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Exploring the role of elevated antioxidants and antioxidant enzymes in conferring resistance against *Alternaria porri* in onion germplasm

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ABSTRACT

The antioxidants and defense associated antioxidant enzymes associated with the entry of foreign bodies signal the first line of defense. In the present study, onion germplasm was analyzed for various biochemical parameters in response to inoculation *Alternaria. porri* for establishing role of antioxidant defense mechanism in onion for understanding their role in antioxidant-associated defense mechanism. The genotypes were categorized as resistant, moderately susceptible, susceptible and highly susceptible based on percent disease index (PDI). There were significant differences for all the parameters studied before and after inoculation. As compared to resistant genotypes, the susceptible and highly susceptible genotypes showed minimum total chlorophyll content at 3 weeks post-inoculation. The total phenol content in the leaf of resistant genotypes was high (246.4 mg GAE/100g FW) as compared to moderate susceptible (115.99 mg GAE/100g FW), susceptible (151.14 mg GAE/100g FW) and highly susceptible (146.60 mg GAE/100g FW) genotypes at 3 weeks post-inoculation (WPI). The antioxidant enzymes namely catalase, peroxidase and superoxide dismutase production increased upto 3 WPI. The activity of antioxidant enzymes (catalase, peroxidase and superoxide dismutase) was significantly high in resistant genotypes (9.80, 12.80 & 9.39 µmole/min/mg protein, respectively) as compared to moderately susceptible, susceptible and highly susceptible genotypes at 3 WPI. The study supports that antioxidant enzymes play a significant role in defense mechanism in conferring resistance against purple blotch and can be used as an effective bio-marker in resistance breeding against *Alternaria porri*.

Key words: *Allium cepa L., Alternaria porri, Antioxidant enzymes, Bo-marker.*

Introduction

Onion (*Allium Cepa* L.) an important food item worldwide and India produces 22.81 million tonnes annually from 1.22 million ha area (NHB Database, 2020). The low productivity problem of onion in India (18.10 t/ha) is aggravated due to occurrence of the myriads biotic and abiotic stresses. Purple blotch is a devastating diseases incited by *Alternaria porri.* Its severity increases at maximum day temperature around 28-32 °C with maximum relative humidity >85 %. It not only affect foliage but bulb formation also and thereby deteriorate both yield and quality of the crop. Report on immune response to purple blotch in primary gene pool of onion is vary but some lines have been identified that shows moder-

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ate to complete resistance against purple blotch (Yadav *et al.,* 2017). Field screening is still common but identification of useful biomarkers (i.e. biochemical constituents associated with particular diseases phenotype) were needed to strengthen the screening observations.

In onion there is no information on changes of leaf biochemical compounds particularly antioxidants and antioxidant enzymes upon infection with *Alternaria porri* which serves as first line of defence. The information may help in identifying resistance mechanism and can be employed in future resistance breeding program. The study aimed at determining the changes in the leaves antioxidants and antioxidant enzymes upon challenge inoculation with *Alternaria porri* and studying the relationship of these changes to host resistance.

Materials and Methods

Thirty-five onion genotypes were screened against *Alternaria porri* under polyhouse condition in main research farm of Division of vegetable Science, ICAR-IARI New Delhi. Random block design was used for experiment with three replications.

Five plants from each replication of individual genotype was randomly tagged for disease assessment on the 0 to 5 rating scale given by Sharma (1986) The disease scoring was done at 7 day's intervals starting from one week (7 days) after inoculation to five week (35 days) post inoculation (WPI). Based on individual plant scoring, Percent Disease Index (PDI) of each genotype was calculated by the formula given by Wheeler (1969). The genotypes were classified into different disease reaction class following Pathak *et al*. (1986).

Five plants in each genotype per replication were tagged for leaf biochemical analysis at different time point. Two important leaf antioxidant, namely chlorophyll and total phenol contents were measured at 4 different time points before and after inoculation. The disease has direct impact on leaf which is associated with chlorophyll content breakdown and damage. Leaf chlorophyll content was estimated using Hiscox and Israelstam (1979). For determination of phenol content standard Folin-ciocalteu method of Singleton *et al*. (1999) was followed.

The leaves (500 mg) were ground with the liquid nitrogen in pre-chilled mortar and pestle and 5 ml of chilled phosphate buffer (50 mM: pH 7.0)/g was added and mix thoroughly. The sample were centrifuge at 15000 g for 20 minutes at 4 °C and supernatant collected in amber coloured bottle after sieved through two layers of cheesecloth and stored at 4 °C and used as plant extract for estimation of antioxidant enzymes.

