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Enzymatic response of mungbean (*Vigna radiata*) genotypes against *Cercospora* leaf spot disease

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ABSTRACT

Mungbean (*Vigna radiata* L.) is one of the most valuable pulse crops grown in India. Mungbean suffers from several diseases with substantial losses in yields. Among the diseases, mungbean leaf spot disease is the most destructive which is caused by *Cercospora canacens* and cause heavy loss (0-61%) in yield in Indian subcontinent and adjacent areas of South-East Asia. An experiment was conducted to find out the enzymatic response of various genotypes of mungbean against *Cercospora* leaf spot. The level of antioxidant enzyme SOD, peroxidase and catalase increase in both susceptible and resistance cultivar, but in resistance cultivar the level these enzymes increases very rapidly as compare to susceptible cultivar. Based on the various observations, it can be interprets that in resistant cultivar, the higher level of ROS produced after inoculation is minimized up to optimum level by the action of these enzymes SOD, peroxidase and catalase, but not in susceptible cultivar. Plants show resistance or susceptibility for disease is due to the activity of SOD, peroxidase, and catalase. In F2 generation plants show segregation pattern in the ratio of 1:2:1 which depicts that the gene governing the enzyme activities are partial dominant in nature.

Key words: Catalase, *Cercospora,* Mungbean, Peroxidase, SOD, Superoxide

Pulses are an important source of proteins, vitamins and minerals in human diet. Pulses contain protein ranging from 20 to 40 per cent, which is almost 2.5 to 3.0 times more than normally found in cereals (Chaturvedi and Ali 2002). Besides their high nutritional value, they have a unique characteristic of maintaining and restoring soil fertility through biological nitrogen fixation and thus play a vital role in maintaining soil fertility and sustainable agriculture (Asthana 1998). The important pulse crops growing in India are Bengal gram, lentil, mungbean, blackgram, cowpea, red gram and pea. Among these, mungbean (*Vigna radiata* L.) is an ancient and well known leguminous crop of all over world. It is grown throughout the southern Asia including India, Pakistan, Bangladesh, Sri Lanka, Thailand, Cambodia, Vietnam, Indonesia, Malaysia and China, etc.

The lower productivity in mungbean is mainly due to low genetic yield potentiality, indeterminate growth habit, canopy architecture, low partitioning efficiency, cultivation in marginal land and biotic and abiotic stresses (Khare *et al*. 1998). Among the diseases of mungbean *Cercospora* leaf spot is the most important disease. Maximum loss of 61% was observed in case of grain yield (Iqbal *et al.* 1995). Several workers had reported the effective control of the disease with the application of fungicides (Singh and Naik 1977, Singh and Singh 1978). The cheapest, practical and economical control of the disease can be achieved by the genetic stock resistant to the disease (Hossain *et al*. 1981 and Iqbal *et al*. 1990). Hence this present investigation was carried out on different enzymatic aspects of *Cercospora* leaf spot, viz. superoxide dismutase, peroxidase, and catalase, activity in four populations, parent 1, parent 2, F_1 and F_2 of two crosses KOP × ML 1720 and KOP × HUM 12.

MATERIALS AND METHODS

In the present experiment, data on three enzymes namely superoxide dismutase, peroxidase and catalase were recorded on six genotypes Kopergoan (P1, Susceptible), ML1720 (P2, Resistant), KOP × ML 1720 (F1), HUM 12 (P2), KOP × HUM 12 (F1), and two segregating population KOP × ML 1720 (F2), KOP × HUM 12 (F2) of two crosses HUM 12 × ML 1720 and KOP × HUM 12. Mung bean plants taken in the experiment were of parent 1 ($P_{1)}$ parent 2 (P_2), plants of F_1 and F_2 generation. 15 days after inoculation, i.e. 45 days after planting. The three different enzymes namely superoxide dismutase, peroxidase and catalase were extracted in laboratory from diseased and non-diseased leaves among three replications. Obtained data were analyzed by two factor analysis method using

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OPSTAT of HAU, Hisar. Superoxide dismutase activity (SOD) activity was assayed in infected leaf of mungbean with *Cercospora canescans* and healthy leaf of mungbean. It was assayed by the method of Dhindsa *et al*. (1981). Catalase assay was done as described by Beers and Sizer (1952) in which disappearance of peroxide was followed by Spectrophotometry at 240 nm.

