kashi Anupama | 1.69 | 0.16 | 0.60 | 0.82 |
| Arka Rakshak | 0.42 | 1.07 | 0.38 | 0.62 |
| Arka Samrat | 1.01 | 0.37 | 0.74 | 0.71 |
| Arka Meghali | 0.09 | 1.14 | 0.13 | 0.46 |
| Arka Abha | 0.54 | 0.50 | 0.42 | 0.49 |
| Arka Saurabh | 0.00 | 0.55 | 1.01 | 0.52 |
| Vaibhav | 0.00 | 1.10 | 0.96 | 0.69 |
| 2809 | 1.54 | 1.79 | 1.95 | 1.76 |
| NCEBR-4 | 1.14 | 0.00 | 0.00 | 0.38 |
| NCEBR-1 | 1.17 | 0.59 | 0.22 | 0.66 |
| Arka alok | 0.75 | 0.17 | 0.41 | 0.44 |
| H-335 | 0.53 | 0.14 | 0.54 | 0.40 |
| 5.3.7 | 0.14 | 0.78 | 0.81 | 0.58 |
| H-2805 | 1.70 | 1.10 | 0.61 | 1.14 |

**Table 3. AUDPC for different genotypes of cucumber screened under natural condition for downy mildew disease**

| Genotype | Between 4th and 5th day | Between 5th and 6th day | Between 6th and 7th day | Mean AUDPC |
|---|---|---|---|---|
| Punjab chhauraha | 45.00 | 65.83 | 76.67 | 187.50 |
| CO-3 | 56.67 | 86.67 | 100.00 | 243.33 |
| Pusa Ruby | 40.83 | 68.33 | 90.00 | 199.17 |
| PKM-1 | 32.50 | 60.00 | 76.67 | 169.17 |
| 8.3.3 | 5.00 | 7.50 | 10.83 | 23.33 |
| 4.3.3 | 10.83 | 16.17 | 28.67 | 55.67 |
| PED | 15.00 | 31.67 | 58.33 | 105.00 |
| Arka vikas | 9.17 | 18.33 | 23.33 | 50.83 |
| LA-1670 | 51.67 | 73.33 | 86.67 | 211.67 |
| LA-2656 | 34.17 | 58.33 | 80.00 | 172.50 |
| LA-2093 | 26.67 | 51.67 | 73.33 | 151.67 |
| L1-1777 | 0.00 | 2.50 | 5.83 | 8.33 |
| H-369 | 9.17 | 16.67 | 25.83 | 51.67 |
| H-917 | 20.83 | 38.33 | 66.67 | 125.83 |
| H-331 | 11.67 | 35.83 | 51.67 | 99.17 |
| H-329 | 21.67 | 29.17 | 46.67 | 97.50 |
| Kashi Anupama | 50.00 | 71.67 | 78.33 | 200.00 |
| Arka Rakshak | 4.17 | 9.17 | 15.83 | 29.17 |
| Arka Samrat | 14.17 | 23.33 | 35.00 | 72.50 |
| Arka Meghali | 25.83 | 40.00 | 55.00 | 120.83 |
| Arka Abha | 19.17 | 28.33 | 38.33 | 85.83 |
| Arka Saurabh | 5.00 | 6.67 | 14.17 | 25.83 |
| Vaibhav | 10.00 | 17.50 | 35.83 | 63.33 |
| 2809 | 51.67 | 81.67 | 96.67 | 230.00 |
| NCEBR-4 | 2.50 | 5.83 | 8.33 | 16.67 |
| NCEBR-1 | 6.67 | 13.33 | 18.33 | 38.33 |
| Arka alok | 7.50 | 10.83 | 14.17 | 32.50 |
| H-335 | 10.83 | 14.17 | 19.17 | 44.17 |
| 5.3.7 | 4.17 | 10.83 | 21.67 | 36.67 |
| H-2805 | 20.83 | 46.67 | 66.67 | 134.17 |

