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Activity Profile of Defense Related Enzymes in Pearl Millet Against Downy Mildew (*Sclerospora graminicola)*

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The activity profile of defense related enzymes viz., peroxidase, polyphenol oxidase, catalase, β-1, 3 glucanase and phenylalanine ammonia-lase (PAL) with and without inoculation of pathogen *Sclerospora graminicola* (Sacc.) were evaluated in downy mildew resistant and susceptible parents and the crosses (RxS, SxR, RxR) of the pearl millet. The activity of all these enzymes significantly increased post inoculation of the seedlings suggesting induction of the defense response. The level of induction in these enzymes was significantly higher in resistant parent and also in crosses involving resistant genotype as a male parent. The pattern of inheritance as observed in the present investigation is indicative of a dominant mode of inheritance. When the data was analyzed and compared with the field data of downy mildew incidence it was also noticed that SxR crosses recorded comparatively low incidence of downy mildew.

Key words: Downey mildew, defense related enzymes, PAL, pearl millet.

Downy mildew disease of pearl millet caused by the oomycetous, biotrophic fungus *Sclerospora graminicola* (Sacc.) Schroter, causing huge yield losses of 10–60% in various countries of Asia and Africa[1]. The exact reasons for the breakdown of resistance are not known as there is a lack of complete understanding of the biochemical basis of resistance of pearl millet to downy mildew. However, since the introduction of high yielding single cross hybrids in India, in the late 1960's, downy mildew has been a major production constraint and a major focus of pearl millet improvement research both by ICRISAT and the Indian National Program [2].

Plants have different defense mechanisms to evade fungal invasion. In pearl millet, Downy mildew showed a typical case of inflorescence malformation and conversion of florets into green leafy structures. Earlier investigations suggested that in abnormal growth of ear heads, PPO activity always remained higher in comparison to completely proliferated suppressed and normal ear heads[3]. Phenols and oxidizing enzymes such as PPO and POX have an active role in resistance mechanism of plant diseases[4]. It has been reported that resistant cultivars have higher amount of total and OD phenols (auxin protectors) than susceptible ones. Phenolic compounds are among the most influential and widely distributed secondary products in the plants. Such compounds govern disease resistance in many crop plants. Increased activity of polyphenol oxidase (PPO), peroxidase (POX), and phenylalanine ammonia lyase (PAL) has been reported in plants treated with various biotic and abiotic inducers of resistance [5, 6]. Development of defense responses in plants is complex and both structural and biochemical barriers [7]. These barriers have direct or indirect action on the course of pathogenesis and has the major effect on plant resistance to a given pathogen. The pathogenesis-related (PR) proteins, hydrolases such as β-1,3-glucanases and chitinases have been suggested to be involved in

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plant resistance against fungal pathogens[8]. The β-1,3-glucanases and PAL are induced by pathogens and inhibit fungal growth by release of oligosaccharide elicitors inducing the production of phytoalexins[9] while induction of PAL as a response to pathogen infection is well documented in various host interaction [10].

Downy mildew has been recognized as a potentially important disease of pearl millet since a long time, the disease continues to be a major threat, as a basic understanding of host– pathogen interactions and of resistance and susceptible mechanisms is lacking. Development of resistant cultivar through breeding is the only method of choice for checking the yield losses. The differences in the biochemical constituents and certain enzymes which are involved in imparting resistance can be a great aid in rapid and effective screening of the germplasm. However, this biochemical constituents/enzymes need to be confirmed and confirmation warrants undertaking biochemical studies specifically in the divergent parents and validation needs analysis of the breeding population involving crosses like R × R, R × S , and S × R. In order to generate biochemical information on these aspects for practical utility the current investigation entitled "Activity profile of defense related enzymes in pearl millet against downy mildew was undertaken to analyze the induction level of metabolites and specific enzymes in resistant/susceptible parents and their crosses, post inoculation by *Sclerospora graminicola*.

