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GENETICS OF CERCOSPORA LEAF SPOT RESISTANCE IN MUNG BEAN

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SUMMARY

Pulses are notably good nutritive complements of carbohydrate-rich staple diets, such as, wheat, maize, and rice. Mung bean is an essential pulse crop with different proteins and antioxidants proven beneficial for health. The yield of mung bean in Pakistan is comparable to the world average, but overall production is low because of several biotic and abiotic factors. Cercospora leaf spot (CLS) is one of mung bean's most damaging diseases, limiting its productivity, causing significant losses in yield and an overall gap in production. The presented investigation progressed to comprehending the genetics of resistance to CLS in mung bean. A minicore set of 293 mung bean genotypes developed and maintained by the World Vegetable Center, Taiwan, served as samples in the study. Observed CLS attacks occur during flowering and reduce the yield by decreasing the number of pods per plant. The genetics to resistance against CLS has a single recessive gene controlling it; hence, homozygous recessive plants will be CLS-resistant. Therefore, single gene transfer methods, such as, backcross breeding, are recommendable for incorporating CLS resistance in high-yielding mung bean genotypes.

Keywords: Cercospora, CLS, generation mean analysis, mung bean, minicore

Key findings: Cercospora leaf spot (CLS) negatively correlates with number of pods per plant (PPP), and PPP positively correlates with seed yield per plant (SYPP). Thus, ultimately, CLS reduces plant yield by reducing PPP. Genotype VI000105 BG was the most susceptible, and genotype VI004954 BG was the most tolerant to CLS. Both genotypes can be valuable in different breeding programs to focus on CLS resistance as an objective. None of the checks were resistant to CLS. Resistance to CLS has the control by a single recessive gene.

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INTRODUCTION

Food insecurity is a massive threat to an exponentially increasing most of the population on the globe. The global population grows at a rate of 1.09% per year, with predictions to reach 10 billion by the year 2050 (Dorling, 2021). Developing nations have a substantially higher percentage of population growth compared with developed regions worldwide. The population growth rate in Pakistan is 2.4% yearly, which significantly amplifies the strain on agriculture. In addition to boost the production of major food crops, it is also a current need to concentrate on maximizing the yield potential of minor crops to cope with the challenges of food insecurity (Government of Pakistan, 2022). With their high digestibility and high bioavailability of nutrients, which are significantly fewer in cereals, pulses are considerably an excellent complement to brans. Being small crops, most of the world's pulses have cultivation in remote regions, taking up 5.8% of all the arable lands. The mung bean (*Vigna radiata* L.), a member of the Fabaceae family, also known as the Leguminosae, is a significant pulse crop. Around the world, it has a variety of names, including moongi, mung, moong, mungo, green gram, chicksaw pea, golden gram, and Oregon pea.

Approximately 60% to 65% of mung bean's dry weight contained carbs, whereas 22% to 28%are proteins, 3.5% to 4.5% fiber, 1% to 1.5% fat, and 4.5% to 5.5% ash. Mung bean sprouts are an excellent source of minerals, such as, iron, phosphate, and calcium, plus vitamins A, B, C, and E. Sprouts are also low in cholesterol and have a lot of digestible fiber. Additionally, mung bean is an excellent source of many essential and nonessential amino acids, including phenylalanine, isoleucine, leucine, lysine, glutamic acid, and aspartic acid (Lambrides and Godwin, 2007). Moreover, mung bean protein has a high digestion coefficient when compared with other pulses. Stachyose and raffinose, two anti-nutrient chemicals found in minute amounts in mung bean, may induce stomach issues (Popova and Mihaylova, 2019). Likewise, the mung bean's tannins, trypsin inhibitors, phytic acid, and hemagglutinin all serve crucial biological purposes like promoting digestion and getting rid of toxins. People of all ages need to eat mung beans because of the mentioned facts (Kumar and Pandey, 2020; Marwiyah *et al*., 2021; Papan *et al*., 2021).

Mung bean cultivation covers an area of around 7.3 million ha globally, producing 5.3 million t annually with an average of 0.73 t/ha (Nair and Schreinemachers, 2020). It has broad cultivation across the tropical and subtropical regions of the world, with Asia accounting for more than 90% of the world's mung bean production. Mung bean production is mainly in India, Myanmar, Pakistan, Thailand, the Philippines, and Bangladesh. India is the world’s largest producer of mung bean, accounting for about 50% of global production, or 1.04 million t annually. Given the numerous existence of wild species and wild relatives, the Indo-Pak subcontinent has various experts indicating it as the mung bean's origin and first domestication region (Singh *et al*., 2011).

Pakistan has 161,800 ha of mung bean cultivation, producing 118,800 t, with an average yield of 734.2 kg/ha (Government of Pakistan, 2022). The average mung bean yield in Pakistan is roughly on par with the global average; however, production is relatively low due to several issues. Mung bean output is less due to cultivation on marginal grounds and several biotic and abiotic stressors that prevent the crop from realizing its full genetic potential (Ullah *et al*., 2020). Drought, salt, and water-logging are three abiotic stresses contributing to decreased output (Kumar *et al*., 2013). The two most harmful biotic stressors are Cercospora leaf spot and mung bean yellow mosaic disease (Singh and Gurha, 2007; Mohan *et al*., 2014). Mung bean is very susceptible to Cercospora leaf spot (CLS), which comes from *Cercospora canescens*, an ascomycete fungus reproduced by conidiophores. The start of blossoming is when the disease first occurs. On the surface of the leaf and stem, it leaves circular to irregularly shaped scars that range in size from millimeters to 1.5 cm in diameter and have a pale brown or grayish appearance. Various measures, such as, the severity rating, percent

disease index, and infection percentage, have sought to estimate the disease severity. So far, disease intensity in mung bean has ranged from 7.83% to 43.73%, with yield losses of between 23% and 96% (Kaur, 2007; Bhat *et al*., 2008). Therefore, a study intended to help boost mung bean production by filtering the available germplasm for genotypes resistant to Cercospora and those vulnerable, identifying the gene action contributing to mung bean resistance to CLS. In light of the above, the research advances for studying CLS resistance in mung bean must include identifying resistant cultivars, marker-assisted breeding, genomic studies, and integrated disease management, which could be valuable for enhancing mung bean production in the country.

