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Screening of Lentil Germplasm against Stemphylium Blight and Studies on Association between Disease and Biochemical Parameters

A. Roy[1,2], A. Sarkar[2], S. Pavithra[2], P. Bhaumik[3], P.M. Bhattachraya[3], N. Sahana[4]

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ABSTRACT

Background: Stemphylium blight, an important fungal disease of lentil caused by *Stemphylium botryosum* Wallr, has been reported to cause an estimated yield loss of 62% in India and Bangladesh. Considering the importance of the crop *vis-à-vis* the severity of the disease under *terai* agro-climatic region of West Bengal, the present study was undertaken to evaluate the germplasm of lentil for Stemphylium blight resistance for their utilization in future resistance breeding programme.

Methods: The present experiment was carried out during the rabi season of 2019-20 and 2020-21 with forty lentil germplasm including two checks *viz;* WBL77 and IPL220 in an alpha lattice design under field condition and in completely randomized design under artificial inoculation respectively. Statistical analysis was carried out using R v. 4.1.1 software.

Result: ANOVA revealed significant variation among the genotypes for the studied characters. PCV was found to be higher than GCV for all the studied characters. High heritability (broad sense) accompanied with high genetic advance as percent of mean indicated the presence of additive type of gene action. Based on per cent disease index germplasm were categorized as resistant or moderately resistant types for both under field condition and artificial inoculation. AUDPC was significantly but negatively associated with change in phenol and change in OD phenol under field condition same as that was observed between AUDPC and PPO content under artificial inoculation. Above study state that selection based on disease reaction and their associated parameters gives fruitful result to provide disease resistance parent against Stemphylium blight.

Key words: Area under disease progress curve, Lentil, Per cent disease index, Stemphylium blight.

INTRODUCTION

Lentil (*Lens culinaris* Medikus) is one of the most important *rabi* pulse crops globally covering a total area of 6.10 million hectare and a production of about 6.33 million tones (FAOSTAT, 2020). The crop is mainly grown for its nutritious value being mostly consumed by South Asian as 'dal'. Among the other factors for declining productivity of the crop disease is considered to be an important one and in recent times the disease Stemphylium blight caused by the fungal pathogen *Stemphylium botryosum* has been reported in many countries including India, Bangladesh, Nepal, Canada, Australia, USA and Syria under favorable environment condition (Taylor *et al.,* 2007; Baya and Erskine, 1998). The disease has been reported to occur in the late flowering stage and spread more quickly in dense population with pin head or light brown spots forming on the leaflet killing them 2-3 days. During unfavorable condition, the disease severity may take higher proportion with leaves getting blighted and becomes defoliated with some green leaves and immature seeds (Alam *et al.,* 2017).

Many authors have emphasized the association between the degree of resistance and phenolics present in the plant tissue (Nicholson and Hammerschmidt, 1992; Bhagat and Chakraborty, 2010) Ortho dihydroxy Phenols are important in disease resistance reaction as they are easily oxidized and the resultant quinones are highly reactive and toxic to pathogens and their enzymes. The distribution of polyphenol oxidase activity across the plant kingdom depends upon on a number of variables including the age, species, variety, maturity and stress of the plant. Because of their conspicuous reaction products and induction by wounding and pathogen attack, polyphenol oxidase activity has frequently been suggested to assess the plant defense

[1]Department of Genetics and Plant Breeding, M.S Swaminathan School of Agriculture, Centurion University of Technology and Management, Paralakhemundi, Gajapati-761 211, Odisha, India.
[2]Department of Genetics and Plant Breeding, Uttar Banga Krishi Viswavidyalaya, Pundibari, Cooch Behar-736 165, West Bengal, India.
[3]Department of Plant Pathology, Uttar Banga Krishi Viswavidyalaya, Pundibari, Cooch Behar-736 165, West Bengal, India.
[4]Department of Biochemistry, Uttar Banga Krishi Viswavidyalaya, Pundibari, Cooch Behar-736 165, West Bengal, India.

Corresponding Author: A. Roy, Department of Genetics and Plant Breeding, M.S Swaminathan School of Agriculture, Centurion University of Technology and Management, Paralakhemundi, Gajapati-761211, Odisha, India. Email: royanjanag328@gmail.com

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against pest and pathogens (Constabel *et al.,* 1995; Thipyapong *et al.,* 1995; Thipyapong and Steffens, 1997).

