Research Article

Biochemical studies in lentil (*Lens culinaris* Medik.) genotypes under *Stemphylium* blight stress

*S. PAVITHRA, [1]A. SARKAR AND [2]P. M. BHATTACHARYA

Department of Genetics and Plant Breeding, Faculty of Agriculture
Uttar Banga Krishi Viswavidyalaya, Cooch Behar-736165, West Bengal, India

ABSTRACT

Stemphylium blight, caused by the fungal pathogen Stemphylium botryosum, is one of the important diseases in lentil, an important rabi pulse grown in the plains of West Bengal. Considering the severity of the disease in the existing cropping system, an experiment was undertaken with the objective to study the change in the patterns of various biotic stress related biomolecules among fifty (50) lentil genotypes under the incidence of Stemphylium blight. The genotype IC620839 recorded the lowest PDI (11.25) with the AUDPC score of 202.41 while IC54456 had the highest PDI (79.37) and AUDPC score (1598.60), respectively. The genotype IC241072 with the lowest disease score (1.5) and PDI (11.87) along with the highest Ortho-Dihydric Phenol and Super Oxide Dismutase content under artificial inoculum was in a separate cluster. The observation could thus be helpful in the future crop improvement schemes as far as disease resistance in lentil is concerned.

Keywords: AUDPC, correlation, lentil, pirate plot, phenol and Stemphylium blight

Lentil (*Lens culinaris* Medik.) is a low-cost legume crop and is an excellent source of protein, micronutrients and vitamins, grown to serve the diets of a larger section of population (Singh and Singh, 2022). Yield fluctuation in lentil has been witnessed at global level in recent years. The reason for unsatisfactory production trend in lentil has been attributed to the occurrence of diverse biotic and abiotic stresses (Mandal *et al*., 2019). Wide range of pathological threats have arisen for lentil production recently and Stemphylium blight caused by *Stemphylium botryosum* being one among them. *Stemphylium* blight is a devastating foliar disease and causes 60-90% yield loss and sometimes a total crop failure (Pant *et al*., 2022). The poor performance of the crop under such stress situations is because of the altered biochemical events happening inside the plant system leading to the oxidative stress. Plants under oxidative stress usually produce free radicals in the form of reactive oxygen species (ROS) which cause harmful effects. To counter this problem plants tend to synthesize ROS scavenging biochemicals which neutralize the cellular ROS and give relief from the stress (Akram *et al*., 2017). Studies on biochemicals, associated with

the stress can be helpful in selection for biotic stress tolerance. Therefore, a study was undertaken to evaluate the biochemical pattern of diseased lentil genotypes in comparison with the healthy (nee resistant) ones. The experimental data were subjected to Pearson's correlation coefficient analysis to study the relationship between the biochemicals and the disease incidence. Advanced RID plotting technique was used to visualize the data efficiently. Finally Euclidean method of hierarchical clustering was done to group the genotypes based on assessed biochemical parameters and disease reaction.

MATERIALS AND METHODS

Field experiment

The experimental material comprised of 50 lentil accessions collected from NBPGR and ICARDA. The genotypes were grown in field and pots following Alpha-lattice design and randomized block design respectively with two replications at Uttar Banga Krishi Viswavidyalaya, West Bengal (26.4035°N, 89.3850°E) during rabi 2021-2022. Natural disease incidence was noticed in field condition during peak flowering stage.

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*Email: dtpavi@gmail.com

The extent of natural disease incidence in field was assessed following Hashemi's disease score (Hashemi *et al.*, 2005) and subsequent analysis of Artificial inoculation with *S. botryosum* culture was done in plants grown under pot condition simultaneously.

Lab experiment

Leaf samples collected from healthy plants before disease incidence and 72 hours after artificial inoculum spray were subjected to biochemical analysis. Estimation of biochemicals like Phenol (PHE) (Bray and Thorpe, 1954), Ortho-dihydric phenol (ODP) (Mahadevan and Sridhar, 1986), Polyphenol oxidase (PPO) (Mayer *et al.*, 1965), Peroxidase (POD), Superoxide dismutase (SOD) and Ascorbate peroxidase (APX) (Senthil Kumar *et al.*, 2021) were performed at the Central Instrumentation Center, UBKV, West Bengal.