MATERIALS AND METHODS

Germplasm collection

A minicore set of 293 mung bean genotypes developed and collected from the World Vegetable Center (WorldVeg), formerly the Asian Vegetable Research and Development Center (AVRDC), Taiwan, served as the samples in the pertinent investigation. These genotypes come from the germplasm worldwide and contain maximum diversity in a minimum possible number of genotypes. The minicore set is obtainable for further studies from the germplasm storage of WorldVeg-affiliated research institutes.

Inoculum preparation

The inoculum construction used the methodology of Chand *et al*. (2013). Taking samples of diseased leaves from the field, they underwent surface sterilization with a 70% ethanol solution. Afterward, under a laminar airflow cabinet, removing 4 mm^2 portions of the infected leaf lesions ensued and put onto petri plates with 2% agar. Petri plates gained incubation for 24 h at room temperature. The fungus that had begun to sprout on the dish gained transfer to petri plates with growth media earlier developed from a 2% solution of agar, glucose, and fine mung bean powder. Then, after further purification and proliferation, allowed the fungus to spread across several petri plates for a week. Then, combining the fungus with distilled water and shaking for 24 h at 150 rpm created the suspension. This suspension served as an inoculum, administered twice as a foliar spray on the mung bean genotypes during flowering, separated by a week, to ensure the development of disease symptoms in the healthy genotypes (Figure 1).

Evaluation against CLS

The screening experiment consisted of two parts, one under protected conditions and the other under unprotected. The mung bean minicore under investigation was grown in May 2018 under the tunnels with a 1 mm fly net to protect against the attack of white flies (carrying MYMV) and other insect pests (Figure 2). The planting of genotypes had the augmented design in 3-m rows as guided by Sahoo *et al.* (2022). The design consisted of five blocks, each containing replications of the three local mung bean cultivars NM-92, NM-98, and NM-2016 (all perceived as CLS-tolerant). Following Birhanu *et al*. (2018), maintaining a plant-to-plant and row-to-row spacing must be at 15.24 cm and 45.72 cm, respectively. All advised agronomic and cultural measures employed by Mmbando *et al.* (2021) to promote improved crop stand and growth. Under unprotected conditions, the genotypes had the disease's inoculum administered using a hand sprayer before flowering. However, under protected conditions, no disease inoculum was used; instead, applying a common fungicide called Billa^{TM} at the prescribed amount minimized the severity of the disease.

Disease development and scoring of genotypes

In unprotected conditions earlier described, fungal inoculum application on each plant ensured maximum disease attack. In addition, no fungicide spraying transpired to suppress the fungal growth. At maturity, the recording of disease scores analyzed the disease effects.

Figure 1. Inoculum preparation and CLS proliferation.

Figure 2. Tunnel covered with net for evaluation of genotypes against CLS.

Table 1. Disease severity ratings given by Shahbaz *et al*. (2014).

Severity rating	"Symptoms on plants at flowering and pod-formation stage"
0	"No visible symptoms on plants"
1	"1-10% foliage or pod area affected with small pinhead lesions"
3	"11-20% foliage or pod area affected with small round brown spots"
5	"21-30% foliage or pod area affected with large spots"
7	"31-50% foliage or pod area affected with bigger coalescing spots"
9	"51-100% foliage or pod area affected with bigger coalescing spots"

It employed two scales, i.e., Infection Percentage (IP) and Percent Disease Index (PDI). Infection percentage calculation followed the formula presented by Shahbaz *et al.* (2014).

$$\text{IP} = \frac{No.\,of\ infected\ plants\ per\ genotype}{Total\ number\ of\ plants\ per\ genotype} \text{ X } 100$$

For the calculation of the percent disease index, a disease rating from "0" to "9" was given to individual plants per genotype based on disease severity, as prescribed by Shahbaz *et al.* (2014) (Table 1). PDI estimation applied the formula given by Kumar *et al.* (2011) and Shahbaz *et al.* (2014) as follows:

$$\text{PDI} = \frac{Sum\ of\ all\ disease\ severity\ ratings\ of\ a\ genotype}{(Total\ no.\ of\ observations)\,\text{X}\,(Maximum\ rating\ in\ scale)} \text{ X } 100$$

Growth and yield contributing traits

The data recorded for the following parameters helped select one high-yielding CLS-resistant parent (P_1) and one low-yielding CLS-susceptible parent (P_2). Measuring plant height (PH) had three plants per genotype randomly tagged, with the height measured using a meter rod, then taking the average. Total pods from three randomly selected plants counted and averaged gained the number of pods per plant (PPP). Pod length (PL) measurement took the average stretch of five healthy and vigorous pods from already harvested pods of each selected plant. Harvested pods threshed separately attained the average number of seeds per pod (SPP). The 100 seed weight (100SW) determination used already threshed pods with 100 representative seeds from each entry randomly picked and weighed separately using an electric balance, then calculating the mean weight of 100 seeds. At crop maturity, the pods harvested from each plant per entry received separate threshing, followed by seed weighing. Weighing five samples of bulked seed from each entry used the electric balance, with the average computed for the seed yield per plant (SYPP). Harvested plants underwent sun-drying to remove any moisture. Then dry weight measurement of each plant using an electric balance had the ratio of seed yield per plant with total plant biomass calculated as harvest index (HI) per genotype (Pratap *et al.,* 2021).

