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Short Communications

Response of plant defense enzymes against tomato early blight disease

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Tomato (*Lycopersicon esculentum*) is one of the important commercial crops in the world. The fruit production is reduced by a number of abiotic and biotic factors. The tomato plant is prone to attack by several diseases that hamper its cultivation. Among the plant pathogens, *Alternaria solani* (Sorauer) causing early blight of tomato is one of the most common and destructive diseases of the cultivated tomato in tropical and sub-tropical areas having heavy dew, rainfall and high relative humidity (Nash and Gardner 1988). Early blight causes pre and post-harvest losses in tomato (Wolters *et al*. 2019). The disease severity may range from 18-87.5% (Maghaddam *et al.* 2020). The fruit yield loss up to 79% has been reported in major tomato growing countries (EL-Tanany *et al.* 2018). The disease is checked by fungicides application (Odilbekov *et al*. 2019). The regular spray of copper-containing fungicides and certain new generation fungicides like QoI (Quinone oxidation inhibitor) are required to check the disease. Disease resistant cultivars may be the better alternative to manage the disease (Hashemi *et al*. 2019). The pathogen reproduces asexually, but virulent isolate overcome existing resistance genes (Adhikari *et al.* 2017). The plants develop antioxidant defense systems to control ROS (reactive oxygen spp.), consisting of superoxide dismutase, peroxidase and catalase (Poli *et al.* 2018). Bradley *et al.* (1992) showed a correlation between increased peroxidase (PO) activity and resistance in plants. Phenylalanine ammonia lyase (PAL) activity is also essential for the accumulation of phenolics in an infected plant (Klessig and Malamy 1994). The resistant varieties and enzymes play important role in plant defense against pathogens. The research work in this field is limited and there is a need for more work in this area. Keeping these points into consideration, the objective was to study the response of biochemical defense enzymes against *A. solani* in tomato cultivars.

Tomato early blight resistant (H-88-78-1) and susceptible (Punjab Chhuhara) cultivars of tomato obtained from ICAR-Indian Institute of Vegetable Research (ICAR-IIVR), Varanasi (2015). The chemical azoxystrobin was obtained from Syngenta India Pvt Ltd and the fungus *A. solani* was isolated from infected plant parts and tested for pathogenicity. Isolated cultures were maintained on Potato Dextrose Agar (PDA) slants and stored in a refrigerator at 4^0C. The mixture of sterilized soil, sand and FYM (1:1:1) were taken in pots (12″ dia). Seeds of resistant (H-88-78-1) and susceptible (Punjab Chhuhara) cultivars were sown in pots. Thereafter light and frequent irrigation were given at regular intervals. For each treatment (Table 1) four replications were maintained. The study was conducted at Research farm of Institute of Agricultural Sciences, Banaras Hindu University, Varanasi during 2015 crop season.

The spore suspension was prepared from 30 days old *A. solani* culture. The plants were inoculated with spore (4×10^6/ml) suspension using hand atomizers. One set of tomato plants was treated with azoxystrobin 25 SC (0.1%) to induce the defense reaction. One day after treatment, one set of treated plants was inoculated with *A. solani* and

Table 1 Varieties of tomato and inoculants used in the study during 2015 crop season

Treatment	Varieties	Response	Inoculants
T1	H-88-78-1	Resistant	Azoxystrobin + *A. solani*
T2	H-88-78-1	Resistant	Azoxystrobin
T3	H-88-78-1	Resistant	H-88-78-1 (R) + *A. solani*
T4	H-88-78-1	Resistant	H-88-78-1 (R) - control
T5	Punjab Chhuhara	Susceptible	Azoxystrobin + *A. solani*
T6	Punjab Chhuhara	Susceptible	Azoxystrobin
T7	Punjab Chhuhara	Susceptible	*A. solani*
T8	Punjab Chhuhara	Susceptible	Control

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another set of treated tomato plants was not inoculated with *A. solani*. The plants not treated with chemical and pathogen was kept as control. Four replications were maintained in each treatment at 80% humidity and 26°C temperature. The leaves from sprayed and unsprayed tomato plants were collected at 0, 1, 3, 5, 7 and 9 days intervals and stored at -80°C. Enzyme extract prepared from 60 days old tomato leaves were used for the estimation of catalase, peroxidase, total phenol and phenyl ammonia lyase.

