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Estimation of total phenols and peroxidase isozyme in plants infected with blackeye cowpea mosaic disease*

Cowpea (*Vigna unguiculata* (L.) walp.,) commonly known as Lobia or Chowli in Hindi, Barbati in Bengali and Alasande in Kannada belongs to the family Leguminaceae and subfamily Papilionaceae. It is one of the important pulse crops cultivated in tropical and sub-tropical countries for vegetable, fodder and green manure. The seeds are rich in nutritive value, containing 24.0% protein, 1.8% fat, and 60.3% carbohydrates. Seeds are also good source of vitamins and phosphorus. It is widely grown all over India more particularly in the central and peninsular regions. Viruses constitute the major group of pathogens infecting cowpea, of which blackeye cowpea mosaic virus (BlCMV) is the most destructive viral disease of cowpea, causing characteristic symptoms like alternate dark and green patches, showing severe mosaic mottling with blisters on trifoliate leaves. Affected plants produce fewer flowers and pods with few immature and deformed seeds, affecting yields both quantitatively and qualitatively. It can cause a yield loss of 13-87 per cent under field conditions.

Total phenols and isozyme studies were carried out in BlCMV resistant and susceptible genotypes. Susceptible genotype GC3 and resistant genotype DCS-6 were used and seedlings were raised in the green house. Samples were collected at an interval of 30 and 60 DAS. Estimation of total phenols present in plant samples was done by following Folin-ciocalteau reagent (FCR) method (Bray and Thorpe, 1954).

Reagents 1. Folin – ciocalteau reagent (1%) and 2. Sodium carbonate (2%)

The isozyme bands of Peroxidase were localized by incubating the gel in guiacol (0.25%) for 30 min followed by incubating in 0.3 per cent hydrogen peroxide for 15 min, which shows the appearance of bands of peroxidase enzyme. The migration distance of each band and tracking dye was recorded. The migration distances were expressed as the ratio of the migration distance of isozyme to the tracking dye. This factor is known as relative mobility (Rm) and calculated as follows:

$$Rm = \frac{\text{Distance travelled by the isozyme (cm) from the point of origin}}{\text{Distance travelled by the tracking dye from the point of origin}}$$

Using the Rm values, Zygrograms were also prepared pictorially.

Total phenol estimated at 30 and 60 days after sowing, are presented in Table 1. In the susceptible GC3 genotype, diseased leaf showed more phenol (0.51) compared to healthy (0.33), whereas not much difference was observed between healthy (0.68) and diseased leaf (0.71) in case of resistant genotypes DCS-6 at 30 days after sowing.

At 60 days after sowing, the diseased leaf showed increased total phenols (0.57 mg/g fresh wt) compared to healthy leaf (0.29 mg/g fresh wt) in case of susceptible genotype GC_3, whereas the total free phenols were more in diseased (0.52 mg/g fresh wt) when compared to healthy (0.40 mg/g fresh wt) in case of resistant genotype DCS-6. Resistant genotypes recorded higher phenol content than susceptible genotypes. At 30 days after sowing resistant genotypes recorded higher phenols both in healthy (0.68 mg/g fresh wt) and diseased leaves (0.71 mg/g fresh wt), whereas at 60 days after sowing, in healthy leaf the phenol content was more (0.40 mg/g fresh wt) and there was a little reduction in case of diseased leaf (0.52 mg/g fresh wt). With increase in age of the plant from 30 to 60 days, the phenol content increased (0.51 to 0.57 mg/g fresh wt) in the diseased leaf of susceptible genotype GC3, whereas in resistant genotypes there was depletion of total phenols (0.71 to 0.52 mg/g fresh wt). Similar findings were reported by Dantre (1983) and Singh and Srivastav (1999), who reported that virus infection increased the total phenols in diseased leaf compared to healthy ones.

