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ISOZYMIC VARIATION IN RELATION TO YELLOW MOSAIC DISEASE IN SOYBEAN

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ABSTRACT

Electrophoretic variations were used to identify yellow mosaic resistant, tolerant and susceptible soybean lines at early stages of the plant growth. Five enzyme systems, i.e., peroxidase (Po), catalase (Cat), polyphenoloxidase (Ppo), esterase (Est) and ribonuclease-1 (RNase) were studied. The Po bands numbers 5, 7, 8 and 12 in 6-day-old cotyledons; Cat 1, 2, 3 and 4 in 3, 6 and 12-day-old tissues; Ppo 6, 7, 9 in 3 and 6-day-old tissues; Est 1, 3, 5, 6 and 8 in 3 and 6-day-old seedlings and RNase 1, 2, 3, 4 and 5 in 3, 6 and 12-day-old tissues were the most prominent bands which can be used as biochemical markers for identification of resistant, tolerant and susceptible soybean lines at these specific stages.

Key words: Isozyme, soybean, electrophoretic analysis, peroxidase, catalase, esterase, ribonuclease-1.

Resistance is determined by genes of the host. Isozymes are gene products which can be detected using electrophoresis. If differences in isozymes could be correlated with resistances and susciplibility, then they could be used as markers for the identification of resistance at the early stages of plant development. Isozymic differences for resistant and susceptible plant tissues were observed in potato [1], barley [2] and soybean [3, 4]. Differences in the banding pattern and intersity of peroxidases and esterases in the lines resistant and susceptible to bean anthracnose in 3 and 40 days old seedlings has also been reported [5]. This report presents the possible isozymic differences between soybean lines resistant, tolerant and susceptible to yellow mosaic disease.

MATERIALS AND METHODS

Two each of yellow mosaic resistant (*Glycine formosana* and UPSM-534), tolerant (UPSM-533 and Hordel) and susceptible (Jupitor and Bragg) soybean varieties were used

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for isozyme analysis by polyacrylamide gel electrophoresis (PAGE). Enzymes were extracted from samples collected at 4 stages of seed germination, i.e., 3, 6, 12 and 18 days. The seedlings were separated into cotyledons and shoots 6 and 12 days after germination. The radical portion was discarded.

Samples were crushed in chilled mortor and pestle with cold phosphate buffer (pH 7.0). The homogenates were kept over-night at freezing temperature and centrifuged at 14,000 rpm. The PAGE (7.5%) analysis was done according to the method described by Davis [6]. Ammonium persulphate was used as polymerizing agent. Sample and spacer gels were omitted in all the cases. After running, the gels were developed with different stains.

Peroxidases were localized with a mixture of equal amount of benzidine solution and 3% hydrogen peroxide. Catalase was stained by first incubating the gels in 0.5% KI solution acidified with glacial acetic acid (12 drops/30 ml). Polyphenol oxidase was localized by incubating the gel first in phosphate buffer (pH 7.4) for 30 min and then in 0.01 M DOPA (DL-3-4, dihydroxy phenylalanine) solution for 1 h in the same phosphate buffer. Esterases were localized using α-naphthyl acetate as the substrate and Fast Blue RR as a dye coupler. The gels were put in incubator at 37°C for 1 h and then washed in running distilled water. For ribonuclease-I, the gels were incubated in acetate buffer (pH 5.0) in which yeast RNA was mixed @ 4 mg/ml at 37°C for 10–20 min and then stained with 0.2% toluidine Blue in 5% acetic acid (pH 3.0) for 30 sec. Excess stain was washed off with 0.5% acetic acid solution.

RESULTS

Among the four different stages of seed germination studied only 6 and 12 days old cotyledons, 6, 12 and 18 days old shoots and 3 days old seedlings displayed differences in band patterns.

PEROXIDASES

Differences were observed in the cotyledons of 3 and 6 days old seedlings and in shoots at the age of 12 and 18 days. In 3-days-old seedlings, band number 12 was expressed in both the tolerant and susceptible lines. In 6-days-old cotyledons, bands 5 and 12 were present in the tolerant lines in which band 7 was absent, while band 8 was present in the tolerant and susceptible lines. In the shoots of 12-day-old seedlings band 15 was present in the tolerant lines, while at the age of 18 days it appeared in the shoots of susceptible varieties only. In the 18-day-old shoots band 4 and 5 were present both in tolerant and susceptible lines, but absent in resistant (Fig. 1).