Given the above stated facts, it is therefore prudent to screen the germplasm to assess the incidence of disease and thereby to identify the lines showing variable response to the disease so that they can be effectively utilised in future breeding program. Besides identifying a resistant source is equally important as it may very well be used in any breeding programme to transfer the disease resistance character to an otherwise well adapted and high yielding variety. Therefore, evaluation of germplasms for Stemphylium blight resistance in lentil besides other morphological characters need to be done. Screening of advanced breeding lines showing variation in resistance or susceptibility for this disease shall allow for selection of resistant lines for Stemphylium blight resistance breeding.

MATERIALS AND METHODS

Forty lentil germplasm including two checks *viz*; WBL77 and IPL220 were evaluated in *rabi* season over two successive years 2019-20 and 2020-21 in the instructional farm of Uttar Banga Krishi Viswavidyalaya, Pundibari, Cooch Behar located at 26°24′09.1″ N latitude and 89°23′08.3″E longitude and at 43 meter above the mean sea level. The details of germplasm have been presented in Table 1.

Alpha Lattice design implied with three replications under field condition and for artificial inoculation under pot condition completely randomized design was used with three replications. Standard recommended fertilizer doses were applied to the crop and intercultural operations were carried out as per the schedule. To prepare the inoculum few previously prepared fungal culture of Pundibari isolate of *Stemphylium botryosum* obtained from the Department of Plant Pathology, UBKV was kept with sterilized lentil seeds (100 gm) in a conical flask and kept for 10-15 days in BOD at 26±1°C for the growth of the fungus. Lentil seeds were covered with mycelium of the fungus mixed with 900 ml of distilled water. The combination was mixed properly and then collected in a sprayer for spraying over plants in pot in the evening hours. Observation for each of the morphological characters was recorded as the average of the same in five randomly selected plants in each plot and for disease reaction after the appearance of the disease at seven days interval. Disease severity was assessed after Hashemi *et al.* (2005) using a 0-10 scale as given in Table 2. Per cent disease index (PDI) was worked out using the formula:

$$PDI = \left[\frac{\text{Sum of numerical rating}}{\text{Total number of observations taken}} \times \text{Maximum disease score} \right] \times 100$$

Based on disease severity, genotypes were classified into different groups. Further area under disease progress curve (AUDPC) was calculated after Simko and Piepho (2011) using simple trapezoidal rule to detect the gradual progress of the disease.

Extraction and estimation of total phenol was done after Bray and Thorpe (1954) and the absorbance was recorded at 650 nm using spectrophotometer (Model UV-1900I, Shimadzu, Japan) against a blank. Presence of total phenol was calculated from the standard curve prepared using catechol. Ortho-dihydroxy phenol estimated after Mahadevan and Sridhar (1986) employing Arnow's reagent which is specific to ortho groups (Johnson and Schaal, 1957). The light pink colour developed in the estimation was measured colorimetrically at 515 nm using spectrophotometer (Model UV- 1900I, Shimadzu, Japan). OD phenol concentration was calculated from the standard curve which was prepared using catechol. Polyphenol oxidase (PPO) activity was assessed after Mayer *et al.* (1965) and the absorbance was measured at 495 nm in a spectrophotometer (Model UV- 1900I, Shimadzu, Japan) up to 2 minutes at an interval of 30 seconds against a blank.

RESULTS AND DISCUSSION

Per cent disease index (PDI) assessed 109 days after sowing (DAS) for the year 2020-21 under field condition ranged from 13.00% to 40.67% whereas the same estimated 115 DAS under artificial inoculation varied from 13.50% to 38.89% (Table 3). Based on PDI estimation none of the genotypes in the experiment was fond to be of either immune (disease reaction- 0), moderately susceptible (disease reaction- 5), susceptible (disease grade-7) or highly

Table 1: List of lentil germplasm.

Sl. no.	Genotypes	Sl. no.	Genotypes	Sl. no.	Genotypes	Sl. no.	Genotypes
1	IC241067	11	IC78540	21	IC199779	31	IC614827
2	IC241090	12	EC223188	22	IC78486	32	IC201778
3	IC241119	13	IC78518	23	EC225484	33	EC223219
4	IC241072	14	IC78547	24	IC241071	34	EC267544
5	IC565035	15	WBL77	25	IC78513	35	EC267563
6	IC241082	16	IC78454	26	IC610426	36	EC267598
7	IC78535	17	IC78462	27	IC241097	37	EC267604
8	IC78531	18	EC33920	28	IC241061	38	EC267636
9	EC16391	19	EC223244	29	IC620839	39	IC78408
10	IC78545	20	EC225486	30	IC544556	40	IPL220

susceptible (disease reaction- 9) type both under field as well as under artificial inoculation. The germplasm were thus found to be showing resistance (disease reaction- 1 and 2) to moderately resistance (3 and 4) disease reaction (Table 4). The above results were in conformity with observation made by Yadav *et al.* (2017), Das *et al*. (2017) and Kant *et al*. (2017) with lentil genotypes showing variable response towards *S. botryosum* in field condition thereby establishing the variability over the genotypes in terms of the response to the disease-causing organism as it was found in the present experiment. Subedi and Neupane (2018) while studying Stemphylium blight in lentil reported a higher crop yield with lower disease index.