Table 1. Changes in total free phenol content (mg of fresh wt^{-1}) in cowpea genotypes due to infection of BlCMV at different crop growth stages

	Leaves at 30 DAS		Leaves at 60 DAS	
Genotypes	Healthy	Diseased	Healthy	Diseased
GC_3	0.33	0.51	0.29	0.57
DCS-6	0.68	0.71	0.40	0.52

The difference in the isozyme pattern can be used in the biochemical characterization of resistance and susceptibility of host plant. The results of the study are presented in Table 2 and Fig. 1. The study revealed that at 30 days after sowing,

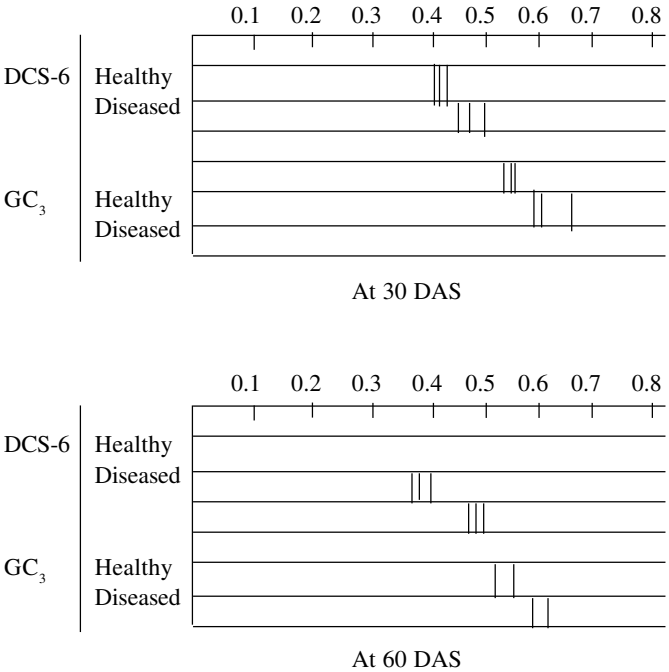

Fig. 1. Relative mobility of peroxidase in cowpea genotypes due to infection of mosaic at different crop growth stages

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Table 2. Relative mobility (Rm) values of peroxidase at different crop growth stages of cowpea genotypes infected by BlCMV

Genotypes		Peroxidase					
		30 DAS			60 DAS		
DCS-6	Healthy	0.40	0.42	0.43	0.38	0.36	0.37
	Diseased	0.46	0.48	0.50	0.41	0.42	0.43
GC_3	Healthy	0.53	0.51	0.52	0.48	0.45	-
	Diseased	0.65	0.58	0.59	0.52	0.51	-

in DCS-6 genotype both healthy and diseased leaves showed three bands. The Rm values of healthy leaf were 0.40, 0.42 and 0.43 and that of the diseased leaf were 0.46, 0.48 and 0.50, but both showed polymorphism. In GC_3 genotype, both healthy and diseased leaves showed three bands. The Rm values of healthy leaf were 0.53, 0.51 and 0.52 and that of the diseased leaf were 0.65, 0.58 and 0.59, but both showed polymorphism.

At 60 days after sowing in DCS-6 genotype both healthy and diseased leaves showed three bands. The Rm values of healthy leaf were 0.38, 0.36 and 0.37 and that of the diseased leaf were 0.41, 0.42 and 0.43, but both showed polymorphism. In GC_3 genotype, both healthy and diseased leaves showed two bands. The Rm values of healthy leaf were 0.48 and 0.45 and that of the diseased leaf were 0.52 and 0.51, but both showed polymorphism.

The infected leaves showed greater intensities of the isoperoxidase bands in comparison with extracts of the healthy leaf. Similar results were reported by Wood and Barbara (1971) and Suteri (1985). In diseased samples the peroxidase activity was higher in the early stage of symptom expression. It may be due to oxidation of phenolic compounds and other metabolites, but decreased activity at later stage of infection was due to destruction of cell organelles, which is associated with deficiency of metabolites. The above results were in agreement with the results of Sarkar and Joshi (1977) who worked on MLO infected brinjal plant.

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