Catalase activity was assayed following Aebi (1984) Enzyme activity was calculated by computing amount of hydrogen peroxide decamped and as concentration of hydrogen peroxide reduced/minutes/mg protein.

The increase in optical density due to the oxidation of guaiacol to tetra-guaiacol at 470 nm was measured at Eppendorf Biospectrometer and used for quantification of peroxidase activity. Enzyme activity was calculated based on extinction coefficient of tetra-guaiacol " = 26.6 mM-1 cm-1 and expressed as µmol tetra-guaiacol formed per minute/mg protein (µmol/mg protein/min) (Castillo *et al*., 1984).

Super oxide dismutase was estimated following Dhindsa *et al*. (1981) with minor modifications. The SOD activity was assayed by monitoring the inhibition of photochemical reduction of nitro blue tetrazolium at 560 nm.

The significant difference in antioxidants and antioxidant enzymes at different time points were measured through 'paired t-test'. The degree and direction of trait association was estimated through correlation analysis. The SAS software ver 9.3 was used for the above analyses.

Results and Discussion

Significant differences for disease severity among the genotypes were observed (Table 1). The percent disease index ranges from 10 % (Pusa Soumya) to 90.0 % (2017 KhB Sel-4) at five weeks post inoculation. Based on PDI values genotype Pusa Soumya found resistant (R) and four genotypes (Arka Kirtiman, Arka Niketan, Bhima Super and Bhima Red) showed moderate susceptible (MR), 20 genotypes were susceptible (S) and 10 are highly susceptible (HS) to purple blotch disease. From these four groups (R, MS, S & HS), individually antioxidants and antioxidant enzyme activity before and after inoculation were calculated and also correlated with PDI value of each group to relate their role in plant defence mechanism (Fig 4A-E).

The resistant and moderate susceptible onion genotypes (less PDI value) has showed presence of more total chlorophyll content at 3 weeks post inoculation but at same the time susceptible and

highly susceptible genotypes (high PDI values) showed great reduction in total chlorophyll content. The total chlorophyll content in resistant genotype (Pusa Soumya) before inoculation was 6.60 µg/ml which was increased to 8.49 µg/ml at 3 weeks post inoculation, the average of total chlorophyll content in moderately susceptible genotypes were increased in lesser rate from 4.16 (before inoculation) to 4.79 µg/ml at 3 weeks post inoculation. While, susceptible and highly susceptible genotypes having high PDI values were showed higher rate of decrease in the total chlorophyll content post inoculation (Fig 4A). Meena *et al.* (2016) also observed gradual decrease in leaf chlorophyll content in tomato plants after infection with fungal pathogens (*Alternaria alternata* and *Xanthomonas oryzae,* respectively).

A resistant (Pusa Soumya) and moderate susceptible genotypes with less PDI value showed presence of higher amount of total phenol content after challenge inoculation as compared to susceptible and highly susceptible genotypes (Fig 4B). Compounds like total phenol are toxic substances to pathogens, thus the accumulation of phenolic compounds at the region of infection showed an associa-

Table 1. Percent disease index (PDI) and disease reaction of different onion genotypes after challenge inoculation.