RESULTS AND DISCUSSION

It is clear from the Table 1 that the SOD activity increases after inoculation of the pathogen *Cercospora canescens* to mungbean plant. The segregating pattern in F2 population nearly shows the three different groups. In the susceptible type P1 there are three plants. Similarly there are three resistant plants showing enzymatic activity above greater than 0.756 and, twelve plants out of F1 type had enzymatic range from 572 to 6.683. This shows the segregating ratio 1:2:1 and it indicates single gene governed trait in mungbean. This shows that F1 is not behaving like parent it is little tilted towards resistant parent. The Table 2 revealed that the SOD activity increases after inoculation of the pathogen *Cercospora canescens* to mungbean plant. There is significant increase in SOD activity in parent 1, parent 2, plants of F1 generation and twelve plants of segregating population of F2 generation of two crosses

Table 1 Superoxide dismutase activities (SOD) in cross of KOP × HUM 12 of mungbean in responses to pathogen *Cercospora canescens*

Mungbean genotype	Superoxide dismutase activity (EU/g fresh wt)		
	Non infected	Infected	Genotype
	Mean	Mean	Mean
KOP (P1)	0.438	0.577	0.508
ML1720(P2)	0.778	1.063	0.921
KOP × ML1720 (F1)	0.610	0.930	0.770
F2:1	0.452	0.595	0.524
F2:2	0.472	0.608	0.540
F2:3	0.491	0.617	0.554
F2:4	0.678	0.902	0.790
F2:5	0.663	0.953	0.808
F2:6	0.683	0.946	0.815
F2:7	0.647	0.965	0.806
F2:8	0.678	0.987	0.833
F2:9	0.572	0.930	0.751
F2:10	0.756	1.039	0.898
F2:11	0.764	1.047	0.905
F2:12	0.747	1.025	0.886
Mean enzyme	0.629	0.879	
CD (P = 0.05)			
Genotype	0.050		
Enzyme	0.018		
Interaction	0.071		

Table 2 Superoxide dismutase activities (SOD) in cross of KOP × HUM 12 of mungbean in responses to pathogen *Cercospora canescens*

Mungbean genotype	Superoxide dismutase activity (EU/g fresh wt)		
	Non infected	Infected	Genotype
	Mean	Mean	Mean
KOP (P1)	0.431	0.600	0.515
HUM 12(P2)	0.878	1.093	0.986
KOP × HUM 12 (F1)	0.790	0.880	0.835
F2:1	0.469	0.600	0.534
F2:2	0.475	0.608	0.542
F2:3	0.483	0.625	0.554
F2:4	0.810	0.892	0.851
F2:5	0.810	0.913	0.861
F2:6	0.792	0.878	0.835
F2:7	0.834	0.902	0.868
F2:8	0.846	0.838	0.842
F2:9	0.820	0.930	0.875
F2:10	0.855	1.021	0.938
F2:11	0.822	1.074	0.948
F2:12	0.807	1.039	0.923
Mean enzyme	0.728	0.859	
CD (P = 0.05)			
Genotype	0.045		
Enzyme	0.016		
Interaction	0.064		

KOP × ML 1720 and KOP × HUM 12 between non-infected and infected mungbean plant. In KOP × ML 1720 the critical difference of SOD activity between non infected and infected leaf was 0.016 and between gentotypes was 0.045. Out of twelve plants of F2 generation, three plants shows SOD activity as their parents P1, i.e. Kopergoan and six plants shows as that of F1 generation and three plants as P2, i.e ML1720.

