Role of Isozymes in Rust (*Phakopsora pachyrhizi* Syd.) Resistance in Soybean

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SUMMARY

In the present investigation two known rust resistant (EC241778 and EC241780) and two susceptible (JS335 and DSb1) soybean genotypes have been used in order to know the differences in isozyme pattern. It revealed that at 45 DAS, PO peroxidase and polyphenol oxidase PPO isozymes shown monomorphism in all the genotypes (except in JS335 for peroxidase activity) irrespective of healthy and diseased leaves. At 75 DAS, both PO and PPO isozyme shown polymorphic activities under diseased condition indicating the PO and PPO isozyme synthesis is need based and polymorphic activities appear in response to infection by *Phakopsora pachyrhizi*. However, in resistant genotypes, bands with higher Rm values were found when compared to susceptible genotypes.

Key words : Peroxidase, Polyphenol oxidase, Soybean, *Phakopsora pachyrhizi*, Electrophoresis.

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Soybean [*Glycine max* (L.) Merrill] has been referred as gold due to its high protein content (40-43%) besides high oil content of 21-23 per cent. But the soybean production is largely influenced by repeated outbreak of the rust caused by *Phakopsora pachyrhizi* Syd. which can cause losses from 15 to 80 per cent depending on the locality, season and cultivar (Bromfield and Yang, 1976). The disease is appearing every year in epiphytotic form in rust endemic areas and causes substantial yield losses (Anonymous, 1995). Three triazole fungicides have been found effective in the control of soybean rust (Patil and Anahosur, 1998). However, continuous use of these chemical fungicides may pose the problem of development of resistance to pathogen. Therefore, the development of high yielding rust resistant varieties is of prime importance. In nature, some genotypes exhibit resistance to foliar diseases in any crop. The degree of resistance has been attributed to the genetic and biochemical make up of particular genotype, which needs to be investigated. Therefore, it is utmost important to study and find out the factors of resistance. Isoenzymes occurring within the same organism can be the rapid, sensitive laboratory tool for characterization of resistance in the genotypes. Keeping this in view, electrophoresis analysis of enzyme protein *i.e.* peroxidase (PO) and polyphenol oxidase (PPO) was attempted in four soybean genotypes.

MATERIALS AND METHODS

The investigations were carried out during *kharif* 2004 at Main Agricultural Research Station, University of Agricultural Sciences, Dharwad. At the same time, pot experiment was conducted for maintaining the disease free plants in the glasshouse conditions. Two rust susceptible genotypes (JS335 and DSb1) and two rust resistant (EC241778 and EC 241780) soybean genotypes were used in this study. All the four genotypes were sown in the field in three replications in completely randomized block design. Uredospore suspension of *P. pachyrhizi* (10^5 spores/ml) was sprayed at 25 days after sowing. The sampling was done at 45 and 75 DAS and assessed the rust severity using 0 to 9 scale of Mayee and Datar (1986) and further per cent disease index (PDI) was calculated using the formula given by Wheeler (1969). For comparison, leaves of same age from healthy plants maintained in the glass house were used. Acetone powder of fresh composite leaf samples was prepared as per the procedure described by Brynt and Forrest (1979). The isozyme analysis of PO and PPO was done by using vertical slab gel electrophoresis technique with non discontinuous buffer system (Hames, 1990). Peroxidase isozyme was extracted by homogenizing 300mg of acetone powder of each sample in 5ml of chilled extraction buffer, pH 5.0 (Farkas and Stahmann, 1966). Electrophoresis was performed according to Davis (1964). The isozyme bands of peroxidase were localized by incubating the gel in guiacol (0.25%) for 30 min. followed by incubation in 0.3% hydrogen peroxicide for 15 min, which

shows the appearance of bands of peroxidase enzyme. The isozymes of polyphenol oxidase were localized on polyacrylamide gels as per the procedure suggested by Park *et al.* (1980). The enzyme source was prepared by extracting 300mg of acetone powder of each sample in 5ml of chilled 0.2M sodium acetate buffer, pH 5.6. The migration distance of each band and tracking dye was recorded. Further, relative mobility (Rm) was calculated using the following formula–

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

RESULTS AND DISCUSSION

Studies on peroxidase activity revealed that at 45 DAS in both healthy and diseased leaves, two bands appeared in all the genotypes except in healthy leaves of JS335 in which the bands were not appeared. But in diseased leaves, all the two bands appeared in both resistant (EC241778 and EC241780) and susceptible (JS335 and DSb1) genotypes. In healthy leaves, monomorphism with Rm values of 0.81 and 0.83 were recorded. But in diseased leaves, both susceptible genotypes showed polymorphic activities (Table 1) and monomorphism in resistant genotypes with Rm values 0.83 and 0.85. At 75 DAS, only one band appeared under both healthy and diseased leaves. However, polymorphic isozyme bands were observed under diseased condition with Rm values of 0.82, 0.84, 0.85 and 0.86 whereas in case of healthy leaves, monomorphism existed with Rm value of 0.83. In diseased leaves, the polymorphic activities appeared in response to infection by *P. pachyrhizi* but not in healthy leaf (Table 1). The reason that can be coated for the above result is that the isozyme peroxidase synthesis is need based in response to infection by *P. pachyrhizi*. The results are in agreement with Chahal *et al.* (1988).

Polyphenol oxidase isozyme also indicated the similar activities as that of peroxidase. At 45 DAS, the band with Rm value of 0.87 appeared in all the genotypes under both healthy and diseased conditions. Later, at 75DAS band with Rm values of 0.91 in JS335, 0.91 in DSb1, 0.92 in EC241778 and 0.93 in EC241780 genotypes appeared in diseased leaves (Table 2) but not in healthy leaves of same genotypes indicating that these isozymes are synthesized against the invasion by *Phakopsora pachyrhizi*. The results are in consonance with Birecka and Catalfamo (1975).

Table 1: Relative mobility (Rm) values of peroxidase at different crop growth stages of soybean genotypes

Genotypes	Peroxidase					
	45DAS				75DAS	
	Healthy		Diseased		Healthy	Diseased
	B_1	B_2	B_1	B_2		
JS335	-	-	0.81	0.83	0.83	0.82
DSb1	0.81	0.83	0.82	0.84	0.83	0.84
EC241778	0.81	0.83	0.83	0.85	0.83	0.85
EC241780	0.81	0.83	0.83	0.85	0.83	0.86

Table 2: Relative mobility (Rm) values of polyphenol oxidase isozyme at different crop growth stages of soybean genotypes

Genotypes	Polyphenol oxidase			
	45DAS		75DAS	
	Healthy	Diseased	Healthy	Diseased
JS335	0.87	0.87	0.86	0.91
DSb1	0.87	0.87	0.87	0.91
EC241778	0.87	0.87	0.89	0.92
EC241780	0.87	0.87	0.89	0.93

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