MATERIAL AND METHODS

Plants, growth condition and experimental treatments

The investigation was carried out using three downy mildew (DM) resistant parents (DHLB -16B, DHLB–17B, DHLB-18B), one DM susceptible parent (7042S), two Susceptible × Resistant Crosses (7042S × DHLB-17 B, 7042S × DHLB-18B) and two Resistant × Resistant Crosses–DHLB-16B×DHLB-17B and DHLB-17B×DHLB-18B of pearl millet. The seeds were procured from the Bajra Breeder, College of Agriculture Dhule, under MPKV, Rahuri, Maharashtra. The pathogen *S. graminicola* pathotype 1 was maintained on its susceptible host (7042S cultivar of pearl millet) under greenhouse conditions.

Screening of pearl millet cultivars for downy mildew reaction

Cultivar resistance of pearl millet for downy mildew reaction was screened in the downy mildew sick plot of the Department of Botany, Agriculture College Dhule, Maharashtra, India. Seeds were sown in downy mildew nurseries containing heavy load of soil-borne oospores and sporangial inoculation provided from infector rows[11] . The test entries were sown in a randomized block design with three replicates. Normal agronomic practices were followed to raise the crop. Evaluation of cultivar resistance to downy mildew was carried out by recording disease incidence 30 days after sowing and also at the dough stage (60 days). Plants were rated as diseased when they showed any of the typical symptoms of downy mildew, i.e. stunting, chlorosis, downy growth of asexual spores on the under surface of the leaves and malformed ear heads. Percentage disease incidence was rated from the number of systemically-infected plants.

Inoculation of the plant material

The seeds were surface sterilized in 0.1% (v/v) sodium hypochlorite solution for 15 min, washed thoroughly with sterile distilled water and germinated on moist filter paper under aseptic conditions at 25 ± 1^0C in dark for 14 days. The fourteen-day-old seedlings were root dip inoculated with zoospores suspension of *S. graminicola* [12]. Seedlings dipped in sterile distilled water served as control. The seedlings were analyzed 72 h after inoculation for enzyme assays. Experiment conducted in the Dept. of biochemistry during 2012-2013.

Estimation of total phenols and ortho-dihydric phenols

For estimation of total phenol and O.D phenol exactly 0.250 g of the sample from inoculated and control leaf was weighed and macerated with 5-time volume of 80% ethanol in a pestle and mortar. The homogenate was centrifuged at 10,000 rpm for 20 min. The supernatant was collected and the residue was re-extracted two more times with 80% ethanol. The combined supernatant was evaporated to dryness. The residue concentrate was dissolved in a known volume of

distilled water (5 ml) and aliquot was used to estimate the phenol and OD phenol separately.

Estimation of total phenols was carried out with Folin-ciocalteu reagent[12]. 0.1ml aliquots was pipetted into test tubes and made up the 3ml with water. To this 0.5 ml of Folin-ciocalteu reagent was added and after 3min, 2 ml of 20% Na_2Co_3 solution was added to each tube mixed thoroughly and the tubes were placed in boiling water for exactly one min. The tubes were cooled under tap water and absorbance was measured at 650nm against a reagent blank. The concentration of phenols in the test sample was expressed as mg /100g material from standard curve prepared using different concentration of catechol.

Estimation of ortho-dihydric phenols was carried out with Arnow' reagent[13.] 0.1ml aliquots was pipetted into test tubes and made up to 1ml with water. To this 1ml of 0.05 N HCl, 1ml of Arnow's reagent, 10 ml of water and 2 ml of 1N NaOH was added. The content were mixed thoroughly (pink colour appeared). The absorbance was measured at 515nm. The amount of ortho-dihydric phenols present in the sample was calculated from the standard curve prepared using catechol and expressed as mg g^{-1} fr.wt