The mean of peroxidase activity of cross KOP × ML1720 and KOP × HUM 12, between infected and non-infected leaves. For cross KOP × ML1720 the critical difference of peroxidase activity of non-infected and infected leaves is 0.050. and between the plants of P1, P2 , F1 and plants of F2 generation is 0.137, similarly for cross KOP × HUM 12 the critical difference of peroxidase activity of non-infected and infected leaves is 0.056 and between the plants of P1 , P2, F1 and segregating population of F2 was 0.152 in Table 3. From the Table 5 and 6 it is observed that the activity of catalase enzyme between infected and non-infected plant leaves of crosses KOP × ML 1720 and KOP × HUM 12. The critical difference of catalase activity in non-infected and infected leaves of crosses KOP × ML1720 and KOP × HUM 12 is 0.022 and 0.016 respectively. The critical difference between all plants of P1, P2, F1 and segregating population of F2 generation of crosses KOP

Table 3 Peroxidase activity in different plants of cross of KOP × ML 1720 mungbean in responses to pathogen *Cercospora canescens*

Mungbean genotype	Peroxidase activity (U/mg)		
	Non-infected	Infected	Genotype
	Mean	Mean	Mean
KOP (P1)	0.698	0.915	0.807
ML1720(P2)	1.725	1.893	1.809
KOP × ML1720 (F1)	1.318	1.606	1.462
F2:1	0.728	0.942	0.835
F2:2	0.717	0.986	0.851
F2:3	0.751	0.965	0.858
F2:4	1.333	1.591	1.462
F2:5	1.263	1.621	1.442
F2:6	1.273	1.691	1.482
F2:7	1.280	1.611	1.445
F2:8	1.387	1.567	1.477
F2:9	1.339	1.576	1.458
F2:10	1.580	1.851	1.716
F2:11	1.650	1.849	1.749
F2:12	1.560	1.866	1.713
Mean enzyme	1.240	1.502	
CD P=(0.05)			
Genotype	0.137		
Enzyme	0.050		
Interaction	0.167		

Table 4 Peroxidase activities in of mungbean in cross KOP × HUM 12 in responses to pathogen *Cercospora canescens*

Mungbean genotype	Peroxidase activity (U/mg)		
	Non infected	Infected	Genotype
	Mean	Mean	Mean
KOP (P1)	0.672	0.800	0.736
HUM 12 (P2)	1.823	1.957	1.890
KOP × HUM 12(F1)	1.203	1.423	1.313
F2:1	0.735	0.830	0.783
F2:2	0.755	0.887	0.821
F2:3	0.741	0.880	0.810
F2:4	1.203	1.423	1.313
F2:5	1.099	1.498	1.298
F2:6	1.224	1.310	1.267
F2:7	1.279	1.331	1.305
F2:8	1.143	1.385	1.264
F2:9	1.188	1.422	1.305
F2:10	1.720	1.449	1.584
F2:11	1.703	1.865	1.784
F2:12	1.684	1.838	1.761
Mean enzyme	1.211	1.353	
CD (P = 0. 05)			
Genotype	0.152		
Enzyme	0.056		
Interaction	0.193		

× ML 1720 and KOP × HUM 12 are 0.059 and 0.042 respectively. The critical difference of interaction between all plants of crosses KOP × ML 1720 and KOP × HUM 12 generation are 0.084 and 0.060 respectively. The Table 7 showed that the genetic model which is generally used for the identification of number of genes present in the genotype governing SOD, peroxidase and catalase activity. It is quite evident that in F2 generation plant shows segregation pattern in the ratio of 1:2:1 which depicts that the gene governing the enzyme activities are partially dominant in nature.