Catalase activity was assayed according to the method given by Beers *et al.* (1952). A decrease in H_2O_2 was monitored at 240 nm (extinction coefficient of 0.036 mM/ cm). Enzyme specific activity was expressed as µmol of H_2O_2 oxidized/min/mg protein. Peroxidase activity was assayed according to Egley *et al.* (1983). Absorbance was measured at 420 nm (extinction coefficient of 26.6 mM/ cm at 30 sec intervals up to 2 min using a Bausch and Lomb Spectronic-20 spectrophotometer. Enzyme specific activity was defined as µmol of H_2O_2 reduced/min/mg protein. Total phenols were estimated following the procedure given by Bray *et al.* (1954). The amount of phenolics was expressed as µg catechol/mg protein. PAL activity was determined as the rate of conversion of L-phenylalanine to trans-cinnamic acid at 290 nm as described by Dickerson *et al.* (1984). The amount of trans-cinnamic acid formed was calculated using its extinction coefficient of 9630/m (Dickerson *et al.* 1984). Enzyme activity was expressed as nmol trans-cinnamic acid/ min/ mg protein. The analysis was done using Microsoft Office Excel 2007 software. The treatments were arranged in a completely randomized design (CRD). The data were subjected to analysis of variance (ANOVA) using CPCS1 software and means were separated using Critical Difference (CD) test at ($P<0.05$).

The highest catalase activity (1.70) was observed on 5th day after *A. solani* inoculation and thereafter, enzyme activity (1.47) was decreased on azoxystrobin treated tomato cv. H-88-78-1. Among all the treatments, azoxystrobin pretreated resistant cultivar challenged with *A. solani* showed higher enzyme activity. The catalase activity in susceptible cultivar Punjab chhuhara was 1.39 which is more than in control (0.76) after 5 days of inoculation with *A. solani* alone and thereafter, it started to decline (1.36). The higher-level expression of defense-related enzymes and proteins and action of chemicals at the infection site certainly prevent the infection of pathogen attack. Nafisa *et al.* (2020) observed the response of 29 different tomato genotypes toward early blight disease by studying the biochemical attributes. The results showed that CAT contributed the maximum to resistance in tomato genotypes.

Increased activity of peroxidase was observed in both resistant and susceptible tomato cultivars upon challenged inoculation with *A. solani* in azoxystrobin (0.1%) pretreated plants. The maximum enzyme activity (1.48) was observed on 5th day after inoculation on azoxystrobin treated tomato cv. H-88-78-1 thereafter, enzyme activity (1.44) found decreased on 9th day. The enzyme activity in control plants was 0.72. Peroxidase is involved in the production of ROS, which are directly toxic to the pathogen or indirectly reduce the spread of the pathogen by increasing the cross-linkage and lignifications of the plant cell walls (Hammond *et al.* 1996). In these plants, disease incidence was less under glasshouse conditions. Thus, enhanced induction of peroxidase in fungicide treated plants may have been part of SAR which reduced the pathogen infection.

Enhanced activity of total phenol was observed in azoxystrobin (0.1%) pretreated plants of both resistant and susceptible tomato cultivars upon challenged inoculation with *A. solani*. The accumulation of total phenol activity (12.72) was observed on 5th day after inoculation and thereafter enzyme activity (11.76) was decreased on azoxystrobin treated resistant tomato cv. H-88-78-1. The enzyme activity on 5th day was 8.40 in susceptible cultivar Punjab chhuhara and further decreased to 8.16 on 9th day under similar conditions. The enzyme activity in control plants was 5.52. Resistance in pea to *Pythium ultimum* and *F. oxysporum* f. sp. *pisi* due to the accumulation of phenolics by prior application of *P. fluorescens* has also been reported (Benhamou *et al.* 1996).

In tomato plants treated with azoxystrobin, PAL was synthesized, whereas an additional increase in the synthesis was observed in azoxystrobin treated plants challenged inoculated with *A. solani*. The maximum enzyme activity (13.83) was observed on 5th day after *A. solani* inoculation on azoxystrobin (0.1%) treated tomato cv. H-88-78-1. The PAL activity decreased to (13.58) after 9 days of inoculation under similar conditions. The enzyme activity in control plants was 7.41. In all the sets of treatments, infected plants showed a significantly increased level of PAL activity than the control treatment. The timing and expression patterns of the defense enzymes are important for the suppression of pathogens.

The studies on systemic resistance revealed the induction of catalase, peroxidases, total phenol and phenylalanine ammonia lyase in tomato plants that were treated with novel QoI fungicide azxoystrobin after inoculation with *A. solani*. Systemic acquired resistance (SAR) is an induced plant defense method that provides protection against many diseases (Chaudhary 2018). Meena *et al*. (2016) found an increase in biochemical enzymes after *Alternaria solani* infection in tomato.

