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Biochemical evaluation of cluster bean cultivars for resistance to *Alternaria* leaf spot disease

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Abstract

Cluster bean (*Cyamopsis tetragonoloba* L. Taub.) is an important leguminous crop grown in the arid and semi-arid regions of India. The present study was conducted during Kharif 2022 at Sri Karan Narendra Agriculture University, Jobner, Rajasthan, to investigate biochemical changes associated with resistance to *Alternaria* leaf spot caused by *Alternaria cyamopsidis* in twenty cluster bean varieties/genotypes. Biochemical parameters, viz., total phenol, peroxidase (PO), polyphenol oxidase (PPO), total soluble sugar and protein content, were estimated in healthy and diseased leaves. Resistant genotypes exhibited higher levels of phenol, PO activity, PPO activity, sugar, and protein content than susceptible genotypes under both healthy and infected conditions. Disease infection increased phenol content and the activities of PO and PPO, while sugar and protein contents decreased. Among all genotypes, RGC-986 recorded the highest phenol content (9.03 mg g⁻¹), PO activity (10.94 unit activity min⁻¹ g⁻¹), PPO activity (5.83 unit activity min⁻¹ g⁻¹), sugar content (8.79 mg g⁻¹), and protein content (22.93 mg g⁻¹), whereas RGC-936 showed the lowest values. The results indicate that increased phenol content and antioxidant enzyme activities are closely associated with resistance to *Alternaria* leaf spot and may serve as useful biochemical markers for screening resistant cluster bean genotypes.

Keywords: Cluster bean, phenol, peroxidase, polyphenol oxidase (PPO), total soluble sugar, protein

1. Introduction

Cluster bean [*Cyamopsis tetragonoloba* (L.)] (2n = 14), commonly known as guar, is an underexploited leguminous vegetable crop belonging to the family *Fabaceae*. It is also known by local names such as Chavli Kayi and Khutti. Guar is mainly cultivated during the kharif season in the arid and semi-arid regions of India. It is a drought-tolerant, deep-rooted annual legume adapted to hot and dry conditions. Besides its use as a vegetable and fodder crop, guar is widely grown for the extraction of guar gum from the seed endosperm, which contains about 28-33% gum. Guar gum serves as an important raw material in various industries, including textiles, paper, petroleum, pharmaceuticals, food processing and cosmetics (Joshi and Arora, 1993) [5]. Cluster bean grows best under a long photoperiod with humid conditions during the vegetative stage, followed by a short photoperiod with cool and dry weather during flowering and pod formation. It can be cultivated on a wide range of soils, from sandy to loamy types. However, compact soils adversely affect root development and reduce the activity of nitrogen-fixing bacteria. Although cluster bean is primarily a rainy-season crop, some varieties can also be grown from March to June, while others are cultivated from July to November under South Indian climatic conditions. Cluster bean is a photosensitive crop and a soil temperature range of 21-25 °C is considered optimum for its seed germination. (Hymowitz and Matlock, 1963) [4]. Tender cluster bean pods are highly nutritious. Per 100 g of edible portion, they provide approximately 16 kcal of energy, 81 g of moisture, 3.2 g of protein, 1.4 g of fat, 10.8 g of carbohydrates, 49 mg of vitamin C, 57 mg of calcium and 4.5 mg of iron. (Kumar and Singh, 2002) [6]. Among the various diseases affecting cluster bean, *Alternaria* leaf spot caused by *Alternaria cyamopsidis* is a major foliar disease. It adversely affects plant growth and pod production, leading to considerable yield losses, particularly in the cluster bean-growing regions of Northern India. (Rangaswami and Rao., 1957) [13]. In the initial stage of infection, small water-soaked spots appear on the leaves. These spots later develop into greyish to dark brown lesions with concentric rings.

Under severe infection, the lesions coalesce and cover a large portion of the leaf blade. The affected leaves become chlorotic and may drop prematurely. Infection at the early growth stage can reduce or prevent flowering and pod formation. The disease is most severe between the flowering and pod-setting stages. Yield losses ranging from 43 to 78 percent have been reported when infection occurs at the seedling stage. (Sharma, 1983) ^[15].