Assay of peroxidase, polyphenol oxidase

Extraction of sample for estimation of peroxidase activity and polyphenol oxidase and for catalase activity was carried out from inoculated and control seedlings. About 0.250 g of sample was weighed separately and macerated with 2ml of 0.1 M sodium phosphate buffer (pH 6.8) in prechilled mortar and pestle. The homogenate was then centrifuged at 10,000 rpm at 4^0C for 20 min. The clear supernatant was taken as the enzyme source. The peroxidase activity was assayed by the method of Shanon *et al*[15]. The assay mixture of POX contained 2.3 mL of 0.1M of phosphate buffer (pH 6.8) at 4°C. The reaction mixture (0.7 ml) consisted of 0.5ml 0.01 M pyrogallol and 0.1 ml of 0.025 M hydrogen peroxide. The addition of 0.1 mL of crude enzyme extract initiated the reaction, which was measured spectrophotometrically at 420 nm. The polyphenol oxidase activity was assayed by the method of Kar and Mishra[16]. The assay mixture of Polyphenol oxidase (PPO) contained 1.5 ml of 0.1 M phosphate buffer (pH 6.8) at 4°C. The reaction mixture (1.5 ml) consist 0.5ml of 0.01 M pyrogallol, 0.9 ml of distilled water and the addition of 0.1 ml of crude enzyme extract initiated the reaction, which was measured spectrophotometrically at 420 ·m at 30 s interval for 3 min.

PAL activity (Phenylalanine ammonia-lyase)

The PAL activity (E.C. 4.1.3.5) was assayed by the method of Campos *et al*[17]. Enzyme was extracted from inoculated and control seedlings separately. Exactly 0.250 g sample were weighed and macerated with 2ml of 50mM borate buffer (pH 8.5) containing 5mM of 2-mercaptoethanol and 0.4 g insoluble polyvinylpolypyrrolidone. The homogenate was centrifuged at 20,000 xg at 4^0C for 20 min. The supernatant was used as a enzyme source. The assay mixture containing 0.5 ml of borate buffer, 0.1 ml aliquots of the supernatant, 0.9 ml of distilled water and 1 ml of 100mM L-phenylalanine were incubated at 32^0C for 60 min. Then, 0.5 ml of (TCA) trichloroacetic acid at 4% (w/v). was added to terminate the reaction. The absorbance of the supernatant was read at 290 nm. PAL activity was calculated as nmol of trans cinnamic acid per gm of tissue produced under the specific conditions, or as nmol of cinnamic acid produced per mg of protein per min for fractions of purified min protein.

β-1, 3- glucanase activity and Protein content

The assay of β-1, 3- glucanase was carried out as per the method described by Rakshith *et al.*, [18]. Inoculated and control seedlings were separately weighed and about 0.250 g of leaf tissues were macerated with 2ml of 0.2m Tris-HCl buffer (pH 7.5) in pre-chilled mortar and pestle. The homogenate was centrifuged at 11,000 x g at 4^0C for 30 min and the supernatant was used as the crude enzyme source. The assay mixture containing 1 ml reaction mixture contained 950 µl of laminarin and 50 µl of crude enzyme extract and was incubated at 37^0C for 30 min and the reducing sugar released in the solution at the end of reaction was estimated by Nelson-somogyi's method [19]. The absorbance was read at 620 nm. The β-1, 3- glucanase activity was expressed as µgmol of glucose released mg^{-1} soluble protein min^{-1}. The protein content in the crude enzyme extract of different enzyme assays was estimated according to the method of Lowry *et al.* [20].

Statistical analysis

All the biochemical parameters were analyzed in three replications. The data obtained for biochemical constituents and enzymes determination were subjected to simple completely randomized design (CRD) for the significance of various data using ''F'' test [21].

RESULTS AND DISCUSSION

Resistance of pearl millet cultivars to downy mildew disease

Differential downy mildew disease incidence in pearl millet cultivars was recorded in the field screening experiment. Seeds were sown in downy mildew nurseries containing heavy load of soil-borne oospores and sporangial inoculation provided from infector rows under downy mildew sick plot, confirmed the occurrence of resistance variation among cultivars. The cultivars DHLB-16B, DHLB-17B, DHLB-18B did not exhibit any downy mildew symptom expression despite exposure to continuous supply of inoculum for over 30 and 60 days. Maximum disease of over 82% was observed in susceptible cultivars 7042'S both at 30 and 60 days after infection while RxR showed very least mean diseased plants i.e. 5.12 and 7.57 % . The S x R crosses 7042 S xDHLB-17B, 7042S X DHLB-18 recorded comparatively low incidence of downy mildew (Table 1). The results of the present investigation are in agreement with the previous results recorded in pearl millet [7].