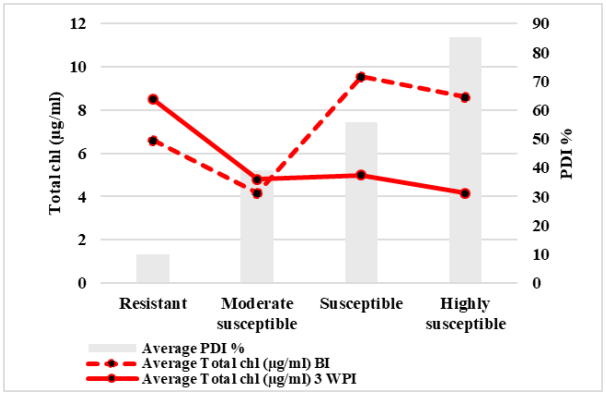
Genotypes	Source	PDI (%) at 1 WPI	PDI (%) at 3 WPI	PDI (%) 5 WPI	Disease reaction at 5 WPI
Pusa Soumya	IARI, New Delhi	0	10.00	10.00	R
Bhima Red	DOGR, Pune	22.50	35.00	38.33	MS
Bhima Super	DOGR, Pune	20.00	40.00	38.75	MS
Arka Kirtiman	IIHR, Bangalore	25.00	30.00	39.16	MS
Arka Niketan	IIHR, Bangalore	25.00	34.16	40.00	MS
Bhima Shweta	DOGR, Pune	25.00	45.00	45.00	S
2017 KhB Sel-17	Nashik, Maharashtra	20.00	40.00	50.00	S
2017 Kh Sel-2	Jalgaon, Maharashtra	25.00	48.33	51.33	S
2017 KhB Sel-15	Pune, Maharashta	30.00	40.00	51.66	S
2017 Kh Sel-25	Ahmedabad, Gujrat	25.00	43.52	51.90	S
Bhima Shakti	DOGR, Pune	33.33	40.00	53.28	S
Bhima Kiran	DOGR, Pune	30.00	46.66	55.00	S
Bhima Dark Red	DOGR, Pune	27.50	48.33	55.47	S
2018 KPBS-50	Jaipur, Rajasthan	22.50	42.5	55.71	S
Pusa Madhavi	IARI, New Delhi	22.50	46.66	56.33	S
Bhima Safed	DOGR, Pune	30.00	46.00	57.14	S
Arka Lalima	IIHR, Bangalore	27.50	42.20	57.85	S
2017 Kh Onion Sel-1	Local market, Delhi	20.00	41.66	58.33	S
2017 KhB Sel-23	Local market, Delhi	26.66	55.00	59.00	S
Early Grano	IARI, New Delhi	21.66	41.66	59.28	S
Bhima Raj	DOGR, Pune	31.66	45.00	59.60	S
2017 Kh Sel-13	Sonipat, Haryana	21.66	43.75	59.64	S
Arka Pragati	IIHR, Bangalore	31.66	47.50	60.00	S
2017 KhB Sel-11	Local market, Delhi	25.00	43.33	60.00	S
Sel-153-1	IARI, New Delhi	32.50	46.66	60.00	S
Pusa Red	IARI, New Delhi	30.00	67.00	81.66	HS
2017 Kh Sel-10	Local market, Delhi	25.83	65.65	82.85	HS
2017 Kh Sel-20	Local market, Delhi	30.00	62.00	83.33	HS
Pusa Riddhi	IARI, New Delhi	32.50	75.00	84.00	HS
Bhima Shubhra	DOGR, Pune	27.50	70.00	84.16	HS
Pusa Shobha	IARI, New Delhi	27.50	75.00	85.00	HS
2017 KhB Sel-21	Junagadh, Gujrat	27.50	73.75	86.19	HS
2017 Kh Sel-3	Pune, Maharashtra	25.00	75.00	87.33	HS
2017 KhB Sel-19	Local market, Delhi	27.50	68.75	88.33	HS
2017 KhB Sel-4	Rohtak, Haryana	27.50	75.00	90.00	HS

R-Resistant, MS-Moderately susceptible, S-Susceptible, HS-Highly susceptible, WPI- Week post inoculation.

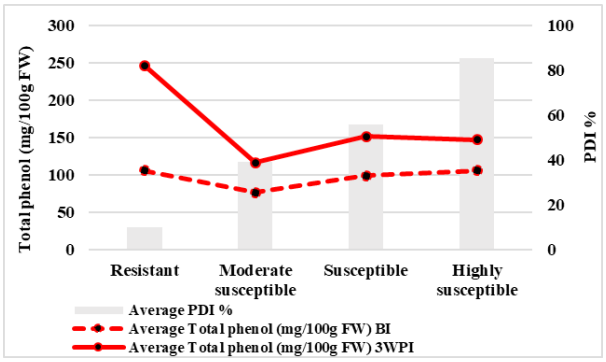
tion of phenolic compounds with the restriction of pathogen development after infection. Based on Benhamou *et al*. (2000) phenolic compounds may halt pathogen infection by increasing the mechanical strength of the host cell wall.

The resistant genotype showed high rate of increase in catalase activity post inoculation as compared to moderate susceptible, susceptible and highly susceptible genotypes (based on PDI). Along with this the catalase activity in resistant (Pusa Soumya) (9.80 µmole/mg protein/min) was found high as compared to catalase activity in moderate susceptible (5.51 µmole/mg protein/min), susceptible (5.94 µmole/mg protein/min) and highly susceptible genotypes (4.31 µmole/mg protein/min) at 3 weeks post inoculation (Fig 4C). Hanifei *et al*. (2013) was also found the positive correlation between catalase activity and resistance in melon against pathogen *Fusarium oxysporum* f. sp. *melonis* and *Trichothecium roseum*.