ROS are produced in aerobic organisms within the cell and are normally in balance with antioxidant molecules. Oxidative stress arises from an imbalance between generation and elimination of ROS. These cytotoxic activated ROS can seriously disrupt normal metabolism through oxidative damage to lipids, protein and nucleic acids (Mittler *et al*. 2004). This can lead to changes in the selective permeability of bio-membranes, causing membrane leakage and changes in the activity of membrane-bound enzymes. In plant cells, antioxidant enzymes, such as superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) are considered to form a defensive team, whose combined purpose is to protect cells from oxidative damage during growth, development and senescence. Catalases play a key role in removing hydrogen peroxide from cells (Lehninger

Table 5 Catalase activities in of mungbean in plants of KOP × ML1720 (F2) in responses to pathogen *Cercospora canescens*

Mungbean genotype	Catalase activity (U/mg)		
	Non-infected	Infected	Genotype
	Mean	Mean	Mean
KOP (P1)	0.557	0.794	0.675
ML1720 (P2)	1.184	1.791	1.487
KOP × ML1720 (F1)	0.934	1.318	1.126
F2:1	0.594	0.867	0.731
F2:2	0.616	0.913	0.765
F2:3	0.635	0.923	0.779
F2:4	0.908	1.354	1.131
F2:5	0.885	1.342	1.114
F2:6	0.950	1.285	1.117
F2:7	0.910	1.267	1.089
F2:8	0.954	1.312	1.133
F2:9	0.853	1.257	1.055
F2:10	1.134	1.696	1.415
F2:11	1.045	1.681	1.363
F2:12	1.118	1.675	1.397
Mean enzyme	0.885	1.298	
CD (P = 0.05)			
Genotype	0.059		
Enzyme	0.022		
Interaction	0.084		

Table 6 Catalase activities in of mungbean in plants of KOP × HUM 12 in responses to *Cercospora canescens*

Mungbean genotype	Catalase activity (U/mg)		
	Non infected	Infected	Genotype
	Mean	Mean	Mean
KOP (P1)	0.690	0.764	0.727
HUM12 (P2)	1.118	1.791	1.454
KOP × HUM12(F1)	1.020	1.260	1.140
F2:1	0.741	0.832	0.787
F2:2	0.725	0.824	0.775
F2:3	0.721	0.841	0.781
F2:4	1.027	1.238	1.133
F2:5	1.057	1.184	1.121
F2:6	0.974	1.265	1.119
F2:7	1.185	1.260	1.223
F2:8	1.102	1.757	1.430
F2:9	1.051	1.778	1.414
F2:10	1.087	1.785	1.436
F2:11	1.078	1.725	1.402
F2:12	1.083	1.709	1.396
Mean enzyme	0.977	1.334	
CD (P = 0.05)			
Genotype	0.042		
Enzyme	0.016		
Interaction	0.060		

Table 7 Genetic model used for the identification of number of genes

Enzyme		P1	P2	F1
Sod	KOP × ML1720	3	6	3
	KOP × HUM12	3	5	4
Peroxidase	KOP × ML1720	3	5	4
	KOP × HUM12	3	7	2
Catalase	KOP × ML1720	3	6	3
	KOP × HUM12	3	4	5

et al. 2005). The catalase activity apparently occurs in both the resistant and susceptible genotypes, but increases after inoculation of *Cercospora canacens* in the resistant genotype, but not in susceptible plants. In the experiment catalase activity was also increases rapidly in the resistant cultivar of ML 1720 and HUM 12 after inoculation. In suceptible cultivar there was also increase of catalase activity but it is very low compare to the resistant cultivar ML 1720 and 12. Resistance of inoculated mungbean crop plants with *Cercospora canacens* could be interpreted as a result of increase in level of catalase which causes effective detoxification of ROS compounds, especially H_2O_2.

It is possible that interaction between mungbean leaf and *Cercospora canacens* induces hydrogen peroxide accumulation. Since hydrogen peroxide also serves as a secondary messenger, it could alter gene expression of ROS-related enzymes both at the site of infection and distally through movement in the transpiration stream. The control of steady-state ROS levels by SOD is an important protective mechanism against cellular oxidative damage, since O_2^- acts as a precursor of more cytotoxic or highly relative ROS (Mittler *et al.* 2004). SOD has been established to work in collaboration with POD and CAT which act in tandem to remove O_2 and H_2O_2, respectively.

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