2. Materials and Methods

2.1 Experimental site

Laboratory investigations were conducted under controlled conditions in the Department of Biochemistry, SKN College of Agriculture, Jobner (Jaipur), to ensure the accuracy, consistency and reliability of the results. Field experiments were carried out during the *Kharif* 2022 season at the Instructional Farm of Sri Karan Narendra Agriculture University, Jobner, Jaipur, Rajasthan, following recommended agronomic practices.

2.2 Biochemical changes in Cluster bean induced by *Alternaria* leaf spot disease

The procedures followed for the estimation of different biochemical parameters are presented below:

2.2.1 Estimation of phenol

Total phenol was estimated by Folin-Ciocalteu Reagent method (Malick and Singh, 1980) ^[9].

Reagents

1. 80% ethanol
2. Folin-Ciocalteu reagent
3. 20% Sodium carbonate
4. Standard Stock solution: 100 mg catechol was dissolved in 100 ml water
5. Working solution: 2 ml of stock diluted to 100 ml water.

Procedure for estimation

Weighed, 0.5 g of sample and ground with pestle and mortar in 5 ml of 80% ethanol. The homogenate was centrifuged at 10,000 rpm for 20 minutes, supernatant was collected. The residue was re-extracted with 2.5 ml of 80% ethanol, centrifuged at 5000 rpm for 10 minutes and the supernatants

were pooled. The supernatants were evaporated to dry. The residue was dissolved in 5 ml of distilled water and vortex, from that 0.2 ml of sample was pipetted out into test tube. The volume was made up to 3 ml with distilled water then 0.5 ml of Folin-Ciocalteu reagent was added to test tube. After three minutes 2 ml of 20% sodium carbonate was added. The contents were mixed thoroughly, the tubes were placed in boiling water for exactly one minute, cooled and absorbance were taken at 650 nm against blank. Standard curve was prepared using different concentrations of catechol. Amount of phenol present in the sample were calculated from the graph.

$$\text{Total phenol (mg/g)} = \text{Dilution factor} \times \text{graph factor} \times \text{optical density}$$

2.2.2 Estimation of peroxidase

Peroxidase was estimated by Costa (2002) ^[2] method.

Reagents

1. Sodium phosphate buffer 0.1 M pH (7.0): Prepare 0.1 M solution of mono sodium phosphate and 0.1 M di sodium phosphate and then mix 390 ml of mono sodium phosphate and 610 ml of di sodium phosphate.
2. Pyrogallol 20 mM: Dissolve 0.63 g of 1 M pyrogallol in 250 ml of distilled water to obtain desired molar substrate.
3. Hydrogen peroxide solution 12.3 mM: Dilute 0.14 ml of 30% H₂O₂ to 100 ml with water. The extinction of this solution should be 0.485 at 240 nm, should be prepared freshly.

Procedure

The experiment needs to be carried out in cold condition. A known quantity (0.5 g) of leave was macerated in 5 ml of 0.1 M phosphate buffer pH 7 in pre-chilled mortar and pestle. The homogenate was centrifuged at 15,000 rpm for 15 minutes at 4 °C, supernatant was used as enzyme source. The assay mixture contained 2 ml of buffer solution, 50 µL of pyrogallol, 100 µL of enzyme extract and 30 µL of H₂O₂. The absorbance was recorded at 420 nm in UV spectrophotometer at 30 second interval for 3 minutes. The enzyme activity was calculated for 1 g of sample and expressed as units per minute per g of sample.

$$\text{Enzyme activity (unit – activity/min/g)} = \frac{\text{Change in absorbance} \times \text{dilution factor}}{\text{Weight of the sample} \times \text{dilution factor}}$$

2.2.3 Estimation of polyphenol oxidase (PPO)

Polyphenol oxidase was estimated by Mayer *et al.* (1965) ^[11] method.