Catalase band 1 was observed in the shoots of susceptible varieties at the age of 3, 6 and 12 days. Band number 2 appeared in the shoots of tolerant lines 6 and 12 days after

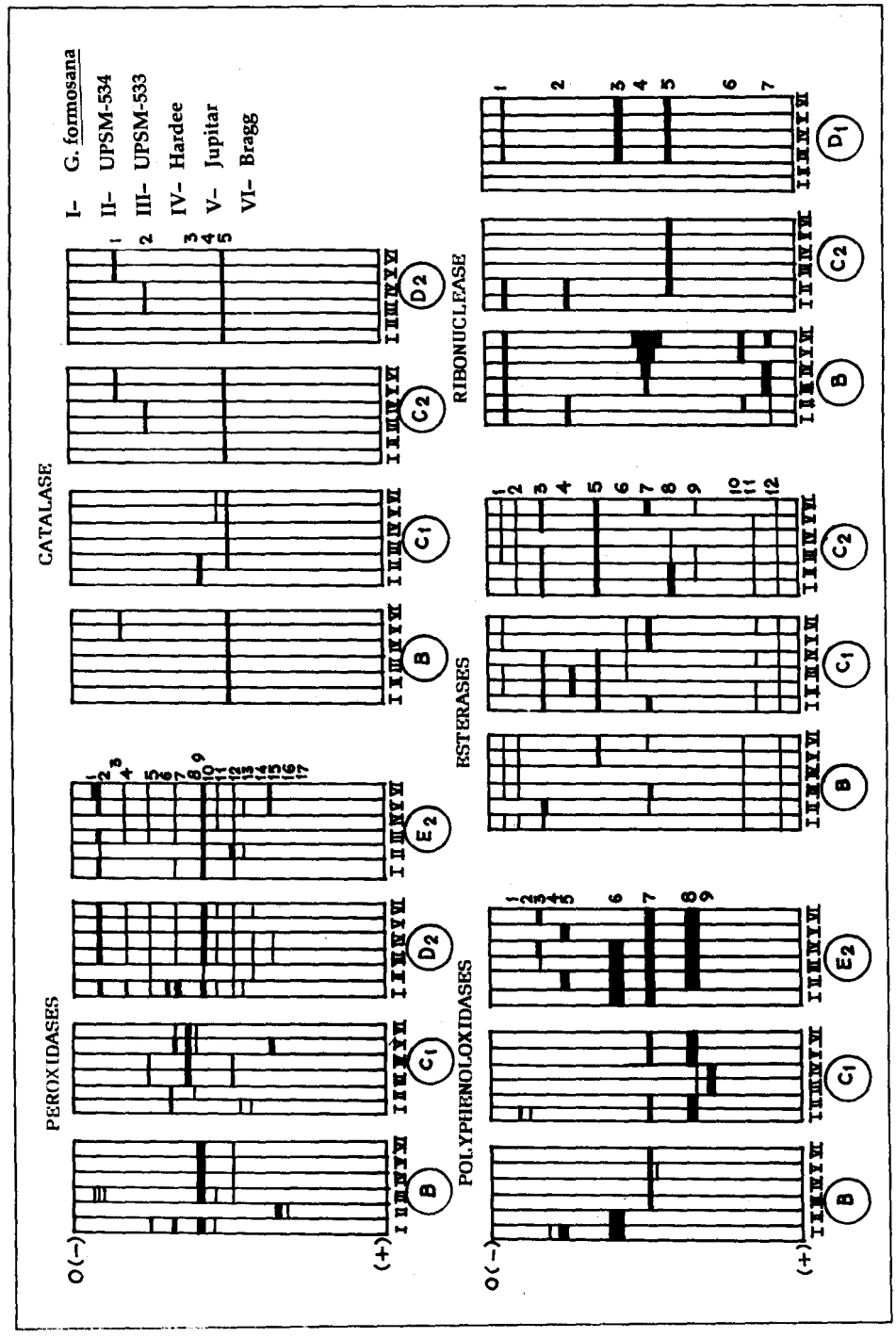

Fig. 1. Isozymic differences for yellow mosaic resistance/tolerance/susceptibility in soybean.

B — 3-day-old seedling
C1 — 6-day-old cotyledons
C2 — 6-day-old shoot
D1—12-day-old cotyledons
D2 — 12-day-old shoot
E2 — 18-day-old shoot
Numbers I to VI show varieties.
Numbers 1 to 17 show bands.

germination. Bands 3 and 4 were expressed in the cotyledonous tissues of 6-days-old seedlings of the resistant and susceptible lines, respectively (Fig. 1).

POLYPHENOLOXIDASE

Differences for this enzyme were observed in 3 and 6 days old cotyledons and 18-day-old shoots. In 3-day-old seedlings, band 6 was observed in resistant lines, and band 7 in tolerant as well as susceptible lines. In 12-day-old cotyledons, band 7 was present in the resistant as well as susceptible lines but absent in tolerant lines in which band number 9 was present. In 18-day-old shoots, band 6 was present in the resistant and tolerant lines, although it was very faint in *G. formosana*.