Phenol and OD phenol estimated at 70 DAS and 103 DAS under field condition revealed an increment in their concentration following the appearance of the disease in all the studied germplasm. On the contrary polyphenol oxidase (PPO) concentration measured at 102 DAS after disease appearance under field condition and 48 hrs after inoculum sprayed in pot condition revealed the highest absorbance (6.60 and 4.95 abs min^{-1} g^{-1}) as against the lowest reading of 1.35 and 0.90 abs min^{-1} g^{-1} under field and pot condition respectively. The ANOVA (Table 5 and Table 6) revealed significant variation among the genotypes for all the studied characters.

The mean, range, genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability (broad sense) and genetic advance as per cent of mean (GAM) have been presented in Table 7. The PCVs were observed to be higher than the corresponding GCV ranging from 18.04 to 49.30 for GCV and 18.74 to 51.38 for PCV respectively with high GCV being noted for change in phenol (49.30) coupled with high PCV (51.38) indicating the environmental influence for the studied character. Except

Table 2: Disease scoring scale (0-10) for Stemphylium blight (Hashemi *et al.*, 2005).

Scoring	Significance/Description
0	Healthy plant; free of disease.
1	Dull leaves or few tiny tan spots.
2	A few small to large chlorotic spots.
3	Expanding lesions on leaves and leaf drop starting.
4	20% nodes on main stem showing chlorotic/necrotic symptoms and/or leaf drop.
5	40% nodes on main stem showing chlorotic/necrotic symptoms and/or leaf drop.
6	60% nodes on main stem showing chlorotic/necrotic symptoms and/or leaf drop.
7	80% nodes on main stem showing chlorotic/necrotic symptoms and/or leaf drop.
8	100% leaves dried up/defoliated but small green tip recovering.
9	100% leaves dried up/defoliated including tip but stem still green.
10	Whole plant dies and completely dried up.

Table 3: Per cent disease index (PDI) disease reaction of lentil germplasm against Stemphylium blight during *rabi* season (2020-21).

Genotype	Field condition		Artificial inoculation	
	PDI (109 DAS)	Disease reaction	PDI (115 DAS)	Disease reaction
IC241067	29.33	2	32.83	3
IC241090	21.33	2	23.33	2
IC241119	37.33	3	29.17	3
IC241072	24.67	2	25.06	2
IC565035	31.78	3	29.17	2
IC241082	28.00	2	31.11	3
IC78535	39.33	3	30.83	3
IC78531	34.00	3	33.61	3
EC16391	38.67	3	30.83	3
IC78545	29.17	2	30.83	3
IC78540	36.00	3	34.17	3
EC223188	40.00	4	30.83	3
IC78518	31.33	3	36.67	3
IC78547	38.67	3	38.06	3
WBL77	40.00	4	34.00	3
IC78454	31.33	3	35.44	3
IC78462	28.00	2	30.00	3
EC33920	28.67	2	30.83	3
EC223244	32.67	3	25.83	2
EC225486	28.67	2	27.50	2
IC199779	34.67	3	29.72	2
IC78486	35.17	3	32.22	3
EC225484	25.33	2	30.00	3
IC241071	23.33	2	25.83	2
IC78513	32.00	3	31.67	3
IC610426	37.22	3	32.22	3
IC241097	40.67	4	33.06	3
IC241061	26.67	2	22.78	2
IC620839	14.67	1	22.61	2
IC544556	35.00	3	34.17	3
IC614827	15.33	1	24.72	2
IC201778	32.67	3	30.28	3
EC223219	30.67	3	38.89	3
EC267544	30.00	3	29.72	2
EC267563	34.67	3	33.33	3
EC267598	30.67	3	31.67	3
EC267604	37.33	3	32.22	3
EC267636	26.67	2	25.00	2
IC78408	23.33	2	28.00	2
IPL220	13.00	1	13.50	1
Mean	30.70	-	30.04	-
CV	7.06	-	13.13	-
SE m (±)	1.25	-	2.28	-
CD (P=0.05)	3.53	-	6.41	-

Table 4: Disease scale and grouping of germplasm on the basic of disease reaction on Stemphylium blight for 2020-21.