Reagent

1. Sodium phosphate buffer 0.1 M (pH 6.5): Prepare 0.1 M solution of mono sodium phosphate and 0.1 M di sodium phosphate and then mix 685 ml of mono sodium phosphate and 315 ml of di sodium phosphate.
2. 0.01 M Catechol: Dissolve 0.27 g of catechol in 250 ml of distilled water to obtain desired molar concentration of catechol.

Procedure

Enzyme extract was prepared by homogenizing 0.5 g of leave sample in 3 ml of 0.1 M of sodium phosphate buffer (pH 6.5). The homogenate was centrifuged at 15,000 rpm for 15 minutes at 4 °C and the supernatant was used for the assay. The reaction mixture contained 1.5 ml of 0.1 M sodium phosphate buffer 200 µL of the enzyme extract and 200 µL of 0.01 M catechol and the absorbance was recorded at 495 nm in UV spectrophotometer at 30 second interval for 3 minutes. The enzyme activity was calculated for 1 g of sample and expressed as units per minute per g of sample.

$$\text{Enzyme activity (unit – activity/min/g)} = \frac{\text{Change in absorbance} \times \text{dilution factor}}{\text{Weight of the sample} \times \text{time duration}}$$

2.2.4 Sugar estimation

Total soluble sugar

Leaf material (0.1-0.5 g) without midrib were extracted in 10 ml of 80% ethanol in a mortar pestle, the extract obtained was centrifuged at 8000 rpm for 10 min. Extraction was repeated four times with 5 ml of 80% ethanol each time and supernatants were collected into same beaker. Volume of the extract was made to 50 ml with 80% ethanol. The 0.1 to 0.5 ml of aliquot from supernatant was added to 4.0 ml of Anthrone reagent (0.2%: 200 mg anthrone dissolved in 100 ml H₂SO₄, prepared fresh before use) and the test tubes were placed in ice cold water. The intensity of colour was read at 620 nm on spectrophotometer (Dubois *et al.*, 1956) [3].

2.2.5 Estimation of soluble protein content

The soluble protein content of the leaves samples was determined by using the method of Lowry *et al.*, (1951). 0.5 g of leaves sample was macerated in mortar with 5 ml 0.1 M sodium phosphate buffer (pH 7.0). The homogenate so obtained was centrifuged at 16000 rpm for 20 minutes. The supernatant was used for estimation of soluble protein content. For this purpose, 2 percent sodium carbonate (anhydrous) in 0.1 N NaOH (Solution-A) was prepared. Similarly, 0.5 percent copper sulphate (CuSO₄. 5H₂O) in 1 percent sodium potassium tartrate (freshly made) was prepared and was regarded as solution-B. From these two reagents, solution-C (alkaline copper sulphate) was prepared by mixing 50 ml of solution-A with 1 ml of solution-B just before use. An aliquot of 0.1 ml supernatant was taken in test tube and the volume was made to 1 ml with distilled water followed by addition of 5 ml solution-C mixed well and incubated at room temperature for ten minutes. A 0.5 ml of folinciocalteu reagent (FCR) was diluted to 1N, mixed well and incubated at room temperature in dark for 30 minutes. The absorbance was recorded at 660 nm against blank. The amount of protein in sample was determined from the standard curve prepared by using different concentrations of bovine serum albumin. It was expressed as part per million (ppm) fresh leaves.

3. Results and Discussion

3.1 Total phenol content in healthy and infected leaves of cluster bean varieties/genotype

Phenol content of twenty varieties/genotype was estimated before and after disease occurrence. The data pertaining to total phenol content are presented in Table 1. The concentration of total phenol (mg/g) showed variation both before and after infection of leaves by *Alternaria* pathogen. The concentration of total phenol ranged from 7.85-4.85 mg/g in healthy leaves and from 9.03-5.05 mg/g in diseased leaves. The higher amount of total phenol content was found in RGC-986 (7.85 mg/g) and lower amount was found in RGC-936 (4.85 mg/g) before the occurrence of disease. After disease appearance, the total phenol content was increased in all the varieties/genotypes however; higher total phenol content was found in resistant RGC-986 (9.03 mg/g) and lower total phenol content in susceptible RGC-936 (5.05 mg/g). It was found that the moderately susceptible and susceptible lines had significantly lower total phenol contents than the resistant and moderately resistant lines. Results obtained are in line with findings of Meena *et al.* (2012) [12] and Mallick *et al.* (2015) [10] who observed that resistant genotypes had higher amounts of all biochemical components than susceptible ones. Kumar *et al.* (2021) [7] reported that infection by *Cercospora arachidicola* caused

significant biochemical alterations in groundnut leaves. Total phenol contents increased after infection. Among the cultivars studied, HNG-10 exhibited the highest accumulation of phenol followed by HNG-123 and HNG-69.