The genotypes with high rate of increase in peroxidase activity at one & three weeks post inocula-



A



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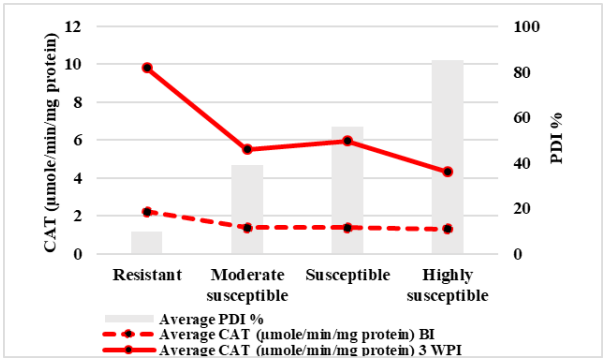

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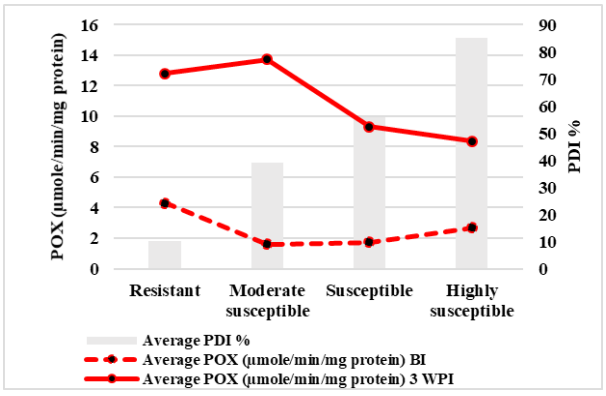

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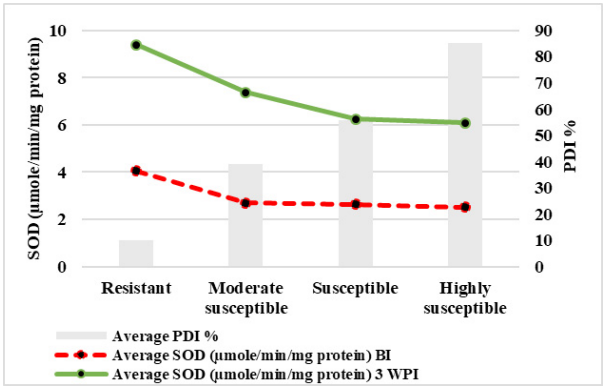

E

Fig 4A-E. Mean performance of onion genotypes before inoculation (BI) and 3 week post inoculation (WPI) for PDI (%) and total chlorophyll (µg/ml) (A), total phenol (mg/100 g FW) (B), catalase activity (µmol/mg protein/min) (C), Peroxidase (POX) activity (µmol/mg protein/min) (D) and Superoxide dismutase (SOD) activity (µmol/mg protein/min) (E).

tion were Arka Kirtiman, Arka Niketan and Pusa Soumya respectively. Pusa Soumya was found resistant to purple blotch with only 10 % PDI, Arka Kirtiman and Arka Niketan found moderately susceptible. Minimum peroxidase post inoculation was observed in Bhima Shubhra was found highly susceptible to disease. The peroxidase activity in resistant (12.80 µmol/mg protein/min) and moderate susceptible (13.78 µmol/mg protein/min) genotypes were found high as compared to susceptible (9.32 µmol/mg protein/min) and highly susceptible (8.37 µmol/mg protein/min) genotypes at 3 weeks post inoculation (Fig 4D). The increased peroxidase activity was found to be associated with the induced systemic resistance in plants against several pathogens (Baysal *et al*., 2005) and induced several plant defense mechanisms, such as lignin biosynthesis and oxidative cross-linking of plant cell walls, as well as the generation of reactive oxygen species (Bestwick *et al*., 1998).

The average of peroxidase activity in resistant (9.39 µmol/min/mg protein) and moderately susceptible (7.37 µmol/min/mg protein) genotypes were higher than average of superoxide dismutase activity in susceptible (6.24 µmol/min/mg protein) and highly susceptible (6.09 µmol/min/mg protein) genotypes at 3 weeks post inoculation (Fig 4E). Hanifei *et al*. (2013) also observed a significant rise in the activity of SOD in melon after infection with *Fusarium oxysporum* as compared to non-infected seedling in control group. SOD plays a major role in plant defense mechanism against plant pathogens. SOD is considered as a major scavenger of superoxide anion radical through dismutations of superoxide anion radical (O_2^-) into H_2O_2 and O_2 (Khan and Panda, 2008). Generation of H_2O_2 generation in infected plants may be one of the strategies to their defense against the invading necrotrophic pathogen.