Total phenol and O.D content

The total phenol content was significantly higher in all the three resistant genotypes and the two crosses involving resistant parents .The total phenol content also differed between resistant genotypes (DHLB-16B, DHLB-17B, and DHLB-18B). The total phenol content of the susceptible genotype 7042S was much less 3.08 mg g^{-1} fr.wt.

Table 1. Field Screening of pearl millet parent and crosses for downy mildew reaction.

Cultivar/Crosses	No of plants	No of plants infected in30 DAS	No of plants infected in 60 DAS	Mean DM incidence in field
DHLB-16 B (Resistant)	10	0(0.0)	0(0.0)	0.0
DHLB–17 B (Resistant)	20	0 (0.0)	0 (0.0)	0.0
DHLB–18 B (Resistant)	53	0 (0.0)	0 (0.0)	0.0
7042'S (susceptible)	50	38 (78)	44 (86)	82
DHLB-18 B × 7042'S (R × S)	41	9 (21.3)	9 (21.9)	21.6
DHLB-17 B × 7042 S (R × S)	28	2 (7.14)	5 (17.8)	12.5
7042 S × DHLB - 17 B (S × R)	13	1 (7.6)	1 (7.6)	7.6
7042 S × DHLB -18 B (S × R)	19	2 (10.5)	2 (10.0)	10.5
DHLB-16 B × DHLB- 17 B (R × R)	39	0 (0.0)	2 (5.1)	5.12
DHLB-17 B × DHLB –18 B(R × R)	33	2 (6.06)	3 (9.0)	7.57

(Figures in parenthesis are percent increase or decrease over control)

Total phenols content were less in control seedlings of both resistant and susceptible parents, though they were significantly more in resistant genotypes while a significant increase in total phenol content after inoculation was recorded in seedlings of both resistant and susceptible genotypes and their crosses (R×S, S×R and R×R) . The percent increase in total phenol content varied from as low as 8.44%in DHLB-18B to as high as 29.02% in DHLB-16B. In susceptible genotype the total phenol content increased from 3.08 to 3.26 mg g^{-1} fr.wt an increase of 5.84% (Table 2).Whereas in crosses a marginal increase was recorded in total phenol content of 2.27 and 2.41 % respectively on inoculation with the pathogen in DHLB-17B × 7042S and DHLB-18B × 7042S. It is interesting to note that crosses involving resistant genotype as a male parent had considerable higher total phenol content both under non inoculated and inoculated conditions. S×R crosses *viz.*, 7042S × DHLB-17B and 7042S × DHLB-18B which recorded an increase

in the total phenol content of 10.12 and 20.34% respectively. The total phenol content of the R×R crosses *viz.*, DHLB-16B × DHLB-17B and DHLB-17B × DHLB-18B was much higher both under non inoculated and inoculated condition, however, the level of induction as reflected by % increase over non inoculated control was less than 10% in both the crosses.

This result clearly indicates correlation between higher levels of phenols and resistance to downy mildew pathogen and our results are in conformity to those reported earlier in pearl millet and other crops like onion, sorghum, and grape vine [22, 23, 24]. A pearl millet variety resistant to *S.graminicola* has been reported to contain more total phenols than the susceptible varieties [25, 26]. An increase in total phenol content after inoculation and higher total phenols in infected pearl millet leaves has been reported earlier [27].

Ortho-dihydric phenols were estimated by employing Arnow's reagent which is specific to ortho-groups [28].The persual of the data revealed higher levels of O.D. phenols in resistant parents and crosses involving resistant parents (R×R) than the susceptible parents. The level of O.D. phenols in downy mildew resistant genotypes varied from 3.41 mg g^{-1} fr.wt to 3.96 mg g^{-1} fr.wt as against the conc. of 2.71 mg g^{-1} fr.wt in susceptible genotype (Table 2). Inoculation of seedlings with pathogen however caused decrease in the level of O.D.phenols in all the resistant parents, susceptible parents and the crosses (S×R R×S and R×R).There was no definite trend in the level of reduction of O.D. phenols in resistant or susceptible parents, although the susceptible parents 7042S recorded comparatively more reduction in the level of O.D. phenols (12.18%).The crosses involving susceptible genotype as a male parent also