ESTERASES

Differences were observed only in 3 and 6 days old seedlings for this enzyme. In 3-days-old seedlings, band 3 was present in the resistant lines, while band 5 expressed in the susceptible lines. In 6-day-old cotyledons, band 3 and 5 were found in resistant and tolerant, and band 6 in susceptible lines. Band 8 was present in the resistant and tolerant, while band 1 was observed in the shoot of tolerant and susceptible lines at the age of 6 days (Fig. 1).

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In 3-day-old seedlings the resistant line exhibited band 2, while band 4 appeared in the remaining lines. Bands 1, 3 and 5 were absent in the 12-day-old cotyledons of resistant varieties (Fig. 1).

DISCUSSION

The present study shows marked differences between resistant, tolerant and susceptible lines in peroxidases, catalase, polyphenoloxidase, esterase and RNase isozyme pattern at different stages of seedling development. The most important stage was of 3-day-old seedlings, when differences were observed in all the enzymes studied. Band 3, 6 of polyphenoloxidase, band 3 of esterase and band 2 of RNase were helpful in distinguishing the resistant lines from the others.

In contrast, presence of band 1 of catalase and band 5 of esterase were helpful in identifying susceptible lines at this stage.

Six-day-old cotyledons also showed marked differences in all enzyme system except RNase. At this stage differences in bands of the shoot tissues were observed for catalase, esterase and RNase. Clear bands 5 and 12 of peroxidase and 9 of polypheoloxidase were

present in the tolerant lines, bands 3 and 4 of catalase were conspicuous in the resistant and susceptible lines respectively, in 6-day-old cotyledons. Similarly, shoot tissue at this stage also showed band 1 and 2 of RNase in the resistant and bands 1 and 2 of catalase in the susceptible and tolerant lines, respectively, which may be used as markers for identification of these lines in 6-day-old seedling. However, differences were also observed in 12-day-old shoots as band 15 of peroxidases and band 2 of catalase, which appeared in the tolerant lines and band 1 of catalase, were specific for susceptible lines but the cotyledon tissue at this stage showed marked differences in RNase as bands 1, 3 and 5 were absent in the resistant lines, which could be related with the resistance mechanism. At the age of 18 days, very few differences existed for peroxidase and polyphenoloxidase in the seedlings and were not enough to screen the resistance, tolerant and susceptible lines with high degree of reliability. Some of the proteins are associated to susceptibility but did not appear in the resistant lines. These results show that electrophoretic analysis may be used for screening plant population for resistances at 3-day-old seedling stage in the laboratory by selecting the band 12 for peroxidases, 1 for catalase, 6 and 7 polyphenoloxidases, 3 and 5 for esterase and 2 and 4 for RNase as markers. Our findings are in agreement with those of others [5, 7]. Difference in RNase activity of the resistant and susceptible seedlings in wheat has been reported [8]. Zuhua He et al. [9] obtained isozymic differences for herbicide tolerance and susceptibility in rice varieties at early seedling stage.

From these findings it is clear that these isozymes are very useful for early identification of the resistant and susceptible lines. Use of more enzyme systems at a time may be even more effective in screening the resistant lines.

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REFERENCES

1. N. Kedar. 1959. The peroxidase test as a tool in the selection of potato varieties resistant to late blight. Am. Potato J., **36**: 315–324.

2. G. B. Orlob and D. C. Arny. 1961. Some metabolic changes accompanying infection by barley yellow dwarf virus. Phytopathology, **51**: 768–775.

3. S. Gangopadhyay and T. D. Wyllei. 1974. Biochemical comparison of soybean varieties in relation to susceptibility to *Marcophomins phascolins*. Indian J. Mycol. Plant Pathol., **4**(4): 522–526.

4. J. W. Hitly and A. F. Schithenner. 1966. Electrophoretic comparison of soybean leaf proteins from varieties resistant and susceptible to *Phytophthora megasperma* var. *sofae*. Phytopathology, **56**: 287–291.

5. M. A. Okiror, V. K. Gupta and E. W. M. Van Breukelen. 1982. Genetic and physiological variation among bean lines resistant and susceptible to bean anthracnose. Theor. Appl. Genet., **62**: 355–359.

6. B. J. Davis. 1964. Disc electrophoresis. Method and application to human serum protein. Ann. NY Acad. Sci., **121**: 404–427.

7. J. R. Stavely and E. W. Hanson. 1967. Electrophoretic comparison of resistant and susceptible *Trifolium pratense* noninoculated and inoculated with *Erysphe polygoni*. Phytopathology, **57**: 482–485.

8. A. K. Chakravorty, M. Shaw and L. A. Serubb. 1974. Ribonuclease activity of wheat leaves and rust infection. Nature, **247**: 577–580.

9. Zuhua He, S. Chunhai, F. Bobo and S. Zongtan. 1990. Analysis of isozymes in rice varieties tolerant of and susceptible to herbicide butachlore. Intern. Rice Res. Newsl., **15**(2): 17–18.