3.2 Peroxidase (PO) activity in healthy and infected leaves of cluster bean varieties/genotype

The enzyme peroxidase (PO) activity was estimated in leaves of the cluster bean before and after appearance of the disease and enzyme activity was expressed in unit activity/min/g of the sample. The results (Table 2) of enzyme activity were ranged between 5.92-8.94 (unit activity/min/g) in healthy leaves. The higher peroxidase activity was found in resistant RGC-986 (8.94 unit activity/min/g) whereas; lower peroxidase activity was recorded in susceptible RGC-936 (5.92 unit activity/min/g). After the disease appearance, the enzyme activity was increased in all the genotypes/varieties as compared to healthy. Higher peroxidase activity was found in resistant RGC-986 (10.94 unit activity/min/g) and lowest was susceptible RGC-936 (6.64 unit activity/min/g). It is evident that highly susceptible varieties/genotype displayed lower enzyme activity than resistant varieties/genotype. The present findings are consistent with those reported by Bharti *et al.* (2017) [1], who observed increased peroxidase activity in both susceptible and resistant green gram genotypes following disease infection. However, the magnitude and rate of increase were significantly higher in the resistant genotypes than in the susceptible ones.

3.3 Polyphenol oxidase (PPO) activity in healthy and infected leaves of cluster bean varieties/genotype

The enzyme polyphenol oxidase (PPO) activity was estimated in leaves of the cluster bean before and after appearance of the disease and activity was expressed in unit-activity/min/g of the sample. The data (Table 3) indicated that activity of the enzyme PPO was ranged between 2.99-4.97 (unit-activity/min/g) in healthy leaves. The higher enzyme activity was found in resistant RGC-986 (4.97 unit-activity/min/g) and lower enzyme activity noted in susceptible RGC-936 (2.99 unit-activity/min/g). After the appearance of the disease, the enzyme activity increased in all the genotypes/varieties ranging between 5.83-3.42 (unit-activity/min/g). Higher polyphenol oxidase activity was recorded in resistant RGC-986 (5.83 unit-activity/min/g) and the lower enzyme activity was found in susceptible RGC-936 (3.42 unit-activity/min/g). It is evident that highly susceptible varieties/genotype displayed lower enzyme activity than resistant varieties/genotype.

3.4 Sugar content in healthy and infected leaves of cluster bean varieties/genotype

Sugar content of twenty varieties/genotype was estimated before and after disease occurrence. The data pertaining to sugar content are presented in Table 4. The concentration of total sugar (mg/g) showed variation both before and after infection of leaves. The concentration of sugar ranged from 11.35-6.03 mg/g in healthy leaves and from 8.79-4.08 mg/g in diseased leaves. The higher amount of sugar content was found in RGC-986 (11.35 mg/g) and lower amount was found in RGC-936 (6.03 mg/g) before the occurrence of disease. After infection, the sugar content was decreased in all the varieties/genotypes, but it was higher in resistant RGC-986 (8.79 mg/g) and lower sugar content in

susceptible RGC-936 (4.08 mg/g). Our results are in corroboration with the findings of Waghmare *et al.* (2012) who reported that the plant infected with leaf spot of rose caused by *Alternaria alternata* showed reduction in the quantity of total sugar as compared to the healthy plants. Sharma *et al.* (2020) ^[14] also observed that biochemical contents like total soluble sugar in diseased leaf of different varieties gradually and significantly decreased as compared to healthy leaf of same variety.

