Isozyme Variability in *Phaeoisariopsis personata* (Berk. and Curt.) von Arx Causing Late Leaf Spot of Groundnut (*Arachis hypogaea* L.)

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SUMMARY

***Phaeoisariopsis personata* (Berk and Curt.) von Arx causing late leaf spot (LLS) of groundnut is one of the major constraints for its production in Karnataka. Isozyme studies conducted to know the molecular variability among the isolates. Fifteen commonly growing groundnut varieties infected by *Phaeoisariopsis personata* in the Main Agricultural Research Station (MARS), Dharwad and nine isolates obtained from different locations of North Karnataka were selected. Isozyme studies revealed the variations among the isolates since they produced an extra band with respect to various enzymes, Peroxidase (PO) and Polyphenoloxidase (PPO). Greater peroxidase and polyphenol oxidase activity with similar banding pattern were noticed in the isolates from V14, VIS, VI and V3, V4, V5, V8, V9 and VIS under Dharwad location. Among the nine isolates collected from different locations, revealed that the isolate HAN (Hanumanamatti) and ARA and NIP (from Arabhavi and Nippani) showed higher PO and PPO activity, which produced maximum of three bands with little variation in Rm values.**

Key words : Groundnut, Late leaf spot, Isozymes variability, *Phaeoisariopsis personata*

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Isozymes are defined as multiple molecular forms of a single enzyme. These forms usually have similar if not identical, enzymatic properties, but slightly different amino acid compositions due to differences in the nucleotide sequence of the DNA that codes for the protein. Often the only difference among isozymes is the substitution of one to several amino acids. Isozyme banding patterns obtained from fungi are usually relatively uncomplicated and easy to interpret. Isozyme analysis can be readily performed in most laboratories with relatively little expense. Isozyme analysis has proven particularly useful in situations where it is necessary to differentiate among two (or) more morphologically similar fungi. An isozyme is a direct expression of genotypes and can be used as an indicator of genetic relationships with related population. Each type of enzyme often exists in several forms known as isozymes that carryout the same function but may vary from one another in several properties, requirements and mechanism of action. Several workers examined the possibility of separating fungal taxonomy based on enzyme analysis in gel electrophoresis (Hall, 1971 and Scalo *et al.*, 1981). However, the number of intensity of bands also depends upon the age and type of the organism (Racuson and Foote, 1966). Hence keeping this in view the present investigation on isozyme variability in *P. personata* causing late leaf spot in groundnut was undertaken.

MATERIALS AND METHODS

Groundnut leaf samples showing typical symptoms *of* late leaf spot caused by *P. personata* were collected from nine different locations (Arabhavi, Annigere, Bijapur, Indalgi, Hanumanamatti, Shirahatti, Nippani, Raichur and Dharwad) and also fifteen different varieties *viz.*, (DH-212, DH-86, DH-101, DH-40, DH3-30, GPBD-4, Dh-2001-I, JL-24, TGLPS-3, JSP-2, ICGV-91192, ICGV-86950, LSVT-I-2005-7, LSVT-I-2006-2, TAG-24) in Main Agricultural Research Station (MARS), Dharwad during *kharif* 2007-08. Two hundred fifty gram of infected leaf sample was collected from each location/variety. The sample was placed in polythene bag with appropriate labeling. They were preserved in deep freezer at -20°C and used for the further study.

The possible existence of qualitative variation among pathogenic isolates of *P. personata* was assessed by adopting the vertical Poly Acryl amide Gel Electrophoresis (PAGE). Peroxidase, polyphenol oxidase, and catalase isozyme studies were undertaken as described hereunder.

Isozyme was extracted for individual sample. The leaf tissue with typical late leaf spot symptoms was separated from the leaf tissue with the help of puncture. The material

was crushed with liquid nitrogen, and powdered samples were used for the assay of isozymes.

Fifteen to twenty mg of powdered sample was homogenized in 2.0 ml chilled extraction buffer consisting of 0.1 M tris, 17% sucrose, 0.1 % ascorbic acid, 0.17 per cent cystein hydrochloride and pH 8.0 (Farkas and Stahmann, 1966) in precooled mortar and pestle. The resulting homogenate mixture was centrifuged at 10,000 rpm for 10 min. The supernatant was decanted and used as enzyme source. Gels after the electrophoresis were incubated in the solution containing 0.15 per cent benzidine in 6 per cent NH_4Cl for 30 min in dark. Then drops of 30 per cent H_2O_2 were added with constant shaking till the brown bands appear. After staining, the gels were washed with distilled water and photographed (Sindhu *et al.*, 1984).

