Genetic response of tomato germplasm against early blight and its management through fungicides

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ABSTRACT
The response of fifty one tomato accessions germplasm were tested against early blight under field and walk in tunnel conditions. None of the tested germplasm was immune or highly resistant in both conditions. In the field experiment, among the twenty seven indeterminate varieties and hybrids only one variety (Beefsteak) showed resistant response with PDI 15.55, eleven varieties and five hybrids were moderately resistant, three varieties and five hybrids were moderately susceptible, one variety (Sahel) and one hybrid (Litth-568) showed susceptible response with 68-70 PDL. In the tunnel experiment, among the twenty four determinate varieties and hybrids four varieties (Black Prince, Oregon spring, Zhezha and Bloody Butcher) showed resistant response with PDI 11-16, ten varieties and four hybrids were moderately resistant, four varieties were moderately susceptible and one variety (Napoli) and one hybrid (Lith-174) showed susceptible response with 68.88 PDL. In vitro five fungicides, namely, Cabriotop, Precure Combi, Halonil, Topsin-M and Difenoconazole were tested through poisoned food technique against A. solani. There was a significant decrease in mycelial growth of the fungus with an increase in fungical concentration. At 150 ppm, Difenoconazole (91.95%) gave maximum inhibition of the mycelial growth followed by Cabriotop (64.36%). Least inhibition of mycelial growth was observed in Halonil (45.97%). The fungicides which proved to be most effective in vitro were tested in field, maximum disease control with 11.84 PDI was recorded in the Difenoconazole treated plots followed by Cabriotop sprayed plots with 26.66 PDI. The results of the present studies will be helpful to devise management strategies for the control of tomato early blight.

Key words: Alternaria solani; determinate and indeterminate varieties; fungicides; Resistant; susceptible.

Introduction
Tomato (Lycopersicon esculentum Mill) belongs to the family solanaceae and is one of the most remunerable and widely grown vegetables in the world. Tomato cultivation has become more popular since mid nineteenth century because of its varied climatic adaptability and high nutritive value. Among vegetables, it is the second major vegetable, produced in Pakistan (Mirza, 2007). Its area under cultivation, during 2010-11 was 52.3 thousand hectares, with a total production of 529.6 thousand tonnes and a yield 10.1 tonnes/hectare (Anon., 2010-11). This yield is very low as compared to that of the developed countries, where it can reach up to an average of 15 tonnes/hectare. Its cultivation is limited by various factors including fungi, bacteria, viruses and nematodes. At current more than 200 tomato diseases are known worldwide. Jones et al. (1991) presented major diseases of tomato caused by fungi, bacteria, viruses and various nematodes. But the factors, responsible for its low yield, in Pakistan, the fungal diseases are the most important in them. Among the fungal diseases, Alternaria leaf blight of tomato caused by Alternaria solani is the worst damaging one (Abada et al., 2008) that cause reduction in quantity and quality of the tomato crop. It is an important disease of tropical and sub-tropical areas. Distinctive bulls-eye pattern of leaf spots with concentric rings of spores surrounded by a halo of chlorotic leaf area are the common. The pathogen causes infection on leaves, stem, petiole, twig and fruits as well as leads to the defoliation, drying of twigs and premature fruit drop which ultimately reduce the yield. The disease, if favored by high temperature and humidity (crowded plantation, high rainfall and
Materials and Methods

The present investigations on early blight disease of tomato were conducted at the Department of Plant Pathology UAF and AARI. The material used and methods followed are described below.

Alternaria solani isolates

The fungal pathogen isolates were collected from tomato growing areas of the plants showing typical early blight symptoms on leaves. Then these isolates were further characterized with using Koch postulates on tomato leaves. The early blight pathogen cultures were maintained every 30 days with periodic transfers on potato dextrose agar plates and incubated in darkness at 28°C. These fungal isolates produce many spores and have the highest aggressiveness on all tested materials of tomatoes.