3.5 Protein content in healthy and infected leaves of cluster bean varieties/genotype

Protein content of twenty varieties/genotype were estimated before and after disease occurrence. The data pertaining to

protein content are presented in Table 5. The concentration of protein (mg/g) showed variation both before and after infection by *Alternaria* fungus in leaves. The concentration of protein was ranged from 24.09-17.39 mg/g in healthy leaves and from 22.93-16.81 mg/g in diseased leaves. The higher amount of protein content was found in resistant RGC-986 (24.09 mg/g) and lower amount was found in susceptible RGC-936 (17.39 mg/g) before the occurrence of disease. After disease appearance, the sugar content was decreased in all the varieties/genotypes however, higher total phenol content was found in resistant RGC-986 (22.93 mg/g) and lower total phenol content in susceptible RGC-936 (16.86 mg/g).

Table 1: Total phenol content in healthy and infected leaves of cluster bean varieties/genotype

Varieties/genotype	Total phenol (mg/g)			PDI	Host reaction
	Healthy	Infected	Increased over healthy (%)		
RGC-986	7.85	9.03	13.06	5.50	R
RGC-1003	7.13	8.50	16.11	7.75	MR
RGC-181	6.90	7.82	11.76	22.50	MR
RGC-20-21	7.03	8.29	15.19	8.65	MR
RGC-121	6.28	6.90	8.98	10.00	MS
GD-580	6.67	7.64	12.6	14.35	MS
RGC-207	6.10	6.84	10.81	19.25	MS
EC-76575	6.50	7.45	12.75	18.50	MS
GD-860	6.85	7.55	9.27	21.60	MS
GD-602	6.71	7.00	4.14	13.45	MS
GD-567	6.40	6.90	7.24	25.00	MS
RGC-197	5.92	6.34	6.62	45.00	S
RGC-1066	5.005	5.54	8.84	27.00	S
RGC-1002	5.12	5.49	6.73	33.00	S
GD-571	5.00	5.27	5.12	32.90	S
RGC-1038	4.92	5.17	4.83	45.00	HS
RGC-936	4.85	5.05	3.96	63.00	HS
RGC-1033	5.02	5.25	4.38	48.00	HS
RGC-1055	4.90	5.17	5.22	53.00	HS
RGC-1077	4.94	5.11	3.32	58.00	HS
SEm±	0.16	0.51			
CD(p = 0.05)	0.46	1.57			

Table 2: Peroxidase activity in healthy and infected leaves of cluster bean varieties/genotype

Varieties/genotype	Peroxidase (Unit-activity/min/g fresh wt.)			PDI	Host reaction
	Healthy	Infected	Increased over healthy (%)		
RGC-986	8.94	10.94	18.23	5.50	R
RGC-1003	7.99	9.87	19.03	7.75	MR
RGC-181	7.68	9.86	22.05	22.50	MR
RGC-20-21	7.16	9.73	26.41	8.65	MR
RGC-121	7.28	8.28	12.07	10.00	MS
GD-580	7.40	8.24	10.19	14.35	MS
RGC-207	7.16	8.08	11.37	19.25	MS
EC-76575	7.29	8.18	10.93	18.50	MS
GD-860	6.94	7.85	11.64	21.60	MS
GD-602	7.05	8.05	12.47	13.45	MS
GD-567	6.99	8.13	13.96	25.00	MS
RGC-197	6.76	7.62	11.28	45.00	S
RGC-1066	6.56	7.46	12.06	27.00	S
RGC-1002	6.66	7.39	9.87	33.00	S
GD-571	6.37	7.31	12.79	32.90	S
RGC-1038	6.05	7.19	15.78	45.00	HS
RGC-936	5.92	6.64	10.76	63.00	HS
RGC-1033	5.97	6.37	6.35	48.00	HS
RGC-1055	6.01	6.87	12.59	53.00	HS
RGC-1077	5.99	6.40	6.32	58.00	HS
SEm±	0.09	0.06			
CD(p = 0.05)	0.28	0.18			

Table 3: Polyphenol oxidase activity in healthy and infected leaves of cluster bean varieties/genotype