Scale	Disease severity index	Disease reaction	Number of germplasm	Name of germplasm
0	No infection	Immune	0	-
1	Bellow 10% foliage affected	Resistant	16	IC241067, IC241090, IC241072, IC241082, IC78545, IC78462, EC33920, EC225486, EC225484, IC241071, IC241061, IC620839, IC614827, EC267636, IC78408, IPL220
3	30% of the foliage affected	Moderately Resistant	24	IC241119, IC565035, IC78535, EC16391, IC78540, EC223188, IC78518, EC223188, IC78547, WBL77, IC78454, EC223244, IC199779, IC78486, IC78513, IC610426, IC241097, IC544556, IC201778, EC223219, EC267544, EC267563, EC267598, EC267604
5	50% of the foliage affected	Susceptible	0	-
7	70% of the foliage affected	Moderately Susceptible	0	-
9	More than 70% of the foliage affected	Highly Susceptible	0	-

Table 5: Analysis of variance for biochemical characters and AUDPC in lentil germplasm under field condition.

Year	Sources of variation	d.f	Mean sum of square (MSS)							
			Phenol (70 DAS)	Phenol (103 DAS)	OD Phenol (70 DAS)	OD Phenol (103 DAS)	Change in Phenol	Change in OD phenol	PPO (102 DAS)	AUDPC
2020-21	Genotypes	39	188.26**	190.90**	1.90**	1.98**	134.31**	0.84**	4.87**	21684.20**
	Replication	2	13.20	8.65	0.10	0.02	1.60	0.08	0.40	1954.2
	Block (Replication)	9	5.28	6.84	0.02	0.03	4.59	0.02	0.10	673.2
	Error	69	3.40	4.68	0.04	0.05	3.63	0.03	0.14	1367.1

****, *** Significant at 1% and 5% levels of probability, respectively.

Table 6: Analysis of variance for PPO content and AUDPC in lentil germplasm under artificial inoculation.

Year	Sources of variation	d.f	Mean sum of square (MSS)	
			PPO 48 hrs. after inoculation	AUDPC
2020-21	Genotypes	39	3.07**	14040**
	Error	80	0.08	1245.70

****, *** at 1% and 5% level of significance respectively.

for phenol content at 103 DAS showing moderate GCV (18.04) and PCV (18.74) remaining characters showed high GCV and PCV. High heritability was recorded for all the studied characters ranging from 91.88 to 97.98% and high GAM was observed for all the studied characters ranging from 35.76 to 97.46 as per the classification as suggested by Johnson *et al.* (1955). High heritability coupled with high GAM indicated the characters under study being predominantly controlled by additive gene action and hence direct selection can be resorted to. The characters *viz*; phenol 70 DAS, phenol 103 DAS, OD phenol 70 DAS, OD phenol 103 DAS, PPO 102 DAS under field condition and PPO 48 hrs. after inoculation under artificial inoculation were found to be showing an incremental trend following disease infestation. Ahuja *et al.* (2015) similarly observed significant variation in phenolic compounds among the lentil genotypes.

The genotypic correlation between AUDPC and change in phenol, change in OD phenol and PPO content measured 102 DAS indicated different degree of association under field condition as presented in Table 8. While AUDPC revealed significant and negative association with change in phenol (-0.686) and changes in OD phenol (-0.495) PPO

Table 7: Mean, range, GCV, PCV, heritability (bs) and genetic advance for biochemical characters against Stemphylium blight in lentil germplasm.

Parameters/Character	Mean	Range	GCV (%)	PCV (%)	Heritability broad sense (%)	Genetic advance as per cent of mean
Phenol (70 DAS)	30.33	13.35-48.67	25.86	26.61	98.19	51.78
Phenol (103 DAS)	43.65	24.39-69.58	18.04	18.74	97.54	35.76
OD phenol (70 DAS)	1.89	0.37-3.95	41.67	42.86	97.98	83.46
OD phenol (103 DAS)	3.04	1.46-5.00	26.41	27.32	97.60	52.58
Change in phenol	13.38	3.45-32.86	49.30	51.38	97.29	97.46
Change in OD phenol	1.15	0.21-2.54	45.42	47.61	96.72	89.27
PPO (102 DAS field)	3.42	1.35-6.60	36.69	38.28	91.88	72.45
PPO 48 hrs. after inoculation	2.55	0.90-4.95	39.13	40.76	92.16	77.39

Table 8: Genotypic correlation between biochemical characters and AUDPC in lentil germplasm under field condition.