Establishment of Disease screening Nursery

To evaluate indeterminate and determinate germplasm against early blight screening experiments were conducted in tunnel and field conditions in the Research Area of Plant Pathology section, AARI Faisalabad. These varieties were obtained from Vegetables Research Institute, AARI. 19 varieties and 5 hybrids were grown in field conditions. In tunnel 16 varieties and 11 hybrids were established. The plant to plant distance was maintained 20 cm and row to row distance was maintained 60 cm. The nursery was watered regularly at different intervals. After germination when plants attained about 6-8 inches height, these were inoculated with the spore suspension of A. solani containing spore suspension of 500-800 spores/ml. The inoculations were made until and unless appearance of the disease symptoms. During sunny days simply water was sprayed to develop and maintain suitable humidity for the development of blight symptoms. All the recommended agronomic practices were followed to keep the tomato crop in favorable condition. The percent disease index (PDI) was calculated by using following formula given by Wheeler (1969).

\[
PDI = \frac{\text{Sum of numerical disease rating}}{\text{No. of plants observed} \times \text{Maximum disease rating}} \times 100
\]

The severity of the disease was recorded according to following evaluation scale of Mayee and Datar, 1986.

<table>
<thead>
<tr>
<th>Grade</th>
<th>Nature of infection</th>
<th>Level of resistance/ susceptibility</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No symptoms</td>
<td>Immune</td>
</tr>
<tr>
<td>1</td>
<td>Small circular, scattered, brown spots, covering 1 per cent or less of the leaf area</td>
<td>Highly Resistant</td>
</tr>
<tr>
<td>3</td>
<td>Spots enlarging, dark brown in colour covering 1 to 10 per cent of leaf area and infection on the lower most leaves of the plant</td>
<td>Resistant</td>
</tr>
<tr>
<td>5</td>
<td>Spots enlarging, dark brown in colour covering 11 to 25 per cent of leaf area and infection on the lower most leaves of the plant</td>
<td>Moderately Resistant</td>
</tr>
<tr>
<td>7</td>
<td>Spots dark brown in colour covering 26 to 50 per cent of leaf area and covering one third of the plant</td>
<td>Susceptible</td>
</tr>
<tr>
<td>9</td>
<td>Spots uniformly dark brown, coalescing, covering 50 per cent or more leaf area and severe infection on all leaves</td>
<td>Highly Susceptible</td>
</tr>
</tbody>
</table>

Table 1 . Disease rating scale
In-vitro evaluation of fungicides

The poisoned food technique (Falck, 1907) was followed to evaluate the efficacy of different concentration of five fungicides. All the fungicides were tested in laboratory against A. solani at a concentration of 50, 100 and 150 ppm respectively with three replications each. Molten sterilized potato dextrose agar was used as nutrient medium and required quantity of each fungicide was added separately so as to get a requisite concentration of that fungicide. The fungicides were thoroughly mixed by stirring and about 15 ml poisoned medium was poured to each of the 90 mm glass cavity blocks and allowed for solidification. The actively growing periphery of the culture of A. solani was carefully cut using a sterile cork borer/transfer needle and transferred aseptically to the centre of each glass cavity block containing the poisoned solid medium. Control was maintained by growing the cultures on PDA without the fungicides. The plates were incubated at 24±1°C and the mycelial radial growth was recorded for about one week. The mycelial radial growth was measured 7 days after incubation and percentage of mycelial radial growth inhibition (I) was calculated using the following formula:

\[
\text{Inhibition of the mycelial radial growth} (%) = \frac{C-T}{C} \times 100
\]

Where C and T are average fungal colony diameter (mm) in control and in antifungal treated PDA medium, respectively.