Hybridization

Based on various analyses, two genotypes resulted in parents' selection, i.e., one high-yielding and resistant genotype and the other, low-yielding and susceptible genotype. Growing both genotypes for hybridization followed the recommended agronomic and cultural practices. The tolerant genotype served as the female parent, and the susceptible genotype as the male parent in the hybridization process. The reason for choosing the female as tolerant was to minimize the changes for potential omissions in case of cytoplasmic inheritance. The emasculation of female parents occurred in the evening, while manual cross-pollination happened the next morning to develop the F_1. In the succeeding season, sowing the F_1, P_1, and P_2 generations followed all the recommended practices, and the ensuing breeding scheme had F_1 self-pollinated to develop F_2, then crossed with both parents separately to produce BC_1 and BC_2 generations, respectively.

Final evaluation

In the final trial, all six generations (P_1, P_2, F_1, F_2, BC_1, and BC_2) undertook field sowing following all the methodologies and cultural practices previously done during the screening experiment. The data recording for the mentioned traits ran a generation mean analysis to know the genetics for resistance to CLS.

Statistical methods

Running the augmented analysis of variance will screen out the genotypes against resistance to CLS, as it is efficient in evaluating single replication experiments for genotype evaluation. Further correlation analysis determined the relationship among different variables and found how the CLS contributes to yield reduction. The principal component analysis also helped identify the association of various traits and the allocation of genotypes according to their performance for those traits. Percent change over control distinguished the changes occurring in the genotypes applied with the disease inoculum. It helped in screening the genotypes for resistance to CLS. The genotypes having the minimum alteration, therefore, resulted in resistant genotypes. Lastly, the generations mean analysis defined the pattern of transfer of disease resistance from parent to offspring.

RESULTS AND DISCUSSION

Augmented analysis of variance

The analysis of variance under augmented design was separate on the protected and unprotected conditions. For protected conditions, the results indicated significant variation for blocks (B), entries (E), genotypes (G), and checks vs. genotypes (CvsG) in all parameters except SPP, marked by asterisks. It means no significant difference occurred among all the genotypes studied for SPP. On the other hand, PH, PPP, and SPP had the highest means from blocks 3, 5, and 1, respectively. PPP had the highest averages from block 5, and PL, 100SW, SYPP, and HI had the highest means from block 2. Checks were only significant for HI; otherwise, response of all the controls were similar in all the parameters. Check 1 (NM 92) had the highest means for PH and SPP, check 2 (NM 98) for 100SW, SYPP, and HI, and check 3 (NM 2016) for PPP and PL. The details of ANOVA and means of blocks and checks with the least significant increase (LSI) are in Table 2.

Table 2. Analysis of variance for normal conditions.

Source	d.f.	PH	PPP	PL	SPP	100SW	SYPP	HI
Block (B)	4	475.9**	672.61**	8.5953**	13.448	10.141**	143.145**	1472.5**
Entries (E)	177	60.1**	87.9**	6.4**	4.12	4.56**	84**	473**
Checks (C)	2	62.7	11.2	0.9879	4.749	0.2121	8.676	299.9**
Genotypes (G)	174	58.64**	86.64**	5.9**	3.89	4.51**	79**	412**
C vs. G	1	1456.1**	4512**	245**	89	121**	1443**	9845**
Error	8	132	39.95	0.8227	5.079	0.7984	4.713	297.3
	1	85.3333	19.2223	6.08667	9.04433	3.81067	2.571	18.6533
	2	70.311	9.3777	6.15567	6.26667	5.16567	4.425	36.5497
Block Means	3	93.8667	8.8	5.63333	8.46667	1.954	0.5393	1.6907
	4	79.8333	1.2777	2.16667	3.66667	1.04533	0.0973	11.269
	5	61.6667	39.9657	5.81333	6.67333	5.01233	16.8637	40.347
	1	81.92	15.9134	5.374	7.6006	3.4516	4.6298	11.1678
Check Means	2	77.82	14.148	4.6614	5.73	3.5712	6.3304	25.9302
	3	74.8666	17.1246	5.478	7.14	3.17	3.7376	14.485
	1	97.0331	24.229	6.56726	10.5655	4.62711	7.48578	33.8521
Check Mean + LSI	2	92.9331	22.4636	5.85466	8.6949	4.74671	9.18638	48.6145
	3	89.9797	25.4402	6.67126	10.1049	4.34551	6.59358	37.1693

PH: Plant Height, PPP: Pods Per Plant, PL: Pod Length, SPP: Seeds Per Pod, 100SW: 100 Seed Weight, SYPP: Seed Yield Per Plant, HI: Harvest Index.

Table 3. Analysis of variance for diseased conditions.

Source	d.f.	PH	PPP	PL	SPP	100SW	SYPP	HI	IP	PDI
Block (B)	4	2517**	1314**	4.317	18.86**	3.42**	65.1**	197.3	583**	494.7**
Entries (E)	177	513**	421**	3.6	3.7**	2.43**	7.56**	12	60**	49**
Checks (C)	2	539.7	103	1.912	3.267	0.963	18.54	205.8	5838**	810.9**
Genotypes (G)	174	510**	420**	1.31	3.4**	2.41**	6.59**	11	57**	48**
C vs. G	1	8518**	7754**	54	585**	354.18**	1054.2**	247.6	4345**	4015**
Error	8	364.5	102	0.53	2.148	1.002	3.16	9.6	23	22
Block Means	1	120.667	55.6667	6.0556	9.6943	3.00667	9.0333	15.44	66.666	7.4077
	2	95	10.6667	5.764	6.9723	4.49467	3.2733	25.61	33.333	11.111
	3	52	8.6667	3.3	3.7	2.007	7.9633	21.56	66.666	33.333
	4	65.222	15	5.9	9.1333	3.90333	4.1357	29.94	61.111	30.864
	5	57.8	40.6667	6.1333	5.4333	4.48333	14.9667	36.78	52.777	33.333
Check Means	1	83.08	29	4.735	6.18	3.0948	6.712	18.66	90	31.111
	2	66.2	20.9	5.9184	6.9834	3.9502	6.814	30.97	56.666	30
	3	85.1334	28.5	5.6384	7.7966	3.692	10.0974	27.96	21.666	8.5186
Check Mean +	1	119.456	50.0856	6.5736	8.5143	4.4118	15.8418	45.80	134.39	62.965
LSI	2	102.576	41.9856	7.7570	9.3177	5.2672	15.9438	58.11	101.06	61.854
	3	121.509	49.5856	7.4770	10.130	5.009	19.2272	55.10	66.064	40.373