Statistical analysis

Data analysis was done through R Studio (R Core Team, 2022). Analysis of variance (ANOVA) was computed for six biochemical parameters recorded before and after disease incidence to check the significance of these parameters in relation to the disease among the lentil genotypes. The association between the PDI and AUDPC was observed through marginal plot using the packages "ggExtra" (Attali and Baker, 2022) and "ggplot2" (Wickham, 2016). Pirate Percent Disease Index (PDI) and Area Under Disease Progress Curve (AUDPC) was carried out as suggested by Simko and Piepho (2011). plots, one of the types of RID plot (Raw data Inference Distribution plot) has been constructed using the package "yarrr" (Phillips, 2017) to compare the pattern of biochemicals existing before and after disease incidence in lentil. Pearson's correlation coefficient was computed using the package "metan" (Olivoto and Lucio, 2020) to study the association of biochemicals with disease incidence. Grouping of genotypes based on biochemicals and PDI was carried out by following Euclidean method of hierarchical clustering with the package "stats" (R Core Team, 2022) and "factoextra" (Kassambara and Mundt, 2020).

RESULTS AND DISCUSSION

The ANOVA showed that the genotypes were significantly varying for PDI and all the six biochemical parameters studied before and after disease incidence (Table 1). The Hashemi's disease severity scale grouped the genotypes into five categories *viz.*, moderately resistant (MR), resistant (R), moderately susceptible (MS), susceptible (S) and highly susceptible (HS) (Fig. 1). Among the 50 lentil genotypes maximum number of genotypes were found to be moderately susceptible (22) to *Stemphylium* blight disease.

Table 1: Analysis of variance for biochemicals in healthy and diseased samples

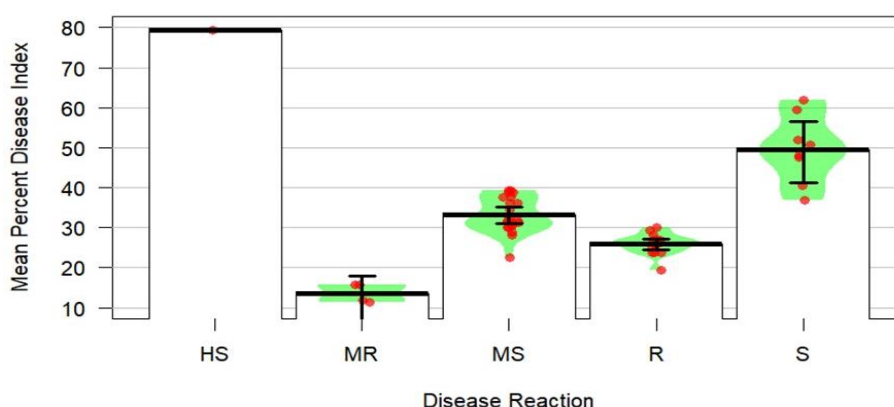| Parameters	Healthy sample		Diseased sample		Significance
	TMSS	EMSS	TMSS	EMSS	P=0.01
Phenol	268.650	9.360	920.660	9.700	**
Ortho-dihydric Phenol	31.060	0.240	13.190	0.200	**
Polyphenol Oxidase	9.350	0.410	1.810	0.000	**
Peroxidase	268.600	3.340	645.850	0.480	**
Superoxide Dismutase	0.004	0.000	0.008	0.000	**
Ascorbate Peroxidase	65.250	17.500	132.390	0.000	**

Fig. 1: Distribution of genotypes under different disease reactions

The percent disease index ranged from 11.25% to 79.37%. Similar range of *Stemphylium* disease severity in lentil was reported at Mohanpur, West Bengal by Mandal *et al.* (2019). The genotype IC544556 (Land race, Bihar) was highly susceptible to the disease with the maximum PDI value of 79.37%. The genotypes IC620839, IC241072, EC223210 and IPL220 were moderately resistant towards the disease with very low PDI values (11.25%, 11.87%, 15.62%, and 15.62%, respectively) (Table 2). Area under disease progress curve was directly proportional to the Percent Disease Index. The linear association of AUDPC with PDI and their range of distribution among the genotypes have been depicted in the marginal plot (Fig. 2).