The polyphenoloxidase was extracted by homogenizing the material in two ml of chilled 0.2 M sodium acetate buffer at 5.6 pH (Park and Young, 1980). In order to stain the polyphenoloxidase isozymes, the gel after the electrophoresis was incubated in 0.03 M catechol containing 0.05 per cent P-phenylene diamine in phosphate citrate buffer (pH 6.0) for one hour.

The banding patterns or the zymograms, so obtained were analyzed based on procedure given for identifying the putative loci as described by Wendel and Weeden (1989). The measurement of band position was made by using the following formula.

$$\text{Rm } Z \frac{\text{Distance moved by the isozyme (cm)}}{\text{Distance moved by the tracking dye (cm)}}$$

The genetic similarity co-efficient was estimated using NTSYS PC-2.0 software programme (Nei and Li, 1979). The clustering was done and dendrograms were drawn by following unweighed pair group with arithmetic mean (UPGMA) routine.

RESULTS AND DISCUSSION

Pathogen variability is one of the main causes of failure of the crop variety or fungicidal control of plant diseases. In nature, new races may arise through mutation, hybridization and different cytoplasmic inheritance, heterokaryosis and parasexuality. Hence, the resistant varieties of today may become susceptible tomorrow for the new races of the pathogen, so in any breeding programme, the detailed knowledge about the existing races of pathogen and their possible occurrence in future is quite essential.

Peroxidase is a key enzyme in the biosynthesis of lignin (Bruce and West, 1989). Increased activity of peroxidases has been elicited by fluorescent Pseudomonads in groundnut (Meena *et al.*, 2000). Peroxidases have been implicated in a number of physiological functions that may contribuk to resistance including exudation of hydroxyl cinnamyl alcohol into free radical intermediates (Gross, 1980), phenol oxidation (Schmidt and Feucht, 1980). In the present study, detection of greater activity and also similar banding pattern was noticed in isolates from Vl4 (LSVT-I-2006-2), Vl5 (TAG-24) and VI (DH-212) under Dharwad location whereas, among the locations greater activity was noticed in isolate HAN (Hanumanamatti). The lower activity was observed in isolates from V5 (DH-3-30) and ANN (Annigere) (Table 1 and 2).

Similarity index values between the isolates ranged from 0.00 to 100. Cluster analysis of the data based on peroxidase gave two distinct clusters. Most of the isolates from Dharwad location were grouped in I cluster with only one isolate in the II cluster V6, GPBD-4 (Fig. 1). Among the isolates from different locations, the isolates SHI and RCH were shown 100 per cent similarity and isolate ANN(Annigere) was grouped in II cluster which was diverse from all others(Fig. 2). It was observed by many workers that the increased peroxidase activity stimulated natural defense mechanism of host plant (Johnson and Cunningham, 1972). Chen *et al.* (I992) and Shalini (2006), who reported that, isozyme analysis for 204 and 12 isolates of *Pythium* sp., respectively from different geographical locations could be differentiated by banding pattern. The cluster analysis showed that isolates within morphological species generally clustered together, but considerable variation existed within certain species, intraspecific cluster and geographic origin were closely related.

Polyphenol oxidase (PPO) is enzyme which uses molecular oxygen to catalyze the oxidation of monophenolic and orthodiphenolic compounds. In the present study, detection of greater activity and similar banding pattern was noticed in isolates from V3 (DH-IOI), V4(DH-40), V5(DH-3-30), V7(DH-2001-l), V8(JL-24), V9(TGLPS-3), and VI 15(TAG-4) (Table 4). All these isolates exhibited two bands with a little variation in Rm values and remaining isolates exhibited only single band. Among the isolates from different locations, higher activity was noticed in isolates ARA and NIP (from Arabhavi and Nippani), which were produced maximum of three bands with little variation in Rm values (Table 3).