PH: Plant Height, PPP: Pods Per Plant, PL: Pod Length, SPP: Seeds Per Pod, 100SW: 100 Seed Weight, SYPP: Seed Yield Per Plant, HI: Harvest Index.

On unprotected conditions, the analysis of variance resulted in significant differences among B, E, G, and CvsG in all the parameters except PL and HI, as indicated by asterisk marks. The case of significance for PL, SPP, and HI under both conditions is a preliminary indication that CLS has affected the pods, thereby reducing the overall yield in the studied genotypes. PH, PPP, and SPP had the highest means from block 1. On the other hand, PL, SYPP, and HI had the highest averages from block 5. For block 2, 100SW has the maximum value. IP showed the highest for blocks 1 and 3. Likewise, PDI showed the highest for blocks 3 and 5. The checks were only significant for IP and PDI. The details of ANOVA and means of blocks and controls with the LSI are available in Table 3.

Considerable variation among the mung bean genotypes had earlier estimation in a field study by Maqbool *et al*. (2017) for yield contributing parameters, including PH, PPP, SPP, 100SW, and SYPP. The molecular research based on SSR markers by Schafleitner *et al*. (2015) found significant variations among the studied mung bean minicore genotypes of the Asian Vegetable Research and Development Center (AVRDC), indicating an elevated amount of genetic variability in mung bean minicore set.

Trait associations

The findings of the correlation analysis appear in Table 4. The correlation analysis indicated that HI has positive and significant correlations with 100SW, PL, SPP, and SYPP. The 100SW exhibited a significant and positive association with HI, PH, PL, PPP, and SYPP. These findings were relevant to earlier studies of Atta *et al*. (2008), Kim *et al*. (2013), and Maqbool *et al*. (2017), who observed similar type of correlations of 100SW with these parameters; however, no relation of 100SW showed with SPP. IP provided a positive and substantial connection with PDI and a negative and significant association with PPP. Similarly, PDI emerged positively and considerably linked with IP and negatively and suggestively related to PPP. PH has a significant positive association with 100SW, PL, PPP, SPP, and SYPP. PL positively and significantly correlated with HI, 100SW, PH, PPP, SPP, and SYPP. The 100SW, PH, PL, SPP, and SYPP had a positive and significant link with PPP while negatively and non-significantly related with IP and PDI. These findings were in agreement with the results of Haritha and Sekhar (2002), Aijaz (2013), and Maqbool *et al*. (2017). However, the outcomes disagreed with Tabasum *et al*. (2010), who observed a negative relation of PPP with SYPP.

Table 4. Interrelationship among studied parameters.

	HI	HSW	IP	PDI	PH	PL	PPP	SPP
HSW	0.4169**							
P value	0.000							
IP	0.0052	0.1705						
P value	0.954	0.645						
PDI	0.035	0.1649	0.6297**					
P value	0.41	.059	0.000					
PH	0.0744	0.3078*	0.2448	0.1733				
P value	0.754	0.000	0.054	0.54				
PL	0.4604**	0.7731**	0.1871	0.1775	0.5723**			
P value	0.000	0.000	0.214	0.654	0.000			
PPP	0.1860	0.2401*	-0.3086*	-0.2758*	0.6659**	0.5743**		
P value	0.214	0.024	0.026	0.0149	0.000	0.000		
SPP	0.3693**	0.1496	-0.1241	-0.1911	0.6190**	0.9228**	0.6040**	
P value	0.000	0.568	0.410	0.654	0.000	0.000	0.000	
SYPP	0.3526**	0.2302*	-0.1563	-0.1755	0.5750**	0.5124**	0.7991**	0.5366**
P value	0.000	0.045	0.3121	0.098	0.000	0.000	0.000	0.000

PH: Plant Height, PPP: Pods Per Plant, PL: Pod Length, SPP: Seeds Per Pod, 100SW: 100 Seed Weight, SYPP: Seed Yield Per Plant, HI: Harvest Index.

SPP showed a significant and positive relationship with all the parameters except 100SW, IP, and PDI. These results contradicted the discoveries of Ghosh and Panda (2006) and aligned with the studies of Gul *et al.* (2008). Finally, SYPP is in significant and positive correlation with all the parameters except IP and PDI. Although, a minor negative (nonsignificant) correlation of SYPP with IP and PDI existed. These results aligned with the conclusions of Ghosh and Panda (2006), Atta *et al*. (2008), Pandey *et al*. (2009), and Maqbool *et al*. (2017), who detected a similar linkage of SYPP with these parameters.

The earlier mentioned results indicate that SYPP is the ultimate criterion for yield in mung bean, which correlates directly with its contributing component characters, including HI, 100SW, PH, PL, PPP, and SPP. Any increase in these parameters contributes directly to the seed yield in mung beans. The 100SW increases as the PL increases, giving more space and nutrients to the seeds. PPP contributes directly to 100SW. If PH is more, then plants will have more PPP and PL, which in turn, contribute to increasing the number and size of the seeds in the form of SPP and 100SW, consequently increasing SYPP. Concerning disease response, the two disease severity scoring implied a positive correlation with each other and a negative correlation with PPP. It means that CLS mainly attacks during flowering and pod formation, thus halting and disturbing pod formation and filling. Reduced PPP will ultimately contribute to reduced SYPP. It means that increased disease severity will eventually reduce the yield of mung bean.