Table 2: Disease parameters for 50 lentil genotypes

G. No.	Genotypes	PDI	AUDPC	MDS	DR	G. No.	Genotypes	PDI	AUDPC	MDS	DR
1.	IC241067	30.00	447.57	4.5	R	26.	EC225486	25.62	469.51	4.5	R
2.	IC241090	28.75	362.27	5.0	MS	27.	IC199779	19.37	195.99	3.5	R
3.	IC551597	36.25	575.55	6.5	MS	28.	IC78486	50.62	875.01	7.0	S
4.	IC241119	26.87	494.10	3.0	R	29.	EC225484	39.37	650.99	5.5	MS
5.	IC241072	11.87	190.85	1.5	MR	30.	EC223191	51.87	832.63	8.0	S
6.	IC565035	26.87	453.67	4.5	R	31.	IC241071	30.00	410.54	5.5	MS
7.	IC614827	23.75	322.16	4.0	R	32.	IC78513	48.12	794.94	7.0	S
8.	IC241082	28.12	487.69	5.0	MS	33.	IC559415	30.00	398.39	6.0	MS
9.	IC78509	31.25	392.80	6.0	MS	34.	IC610426	31.87	453.88	6.0	MS
10.	IC78535	61.87	1125.92	8.0	S	35.	IC241097	59.37	1121.63	7.0	S
11.	EC223226	40.62	550.47	7.0	S	36.	IC241061	23.75	427.80	3.5	R
12.	IC78531	31.25	479.09	5.0	MS	37.	IC620839	11.25	202.41	1.0	MR
13.	EC16391	37.50	556.71	6.5	MS	38.	IC544556	79.37	1598.60	10	HS
14.	IC78545	26.25	422.09	4.5	R	39.	IC614827	22.50	297.21	5.0	MS
15.	EC223210	15.62	263.54	2.5	MR	40.	IC201778	36.87	585.74	7.0	S
16.	IC78540	38.75	605.13	6.5	MS	41.	EC 223219	30.62	483.87	5.5	MS
17.	EC223188	39.37	628.85	6.0	MS	42.	EC 267544A	29.37	481.99	4.5	R
18.	IC78518	25.00	374.18	4.5	R	43.	EC 267563	47.50	806.36	7.0	S
19.	IC78547	38.75	621.38	6.5	MS	44.	EC 267598	32.50	549.25	5.0	MS
20.	WBL77 (C)	28.12	461.08	4.5	R	45.	EC 267604	30.62	526.87	5.5	MS
21.	IC78454	37.50	629.11	6.0	MS	46.	EC 267636	26.25	407.84	4.5	R
22.	IC78462	31.87	438.88	6.0	MS	47.	EC 267657	36.25	642.98	5.0	MS
23.	EC33920	26.87	502.71	3.5	R	48.	EC 78408	31.25	467.70	5.5	MS
24.	EC223244	34.37	451.31	6.5	MS	49.	L-4147 (C)	25.62	404.76	4.5	R
25.	IPL406 (C)	23.75	338.41	4.0	R	50.	IPL 220 (C)	15.62	241.94	2.0	MR

Note: *PDI-Percent Disease Index; AUDPC-Area Under Disease Progress Curve; MDS-Mean disease score; DR-Disease reaction (Hashemi et al., 2005)*

dr-Disease reaction; HS-Highly susceptible; MR-Moderately resistant; MS-Moderately susceptible; R-Resistant; S-Susceptible
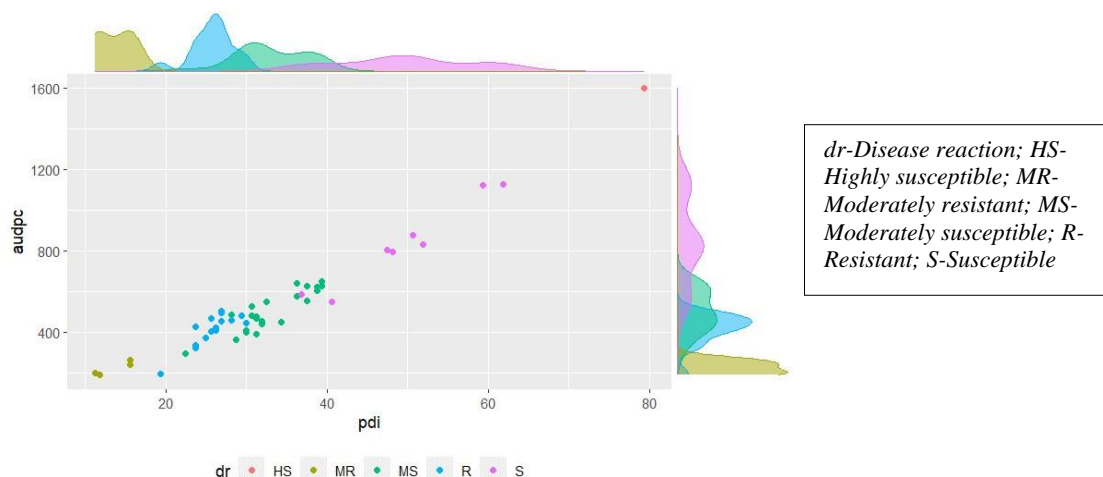
Fig. 2: Marginal plot showing the association between PDI and AUDPC

The comparative analysis of biochemicals through RID plotting technique before and after disease incidence exhibited that the mean content of PHE, POD, SOD and APX were high in artificially inoculated diseased samples than the healthy samples, whereas the mean of PPO and ODP were high in healthy samples. Reduced level of ortho-dihydric phenol during disease development was in accordance with the results reported by Chatterjee and Ghosh (2008) in mesta.