SUMMARY

An experiment was conducted to identify the biochemical defense enzymes, viz. catalase (CAT), peroxidase (POX), phenylalanine ammonia lyase (PAL) and total phenol responsible for resistance against *A. solani* in resistant (cv. H-88-78-1) and susceptible (Punjab Chhuhara) cultivars of tomato after the application of Amistar 25 SC (azoxystrobin 0.1%) during 2015 crop season. Azoxystrobin was applied for their efficacy in inducing defense enzymes in tomato against *A. solani*. Defense-related enzymes were increased in azoxystrobin pre-treated tomato plants. Early blight resistant tomato plants (cv. H-88-78-1) treated with novel QoI (Quinone oxidation inhibitor) fungicide

azxoystrobin inoculated with *A. solani* under glasshouse conditions showed increased accumulation of defense enzymes, v*iz*. CAT, POX, PAL and total phenol than control plants.

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REFERENCES

Adhikari P, Oh Y and Panthee D R. 2017. Current status of early blight resistance in tomato: an update. *International Journal of Molecular Science* **18**: 1–22.

Beers R F and I W Sizer.1952. Colorimetric method for estimation of catalase. *Journal of Biological Chemistry* **195**: 133–39.

Benhamou N, Belanger R R and Paulitz T C. 1996. Induction of differential host responses by *Pseudomonas fluorescens* in Ri TDNA- transformed pea roots after challenge with *Fusarium oxysporum* f. sp. *pisi* and *Pythium ultimum*. *Phytopathology* **86**: 114-18.

Bradley D J, Kjeilborn P and Lamb C J.1992. Elicitor and wound induced oxidation cross linking of a plant cell wall proline rich protein: A novel, rapid defense response. *Cell* **70**: 21-30.

Bray W and V Thorpe.1954. Analysis of phenol compounds of interest in metabolism. *Methods of Biochemical Analysis* **1**: 27-52.

Chaudhary A. 2018. Systemic acquired resistance (SAR) and its significance in plant disease management.https://www.researchgate.net/.../324719141_Systemic_Acquired_Resistance_SAR.

Dickerson D P, Pascholati S F, Hagerman A E, Butler L G and Nicholson R L. 1984. Phenylalanine ammonia-lyase and hydroxycinnamate CoA ligase in maize mesocotyls inoculated with *Helminthosporium maydis*. *Physiological Plant Pathology* **25**: 111-23.

Egley G H, Paul R N, Vaughn K C and Duke S O. 1983. Role of peroxidase in the development of water-impermeable seed coats in Sidaspinosa L. *Planta* **157**: 224-32.

EL-Tanany M M, Hafez M A, Ahmed G A and El-Mageed M A. 2018. Efficiency of biotic and abiotic inducers for controlling tomato early blight. *Middle East Journal* **7**: 650–70.

Hammond-Kosack K E and Jones J D G. 1996. Resistance gene-dependent plant defense responses. *Plant Cell* **8**: 1773-91.

Hashemi L, Golparvar A R, Nasr Esfahani M and Golabadi M. 2019. Correlation between cucumber genotype and resistance to damping-off disease caused by *Phytophthora melonis*. *Biotechnology and Biotechnological Equipment*. **33**: 1494–1504.

Klessig D F and Malamy J. 1994. The salicylic acid signal. *PIant Molecular Biology* **26**: 1439-1458.

Meena M, Zehra A, Dubey M K, Aamir M, Gupta V K and Upadhyay R S. 2016. Comparative evaluation of biochemical changes in tomato (*Lycopersicon esculentum* Mill.) infected by *Alternaria alternata* and its toxic metabolites (TeA, AOH, and AME). *Frontiers in Plant Science* **7**: 1–14.

Nafisa, Shoaib A Iqbal J and Khan K A.2020. Evaluation of phenotypic, physiological and biochemical attributes connected with resistance in tomato against *Alternaria solani*. *Acta Physiologiae Plantarum* **42**: 88.

Nash A F and R G Gardner. 1988. Tomato early blight resistance in a breeding line derived from *Lycopersicon hirsutum* PI 126445. *Plant Disease* **72**: 206–09.

Odilbekov F, Edin E, Mostafanezhad H, Coolman H, Grenville-Briggs L and Liljeroth E. 2019. Within-season changes in *Alternaria solani* populations in potato in response to fungicide application strategies. *European Journal of Plant Pathology* **155**: 953–65.

Poli Y, Nallamothu E, Balakrishnan D, Ramesh P, Desiraju S, Mangrauthia S K, Voleti S R and Neelamraju S. 2018. Increased catalase activity and maintenance of photosystem II distinguishes high-yield mutants from low-yield mutants of rice var. Nagina 22 under low-phosphorus stress. *Frontiers in Plant Science* **9**: 1543.

Wolters P J, Vos L D, Bijsterbosch G, Woudenberg J H C, Visser R G F, Linden G V D and Vleeshouwers V G A A. 2019. A rapid method to screen wild *Solanum* for resistance to early blight. *European Journal of Plant Pathology* **154**: 09–114.