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Degree of Sporulation (SP) and Normalized Difference Vegetation Index (NDVI) as Important Parameter for Characterizing Field Resistance against Cercospora Leaf Spot (CLS) in Mungbean

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A B S T R A C T

A total of 190 recombinant inbred lines (RILs) of mung bean along with parents Kopargaon (susceptible) and ML1720 (resistant) were screened for resistance to *Cercospora canescens* during three consecutive years (2016, 2017, and 2018) at farm as well as polyhouse. Bartlett's test for homogeneity of variance revealed heterogeneity in data (at $p<0.05$). ANOVA revealed significant variation (at $p<0.001$) among the genotypes for various resistance components. Estimates of Correlation coefficient (at $p<0.001$) and multiple regression analysis both concluded that Degree of sporulation (SP) and Normalized Difference Vegetation Index (NDVI) are the important parameter for characterizing field resistance against Cercospora Leaf Spot (CLS) of mungbean. Disease severity calculated as AUDPC varied significantly across the year and location. The genotype × environment interactions exerted significant influence (at $\alpha = 0.001$) which could be reduced by increasing the number of test locations.

Introduction

Around 28.8% (around 0.3 billion) of Indian are vegetarian which equivocally advocates the importance of pulse protein in Indian diet, in particular and Indian economy, in general (Akibode and Maredia, 2012; Singh *et al.*, 2016). The pace of its exploration in terms of research has always been slow. Among the thirteen pulses grown in India, mung bean or green gram [*Vigna radiata* (L.) Wilczek] belonging to subgenus *Ceratotropis* is a self-pollinated, diploid ($2n=2x=22$), short duration pulse/grain legume having579 Mbp genome size (Nair *et al.*, 2019). It is a widely cultivated crop in Southern and Southeast Asia particularly the humid tropics where Cercospora leaf spot disease (CLS) is a key constraint of socioeconomic importance to its production (Mew *et al.*, 1975). Occurrence of disease coincides with reproductive phase of crop (Vakili, 1977) leading to considerable yield loss (Iqbal *et al.*, 1995) which in turn have been compounded by the evolving pathogenic variants of *C. canescens* (Shanmugasundaram, 1987; Chand *et al.*, 2012).

Hence, exploiting the host plant resistance or tolerance to CLS coupled with higher yields are attributes to be explored. In this context, the present study investigates the important components of CLS resistance for its inclusion in resistance breeding programs. Different components of quantitative resistance or partial resistance in different crops to CLS have already been identified (Aquino *et al.,* 1995, Foster *et al.,* 1980, Ricker *et al.,* 1985). Longer latent periods, lower lesion number and reduced capacity for sporulation (Parlevliet, 1979; Nevill, 1981; Ricker *et al.,* 1985; Rossi *et al.,* 1999) as well as NDVI readings (Alisaac *et al.,* 2018; Robinson *et al.,* 2019) have been identified as components of rate reducing resistance against diseases in various crops.

Characterization of the components CLS disease resistance in mung bean was done, where parents with maximum divergence to CLS reaction along with their 190 RILs (non segregating population) were taken into account. This study would led to better understanding of quantitative resistance in mungbean/Cercospora interaction and pave way for future studies in molecular mapping in mung bean CLS resistance breeding program.

Materials and Methods

Planting materials

Experimental germplasm consisted of two mung bean parents Kopargaon (susceptible) a widely adopted cultivar, susceptible to CLS whereas, ML1720 a mungbean line along with their 190 recombinant inbred lines (RILs)

Experimental design

Experiment was conducted inalpha lattice design with two replications. Field sowing of mungbean line was done in first week of August, while polyhouse sowing was done a week after field sowing. Seeds were sown on mounds with spacing of 20 x 30 cm. The experiment was carried out in field and polyhouse in two replication over three consecutive years to analyze whether significant variation existed for environment, treatment and genotype × environment.

Inoculum preparation and artificial inoculation of CLS

A pathogenic strain of *C. canescens* 'MTCC-10835' has been used in the present study. Mass culturing and artificial inoculation was done as per Chand et al (2013).The inoculum (spore suspension) for artificial inoculation was prepared from 25 days old colonized sorghum grains (200 g) by soaking in 1 liter of sterilized water for 5 minutes. These grains were agitated thoroughly in water to dislodge the spores and filtered through two fold muslin cloths.

The inoculum (10^4 spore ml^{-1}) was delivered on mungbean leaves at flowering stage by spraying with a knapsack sprayer between 16.00 and18.00 hours (Chand *et al.,* 2013). The following morning at 07.00 hours field was irrigated to maintain high humidity. The field was irrigated after every two days in case of no rain, to maintain the humidity.