Screening of genotypes

The change in response of the studied genotypes to any type of stress is a reliable criterion to find out the most and least-performing genotypes for the selection of parents for use in future genetic studies. The lesser the change in response of a genotype, the more stable it will be in diseased conditions. It means that the genotype is tolerant or even resistant to the disease. On the other hand, if more change occurs in the response, especially if the change is negative, it means the infection influences the genotype making it susceptible. With the inoculum application committed during flowering, it did not affect PH. Also, large fluctuations showed in the HI readings, making HI unusable for selection criteria. Therefore, removing these two parameters transpired before calculating the percent change. The results of percent change indicated significant variations in the selected parameters, i.e., PPP, PL, SPP, 100SW, and SYPP with the incidence of CLS.

Table 5. Percent change over control in selected genotypes.

	Genotype	PPP	PL	SPP	100SW	SYPP
Most susceptible	VI000105 BG	-72.95	-30.00	-40.00	-30.40	-83.99
Most tolerant	VI004954 BG	01.90	-22.38	15.31	-20.79	-6.91
Check 1	NM 92	-08.11	-06.87	-05.73	-07.68	-20.67
Check 2	NM 98	-10.43	-03.72	01.50	-07.14	-16.03
Check 3	NM 2016	-12.52	-07.23	-03.92	-08.05	-24.23

PH: Plant Height, PPP: Pods Per Plant, PL: Pod Length, SPP: Seeds Per Pod, 100SW: 100 Seed Weight, SYPP: Seed Yield Per Plant, HI: Harvest Index.

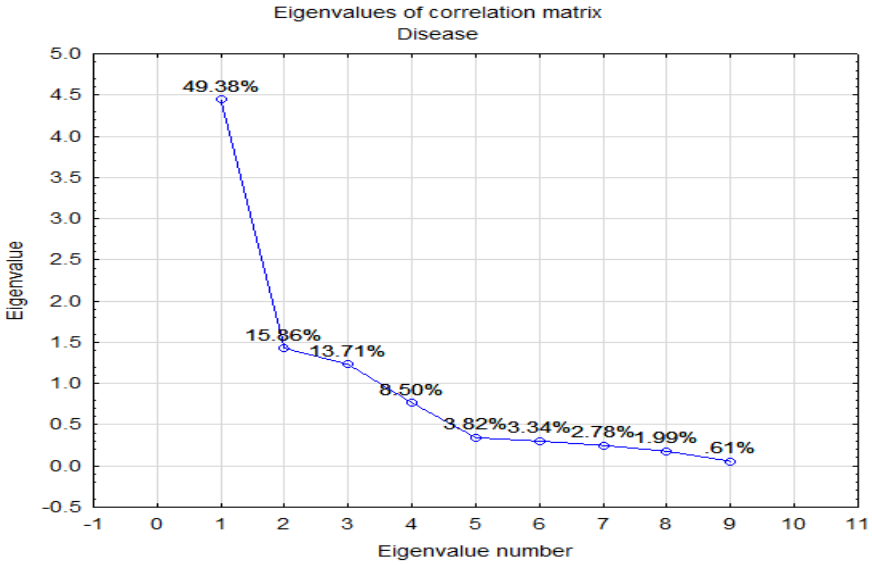

Figure 3. Scree plot for PCA under diseased conditions.

The results of the percent change over control of the studied genotypes show in Table 5. High levels of alterations among the studied genotypes occurred in genotype "VI000105 BG" compared with other genotypes indicating it had the most effects from the disease, including low yield. Inversely, the genotype "VI004954 BG" attained the minimum influence by the sickness having a high output compared with other genotypes. Therefore, the genotype "VI000105 BG" gained selection as a susceptible parent while "VI004954 BG" was the tolerant parent in the generation mean analysis. Another crucial observation noted from the assessment was that none of the checks, perceived as CLS resistant, signified resistance to the disease. Instead, all three genotypes exhibited varied disease appearance, reducing the seed yield from 16% to 24% (Table 5).

Association of genotypes and traits

The principal component analysis (PCA) under unprotected conditions determined the relationship between the studied parameters with the distribution of the genotypes based on those parameters. The PCA for unprotected settings indicated the division of the variation into nine principal components (PCs), and the first two PCs contributed about 65.24% of the total variation (Figure 3). The loading plot indicated that the disease-related parameters were almost 90° from the yield-contributing parameters except for PPP and SYPP. It revealed that apart from the PPP and SYPP,

there is nearly no association among the disease-related and yield-contributing parameters. A more than 90° angle between disease-related parameters and PPP and SYPP occurred, which revealed a rather negative association between the disease and the PPP and SYPP. The closed location of PPP with SYPP indicated a strong positive relation among these characteristics. Also, a strong positive correlation between PDI with IP appeared. Likewise, SPP, PL, 100SW, and HI exhibited a correlation among themselves (Figure 4). The score plot indicated that the genotypes located in the upper left corner along the loading of PPP and SYPP and away from PDI and IP were high-yielding with being tolerant to CLS, i.e., V147, V163, V15, V59, and V44, among others. The genotypes present in the lower right corner tended to be more susceptible to CLS, i.e., V158, V115, V86, and so on. Genotypes located in the upper right corner were disease-tolerant yet low-yielding, such as, V112, V155, and V117, while those present in the lower left corner were inclined to be good-yielding but susceptible to CLS, i.e., V17, V127, V137, and V167, to name a few (Figure 5).