The correlations between PDI and chlorophyll, phenol and antioxidant enzymes are given in Table 2. The PDI at 3 WPI had highly significant negative correlation with total chlorophyll, total phenol content, catalase and peroxidase. Total chlorophyll content at 3 WPI showed highly significant but negative correlation with the PDI at all three stages of observations. Genotypes having low PDI showed increase in antioxidant and antioxidant enzymes after challenge inoculation indicating for their role in disease mechanism. Positive and highly significant correlation observed between phenol with peroxidase, catalase with peroxidase and superoxide dismutase,

Table 2. Correlation in percent disease index (PDI %), total chlorophyll, phenol and antioxidant enzymes in onion genotypes before and after challenged with *Alternaria porri*.

Parameters		PDI (%)			Total chlorophyll (µg/ml)		Phenol (mg GAE/100g FW)		Catalase (µmol/mg protein/min)		Peroxidase (µmol/mg protein/min)		SOD (µmol/mg protein/min)
		1 WPI	3 WPI	5 WPI	BI	3 WPI	BI	3 WPI	BI	3 WPI	BI	3 WPI	BI
PDI (%)	3 WPI	0.535**											
	5 WPI	0.568**	0.960**										
Total chlorophyll (µg/ml)	BI	0.124^{NS}	0.085^{NS}	0.177^{NS}									
	3 WPI	-0.455**	-0.486**	-0.460**	0.544**								
Phenol (mg GAE/100g FW)	BI	0.105^{NS}	-0.058^{NS}	-0.056^{NS}	-0.114^{NS}	0.091^{NS}							
	3 WPI	-0.271^{NS}	-0.360*	-0.371*	0.235^{NS}	0.604**	0.379*						
Catalase (µmol/mg protein/min)	BI	-0.177^{NS}	-0.214^{NS}	-0.197^{NS}	0.348*	0.493**	0.039^{NS}	0.211^{NS}					
	3 WPI	-0.278^{NS}	-0.505**	-0.604**	-0.059^{NS}	0.354*	0.256^{NS}	0.369*	0.246^{NS}				
Peroxidase (µmol/mg protein/min)	BI	-0.211^{NS}	0.165^{NS}	0.112^{NS}	-0.075^{NS}	0.106^{NS}	0.109^{NS}	-0.126^{NS}	0.061^{NS}	0.068^{NS}			
	3 WPI	-0.061^{NS}	-0.352*	-0.368*	-0.081^{NS}	0.398*	0.357*	0.268^{NS}	0.356*	0.466**	0.159^{NS}		
SOD (µmol/mg protein/min)	BI	-0.171^{NS}	-0.373*	-0.388*	-0.224^{NS}	0.239^{NS}	0.112^{NS}	0.155^{NS}	0.302^{NS}	0.266^{NS}	0.181^{NS}	0.455**	
	3 WPI	-0.240^{NS}	-0.291^{NS}	-0.321^{NS}	-0.164^{NS}	0.374*	0.140^{NS}	0.164^{NS}	0.497**	0.390*	0.106^{NS}	0.454**	0.425*

SOD (Superoxide dismutase), before inoculation (BI), week post inoculation (WPI), * (significant), ** & *** (highly significant), non-significant (NS).

peroxidase with superoxide dismutase. Antioxidant and antioxidant enzyme activities increased after challenge inoculation. The correlation between these phytochemicals and PDI highlights their potential for use in disease resistance mechanism.

Considering the positive correlation between PDI and antioxidants, antioxidant enzymes activity, high rate of antioxidants and antioxidant enzymes activity in Pusa Soumya (resistant) and Arka Kirtiman, Arka Niketan, Bhima Super and Bhima Red (moderately susceptible genotypes) and lower enzymatic activity was observed in susceptible and highly susceptible genotypes with some exceptions. Antioxidant enzymes defense plays a significant role in defense mechanism in conferring resistance against purple blotch and can be used as an effective biomarker in resistance breeding programs in onion against *Alternaria porri*. Therefore, the genotypes observed with high antioxidants and antioxidant enzymes activity and low PDI after challenge inoculation can be used for purple blotch resistance breeding of onion.

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Declaration

The authors declare that there is no conflict of interest.

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