Screening for Disease severity

Disease was scored first when most of the lines showed the disease symptoms, second, third and fourth disease scoring was performed at 5, 4 and 3 days interval respectively by adopting the 1-9scale (where, 1= no infection, 2 = upto 10%, 3 = 10.1 to 20%, 4 = 20.1 to 30%, 5 = 30.1 to 40%, 6 = 40.1 to 50%, 7 = 50.1 to 65%, 8 = 65.1 to 80% and 9 = above 80% disease severity) and the Area Under Disease Progress Curve

(AUDPC) was calculated using formula given by Shaner and Finnay (1977):

$$\sum_{i=1}^{n} [\{Yi + (Yi + 1)\}/2\{(ti + 1) - ti\}]$$

Where, Yi = disease level at time ti, (ti+1)-ti = Time (days) between two lesion scores, n = number of observations (score).

Data Collection for resistance components

Incubation period (IP) by subtracting the inoculation day from day to appearance of the first lesion (Aquino *et al.,* 1995); Latent period (LP) by subtracting inoculation day from day to appearance of first Degree of sporulation (Aquino *et al.,* 1995); Degree of Sporulation (SP) by manual counting of sporulated lesion was done 35 days after inoculation on tagged leaves of each plant (Smith, 1980). MPLS (maximum percentage lesion sporulating) = number of lesions sporulated by 35 day after inoculation /total number of lesions counted by 25th day after inoculation (LN) was adopted from Johnston et al (1986). Normalized Difference Vegetation Index (NDVI) value would be taken at three different time point before, during and after biotic stress.

$NDVI=(R_{NIR}-R_{RED})/ (R_{NIR}+R_{RED})$

Where, R_{NIR} and R_{RED} is light reflection at near infra-red and red region of spectrum respectively. Measurements would be taken using hand held NDVI meter (Verhulst *et al.*, 2010a, 2010b)

Data analysis

All the data were tested for the normality using Sapiro –Wilkin test. Non normal data was normalized by square root transformation then Homogeneity of error variances of non-segregating generations was tested by using Bartlett's test (Bartlett, 1937), and when the variances were heterogeneous at 1 % and 5% level of significance the data was transformed by dividing observations of each environment/year by the square root of MSE of that environment/year which makes the error variances homogeneous and pooled analysis was performed on transformed data. SAS 9.3 version was utilized for ANOVA, correlation and regression calculations.

Results and Discussion

Research findings

All the data obtained for different variables showed normal distribution except for Degree of sporulation (SP), hence square root transformation was carried for SL to get normalized data. Bartlett's test for Homogeneity of variance (table 1) revealed that data for all traits were heterogeneous at 5% level of significance when pooled for field and polyhouse trails over three consecutive years. Hence data was transformed and this transformed pooled data was used for further analysis. Mean values along with standard deviation, standard error and normality test are presented in table 1.

Significant treatment x environment interaction

Existence of highly significant variation for year ($f_{(df\ 5,1146;\ \alpha=0.001)}$), treatment($f_{(df191,1146;\ \alpha=0.001)}$), and the treatment x environment ($f_{(df\ 955,1146;\ \alpha=0.001)}$) interactions (table 2) indicated that the treatment interacted considerably with the environmental changes.

Correlation and Regression analysis

The estimates of Correlation coefficient (table 3) among resistance components showed that all the components were moderatly to highly correlation ($p<0.001$) with each other. A

significantly high and positive correlation between AUDPC and SL (r=0.837; p<0.001) while high and negative correlation between AUDPC and NDVI (r=-0.934; p<0.001) was noticed underlining the importance of these traits as components of quantitative resistance in mungbean against CLS. Multiple regression analysis (table 4) clearly enumerated that of AUDPC depended significantly on SP (p< 0.078) and NDVI (p< 0.000) strengthening the fact that traits SP and NDVI are important components of quantitative resistance in mungbean against CLS.

Table.1 Estimates of Mean, SE, Bartlett's test Chi-Square values and Normality test values for resistance components

Traits	Field			Polyhouse			Bartlett's test @ DF(5), p<0.05	Transformed pooled data		
	2016	2017	2018	2016	2017	2018				
	Mean±S. E	Mean±S. E	Mean±S. E	Mean±S. E	Mean± S. E	Mean±S. E	Chi-Square values	Mean±S. E	Shapiro wilk-w	Shapiro wilk-p
AUDPC	346.88±7.78	341.75±8.84	323.69±8.66	327.69±8.94	324.55±9.07	288.59±9.10	849.33**	20.71±0.55	0.979	0.005
IP	12.52±0.18	12.28±0.18	12.30±0.18	12.16±0.18	12.50±0.18	12.75±0.18	778.71**	95.39±1.41	0.984	0.027
LP	23.45±0.19	23.51±0.19	23.37±0.19	23.36±0.19	23.33±0.19	23.69±0.19	221.01**	115.19±0.92	0.987	0.073
LN	77.49±1.88	74.68±2.15	78.39±1.82	76.23±1.79	78.34±2.39	75.91±1.80	1932.44**	24.61±0.62	0.981	0.012
MPLS	43.43±1.35	46.64±1.37	40.37±1.23	43.68±1.27	46.69±1.67	43.62±1.32	1719.95**	12.35±0.37	0.992	0.339
SP(sqrt)	5.67±0.15	5.61±0.15	5.50±0.15	5.71±0.14	5.72±0.14	5.68±0.14	2342.13**	34.14±0.85	0.994	0.615
NDVI	8.28±0.02	8.39±0.03	8.23±0.03	7.37±0.06	7.19±0.06	7.75±0.06	1571.51**	99.05±0.48	0.973	0.001