Table 2. Profile of Phenol, O.D phenol, PPO and PAL activity in 14 days old seedlings of pearl millet (parent /crosses) with and without inoculation

Cultivar/Crosses	Total phenol (mg g^{1} fr. wt)		O.D. phenol (mg g^{1} fr. wt)		PPO activity (ΔA_{420} min^{-1} mg^{-1} protein)	
	Control	Inoculated	Control	Inoculated	Control	Inoculated
DHLB-16 B (Resistant)	4.52	5.84 (29.20)	3.85	3.70 (3.89)	0.314	0.458 (45.86)
DHLB–17 B (Resistant)	4.34	5.40 (24.42)	3.41	3.03 (11.14)	0.294	0.521 (77.21)
DHLB–18 B (Resistant)	5.33	5.78 (8.44)	3.96	3.88 (2.02)	0.270	0.512 (89.63)
7042'S (susceptible)	3.08	3.26 (5.84)	2.71	2.38 (12.18)	0.597	0.785 (31.49)
DHLB-18 B × 7042'S (R × S)	3.73	3.82 (2.41)	2.83	2.61 (7.77)	0.227	0.275 (21.14)
DHLB-17 B × 7042 S (R × S)	3.53	3.61 (2.27)	3.29	2.71 (17.63)	0.234	0.264 (12.81)
7042 S × DHLB -17 B (S × R)	4.05	4.46 (10.12)	2.81	2.67) (4.98	0.364	0.485 (34.34)
7042 S × DHLB-18 B (S × R)	3.49	4.20 (20.34)	2.79	2.65 (5.02)	0.570	0.719 (26.14)
DHLB-16 B × DHLB-17B (R×R)	4.40	4.73 (7.5)	3.05	2.85 (6.56)	0.206	0.244 (18.44)
DHLB-17B ×DHLB–18B (R×R)	4.37	4.67 (6.7)	3.11	2.91 (6.43)	0.342	0.412 (20.46)
Mean	4.084	4.577	3.18	2.93	0.341	0.467
SE +	0.006	0.006	0.005	0.005	0.006	0.005
C.D@ 5%	0.018	0.016	0.015	0.013	0.017	0.015

(The data are means of three replication of experiments and Figures in parenthesis are percent increase or decrease over control)

recorded comparatively higher reduction in O.D. phenols and per say have been reported to impart resistance because they have bactericidal activity. However, contrary to this an increase in O.D.phenols has been reported in groundnut plants as a defense response to *sclerotium rolfsii* [29]. Similar increase in Ortho-dihydric phenols has been reported in many host pathogens interactions [30, 31]. A maximum reduction in level of OD phenol content has been reported earlier in resistant and susceptible genotypes of pearl millet in healthy and diseased tissues [4].

Peroxidase and Polyphenol oxidase activity

The mean peroxidase activity increased from 0.293 min^{-1} mg^{-1} protein in uninoculated control to 0.402 min^{-1} mg^{-1} protein in inoculated seedlings an increase of 137% which signifies induction of peroxidases upon challenges of the pathogen (Table 3).However, it is interesting to note that the peroxidase activity was much higher in the susceptible genotype 7042S even in the absence of the pathogen which was 0.469 min^{-1} mg^{-1} protein as against the resistant genotype which varied from as low as 0.136 min^{-1} mg^{-1} protein in DHLB-16B to as high as 0.234 min^{-1} mg^{-1} protein in DHLB-17B. The crosses involving 7042S a downy mildew susceptible genotype either as male or female parent also exhibited much higher peroxidase activity than the resistant genotypes both in the absence and presence of the pathogen inoculum. It thus appears that higher peroxidase activity as observed in the susceptible genotype in absence of pathogen could be an inbuilt genetic factor of a specific genotype and does not signifies the type of peroxidase isoforms. The cell wall peroxidase isoforms are reported to be involved in oxidative burst leading to hypersensitive cell death. The activity per se in absence of the pathogen could