Inheritance pattern of disease resistance

The information about the inheritance pattern of any trait is highly critical regarding the selection of breeding methodology for improving the attribute. Resistance to CLS is a matter of conflict with many researchers. In the relevant study, two genotypes selected as parents for the study of the inheritance of disease were "VI004954 BG" as P_1 and "VI000105 BG" as P_2. All the six generations developed and grown under diseased conditions had every plant scored for symptoms as described earlier.

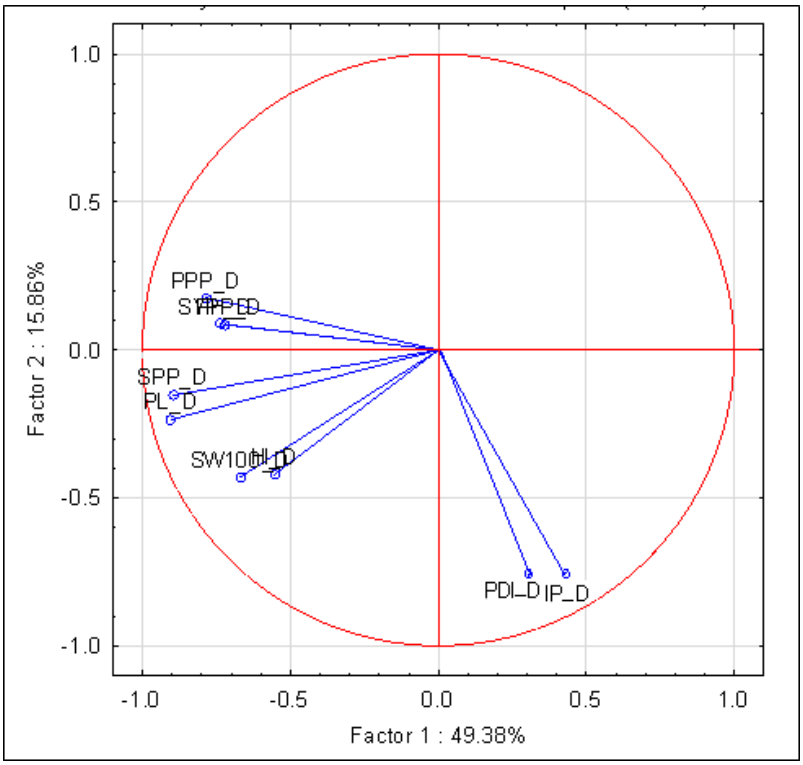

Figure 4. Loading plot for PCA under diseased conditions.

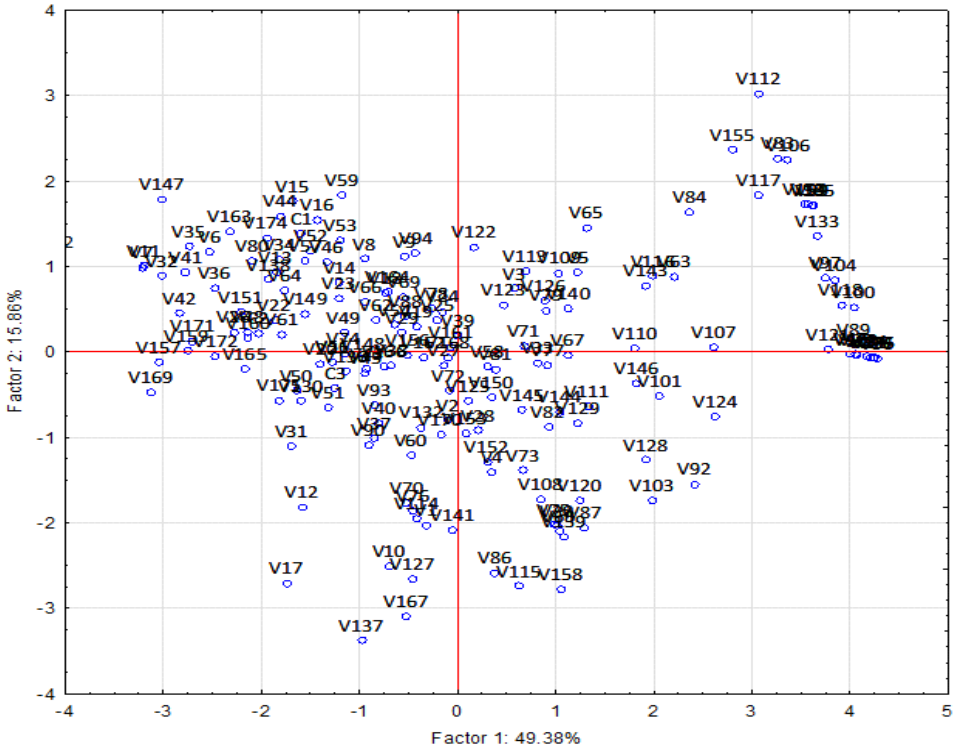

Figure 5. Score plot for PCA under diseased conditions.

Table 6. Segregation ratio of F_2 for CLS resistance.

No. of Plants	Observed		Tested Ratio	Expected		X^2 value
	S	R		S	R	
150	116	34	3:1	112.5	37.5	0.43 ^{NS}
150	116	34	15:1	140.6	9.3	68.99*
150	116	34	63:1	147.6	2.34	434.35*
150	116	34	11:5	103.1	46.8	5.14*

Disease severity analysis employed chi-square for different genotypic ratios to test the goodness of fit. The results of the chi-square for all ratios are available in Table 6. It is evident from the data presented that disease resistance segregation followed a 3:1 proportion, which indicated monogenic inheritance as the genotypes being either tolerant or susceptible. The word tolerant is used here instead of resistant because none of the genotypes proved complete resistance to CLS. The findings of the presented study validate that pooling plants into two categories causes the data to fit in a 3:1 ratio. Tolerance against CLS showed to be recessive to susceptibility. The results agreed with the findings of Mishra *et al*. (1988) and Duangsong *et al*. (2018), while contrary to Singh *et al*. (2017), who found resistance as controlled by a single dominant gene, and Choudhary *et al*. (2021), who concluded it as manipulated by two genes. Therefore, the hypothesis of a susceptibility-causing gene may be any plant factor responsible for the replication or spread of Cercospora. When this gene is present in functional form in any plant, it supports conidial replication or spread by providing a favorable environment for their nourishment resulting in susceptibility; otherwise in its non-functional form, it causes resistance or tolerance in the genotype.