Similar to the POD results in this study Saraswathi and Reddy (2012) have also observed pronounced activity of peroxidase in the diseased leaf samples of groundnut. A study undertaken by Bandeoğlu *et al*. (2004) had shown that SOD and APX activity in lentil got increased under salinity stress. Pirate plots were used to compare the distribution of biochemicals between the samples before and after disease incidence (Fig. 3).
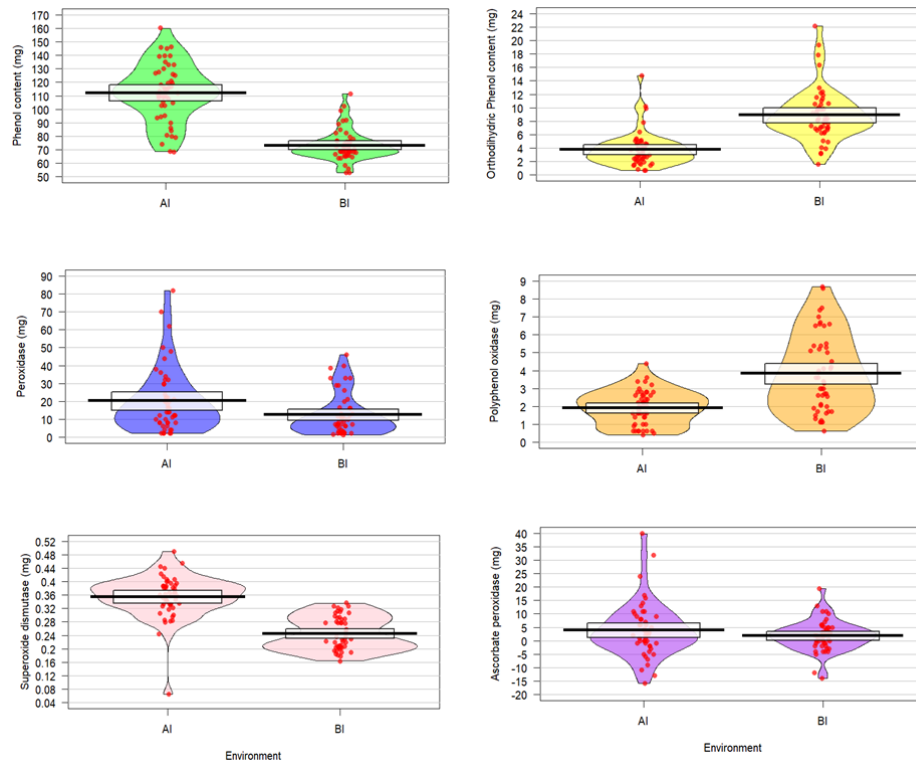

Fig. 3: Pirate plots illustrating the distribution of biochemicals between after disease incidence (AI) and before disease incidence (BI)

Pearson's correlation coefficient studies among the biochemicals and the PDI revealed the existence of significant correlation of disease incidence with PHE and SOD in negative direction. This explained the fact that *Stemphylium* disease severity got reduced with the increase in PHE and SOD in the lentil crop. Similarly, Chandel *et al*. (2022) reported a negative association between PDI and PHE in onion under the same disease. The accumulation of phenolic compounds in the sub-epidermal layer of the plant tissue inhibit the further growth of the plant cell and thus the pathogen also (Tak and Kumar, 2020). Further, the association of SOD and

Stemphylium disease indicate that superoxide radicals (O2-) are the major causal factors for oxidative stress in lentil under *Stemphylium* disease stress. The biochemicals SOD and PPO, ODP and PHE were observed to have significant positive correlation with each other during disease incidence. Rani *et al*. (2018) also reported that ODP and PHE were positively correlated in their study on mung bean. But a significant negative correlation was noticed between POD and SOD. The Pearson's correlation plot clearly illustrated significant association among the parameters (Fig. 4).
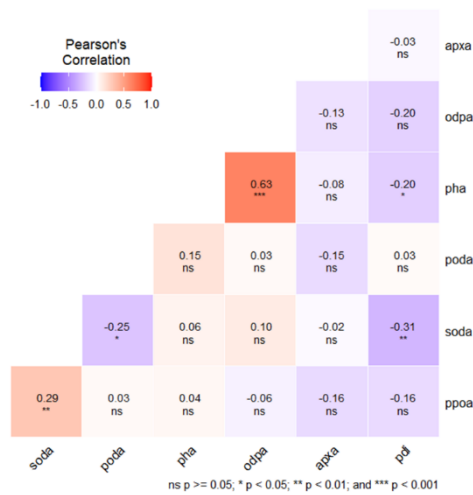
apxa-Ascorbate peroxidase; odpa-Orthodihydric phenol; pha-Phenol; poda-Peroxidase;
sods-Superoxide dismutase; ppoa-Polyphenol oxidase; pdi-Percent disease index