Varieties/genotype	Polyphenol oxidase (Unit-activity/min/g fresh wt.)		Increased over healthy (%)	PDI	Host reaction
	Healthy	Infected			
RGC-986	4.97	5.83	14.75	5.50	R
RGC-1003	3.75	4.23	11.33	7.75	MR
RGC-181	3.66	4.20	12.96	22.50	MR
RGC-20-21	3.56	4.01	11.09	8.65	MR
RGC-121	3.46	3.93	11.95	10.00	MS
GD-580	3.34	3.71	9.95	14.35	MS
RGC-207	3.51	3.83	8.47	19.25	MS
EC-76575	3.26	3.58	9.06	18.50	MS
GD-860	3.23	3.54	8.75	21.60	MS
GD-602	3.60	3.78	4.88	13.45	MS
GD-567	3.39	3.62	6.34	25.00	MS
RGC-197	3.20	3.50	8.57	45.00	S
RGC-1066	3.14	3.39	7.22	27.00	S
RGC-1002	3.12	3.45	9.55	33.00	S
GD-571	3.21	3.50	8.28	32.90	S
RGC-1038	3.07	3.41	10.10	45.00	HS
RGC-936	2.99	3.42	12.57	63.00	HS
RGC-1033	3.13	3.44	8.86	48.00	HS
RGC-1055	3.06	3.48	12.06	53.00	HS
RGC-1077	3.16	3.54	10.71	58.00	HS
SEm±	0.20	0.17			
CD(p = 0.05)	0.61	0.52			

Table 4: Total sugar content in healthy and infected leaves of cluster bean varieties/genotype

Varieties/genotype	Total sugar (mg/g)		Decreased over healthy (%)	PDI	Host reaction
	Healthy	Infected			
RGC-986	11.35	8.79	22.55	5.50	R
RGC-1003	10.89	8.03	26.26	7.75	MR
RGC-181	10.78	7.36	31.72	22.50	MR
RGC-20-21	10.67	7.06	33.83	8.65	MR
RGC-121	8.56	6.09	28.85	10.00	MS
GD-580	8.39	5.99	28.60	14.35	MS
RGC-207	9.01	6.17	31.52	19.25	MS
EC-76575	8.19	5.81	29.05	18.50	MS
GD-860	7.97	5.67	28.85	21.60	MS
GD-602	7.83	5.59	28.60	13.45	MS
GD-567	8.01	5.87	26.71	25.00	MS
RGC-197	7.37	5.06	31.34	45.00	S
RGC-1066	7.09	4.91	30.74	27.00	S
RGC-1002	6.97	4.83	30.70	33.00	S
GD-571	6.89	4.39	36.28	32.90	S
RGC-1038	6.31	4.19	33.59	45.00	HS
RGC-936	6.03	4.08	32.33	63.00	HS
RGC-1033	6.27	4.33	30.94	48.00	HS
RGC-1055	6.21	4.26	31.40	53.00	HS
RGC-1077	6.26	4.23	32.42	58.00	HS
SEm±	0.19	0.57			
CD(p = 0.05)	0.60	1.73			

Table 5: Protein content in healthy and infected leaves of cluster bean varieties/genotype

Varieties/genotype	Protein (mg/g)		Decreased over healthy (%)	PDI	Host reaction
	Healthy	Infected			
RGC-986	24.09	22.93	4.81	5.50	R
RGC-1003	23.37	22.49	3.76	7.75	MR
RGC-181	23.19	22.31	3.79	22.50	MR
RGC-20-21	23.09	20.17	3.98	8.65	MR
RGC-121	21.77	20.80	4.45	10.00	MS
GD-580	21.79	20.77	4.68	14.35	MS
RGC-207	21.37	20.49	4.11	19.25	MS
EC-76575	20.95	19.89	5.05	18.50	MS
GD-860	20.98	19.79	5.67	21.60	MS
GD-602	20.33	19.19	5.60	13.45	MS
GD-567	20.91	17.59	6.31	25.00	MS
RGC-197	18.93	17.99	4.96	45.00	S
RGC-1066	18.82	18.01	4.30	27.00	S
RGC-1002	19.07	18.73	1.78	33.00	S
GD-571	18.76	17.94	4.37	32.90	S
RGC-1038	17.94	17.11	4.62	45.00	HS
RGC-936	17.39	16.86	3.04	63.00	HS
RGC-1033	17.59	16.81	4.43	48.00	HS
RGC-1055	17.77	16.61	6.52	53.00	HS
RGC-1077	17.63	16.68	5.38	58.00	HS
SEm±	0.23	0.37			
CD(p = 0.05)	0.71	1.11			