Similarity index values ranged from 0.00 to 100. Cluster analysis of the data based on polyphenol oxidase gave two distinct clusters. Most of the isolates from

Table 1 : Relative mobility (Rm) values of peroxidase of isolates of *Phaeoisariopsis personata* from different genotypes of groundnut

Isolates / Rm	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	V13	V14	V15
0.08	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.25	+	-	-	-	-	-	-	+	+	+	+	+	+	+	+
0.03	-	+	+	+	+	-	+	-	-	-	-	-	-	+	-
0.066	-	-	-	-	-	-	-	-	+	+	+	+	+	+	-
0.075	-	+	+	+	-	-	+	+	-	-	-	-	-	-	-
0.266	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+
0.308	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.366	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+
0.375	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+
Total no. of bands	4	2	2	2	1	0	3	2	2	2	2	2	2	5	4

Table 2 : Relative mobility (Rm) values of peroxidase of isolates of *Phaeoisariopsis personata* obtained from different locations

Isolates / Rm	ARA	ANN	BJP	IND	HAN	SHI	NIP	RCH	DWD
0.033	+	-	-	+	+	-	-	-	+
0.066	-	-	-	-	+	-	+	-	-
0.075	-	-	+	+	-	+	-	+	+
0.125	-	-	-	-	+	+	-	+	-
0.133	+	-	+	+	-	-	+	-	-
0.142	-	+	-	-	-	-	-	-	-
0.375	-	-	-	-	+	-	-	-	-
0.166	-	-	+	+	+	-	-	-	-
Total no. of bands	2	1	3	4	5	2	2	2	2

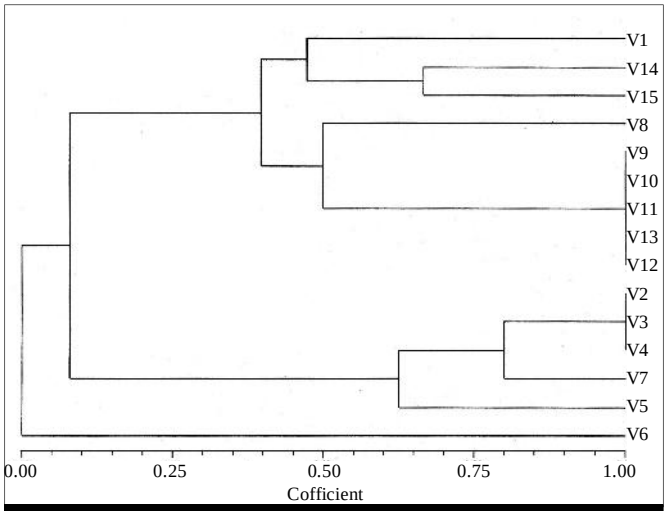

Fig. 1 : Similarity of peroxidase pattern of *P. personata* from different genotypes of groundnut

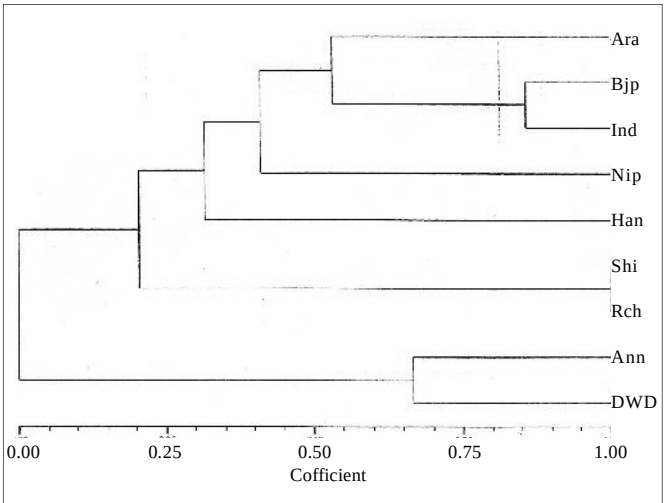

Fig. 2 : Similarity of peroxidase pattern of *P. personata* obtained from different locations

Dhrawad location were grouped in I cluster with only two isolates in II cluster (Fig. 3). Among the isolates from different locations, isolates SHI and IND (Shirahatti and Nippani) were shown 100 per cent similarity (Table 4) and all other isolates shown little variation. Hasabnis (1998) reported that polyphenoloxidase activity was higher

Table 3 : Relative mobility (Rm) values of polyphenol oxidase of isolates of *Phaeoisariopsis persollata* from different genotypes of groundnut grown in Dharawd location

