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BRIEF COMMUNICATION

Effects of citrinin on pigment, protein and nucleic acid contents in maize seeds

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Abstract

Citrinin lowered contents of chlorophyll, carotenoids, proteins and nucleic acids during seed germination of maize cv. Suwan composite. The inhibitory effect was concentration dependent.

Additional key words : carotenoids, chlorophyll, DNA, RNA, *Zea mays*.

Citrinin, a di-hydro-isocoumarin, a potent mycotoxin produced by *Penicillium citrinum* Thom. is a natural contaminant of maize, rice, wheat, barley, oat and decaying tomato fruits (Saito *et al.* 1971, Scott 1977, Nishijima 1984, Betina 1984). However, only a few reports exist on the toxic effects of citrinin on physiological processes of crop plants (Reiss 1978, Bilgrami and Sinha 1985). This is why we studied various biochemical changes induced by citrinin during the germination of seeds of maize, an important cereal crop of India.

Seeds of *Zea mays* L. cv. Suwan composite were obtained from the Cereal Division, Rajendra Agriculture University, Sabour Campus, India. A stock solution of citrinin (*Sigma*, St. Louis, USA.) was initially prepared in 1 cm^3 ethanol from which the dilutions (100, 250, 500, 1000 and 2000 mg m^{-3}) were made in distilled water. The seeds were steeped initially in distilled water for 1 h and subsequently in different concentrations of citrinin for 20 h. For each treatment, 100 seeds were taken in triplicate. The steeped seeds were subsequently placed on moist blotting paper and kept for germination in a seed germinator at 28 ± 2 °C. On the seventh day, chlorophyll (Chl) *a* and *b* contents in leaves of seedlings were estimated

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spectrophotometrically following Arnon (1949). Determination of carotenoid (Car) content of the same leaves was made by the method of Davis (1976). Quantitative estimation of the protein of the seed samples was done by the method of Lowry *et al.* (1951), the qualitative analysis of proteins was done by the disc electrophoretic method (Ornstein and Davis 1964). Gels were scanned by *LKB Ultrascan*-XL-*Enhanced Laser Densitometer* (*LKB*, Sweden). The nucleic acid contents of the control and treated seeds were estimated by the method of Gottlieb and Tripathi (1968). The results were subjected to one way analysis of variance. The least significant differences at 1 and 5 % confidence levels (LSD_{01} and LSD_{05}) were subsequently determined following the procedure of Dospekhov (1984).

A visual examination of the seedlings showed gradual loss of Chl in toxin treated seedlings. The root and shoot lengths of toxin treated seedlings were also reduced in comparison to the normal growth rate of the control seedlings.

The maximum inhibition in Chl *a* concentration (42.34 %) was found at the 2000 mg m^{-3} concentration of citrinin, which was followed by 17.76, 7.96, 5.40 and 3.30 % inhibitions at 1000, 500, 250 and 100 mg m^{-3} concentrations of citrinin, respectively. The concentration of Chl *b* was similarly reduced. The Chl (*a*+*b*) content was reduced by 3.88, 6.62, 9.59, 18.41 and 45.00 % at 100, 250, 500, 1000 and 2000 mg m^{-3} concentrations of the toxin. Reduction in Car concentration was similarly effective, the maximum inhibition (49.40 %) was recorded at the 2000 mg m^{-3} concentration of the toxin. The low concentrations of citrinin (upto 500 mg m^{-3}) did not induce differences significant at the 1 and 5 % levels for both Chl *a* and *b*. Similarly, the differences in Car content were significant only at the 500 · 2000 mg m^{-3} concentrations of citrinin (P = 1 %).

Table 1. Effect of citrinin on protein, DNA and RNA contents of maize seedlings.

Concentration of toxin [mg m^{-3}]	Protein amount [g kg^{-1}(d.m.)]	inhibition [%]	DNA amount [mg kg^{-1}(d.m.)]	inhibition [%]	RNA amount [mg kg^{-1}(d.m.)]	inhibition [%]
0	84.5 ± 1.7		115.2 ± 2.8		365.6 ± 7.8	
100	78.6 ± 1.1	6.98	112.0 ± 1.2	2.78-	359.3 ± 10.8	1.72-
250	74.2 ± 1.2	12.19	107.8 ± 1.4	6.42-	351.4 ± 15.4	3.88-
500	61.4 ± 1.8	27.34	101.2 ± 0.8	12.15	327.4 ± 12.1	10.45-
1000	52.2 ± 0.6	38.22	87.3 ± 1.9	24.22	287.7 ± 7.2	21.31
2000	41.1 ± 0.6	51.36	67.1 ± 1.6	41.75	237.7 ± 15.9	34.98
LSD_{01}		5.28		7.45		16.20
LSD_{05}		3.76		5.44		11.54

non-significant; all other values are significant at both 1 and 5 % levels.

Significant depletion in protein content was due to different concentrations of citrinin (Table 1). Besides reduction in the concentrations of proteins, citrinin also affected their quality. Protein spectra in disc electrophoresis (Fig. 1) revealed eight peaks in the control seeds of which four peaks were significant designated I, III, V and VIII. Their contents were lowered or lost at all concentrations of citrinin, with

the exception of peak III that increased at 250 and 500 mg m^{-3} of citrinin.

The reduction in nucleic acid concentrations (Table 1) also increased with citrinin concentration and the difference was significant at the highest citrinin concentrations.

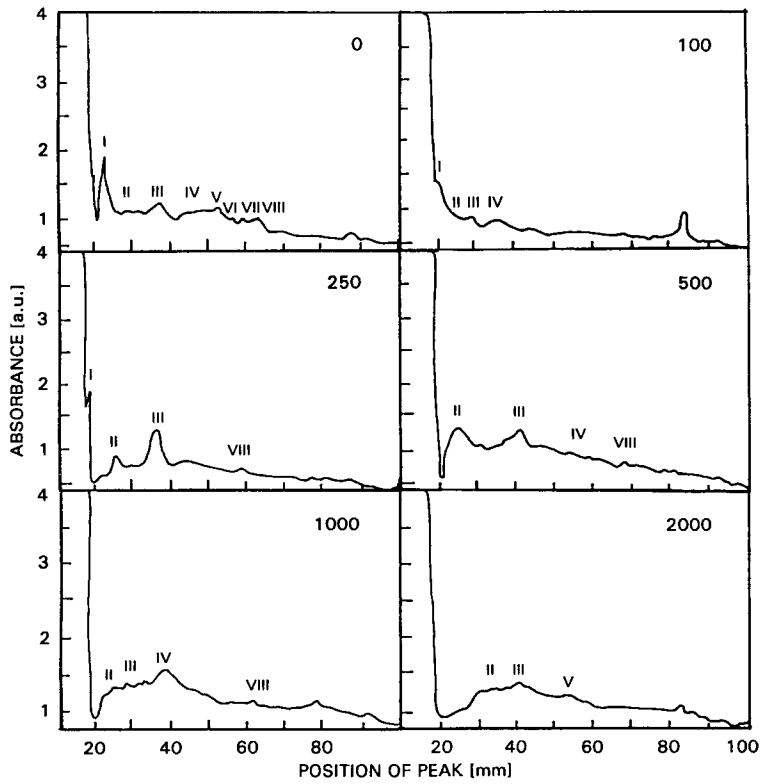

Fig. 1. Effect of citrinin (0, 100, 250, 500, 1000 and 2000 mg m^{-3}) on maize seed protein spectra in disc electrophoresis.

The effects of citrinin shown in our experiments are similar to those induced by aflatoxin B_1. Thus the seeds selected for sowing purposes should be devoid of citrinin contamination.

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