Field evaluation of fungicides

The field experiment was laid out in Randomized Complete Block Design (RCBD) with 4 treatments and 3 replications in the Research Area of Plant Pathology, section AARI Faisalabad. Healthy tomato seedlings were raised in nursery beds and 21 days old seedlings were transplanted into the field with 60 cm inter and 40 cm intra row spacing in plots measuring 2 x 2 m. All other cultural and pest control practices were followed as recommended in package of practices (Anon 1998).

The recommended doses of fungicides – Difenoconazole, Cabriotop, and Precure Combi were tested as three sequential sprays at an interval of 15 days. The first spray was carried out as soon as the first symptom of early blight was seen in the field. Five plants were selected randomly in each plot and observation on the basis of severity of the disease on the foliage was recorded by using 0-9 scale and percent disease index (PDI) was worked out.

Statistical analysis

Data was analyzed by analysis of variance (ANOVA) and the significance of differences within treatments were separated by using LSD test at probability levels P= 0.05 (Steel et al., 1997)

Tomato breeding companies have produced F1-hybrids. These grow from seeds that have been produced by controlled hand pollination of male and female parent lines. These hybrids combine high yield, disease resistance and other plant and fruit characteristics. In Asia, more than 40% of the farmers use hybrids. When using hybrids, new seeds should be purchased each season. This may cost more money, but the resistance against diseases of hybrids means the tomato plants need less spraying with pesticides. The yields are also higher, creating more opportunity to bring tomatoes to the market.

All the indeterminate hybrids showed significant results. Among the eleven hybrids five hybrids Litth-584, Litth-566, Litth-570, Litth-578 P20, Litth-568, Litth-545 & Litth-572 showed moderately resistant to early blight of tomato with 37-43% disease index. Five indeterminate hybrids Litth-539, Litth-589, Litth-559, Litth-592, Litth-545 showed moderately susceptible
response with 46-55% disease index and one hybrid Lith-568 showed susceptible response to early blight of tomato with percent disease index 68.88.

![Response of eleven indeterminate hybrids against early blight](image)

**Figure 2.** Response of eleven indeterminate hybrids against early blight

Short types (determinate) varieties usually support themselves and need no staking. Under severe weather conditions such as typhoons, however, staking may be advisable. Determinate types stop growing after flowering. They require less labour, so they are popular for commercial cultivation. They have a relatively concentrated fruit set which lasts only two or three weeks and the fruits ripen much faster than those from indeterminate types.

All the nineteen determinate varieties showed significant results. Among the nineteen varieties four varieties Black Prince, Oregon spring, Zhezha and Bloody Butcher showed resistant response to early blight with percent disease index 11-16. Ten varieties Bush Beefsteak, Wayahead, Polar beauty, Camp bell, Manatoba, Early Wonder, Alaskan Fancy, Advanta, Caro Rich Tomato and Glacier showed moderately resistant response to early blight with 24-37% disease index. Legend, Black Pear, New Yorker, Taxi, moderately susceptible to early blight of tomato with 50-60% disease index. One determinate variety Napoli showed susceptible response to early blight of tomato with 68.88% disease index.

**Results and Discussions**

The tall and bush types are entirely different kinds of crops. The tall varieties are the best choice for a long harvest period. They keep growing after flowering. This feature is called indeterminate. However, under tropical conditions, diseases and insect attacks will stop growth. The plants generally have more foliage. This will keep the temperature lower within the crop and the fruits grow in the shade of the leaves. Because they are covered, the sun does not damage the fruits and they ripen more slowly. Slower ripening and a high leaf/fruit ratio improve the taste of the fruits and in particular the sweetness. The tall types have to be staked, caged or trellised.