Isolates / Rm	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	V13	V14	V15
0.046	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+
0.053	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-
0.069	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
0.123	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
0.115	+	+	-	-	-	-	+	+	-	-	-	-	-	-	-
0.130	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
0.146	-	-	-	-	+	+	+	+	+	-	-	-	-	-	-
0.153	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+
Total no. of bands	1	1	2	2	2	1	2	2	2	1	1	1	1	1	2

Table 4 : Relative mobility (Rm) values of polyphenol oxidase of isolates of *P. personata* collected from different locations

Isolates / Rm	ARA	ANN	BJP	IND	HAN	SHI	NIP	RCH	DWD
0.05	+	-	+	-	-	-	+	-	-
0.115	-	-	+	+	+	+	+	+	+
0.138	+	+	-	-	-	-	-	+	-
0.176	+	-	-	-	+	-	+	-	+
Total no. of bands	3	1	2	1	2	1	3	2	2

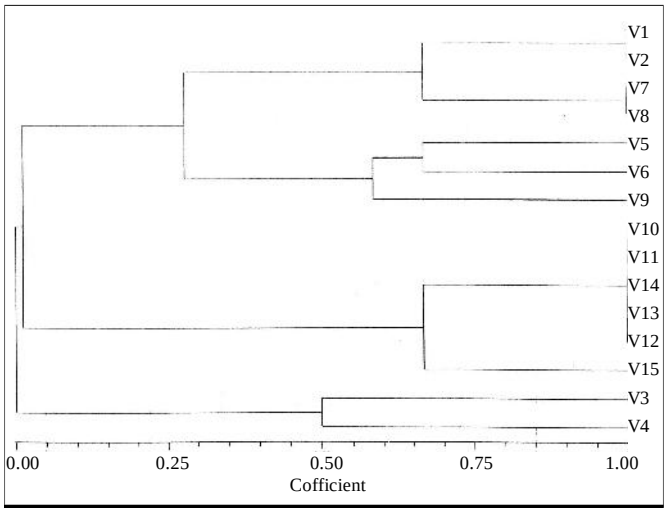

Fig. 3 : Similarity of peroxidase oxidase pattern of *P. personata* from different genotypes of groundnut grown in Dharwad location

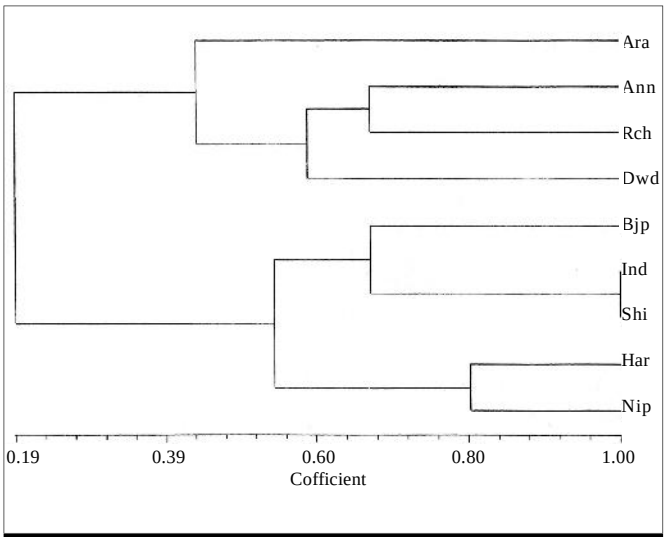

Fig. 4 : Similarity of polyphenol oxidase pattern of isolates of of *P. personata* obtained from different locations

in wheat upon inoculation of leaf rust pathogen and it was expressed by producing more number as well as most prominent bands. Similar observations of increased PPO activity was also reported by Sivakumar and Sharma (2003) while working on banded leaf and sheath blight affected maize plants grown out of seeds treated with *Pseudomonas fluorescens* and Kulkarni *et al*. (2006) on pathogenic variability in *Plasmopara halstedii* on sunflower.

It is clear from present investigation that there is a great molecular variation existing among the isolates of *P. personata* which could be used to distinguish variation among the isolates of *P. personata.* The study also brought out that the pathogen showed molecular variation over locations and also in a location depending on the genotypes grown there.

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