Table 3. Activity profile of defense related enzyme in 14 days old seedlings of pearl millet (Parent/Crosses) with and without inoculation

Cultivar/Crosses	β-13 glucanase (µmol min^{-1} mg^{-1} protein)		POX activity (ΔA_{420} min^{-1} mg^{-1} protein)		PAL activity (nmoles of cinnamic acid min^{-1} mg^{-1} protein)	
	Control	Inoculated	Control	Inoculated	Control	Inoculated
DHLB-16 B (Resistant)	1.05	1.25 (19.0)	0.136	0.190 (39.70)	0.64	1.81 (182)
DHLB–17 B (Resistant)	1.06	1.33 (25.46)	0.234	0.341 (45.72)	0.86	2.55 (196)
DHLB–18 B (Resistant)	1.06	1.24 (19.23)	0.178	0.257 (44.34)	0.68	2.04 (200)
7042'S (susceptible)	1.04	1.06 (1.9)	0.469	0.544 (15.99)	0.98	1.56 (59.18)
DHLB-18 B × 7042'S (R × S)	1.33	1.35 (1.5)	0.312	0.368) (17.94	0.93	1.58 (69.89)
DHLB-17 B × 7042 S (R × S)	1.25	1.36 (8.8)	0.394	0.503 (27.66)	0.94	1.71 (81.91)
7042 S × DHLB - 17 B (S × R)	1.14	1.45 (27.1)	0.430	0.575 (33.32)	0.51	1.62 (217)
7042 S × DHLB -18 B (S × R)	1.16	1.43 (20.95)	0.335	0.497 (48.36)	0.50	1.85 (270)
DHLB-16 B × DHLB- 17 B (R × R)	1.05	1.27 (18.09)	0.233	0.366 (57.10)	0.54	1.67 (209)
DHLB-17 B × DHLB –18 B(R × R)	1.05	1.24	0.212	0.385 (81.60)	0.60	1.63 (171)
Mean	1.119	1.30	0.293	0.402	0.72	1.80
SE +	0.005	0.005	0.005	0.004	0.007	0.007
C.D@ 5%	0.014	0.013	0.015	0.013	0.022	0.020

(The data are means of three replication of experiments and Figures in parenthesis are percent increase or decrease over control)

not be correlated with the downy mildew field incidence. However the levels of induction upon challenge with a pathogen when analyzed revealed that in resistant genotypes the level of induction varied from 39.70% to 45.72%, where as in the susceptible genotype it was 15.99%. The crosses involving susceptible (7042S) genotype as a male parent also exhibited low level induction of peroxidase of 17.94% in DHLB-18B × 7042S (R×S) and 27.67% in DHLB-17B × 7042's (R×S). Induction of specific isoforms of peroxidase has been reported in pearl millet genotypes resistant and susceptible to downy mildew [32]. However contrary to the preset report low activity of peroxidase is reported in highly susceptible genotypes of pearl millet (A7 and L10) and much higher peroxidase activity in immune (L5) and highly resistant genotypes L101,L103 and HR of pearl millet [33]. Thus a total activity of peroxidase is not a good indicator of downy mildew resistance rather a particular isoforms may be more important for synthesis of certain metabolites which may act as a barrier to the spread of invading pathogen.

A significant variation was observed in the polyphenol oxidase activity of the resistant and susceptible parents. The polyphenol oxidase activity was much higher in the downy mildew susceptible genotypes 7042S both in the absence of the pathogen and when the seedlings were dipped in pathogen suspension for 72hrs. The data pertaining to the level of induction as evident from the activity of PPO in absence and presence of the pathogen clearly indicated higher level induction Polyphenol oxidase in resistant genotype from as low as 45.86% increase over non inoculated control in DHLB-16B to as high as 89.83% in DHLB-18B. The activity though higher in susceptible genotype however the level of induction is significantly less i.e. 31.49% (Table 2). It is also evident that the mean of downy mildew incidence in field is correlated with the level of induction of the specific class of polyphenol oxidases. The crosses involving susceptible genotype (7042S) as a male parent exhibited comparatively lower level induction of polyphenol oxidase with higher downy mildew field incidence. Analysis of PPO activity in seedlings of resistant and susceptible pearl millet cultivar with or without inoculation of downy mildew pathogen revealed significantly higher PPO levels in inoculated seedlings[34]. The higher PPO activity as observed in the present investigation in the susceptible genotype both without and with inoculation could probably be attributed to the hypersensitive compatible reaction of the host with the pathogen leading the death. The activity of PPO per se was higher in susceptible genotype however the level of induction was higher in all the resistant genotypes probably suggesting de novo synthesis of Polyphenol oxidases. The results though contradicts with the earlier observations that activity of PPO are linearly related to the degree of resistance[33], however the inoculated seedlings had significantly higher PPO level than control seedlings is in conformity to those reported earlier [35].