All the sixteen indeterminate varieties showed significant results. Among the sixteen varieties one variety Beefsteak showed resistant response with 15.55% disease index and eleven varieties Zarnitza, Carmello, Amish Red, Box Carwillie, Porter Improved, Grighthmires Pride, Manapal, Believe it or not, Super Sioux, Eva Puple Ball and Marmandr showed moderately resistant to early blight of tomato with 28-34% disease index. Three indeterminate varieties Mrs.Max well Big Italian, Marion, Climbing Trips L Crop showed moderately susceptible with 46-52% disease index and one variety Sahel showed susceptible response to early blight of tomato with 69.88% disease index.
Figure 3. Response of nineteen determinate varieties against early blight

All the determinate hybrids showed significant results. Among the five hybrids four hybrids T-1359 F1, Lth-158, Lth-8, Lth-169 showed moderately resistant to early blight of tomato with 33.33% disease index. One determinate hybrid Lth-174 showed susceptible response to early blight of tomato with per cent disease index 68.88.

Figure 4. Response of five determinate hybrids against early blight

It is interesting to note that among the fifty one tomato germplasm, no one was found to be immune or highly resistant in both conditions. However the tested indeterminate and determinate varieties and hybrids which was showed considerable amount of resistance were Beefsteak (indeterminate), Black Prinice, Oregon spring, Zhezha and Bloody Butcher (determinate).

Among the tested germplasm Sahel, LITTH-568 (indeterminate), Napoli, LTH-174 (determinate) were found to be susceptible to early blight disease of tomato. The overall screening results indicate that Beefsteak (indeterminate), Black Prinice, Oregon spring, Zhezha and Bloody Butcher (determinate) are the good source of resistance and it can be useful for the development of tomato hybrid cultivars resistant to early blight. However there is need for further research to evaluate other indeterminate and determinate tomato varieties and hybrids.

Different workers found different results in their respective studies. Islam et al. (2001) observed results in the screening experiment on the bases of early blight intensity, out of sixteen varieties one was found resistant, three were moderately resistant, six were moderately susceptible, four were susceptible, one was highly susceptible and none was found highly resistant.

Kumar and Srivastava in 2010-11 used forty four tomato genotypes to differentiate between early blight resistant and susceptible genotypes. The highest early blight disease incidence was found in PS-1 (73.56%), Kashi Amrit (71.12%), Flap-7171 (69.69%), H-T-4 (61.26%), DT-10 (53.65%) and the lowest in H-88-74-1 (12.04%), EC-520061 (12.29%) and EC-521071.
(25.00%) Floraded (27.00%) and Swarna Naveen (28.61%). Other fifteen genotypes showed moderately resistant and twenty genotypes showed susceptible but two genotypes were found highly resistant on the basis of early blight disease intensity.

Alsafadi et al. (2012) was recorded disease level based on a 1-9 scale. Results showed that cultivars Bosfer and Daher aljabal had a high level of resistance to early blight, compared to cultivars Dara, Gerdi, Haragel and Magdal Mawash which were moderately or highly susceptible to the disease. Moreover, cultivars Wardiat, Breh and Baskanta showed moderate resistance to the disease. Lohith et al. (2011) were found four genotypes EC 251709, EC 251717, EC 164295 and LE 15 showed highly resistant reaction with PDI ranged from 0-10%; whereas LE 44 was resistant (PDI 10.1-25%); EC 165690, EC 163681, EC 136711, EC 163683, LE 16, LE 35, LE 54, LE 85, LE-172 and LE-189 were moderately resistant (PDI 25.1-40%).

Tomato cultivars cultivated in Pakistan have low level of genetic resistance to Alternaria leaf blight disease. Farmers, in pursuance of high yield are inclined to cultivate some varieties which may be less resistant to the disease and rely on fungicide applications for the control of Alternaria solani, the casual organism of Alternaria blight of tomato.

In this study Beefsteak (indeterminate), Black Prince, Oregon spring, Zhezha and Bloody Butcher (determinate) are the good source of resistance against early blight but there is need for further research to evaluate other indeterminate and determinate tomato varieties and hybrids to control this threat.

