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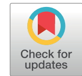
Variations in total phenolics and antioxidant enzymes cause phenotypic variability and differential resistant response in tomato genotypes against early blight disease



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ABSTRACT

Early blight disease caused by *Alternaria solani* is one of the dreadful diseases of the tomato. Existing cultural practices and fungicide applications are not enough to combat with the pathogen while, use of resistant cultivars is one of the most important ways to reduce disease damage. Currently, twenty-five tomato genotypes were screened out against the effect of early blight disease by artificially inoculating the plants with *A. solani*. The four tomato genotypes were kept in highly resistant (HR) group, 4 in resistant (RR), 5 in moderately resistant (MR), 4 in tolerant (TT), 5 in susceptible (SS) and 3 in highly susceptible (HS) on the basis of significant difference (LSD, $p \leq 0.05$) in disease incidence, percent severity index and plant mortality. Growth attributes were significantly ($p \leq 0.01$) decreased in SS and HS as compared to un-inoculated plants (control). Total phenolics, total protein content and activities of the antioxidant enzymes (catalase, peroxidase and polyphenol oxidase) were highly up-regulated in resistant groups than in susceptible groups linked with the induction of resistance against *A. solani*. The Pearson's correlation coefficient revealed a highly significant and very strong association between total phenolics and each biochemical component in HR and RR. The results of the current investigation indicated that phenolic compounds and activities of antioxidant enzymes in infected leaves could be used to assist the screening of resistant genotypes at early stages of early blight development.

1. Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most economically attractive, extensively consumed Solanaceous vegetable after potato (Khan et al., 2014). This fruity vegetable has incredible nutritive value as a rich source of vitamins (A & C), minerals and an antioxidant lycopene that contributes to a well-balanced and healthy diet (Niu et al., 2013). Alternaria blight or early blight of the tomato is one of the most catastrophic disease caused by air-borne, soil inhabiting fungus *Alternaria solani* in temperate, tropical and subtropical regions of the world (Meitei et al., 2014; Sathiyabama et al., 2014). The disease has considerably decreased the crop yield (50–80%) and affected tomato quality (Narendra Babu et al., 2015). Initially early blight disease symptoms appear as small dark brown spots on the lowest leaves surrounded by chlorotic halo which later turns yellow and dry up. When disease progresses, symptoms migrate to the stem and fruit (Rahmatzai et al., 2017) that results in leaf blight, stem collar rot and fruit rot followed by defoliation, drying off twigs and premature fruit dropping (Jambhulkar et al., 2016). The pathogen can overwinter as conidia, chlamydospores and mycelia on plant debris and in the soil. However, atmospheric temperature (27–32 °C), humid condition, wind speed, extended periods of leaf wetness from dew, rainfall and crowded plantation favor the disease prevalence (Rowlandson et al., 2015). The pathogen (conidia) disperses from the host by gusts of wind and rain or overhead irrigation splash through puff or tap mechanisms (van der Waals et al., 2003). Besides, the fungus can survive in soil over a wide range of temperature (4–36 °C) for 5–12 months which make it one of the problematic pathogen to manage (Mamgain et al., 2013).

In response to pathogen infestation, host plant activates a wide variety of protective defense mechanism through production of antimicrobial proteins, accumulation of reactive oxygen species (ROS) and alteration in the cell wall constitution (Agrios 2005; Murphy 2013). The biochemical alterations in host plant due to the higher production of ROS is familiar response that may be defensive in resistant plant while detrimental in case of susceptible host through the signaling factors (Gill and Tuteja, 2010a,b; Wrzaczek et al. 2010). Therefore, information on physiological (chlorophyll and carotenoids) and biochemical attributes (total phenolics, total protein content and antioxidant enzyme) could preferably be associated with resistance attained by host against invading pathogen to compensate the toxic effect of ROS

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(Catanzariti and Jones 2010; Boguszewska and Zagdańska, 2011).

Being an economically important crop, it is necessary to screen out resistant genotypes of the tomato before introducing into the market that would be a successful measure to lessen the dependency on chemical fungicides. Previously various tomato germlines have been screened against *A. solani* for resistance and new germplasms are being introduced (Meitei et al. 2014; Chohan et al. 2015; Kumar et al. 2015). Therefore, the current study was planned to identify and screen out the resistant source of available tomato genotypes against early blight disease on the basis of disease severity, growth and physiology. The study would be useful for commercial cultivation of the tomato genotypes in disease prone areas and would also be employed in development of resistant population for genetic studies.

2. Materials and methods

2.1. Fungal culture and inoculum preparation

Alternaria solani (FCBP-1401) was sub-cultured on Potato dextrose agar (PDA), incubated at 27 ± 2 °C for 5 days and stored at 4 °C. For the pathogenicity trail, cultural suspension (conidia and mycelia) was prepared by scraping and washing off culture into the autoclaved distilled water and conidia per mL were counted and adjusted to constant number (3×10^5 conidia mL^{-1}) for inoculation during experiment.

2.2. Procurement of tomato genotypes

Twenty-five tomato genotypes were procured from Ayub Agricultural Research Institute (AARI) Faisalabad, Pakistan in 2017 (Table 1). The tomato seeds were surface sterilized for ten seconds in 1% sodium hypochlorite solution, after that washed with distilled sterilized water and dried for later use in tomato nursery preparation.

