

Short Communication

**Changes in Carbohydrate Contents and Hydrolysing Enzymes in Cowpea Infected by *Macrophomina phaseolina* (Tassi) Goid**

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Charcoal rot of cowpea (*Vigna unguiculata* (L.) Walp.) caused by *Macrophomina phaseolina* (Tassi) Goid is one of the major diseases which severely hampers the cultivation of cowpea throughout the world (Luttrell and Weimer, 1952; Singh, 1954; Grewal, 1973). The pathogen attacks cowpea at all the growth stages, but somehow charcoal rot of stem and root is more pronounced at the crop maturity in Rajasthan (Sandhu, 1994). The presence of low or high sugar in the stalk has been found to determine predisposition of sorghum to stalk rot (Anahosur and Naik, 1986). As no study has been undertaken in case of charcoal rot of cowpea, the present investigation was undertaken to estimate quantitatively the total carbohydrate content, and activity of hydrolysing enzymes in relation to the healthy and infected stem and root of cowpea plants at different stages.

Samples of healthy and diseased plants were obtained by sowing healthy and inoculated seeds in pots with sterilized soil (15 PSI/45 min.). Seed inoculation was done by fungal mycelium. Inoculum was grown on potato dextrose agar in petri plates, and mycelial mat from 7-day-old culture was gently scraped and crushed in pestle and mortar with a little amount of water. My-

celial paste thus prepared was vigorously shaken with healthy seeds in a flask till the inoculum was uniformly smeared over the surface of the seeds. Generally, 10 to 15 g inoculum paste was used for 50 g of seeds.

Samples of stem at seedling stage (4-10 DAS) and root and stem at maturity (60 DAS) from healthy and diseased plants, 500 mg each, were taken for estimation of total sugars, reducing sugars, starch content and activity of  $\alpha$ -amylase and invertase. Total sugars were estimated by phenol sulphuric acid method (Dubois *et al.*, 1951). Reducing sugars were estimated by dinitrosalicylic acid method (Miller, 1992). The  $\alpha$ -amylase activity was assayed following Bernfeld (1955). Method of Harris and Jeffcoat (1974) was employed for the assay of invertase activity. The residual mass obtained in the ethanolic extraction was used for the estimation of starch as per method suggested by McCready *et al.* (1950).

The levels of total sugars and starch were always more in healthy stem and root tissues as compared to plants infected by *M. phaseolina* (Table 1), but the reducing sugars were more in diseased stem and root tissues. However, difference in the

Table 1. Carbohydrates and hydrolysing enzymes in healthy and diseased stem and root of cowpea

Contents (Fresh weight)	Stem young stage (4-10 DAS)		Stem mature stage (60 DAS)		Root mature stage (60 DAS)		CD at 5%
	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased	
Total sugar (mg g <sup>-1</sup> )	15.43	11.66	10.10	8.40	27.90	15.70	1.55
Reducing sugar (mg g <sup>-1</sup> )	7.53	9.00	4.20	4.30	7.48	7.51	0.42
Starch (mg g <sup>-1</sup> )	3.47	1.80	1.51	0.72	2.78	1.01	0.30
$\alpha$ -amylase (mg starch hydrolysed hr <sup>-1</sup> g <sup>-1</sup> )	0.057	0.072	0.048	0.213	0.048	0.072	0.01
Invertase (mg sucrose hydrolysed hr <sup>-1</sup> g <sup>-1</sup> )	0.50	1.65	0.25	1.10	0.82	1.10	0.17

Av. of three replications.

level of reducing sugars between healthy and diseased stem at young stage was comparatively more than at maturity stage. Further, it is clear from the data (Table 1) that in stem of young plants, the total carbohydrate content, even after infection, was more than that in the healthy stem of mature plants. This indicates the probable reason why the disease is less pronounced in plants at young stage than at mature stage.

Mukhopadhyay and Nandi (1976) also reported decreased carbohydrate level in jute plants infected with *M. phaseolina*. Decrease in carbohydrate levels in infected plants has been ascribed to its utilization for the growth and multiplication of the pathogen (Anahosur and Naik, 1986), enhanced respiration rate (Swamy, 1964) and movements of photosynthates to the infection site (Gauman, 1950).

The activation of carbohydrate hydrolysing enzymes in the infected tissues may also result in carbohydrate depletion (Schipper and Mirocha, 1969). In the present study, the activity of  $\alpha$ -amylase and invertase enzymes was more in stem and

root of diseased plants. However, the activity of  $\alpha$ -amylase increased with the age in the stem of infected plants.

## References

- Anahosur, H.K. and Naik, S.T. 1986. Changes in the sugar and phenol contents of root and stalk of sorghum due to *Macrophomina phaseolina* infection. *Indian Phytopathology* 39: 440-441.
- Bernfeld, P. 1955. Alpha and beta amylases. *Methods in Enzymology* 1: 149-158.
- Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A. and Smith, F. 1951. Colorimetric determination of sugars and related substances. *Analytical Chemistry*. 26: 351-356.
- Gauman, E. 1950. *Principles of Plant Infection*. Hafner, New York.
- Grewal, J.S. 1973. Disease problem in pulse crops. In *Pulses Development*. Souvenir, Lucknow: Directorate of Pulse Development, Govt. of India.
- Harris, G.P. and Jeffcoat, B. 1974. Effect of temperature on the distribution of 14-C-labelled assimilates in the flowering shoot of carnation. *Annals of Botany* 38: 77-83.
- Luttrell, E.S. and Weimer, J.L. 1952. *Macrophomina stem canker and ashy stem blight of cowpea*. *Plant Disease Reporter* 36(5): 194-195.
- McCready, R.M., Guggoli, J., Silveira, V. and Omens, H.S. 1950. Determination of starch and amylase in vegetable, Application to peas. *Annals of Chemistry* 22: 1156-1158.

- Miller, G.L. 1992. Use of dinitrosalicylic acid reagent for determination of reducing sugars. *Annals of Chemistry* 31: 426-428.
- Mukhopadhyay, D. and Nandi, B. 1976. Carbohydrate content of jute plants infected with *Macrophomina phaseolina*. *Indian Phytopathology* 29: 208-209.
- Sandhu, A. 1994. Studies on the charcoal rot of cowpea (*Vigna unguiculata* (L) Walp.) caused by *Macrophomina phaseolina*. Ph.D. Thesis, University of Rajasthan.
- Schipper, A.L. and Mirocha, C.J. 1969. The mechanism of starch depletion in leaves of *Phaseolus vulgaris*, infected with *Uromyces phaseoli*. *Phytopathology* 59: 1722-1729.
- Singh, R.S. 1954. Wilt of lobia in U.P. *Science and Culture* 19: 454-456.
- Swamy, R.N. 1964. Respiration in *Cercospora*-infected groundnut tissues and respiratory stimulation following infection. *Phytopath. Z.* 50: 227-234.