![Figure 5. In vitro effect of different fungicides against A. solani after 9 days](image)

The results revealed in figure 5, clearly indicated that there was difference among the different fungicides in inhibiting the mycelial growth of A. solani. According to statistically Difenoconazole was found significantly most effective inhibited the growth of A. solani that showed 0.8, 0.7 and 0.6 cm at 50, 100 and 150 ppm. While Cabriotop was 2nd most effective followed by Precure Combi, Topsin-M and Halonil at 50, 100 and 150 ppm respectively.

The results revealed that maximum growth of A. solani was in case of 50 ppm. Minimum growth was obtained in case of applying 150 ppm of Difenonconazole. All the concentrations reduced the growth of A. solani but 150 ppm of Difenonconazole gave maximum control over mycelial growth of A. solani after 9 days of incubation at 24 C. Evaluation of fungicides in vitro is a handy tool to screen large number of fungicides.

In previous works, Feriel Issiakhem and Zouaoui Bouznad (2010) also showed effectiveness of Difenoconazole on development of mycelial growth of A. solani and reported that difenoconazole had a strong inhibition effect on A. solani (89%) with a concentration of 0.97 ppm (or 7.81 μl i.a./l) and conidial germination also strongly reduced and reached 92% at 1.95 ppm (7.81μl i.a./l).

Same technique was followed by Arunakumara (2006) but tested fungicides were different. He found copper oxychloride and mancozeb among contact fungicides at all tested concentrations were found to be highly effective in inhibiting the growth of A. solani. Among the systemic fungicides, propiconazole and metalaxyl MZ fungicides at all tested concentrations were found to be highly effective in inhibiting the growth of A. solani.
The details of the treatments are given in material and methods. Four sprays of the fungicides were given at an interval of 15 days starting from 60 days stage of the crop or first appearance of symptom which was earlier. The disease severity expressed as percent disease index (PDI) recorded before the commencement of each spray and a final observation on 90th day (15 days after the final spray) were recorded and the results have been presented in the figure 6. Data revealed that the PDI was in the range of 9.63 to 11.34 in the experimental plots before giving the first fungicidal spray. This range in the disease index did not differ significantly in the plots meant for different treatment. But in subsequent sprays, all the fungicides treated plots recorded significantly less disease index over control on 105th day of planting. Maximum disease control with 11.84% PDI was recorded in the Difenoconazole treated plots followed by (26.66%), in plots sprayed with Cabriotop. Least control of the disease was recorded in Precure Combi treated plots with 35.55 PDI. In the control plot percent disease index was as high as 73.33 per cent.

The data also showed that in all the treatments, there was increase in disease index from 60th day to 105th day. However, the rate of increase in percent disease index was slow in case of fungicides treated plots as compared to the control plots.

Dhamen and Staub (1992) examined the protective and curative activity of difenoconazole against A. solani was superior to that of mancozeb. Treatments 7 days prior to and 1 day after inoculation gave 83-100% disease control. Eradicative treatments applied 2 days after inoculation provided little initial control, but 10 days after inoculation they provided about 60% control. They reported that Difenoconazole is the first sterol inhibitor compound with excellent activity against A. solani.

A lot of work has been done by several workers for controlling early blight. Arunakumara (2006) found under field conditions to control the early blight disease of tomato with nine fungicides during 2005 at K.R.C.C.H. Arabhavi Campus, Gokak. Data on disease severity showed that all the fungicides tested reduced the disease intensity significantly compared to control. However, maximum disease reduction was recorded in 0.1% propiconazole (28.66) followed by 0.2% pyraclostrobin (35.33), 0.1% Amistar (42.66), 0.2% Chlorothalonil (48.00), 0.2% Metalaxyl MZ (46.33), 0.2% Mancozeb (44.66) and 0.2% Kasugamycin (51.66), where as the per cent disease index in control was highest (88.06). Lowest fruit infection of 1.0 per cent was recorded in propiconazole treated plot. Sinha and Prasad, 1991; Choulwar and Datar, 1992; Devanthan and Ramanujam, 1995 reported mancozeb as most effective chemical against A. solani.

References