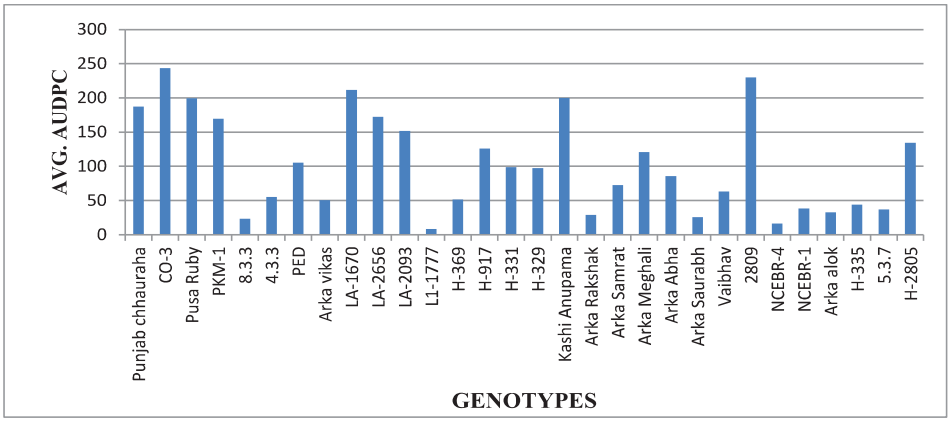
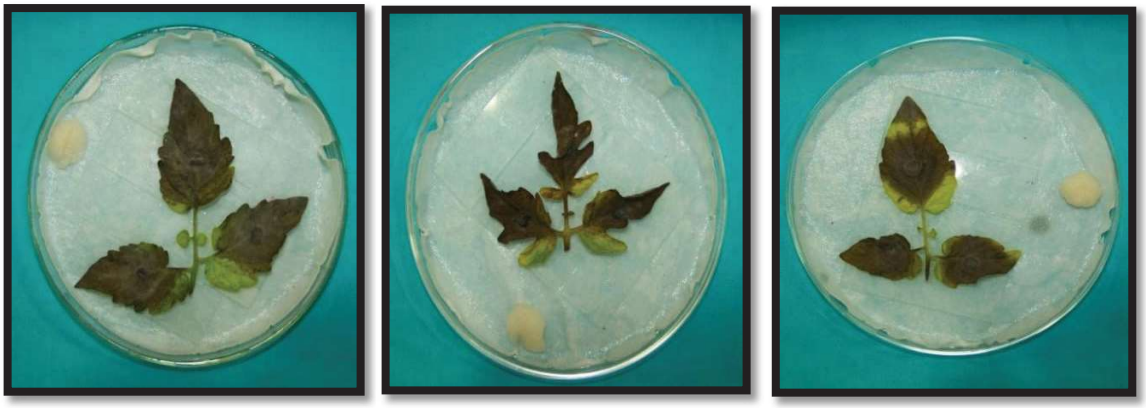



**Fig 3. AUDPC for different genotypes of tomato screened under detached leaf assay condition for early blight disease**


**Resistant genotypes**




**Susceptible genotypes**

**Fig 4. Showing resistance and susceptible genotypes for early blight resistance**

**Apparent infection rate (r)**

The apparent infection rate (r) is a measure of disease development and represents the speed at which an epidemic develops. Disease incidence was recorded daily from third to seventh day after inoculation and the apparent infection rate was calculated with the formula given by (Van der Plank, 1968). The highest average 'r' value was observed in the case of Pusa Ruby (1.90), 2809 (1.76), CO-3 (1.66), Punjab chhauraha (1.19), LA- 2093 (1.16), and 2085 (1.14) that showed highly susceptible response towards early blight disease and the least 'r' value was found in the lines LA- 1777 (highly resistant), NCEBR-4 and Arka Alok showing the resistance against the early blight (Table-2). So the speed of infection was found to be increasing day by day with the daily interval starting from third day. The apparent infection rate 'r' was found to be higher in the case of susceptible and the incidence level increases along with the growth of the plant and the lower level of incidence was found in the case of resistant. And hence, this information will be useful for deciding the resistance level of a genotype with the age of plant. And severity of the disease towards the susceptibility of the genotypes can be due to the environmental factors contributing for the disease development. A similar kind of study were performed and observed by (Wilcoxson *et al*. 1975) and (Patil, 1997).

**Disease Progression and determination of AUDPC**

AUDPC is another criteria for recording the speed of pathogen progression and which differentiates between the resistant and susceptible genotypes. Disease incidences were recorded from three days to seven days and AUDPC were recorded as a measure of quantitative disease resistance involving repeated disease assessments. The disease scoring for AUDPC was calculated by the formula (Jeger and Rollinson, 2001).

The highest AUDPC value was observed in CO-3 (243.33) followed by 2809 (230.00), LA-1670 (211.67), Kashi Anupama (200.00) and Pusa Ruby (199.17) which were high susceptibile and the lower AUDPC was observed in NCEBR-1(38.33), NCEBR-4(16.67) and L1-1777(8.33) which resistant to early blight. The AUDPC values indicate the magnitute of resistance reaction among the genotypes over the time period (Table.3, Fig.3). AUDPC values suggest that resistance source for early blight was present in half of the total tomato genotypes screened. Susceptibility of some genotypes can be due to the environmental factors contributing for the disease development. Pathogen progression is found to be slower in the case resistant compared to susceptible as the rate of colonization and expansion of the disease was slower in the resistant lines. Pathogen colonization and expansion of disease symptoms were also studied by (Mhada *et al*., 2015). Similar study supporting to the present study states that the development and colonization in the leaf tissues, were found to be delayed with the lower temperature and faster with the higher temperature by (Cohen, 1977). This study were further supported by (Neykov and Dobrey, 1987); (Bjoem and Kampmann, 2000) and (Cohen *et al*., 2000).

The information gained out of this investigation based on the screening under artificial condition, apparent infection rate and AUDPC confirmed that the LA-1777 was resistant with less disease progression and can be utilized in breeding programs for disease resistance. The high yielding genotype with early blight resistance and desired agronomic traits can be exploited to develop varieties suitable under conditions of disease epidemics.

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