Genetic studies

Gene action can have two main categories, additive and non-additive effects for quantitative traits. The additive effect, defined as an average effect of genes on the same loci, also includes the additive × additive epistatic effects, whereas the non-additive category includes dominance, additive × dominance, and dominance × dominance effects. Dominance is the interaction of allelic genes, while the interrelation of non-allelic genes is epistasis. The evaluation of crosses ran under normal and diseased conditions. The data of P_1, P_2, F_1, F_2, BC_1, and BC_2 populations had records for rating for CLS, as recommended by Kumar *et al.* (2011) and Shahbaz *et al*. (2014). Data also underwent calculating heritability (broad sense), shown in Table 7. The genetic effects for the tolerance to the CLS indicated that the tolerance is associated with dominance and additive gene action as the simple additive–dominance model was able to adequately reveal the variation among the different studied generations for the disease scores. It disclosed that a single gene with additive and a dominance gene effects ably control the resistance to the CLS disease in mung bean. In addition, it also indicated that there were no non-allelic interaction or epistasis effects for the tolerance to CLS. Population average, additive gene effect, and dominant gene effect estimated for the disease scores caused by CLS were 4.12, 2.15, and 0.64, respectively. The resistance to CLS had a high heritability estimation of 0.93, indicating that genetic factors dominate in determining resistance. It suggests that any typical selection procedures for self-pollinated species, notably backcross breeding, can be helpful in breeding for resistance cultivars utilizing the resistant source, as proposed by Duangsong *et al*. (2018).

Table 7. Generation means for CLS.

Category	Parameter	Value
Generations	P1	4.55
	P2	0.66
	F1	2.30
	F2	1.80
	BC1	3.20
	BC2	1.34
Genetic Effects	[m]	4.12±1.18
	[d]	2.15±0.51
	[h]	0.64±0.26
Chi-square	χ^2	5.54
Broad Sense Heritability (%)	H^2	0.93

CONCLUSIONS

Resistance to CLS is highly imperative to overcome the yield losses of mung bean, especially during the monsoon season in Pakistan. The resistance to CLS has control of a single recessive gene, which indicates that single gene transfer methods, such as, backcross breeding, can be beneficial to incorporate disease resistance into high-yielding mung bean genotypes. Unfortunately, commercial mung bean varieties cultivated in Pakistan are susceptible to CLS. Therefore, research should progress to develop CLS-resistant mung bean varieties to increase its production in the country. Genotype VI004954 BG, which proved a highly tolerant and high-yielding genotype, has a Pakistani origin; therefore, it can serve for various breeding programs and general cultivation in Pakistan. Further investigations can proceed to transfer the CLS resistance gene from resistant genotypes to the commercial varieties grown in Pakistan.

REFERENCES

Aijaz A (2013). Association and inter-relationship among yield and yield contributing characters and screening against Cercospora leaf spot in mung bean (*Vigna radiata* L.). *Sci. Res. Essay* 8(41): 2008-2014.

Atta BM, Haq MA, Shah TM (2008). Variation and inter-relationships of quantitative traits in chickpea (*Cicer arietinum* L.). *Pak. J. Bot.* 40(2): 637-647.

Bhat NA, Maheshwari SK, Ahmad S, Beig BA, Masoodi SD (2008). Field evaluation of mung bean accessions against Cercospora leaf spot. *Ann. Biol.* 24: 1-4.

Birhanu A, Tadesse T, Tadesse D (2018). Effect of inter- and intra-row spacing on yield and yield components of mung bean (*Vigna radiata* L.) under rain-fed condition at Metema District, Northwestern Ethiopia. *Agric. Food Sec.* 7(1): 1-7.

Chand R, Kumar P, Singh V, Pal C (2013). Technique for spore production in *Cercospora canescens*. *Indian Phytopathol.* 66(2): 159-163.

Choudhary P, Chand R, Singh AK (2021). Genetics of Cercospora leaf spot resistance in mung bean [*Vigna radiata* (L.) Wilczek] through generation mean analysis. *Legume Res.* 01: 1-7.

Dorling D (2021). World population prospects at the UN: Our numbers are not our problem? In: The Struggle for Social Sustainability. Policy Press, pp. 129-154.

Duangsong USA, Laosatit K, Somta P, Srinives P (2018). Genetics of resistance to Cercospora leaf spot disease caused by *Cercospora canescens* and *Pseudocercospora cruenta* in yard long bean (*Vigna unguiculata* ssp. sesquipedalis) grain cowpea (*V. unguiculata* ssp. unguiculata) populations*. J. Genet.* 97(5): 1451-1456.

Ghosh A, Panda S (2006). Association and variation studies between grain characteristics and germination of mung bean (*Vigna radiata* L. wilczek). *Legume Res.* 29: 118-121.

Government of Pakistan (2022). Economic survey of Pakistan 2021-2022. Ministry of Food, Agriculture and Livestock (Federal Bureau of Statistics), Islamabad, pp. 22.

Gul R, Khan H, Mairaj G, Ali S (2008). Correlation study on morphological and yield parameters of mung bean (*Vigna radiata*). *Sarhad J. Agric.* 24(1): 37-42.

