SCREENING OF SUGARCANE VARIETIES AGAINST BROWN EYE SPOT BY USING HELMINTHOSPOROSIDE

Y. D. NARAYANA, SUHAS P. WANI AND P. V. RAI

Department of Microbiology, GKVK Campus, University of Agricultural Sciences, Bangalore-560 065

ABSTRACT : A toxic compound, helminthosporoside, produced by *Helminthosporium* sacchari, was used to screen sugarcane varieties against brown eye leaf spot disease. Five microlitre solution (containing $10 \mu g/\mu l$) of helminthosporoside produce characteristic symptoms. Runner appearance and its length were considered as criteria for measuring resistance. Of fifteen leaf strips.

Helminthosporium sacchari (B. de Hann) Butl. causes eye spot disease of sugarcane (Martin, 1961) and infects considerable loss when susceptible varieties are grown. After infection an eye shaped lesion develops on the infected leaf with red centre sorrounded by chlorotic tissue and a reddish brown streak or runner extends towards leaf tip. The fungus can only be isolated from the eye shaped lesion and not from the runner. This observation led Steiner and Byther (1971) to draw the conclusion that a toxic substance was produced by the pathogen. They also suscessfully demonstrated that a semipurified preparation of the culture filtrate of H. sacchari could induce runner formation. The culture filtrate affected only those varieties which were known to be susceptible to the fungus. The most active toxin was identified by Steiner and Strobel (1971) as 2-hydroxy cyclopropyl-D-galactopyranoside and given the name "helminthosporoside". Strobel (1972) and Strobel and Hess (1974) also studied the effect of toxin on ultra structure of cells and plasmamembrane of sugarcane leaves cells. The work on host specific toxins of fungi and their uses has been reviewed by Scheffer and Samaddar (1970). The present study was conducted to develop a simple method for screening sugarcane varieties against brown eye spot disease.

MATERIAL AND METHODS: Infected sugarcane leaves were collected from Regional Research Station, Mandya, Karnataka. The fungus was isolated and purified on potato dextrose Agar medium. The varieties tested were obtained from Mandya (Table 1). The toxin was extracted from 20 day old culture incubated at room temperature (18 to 28°C) and partially purified by the standard procedure (Steiner and Byther, 1969). The reaction of various varieties to the helminthosporoside was tested as mentioned below.

The leaves from various varieties were cut into 22.0 cm. long strips under water and several pin prick made on the lower side of the leaf strip one cm above the cut end. Five microlitre solution of helminthosporoside $(10 \,\mu g/\mu l)$ was placed at each prick point and cut ends of strips were immersed 0.5 cm deep in beakers containing water. The treated strips were exposed to artificial light to hasten absorption of

Received for publication April 12, 1979.

helminthosporoside. The presence or absence of the runner and its length were recorded at intervals of 24 hrs. up to 72 hrs. All treatments were replicated ten times.

RESULTS AND DISCUSSION: The toxin was extracted from *H. sacchari* and partially purified. The toxicity of helminthosporoside was determined by testing it on the leaves of sugarcane varieties namely, Co. 419 Co. 740, IC. 225, HS. 2045, Cheni, Rasdali, Co. 7118, Co. 7116, Co. 6806, B. 37172, Q. 49, Co. 62175, KHS. 3296, KHS 2951 and Co. 1001. The amount of toxin necessary to produce symptoms on sugarcane leaves was 50 μ g.

Steiner and Byther (1969) reported that 0.01 μ g toxin produced streaks over 100 mm long on leaves within 36 hrs. This difference in the quantity of toxin necessary to cause runner might be due to the strain variation in pathogen or variation in atmospheric temperature (Steiner and Byther, 1974).

The results presented in Table 1 indicated that toxin can be used for screening sugarcane varieties against eye spot disease. It also indicate that not only the length of the runner but also the time required for its appearance should be considered while screening the varieties. The runner did not appear at all in resistant varieties even after 72 hrs. of incubation. Out of fifteen varieties tested nine varieties Rasdali, Co. 7118, Co. 7116, Co. 6806, Co. 1001, KHS 2951, B. 37172, Q. 49 and KHS 3296 were highly susceptible, four varieties (viz., Co. 419, Co. 740, HS. 2045 and IC. 225) were moderately susceptible and two varieties viz., Cheni and Co. 62175 were highly resistant. The use of helminthosporoside for screening is more suitable as it gives accurate results in short time with less labour as compared to the inoculation method.

Variety	Runner length (cms)		
	24 hrs	48 hrs	72 hrs
Co. 419	12.25	12.94	13.07
Co. 740	12.67	14.44	17.82
IC. 225		15.59	16.39
HS. 2045		12.34	14.00
Cheni			
Rasdali	8.13	15.28	19.05
Co. 7118	5.00	20.17	20.17
Co. 7116	8.00	20.50	20.50
B. 37172		12.48	16.72
Co. 6806	14.84	15.81	18.79
Q. 49	termine.	13.76	17.10
Co. 62175		_	
KHS. 3296		18.23	18.88
KHS. 2951	2.0	14.67	15.63
Co. 1001		14.28	18.72

TABLE 1 : Effect of helminthosporoside on sugarcane varieties for varying lengths of time

- No runner development

(Resistant varieties)

This method is convenient over the conventional one. Steiner and Byther (1971), Strobel and Steiner (1972) and Byther and Steiner (1972) have successfully used the helminthosporoside for screening of the sugarcane seedlings and varieties.

REFERENCES

- Byther, R. S. and G. W. Steiner (1972). Use of helminthosporoside to select sugarcane seedlings resistant to eye spot. *Phytopathology* 62: 466-470.
- Martin, J. P. (1961). Eye spot. Sugarcane diseases of the world Vol. I (J. P. Martin et al.) pp. 166-202, Elsevier Publishing Co., New York.
- Scheffer, R. P. and K. R. Samaddar (1970). Host specific toxin as determinants of Pathogenicity. Advances Phytochemistry 3: 123-142.
- Steiner, G. W. and R. S. Byther (1969). Host specific toxin of *Helminthosporium sacchari* on sugarcane. *Phytopathology* 59: 1051 (Abst).
- Steiner, G. W. and R. S. Byther (1971). Partial characterisation and use of host specific toxin from *Helminthosporium sacchari*. J. Biol. Chem. 246: 4350-4357.
- Steiner, G. W. and R. S. Byther (1974). Heat induced resistance to helminthosporoside. Proc. Amer. Phytopathol. Soc. 1: 34 (Abst).
- Strobel, G. A., W. M. Hess and G. W. Steiner (1972). Ultrastructure of cells in toxin treated and *Helminthosporium sacchari* infected sugarcane leaves. *Phytopathology* **62**: 339-345.
- Strobel, G. A. and W. M. Hess (1974). Evidence for the presence of Toxin binding protein on the plasma membrane of sugarcane cells. *Proc. Acad. Sci. USA* 71: 1413-1417.
- Strobel, G. A. and G. W. Steiner (1972). Runner lesion formation in relation to helminthosporoside in sugarcane leaves infected by *Helminthosporium sacchari*. *Physiol. Plant. Pathol.* 2:129-132.