AUDPC = Area Under Disease Progress Curve; IP = Incubation Period (Days); LP = Latent Period (Days); LN=Lesion number; MPLS = Maximum percentage sporulating lesion and SP = Degree of Sporulation

Table.2 Estimates of ANOVA and CD for resistance components (transformed pooled data)

Source of Variation	DF	AUDPC	IP	LP	LN	MPLS	SP (sqrt)	NDVI
		M S	M S	M S	M S	M S	M S	M S
Year	5	224756.2**	4785490**	636668.8**	239566.1**	44164.69**	473116.3**	1775328**
Rep within Year	6	34.358	471.513	134.383	3.453	10.615	11.65	126.3741
Treatment	191	702.314**	4564.736**	1953.048**	895.352**	307.615**	1678.733**	530.229**
Year ×Treat	955	164.147**	1063.634**	41.08**	210.186**	48.365**	297.963**	89.091**
Pooled Error	1146	1	1.007	0.995	1.002	1	0.997	0.989
CD (Years)		1.092	4.044	2.159	0.346	0.607	0.636	6.62
CD (Treatments)		13.496	34.355	6.752	15.272	7.326	18.183	9.943
CD (Year × Treatment)		2.58	2.589	2.574	2.583	2.58	2.577	2.567

**significant at p < 0.001

Trait abbreviations as mentioned in Table 1

Table.3 Estimates of Correlation coefficient among the resistance components (transformed pooled data)

Traits	AUDPC	IP	LP	LN	MPLS	SP (sqrt)
IP	-0.722**					
LP	-0.740**	0.991**				
LN	0.689**	-0.715**	-0.723**			
MPLS	0.763**	-0.651**	-0.675**	0.535**		
SP (sqrt)	0.837**	-0.766**	-0.788**	0.846**	0.898**	
NDVI	-0.934**	0.689**	0.710**	-0.690**	-0.742**	-0.823**

**significant at $p < 0.001$

Trait abbreviations as mentioned in Table 1

Table.4 Estimates of Multiple regression analysis among the resistance components with AUDPC as dependent variable (transformed pooled data)

Traits	Regression Coefficients	Standard Error	t-value	Significance
IP	-0.071	0.075	-0.939	0.349
LP	0.045	0.119	0.376	0.707
LN	-0.197	0.133	-1.485	0.139
MPLS	-0.281	0.27	-1.04	0.299
SP (sqrt)	0.327	0.184	1.772	**0.078**
NDVI	-0.85	0.05	-17.025	**0**
Constant	103.633			

Trait abbreviations as mentioned in Table 1

Genotype × Environment interaction

Genotype × Environment interaction accounts for selection of suitable test environments to maximize gain from selection (Yan *et al.*, 2011). For each trait under study there was differential response of the genotype to environmental changes. Presence of significant environment × genotype interaction in response to disease resistance in our study was in accordance with the previously reported works on barley/net blotch interaction studied by Cherif et al (2010) as well as recent works on pea/leaf rust interaction studied by Das *et al.*, (2019) as well as mung bean/ CLS interaction studied by Das *et al.*, (2020).

Trait characterizing resistance to CLS in mung bean

The correlation results among the components of resistance (table 3) were in agreement with that obtained by Aquino *et al.*, (1995) in groundnut for late leaf spot where, AUDPC values were highly correlated with LP, IP, SP and MPLS. Similar results were presented by Johnston *et al.*, (1986), Dwivedi *et al.*, (2002) showing high correlation of AUDPC with LP and MPLS. In accordance with our results, higher negative associations between NDVI and AUDPC have been advocated by Robinson et al (2019) and Alisaac *et al.*, (2018). Hence, selection for longer IP and LP; lower LN, SP and MPLS as well as higher NDVI would effectively delay the CLS

development in the field (Waliyar *et al.*, 1993; Aquino *et al.*, 1995; Dwivedi *et al.*, 2002; Robinson *et al.*, 2019).

In conclusion the evaluating and identifying components of rate reducing resistance *in vivo* is tedious and lengthy process. Existence of variability among the genotypes and significant correlation among all the components of quantitative resistance under study showed that they might be exploited in contributing towards the slowing of epidemics. Resistance resulting in lower AUDPC, higher IP and LP, lower LN, SL and MPLS would contribute to slow disease progression. In our study, SL and NDVI have emerged as main component characterizing the resistance in mung bean/ Cercospora canescens interaction. Furthermore, significant genotype × environment interactions, reveals increment in number of location for effective screening for quantitative disease resistance of large population not only mung bean but other crops also against leaf spot diseases.

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