2.3. Experiment

The experiment was conducted during March-May 2017 in a completely randomized design (CRD) at the research area of Institute of Agricultural Sciences (IAGS), University of the Punjab, Lahore, Pakistan.

Potted soil (12 kg pot^{-1}) was sterilized with 2% formalin solution (Khan et al., 2016) and about twenty days old tomato seedlings (4 seedlings pot^{-1}) of each twenty-five different genotype were transplanted in a plastic pot kept (14″ width × 16″ height) at temperature ± 27 °C and relative humidity 50–70%. Plants were inoculated fifteen days after transplanting with fungal cultural suspension (3×10^5 conidia mL^{-1}) by hand sprayer. After inoculation, inoculated plants were covered with plastic begs for 48 h to maintain humidity 65–70%. Pots of each twenty-five genotypes without fungal inoculation (control) were also maintained in triplicate.

Table 1
Genotypes of tomato procured from Ayub Agricultural Research Institute, Faisalabad. Pakistan.

Sr.	Genotypes	Code	Sr.	Genotypes	Code	Sr.	Genotypes	Code
1	Nadir	NDR	**10**	Faris	FRS	**19**	HT-1570	HT
2	Red pearl	RPL	**11**	Maryum	MRY	**20**	Miracle	MRC
3	TM-1826	TM	**12**	Four season	FRS	**21**	Naqeeb	NQB
4	Avenue	AVN	**13**	Roma	RA	**22**	Rover	RVR
5	Rebecca	RBC	**14**	Kamaal	KML	**23**	Aasba	ASB
6	Solo-I	SL-1	**15**	MDS-Royal slor	MRS	**24**	Nagina	NGA
7	Indigo	IDG	**16**	Betsay	BTS	**25**	Kanon	KNN
8	Wantia	WNT	**17**	1057	1057			
9	Pakit	PKT	**18**	Mehram-670	MHR			

2.4. Disease assessment

The plants were individually evaluated for the disease scoring on the foliage using 0–5 disease scale (Pandey et al., 2003), which is described as 0 = free from infection, 1 = < 5% leaf area affected, 2 = 5.1–10% foliage of plant covered with few isolated spots, 3 = many spots coalesced on the leaves, covering 10.1–25% surface area of plant, 4 = 25.1–50% area of the plants infected, fruits also infected at peduncle end, defoliation and blightening started, 5 = > 50% area of plant part blighted, sever lesions on stem and fruit rotting on peduncle end.

The percent disease incidence (DI) in each pot was calculated as:

$$DI\,(\%) = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100$$

Disease intensity was recorded in terms of percent severity index (PSI) by using following formula (Rahmatzai et al., 2017).

$$PSI\,(\%) = \frac{\text{Sum of all rating}}{\text{Total number of observation} \times \text{grading scale}} \times 100$$

2.5. Physiological and biochemical change assessments

After 15 days of inoculation, different physiological and biochemical changes related to host defensive system were estimated.

2.5.1. Total chlorophyll contents and carotenoids

The tomato leaves (0.5 g) were taken and extract was prepared in 80% ethanol to assess the total chlorophyll contents and carotenoids (Minocha et al., 2009). The absorbance for chlorophyll *a* (645 nm), chlorophyll b (663 nm) and carotenoids (270 nm) were taken and calculated according to the formula given by Lichtenthaler and Wellburn (1983).

2.5.2. Total phenolics

Total phenolics were quantified in a reaction mixture (0.5 mL ethanolic plant extract; 2.5 mL of 20% Na_2CO_3 along with 0.025 mL of Folin-Ciocalteatou's reagent). The absorbance of samples was taken against blank (without ethanolic plant extract) at 765 nm, after incubation at 45 °C for 45 min (Vongsak et al., 2013).

2.5.3. Total protein content and defense related enzymes

The leaf samples were weighed (0.5 g); ground in chilled sodium phosphate buffer (10 mL, 100 mM, pH 7.4) and was centrifuged for 5 min at 10,000 rpm (4 °C). After centrifugation the supernatant of crude enzyme extract was used for the estimation of total protein content by using the modified method of Lowry (Pomory, 2008). This crude extract was also assessed for the activities of defensive antioxidant enzymes viz., catalase (CAT), peroxidase (POX) and polyphenol oxidase (PPO).

2.5.3.1. Catalase activity (EC 1.11.1.6). CAT activity was estimated in the reaction mixture [0.1 mL enzyme extract + 2.9 mL of buffer containing 20 mM H_2O_2 solution in 50 mM sodium phosphate buffer (pH 7.0)]. The CAT activity was measured by monitoring the reduction in the absorbance at 240 nm as a result of H_2O_2 consumption (Lee et al., 2003; Sharma and Ahmad, 2014).

2.5.3.2. Peroxidase activity (EC 1.11.1.7). POX activity was assayed in a reaction mixture consisted of 0.5 mL of enzyme extract, 2 mL of 100 mM phosphate buffer (pH 6.8) and 1 mL of pyrogallol. Solution was filled with 1 mL of 50 mM H_2O_2 (5:5 in H_2O_2 and distilled water), incubated at 25 °C and reaction was terminated by adding 2.5 N H_2SO_4. The quantity of purpurogalline was determined by taking absorbance at 420 nm against a blank (reaction mixture except pyrogallol) (Piotrowska et al., 2010).

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