PAL and β-1, 3- Glucanase activity

Phenylalanine ammonia lyase (PAL) activity was studied in resistant and susceptible genotype of pearl millet and their crosses after inoculating with *S. graminicola* In resistant genotype the enzyme activity significantly increased after fungal inoculation which was 182% in DHLB-16B and 200% in DHLB-18B. In susceptible genotype 7042S although the PAL activity increased after inoculation however the levels of induction was 59.18% The crosses involving susceptible genotype 7042S as a male parents (R×S), the level of induction though slightly higher than in susceptible genotype was significantly less than that observed in resistant genotypes (Table3).

Perusal of the PAL induction level and its correlation with the mean downy mildew incidence in field clearly suggest a good correlation of PAL induction with the degree of host resistance. The results obtained during the present investigations are in accordance with those reported earlier [36, 10]. It has been reported that treatment of resistant seedlings with PAL inhibitor, amino oxy-β-phenyl propionic acid resulted in the enhancement of enzyme activity where as in the presence of 1mM trans-cinamic acid the pathogen induced PAL was completely inhibited. Treatment of pearl millet seedlings with exogenously applied PAL inhibitors induced downy mildew susceptibility in the resistant pearl millet cultivars consistent with increase or decrease of PAL activity has also been correlated with bacterial canker resistance or susceptibility

in tomato[10]. The results obtained are in conformity to those reported earlier and further suggest exploiting downy mildew resistant genotype as a male parent in breeding for downy mildew resistance breeding.

β-1,3- glucanase activity was studied in resistant and susceptible genotype of pearl millet and their crosses after inoculating with *S. graminicola* In resistant genotype the enzyme activity significantly increased after fungal inoculation from 19.0 % in DHLB-16B to as high as 25.46% in DHLB-17B. In susceptible genotype 7042S although the glucanase activity increased after inoculation however the levels of induction was 1.9% The crosses involving susceptible genotype 7042S as a male parents (R×S), the level of induction though slight higher in susceptible genotype was significantly less than that observed in resistant genotypes (Table 3). The results of the present study substantiate this in pearl millet downy mildew interactions. The variation in cultivar resistance studied in field correlated with the activities of β-1, 3-glucanase with higher activity in resistant cultivars and low in highly susceptible cultivars. In S×R crosses where susceptible parent is used as a male parent showed increase activity from 23.2% in7042S × DHLB-17B and 7042S × DHLB-18B 20.95% (Table 3). Similar increases in activity and accumulation of β-1,3-glucanase and chitinase enzymes in incompatible interactions of maize, pepper, barley and wheat with pathogenic fungi have been reported and suggesting a role for these enzymes in determining resistance against fungal pathogens [37.38, 39,40]. Reduction in the activity of β-1, 3-glucanase after inoculation of the host by the pathogen in susceptible cultivars probably indicates pathogen inactivation of the enzyme and/ or reduced host protein synthesis due to pathogen colonization as evidenced by differential induction of the enzyme isoforms.

It is clear from the present investigation that the activity profile of defense related enzymes PPO, β-1, 3-glucanase, PAL and total phenols are upregulated in inoculated seedlings than control plants and the crosses involving resistant genotype as a male parent. This study also showed a dominant pattern of inheritance. Hence the induction of defense related enzymes and total phenols content can be effectively used to screen the host resistance in pearl millet breeding for downy mildew.

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