Fig. 4: Pearson's correlation coefficient plot

The diversity present among the genotypes for biochemicals and disease incidence was assessed by cluster analysis. Euclidean method of hierarchical clustering grouped genotypes into two major clusters. The genotype IC241072 was found to be distinctly placed in a separate cluster and it had shown resistance towards the disease registering the lowest disease score (1.5) and PDI (11.87) along with the high ODP and SOD content under disease condition. Another major cluster was observed to separate into two minor clusters each comprising 46 (Sub-cluster I) and 3 (Sub-cluster II) genotypes, respectively. Most of the disease resistant genotypes were placed in the sub-cluster I. All the genotypes in the sub-cluster II were mostly susceptible to *Stemphylium* disease. The dendrogram of Euclidean hierarchical cluster has been presented in fig. 5.
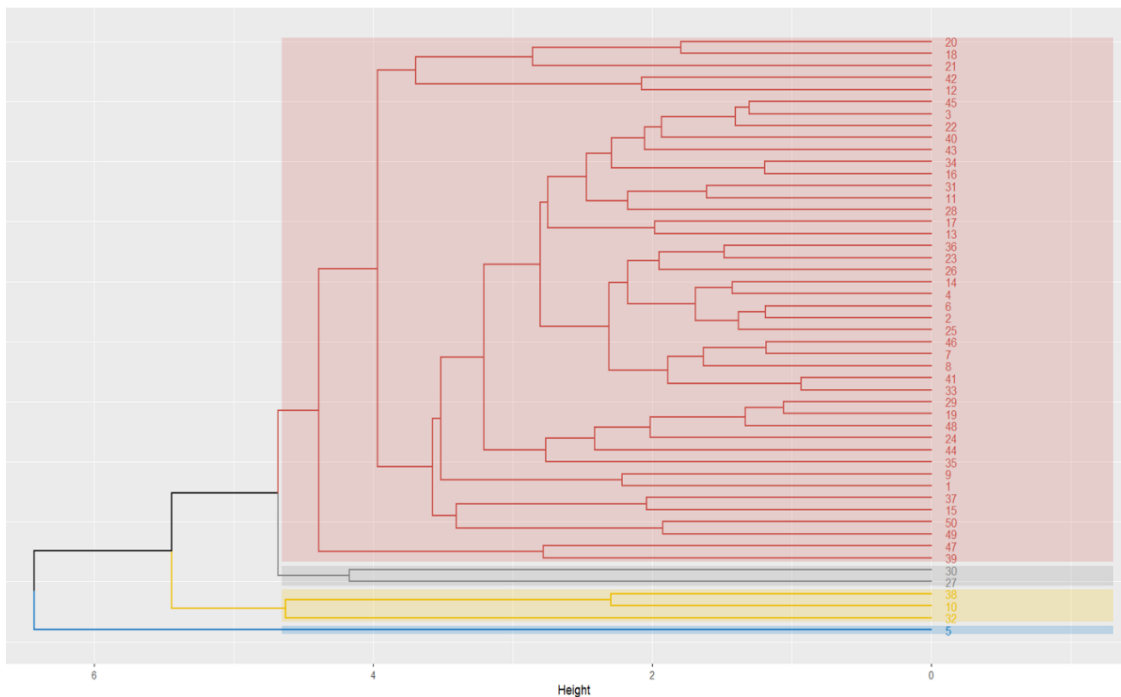

Fig. 5: Dendrogram depicting the Euclidean hierarchical cluster pattern of genotypes

CONCLUSION

Based on the results and their analysis it could be concluded that Phenol and Superoxide dismutase were directly correlated with the disease incidence in lentil crop in the present study. The genotype IC241072 has to be given special consideration during the crop improvement programe since it is placed distinctly in the diversity cluster with resistance to disease. Thus, the study showing the association prevailing among the biochemicals and the same with disease incidence in lentil genotypes will be helpful in the future crop improvement schemes for tailoring not only the concerned disease but also other related biotic stresses in the crop.

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