Character	Change in OD phenol	PPO (102 DAS)	AUDPC
Change in phenol	0.572**	0.278**	-0.686**
Change in OD phenol		-0.033	-0.495**
PPO (102 DAS)			-0.147

**, * at 1% and 5% level of significance respectively.

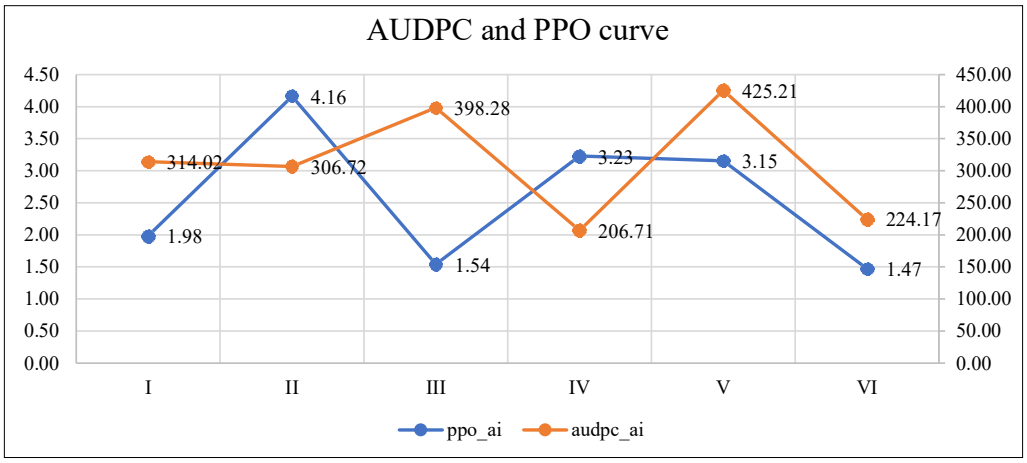


Fig 1: AUDPC and PPO bivariate curve in lentil germplasm under artificial inoculation.

Table 9: Genotypic correlation between PPO and AUDPC under artificial inoculation.

Character	PPO 48 hrs. after inoculation	AUDPC
PPO 48 hrs. after inoculation	1.00	-0.195*

**, * at 1% and 5% level of significance respectively.

activity revealed non-significant but negative (-0.147) correlation with AUDPC. The positive and significant association of change in phenol with change in OD phenol content (0.572) and PPO 102 DAS (0.278) was indicative of the simultaneous manifestation of the biomolecules under the incidence of disease. On the contrary PPO content measured 48 hours after inoculation revealed significant and negative correlation with AUDPC (-0.195) under artificial inoculation (Table 9). Thus, the measured biomolecules can be used as an indicator for screening against the disease.

The bivariate curve, based on two parameters *viz.*, PPO activity 48 hrs after inoculation and AUDPC under artificial inoculation, was derived from the cluster mean values of the said characters as presented in Fig 1. Observation from the same graph indicated that in cluster IV, the PPO content was high (3.23 abs min^{-1} g^{-1}) and the corresponding AUDPC was low (206.71). On other hand in cluster I the concentration of PPO was very minimal (1.98 abs min^{-1} g^{-1}) while higher (314.02) AUDPC was recorded. Similar observation was also recorded in cluster III with 1.54 abs min^{-1} g^{-1} PPO and the AUDPC being recorded was high (398.28). The presence of variable number of germplasm

in different clusters would thus enable to select germplasm based on the relationship of two characters from contrasting groups and could be considered for further study as far as the response of the germplasm towards the disease was concerned. Based on the disease reaction (Table 4) the contrasting germplasm or their combination may be chosen for further crop improvement. Similar results on genetic diversity in lentil have been reported by Maurya *et al.* (2018) and Pandey *et al.* (2017).

CONCLUSION

The germplasm based on disease severity in the present investigation belonged to resistant or moderately resistant category with significant variation for AUDPC, phenol and OD phenol content measured at different interval. The polyphenol oxidase (PPO) content both under field as well as under artificial inoculation also revealed significant variation among the germplasm. The significant but negative association between phenol and OD phenol with AUDPC under field condition and the same between PPO content and AUDPC under artificial inoculation would thus enable to select for germplasm showing variable disease reaction based on the studied biomolecule indicators. The selection of germplasm or their combination based on disease reaction would have been possible as evident from the association between PPO and AUDPC over the clusters. Genotypes of cluster II and III may be considered for further selection to select for sources of resistance against Stemphylium blight in the context of the present experiment.

Conflict of interest

I am Dr. Anjan Roy first and corresponding author stating on behalf of other co-authors that there is no conflict of interest exist for this manuscript.

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