Haritha S, Sekhar MR, (2002). Correlation and path coefficient analysis in mung bean *Vigna radiata* (L.) Wilczek. *Legume Res.* 25(3): 180-183.

Kaur L (2007). Multiple disease resistant sources of mung bean. *Acta Hortic.* 752: 423-426.

Kim JK, Kim EH, Lee OK, Park SY, Lee B, Kim SH, Park I, Chung IM (2013). Variation and correlation analysis of phenolic compounds in mung bean (*Vigna radiata* L.) varieties. *Food Chem.* 141(3): 2988-2997.

Kumar P, Pal M, Joshi R, Sairam RK (2013). Yield, growth and physiological responses of mung bean (*Vigna radiata* L.) genotypes to water logging at vegetative stage. *Physiol. Mol. Biol. Plants* 19(2): 209-220.

Kumar P, Singh V, Chand R (2011). Cercosporin: A photo activated toxin in fungal pathogenesis of plants*. Plant Health Manag.* 12(2): 315-326.

Kumar S, Pandey G (2020). Biofortification of pulses and legumes to enhance nutrition. *Heliyon* 6(3): 1-6.

Lambrides CJ, Godwin ID (2007). Mung beans. In: Pulses, Sugar and Tuber Crops. Berlin, Heidelberg: Springer Berlin Heidelberg, pp. 69-90.

Maqbool MA, Jamil MS, Akhtar MA, Amin N, Habib S (2017). Association and path coefficient analysis of different morphological parameters and yield components in mung bean genotypes*. J. Agric. Basic Sci.* 2(3): 28-35.

Marwiyah S, Sutjahjo SH, Trikoesoemaningtyas, Wirnas D, Suwarno WB (2021). High nonadditive gene action controls synchronous maturity in mung bean. *SABRAO J. Breed. Genet.* 53(2): 213-227.

Mishra SP, Asthana AN, Yadav L (1988). Inheritance of Cercospora leaf spot resistance in mung bean, *Vigna radiata* (L.) Wilczek. *Plant Breed.* 100(3): 228-229.

Mmbando F, Mbeyagala E, Binagwa P, Karimi R, Opie H, Ochieng J, Mutuoki T, Nair RM (2021). Adoption of improved mung bean production technologies in selected East African countries. *Agric.* 11(6): 528.

Mohan S, Sheeba A, Murugan E, Ibrahim SM (2014). Screening of mung bean germplasm for resistance to mung bean yellow mosaic virus under natural condition*. Indian J. Sci. Technol.* 7: 891-896.

Nair R, Schreinemachers P (2020). Global status and economic importance of mung bean. In: The Mung bean Genome. Springer, Cham, pp. 1-8.

Pandey S, Sharma S, Kumari S, Gaur PM, Chen W, Kaur L, Macleod W, Basandrai LK, Bala D, Sandhu A, Tripathi JS, Gowda HS (2009). Integral foliar diseases management of

legumes. In: Genetic Improvement, Management and Trade. Indian Society of Pulses Research and Development, Indian Institute of Pulses Research, Kanpur, India, pp. 143-161.

Papan P, Chueakhunthod W, Poolsawat O, Arsakit K, Tharapreuksapong A, Tantasawat PA (2021). Validation of molecular markers linked to cercospora leaf spot disease resistance in mungbean (*Vigna radiata* [L.] Wilczek). *SABRAO J. Breed. Genet.* 53(4): 749-757. https://doi.org/10.54910/sabrao2021.53.4.16

Popova A, Mihaylova D (2019). Antinutrients in plant-based foods: A review. *The Open Biotechnol. J.* 13(1): 68-76.

Pratap A, Gupta S, Rathore M, Basavaraja T, Singh CM, Prajapati U, Singh P, Singh Y, Kumari G (2021). Mungbean. In The Beans and the Peas. Woodhead Publishing, pp. 1-32.

Sahoo JP, Samal KC, Tripathy SK, Lenka D, Mishra P, Behera L, Acharya LK, Sunani SK, Behera B (2022). Understanding the genetics of Cercospora leaf spot (CLS) resistance in mung bean (*Vigna radiata* L. Wilczek). *Trop. Plant Pathol.* 47(6): 703-717.

Schafleitner R, Nair RM, Rathore A, Wang YW, Lin CY, Chu SH, Lin PY, Chang JC, Ebert AW (2015). The AVRDC – The World Vegetable Center mungbean (*Vigna radiata*) core and mini core collections. *BMC Genomics* 16(1): 1-11.

Shahbaz MU, Iqbal MA, Rafiq A, Batool A, Kamran M (2014). Efficacy of different protective fungicides against Cercospora Leaf Spot of mungbean (*Vigna radiata* L. wilczek*). Pak. J. Phytopathnol.* 26(2): 187-191.

Singh DK, Vaishampayan A, Singh MN, Singh SK, Vennela PR, Varsha G, Jaiswal DK, Vaibhav S (2017). Inheritance studies on Cercospora leaf spot (CLS) resistance in mung and urdbean. *Vegetos* 30(2): 388-391.

Singh G, Singh S, Sheoran OP (2011). Inheritance of mungbean yellow mosaic virus (MYMV) resistance in mungbean [*Vigna radiata* (L.) Wilczek]. *Legume Res.* 36: 131-137.

Singh RA, Gurha SN (2007). Stable sources of resistance to Cercospora leaf spot in mungbean. *Ann. Plant Prot. Sci.* 15(2): 501-502.

Tabasum A, Saleem M, Aziz I (2010). Genetic variability, trait association and path analysis of yield and yield components in mungbean (*Vigna radiata* (L.) Wilczek). *Pak. J. Bot.* 42(6): 3915-3924.

Ullah A, Shah TM, Farooq M (2020). Pulses production in Pakistan: Status, constraints and opportunities. *Int. J. Plant Prod.* 14(4): 549-569.