4. Conclusion

The present investigation demonstrated significant biochemical differences among cluster bean genotypes in response to *Alternaria* leaf spot infection. Resistant genotypes possessed higher levels of total phenol, peroxidase, polyphenol oxidase, sugar and protein content compared to susceptible genotypes. Disease infection enhanced phenol accumulation and the activities of peroxidase and polyphenol oxidase, while reducing sugar and protein contents. The resistant genotype RGC-986 consistently exhibited the highest levels of defensive biochemical constituents, whereas the susceptible genotype RGC-936 recorded the lowest. These findings suggest that phenol content, peroxidase activity and polyphenol oxidase activity play an important role in the resistance mechanism against *Alternaria* leaf spot and can be utilized as reliable biochemical indicators in cluster bean resistance breeding programme.

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6. Conflict of interest: None.

References

- Bharti M, Chandra R, Kumar A, Kumar R, Yadav V, Yadav P. Enzymatic response of green gram (*Vigna radiata*) genotypes against *Cercospora* leaf spot disease. *Indian J Agric Sci*. 2017;87(7):930-933.
- Costa H, Gallego SM, Tomaro ML. Antioxidant defense system in sunflower cotyledons. *Plant Sci*. 2002;162(6):939-945.
- Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F. Colorimetric method for determination of sugars and related substances. *Anal Chem*. 1956;28:350-356.
- Hymowitz T, Matlock RS. Guar in the United States. Oklahoma Agricultural Experiment Station Technical Bulletin No. 611. Stillwater (OK): Oklahoma Agricultural Experiment Station; 1963. p. 1-34.
- Joshi UN, Arora SK. Recent advances in guar gum chemistry and utilization: A review. *Forage Res*. 1993;19:311-328.
- Kumar D, Singh NB. Guar in India. Jodhpur: Scientific Publishers (India); 2002.
- Kumar V, Singh N, Kumar R, Yadav A. Biochemical changes in groundnut cultivars against early leaf spot disease (*Cercospora arachidicola*). *Plant Dis Res*. 2021;36(2):123-128.
- Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin-phenol reagent. *J Biol Chem*. 1951;193:265-275.
- Malick CP, Singh MB. Plant enzymology and histoenzymology. New Delhi: Kalyani Publishers; 1980.
- Mallick SA, Kumari P, Gupta M, Gupta S. Effect of *Alternaria* blight infection on biochemical parameters, quantity and quality of oil of mustard genotypes. *Indian J Plant Physiol*. 2015;20:310-316.
- Mayer AM, Harel E, Shaul RB. Assay of catechol oxidase: Critical comparison of methods. *Phytochemistry*. 1965;5(4):783-789.
- Meena AK, Godara SL, Gangopadhyay S, Ram J. Changes in biochemical constituents in cluster bean due to *Alternaria cucumerina* var. *cyamopsidis*. *Ann Plant Prot Sci*. 2012;20(2):386-388.
- Rangaswami G, Rao AV. *Alternaria* blight of cluster bean [*Cyamopsis tetragonoloba* (L.) Taub.]. *Indian Phytopathol*. 1957;10:13-18.
- Sharma S, Singh R, Kumar A. Biochemical changes in cluster bean leaves due to *Alternaria* blight infection. *Int J Chem Stud*. 2020;8(3):146-153.
- Sharma SR. Effect of fungicides on the development of *Alternaria* blight and yield of cluster bean. *Indian J Agric Sci*. 1983;53(11):932-935.