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Short Communication

Variability in *Neovossia indica*: Based on pathogenecity and isozyme analysis

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ABSTRACT

To identify variability in *Neovossia indica* in the fourteen isolates collected from different agroclimatic areas of Punjab, pathogenecity tests and isozyme analysis based on starch-gel electrophoresis were carried out. Depending upon the pathogenic potential, the isolates were categorized into 5 distinct groups. Based on the analysis of esterase and acid phosphatase the isolates could be grouped into two categories.

Key words: *Neovossia indica,* pathogenecity, isozymes, variability, wheat.

Neovossia (=*Tilletia*) *indica* (Mitra) Mundkur causing Karnal bunt disease of wheat has acquired an international significance because of the quarantine imposed by several nations for the import of wheat seed from the countries where the disease is occurring.

Teliospores of many *Tilletia* spp. usually become incorporated as contaminants in wheat or other grains during harvest, transport or storage and can cause rejection of the shipment and even initiation of costly quarantine sanctions if mistakenly identified as *T. indica*. To identify inter - and intra-generic variability in *Tilletia*, starch/pulse field gel electrophoresis (Tooley *et al.* 1993), column chromatography and polyacrylamide-gel-electrophoresis (Beck *et al.* 1990), polymerase chain reaction based assays and hybridization (Smith *et al.* 1994) etc. have been employed. Variability in *N. indica* has also been documented on the basis of pathogen morphology, cultural characteristics, temperature response and pathogenecity tests (Gill *et al.* 1993).

Present investigations were undertaken to characterize the isolates on the basis of pathogenecity tests, isozyme banding patterns (peroxidase, esterase and acid phosphatase) and to evaluate whether the variability existing in *N. indica* on the basis of pathogenecity also holds good for the isozymes.

Fourteen isolates collected from different districts of Punjab were subjected for pathogenecity tests on a set of 20 wheat strains from 1992 - 1997. Every year, fifteen rows of one meter length of each strain (15 plants) were planted in the field. Ten ear heads of each strain were artificially inoculated using hypodermic syringe with the 7- 10 days old sporidial cultures (> 10,000 sporidia ml^{-1}) of each isolate. Average percent infected grains were calculated after harvesting. As most of the bunted grains were infected partially, depending upon the extent of damage to the grains, numerical values were given for calculating coefficient of infection (Aujla *et al.* 1989). Number of infected grains showing incipient infection, blackening extending up to ½, ¾th and completely infected grains were multiplied with the numerical values 0.25, 0.5, 0.75 and 1.0 divided by 100 to represent the value of coefficient of infection in percent. Overall aggressiveness of the isolates was assigned a numerical value which was the product of average per cent karnal bunt infection, coefficient of infection and number of wheat strains with susceptible reaction.

On the basis of numerical values worked out for disease producing potential (aggressiveness) of the isolates, these were grouped in five categories (Table 1) (i) most aggressive- P4. (ii) aggressive- P7, P8, P11 and P13 (iii) moderately aggressive- P5, P10, P12, P14 (iv) less aggressive- P1, P2 P9 and (v) least aggressive- P3, P6. Similarly, depending upon pathogenecity tests on a set of wheat strains Aujla and associates (1987) grouped the isolates collected from Punjab in to four categories.

Starch gel electrophoresis was carried out to evaluate the isozymes. Cultures of the isolates were maintained in potato dextrose medium incubated at 22± 2°C. The mycelium was harvested after 7, 14, 21 and 45 days of incubation for the analysis of

Table 1. Response of isolates (pathotypes) on the basis of their reaction on wheat varieties (differentials)

Isolates	No. of wheat varieties with susceptible reaction	Average infection (%)	Coefficient of infection (%)	Overall response value[1]
1	2	3	4	5
P 1	7	6.71	2.08	97.70
P 2	7	5.95	3.07	127.87
P 3	5	2.99	1.36	20.33
P 4	17	16.11	10.53	2883.85
P 5	11	6.67	3.85	282.47
P 6	5	3.59	2.19	39.31
P 7	11	8.94	5.18	509.40
P 8	10	10.02	6.64	665.33
P 9	8	3.64	2.23	64.94
P 10	9	7.05	3.93	249.36
P 11	12	11.52	7.24	1000.86
P 12	11	7.41	4.35	354.57
P 13	13	9.75	6.21	783.08
P 14	8	7.04	4.02	226.41

[1]Products of numerical values in the columns 2, 3 and 4 of each isolate.

peroxidase, esterase and acid phosphatase. The isozyme analysis was done following the methods of Brewer (1970), Cardy *et al.* (1981), Scoper (1968) and Shields (1983). The mycelium from the broth medium was simply strained through double layer muslin cloth repeatedly washed with gel buffer (pH 8.65) and incubated under freezing conditions for 3-4 hrs. It was thoroughly ground using pestle and mortar with a few cover slips which helped in thorough maceration of the mycelium. The crude mycelial extract, thus obtained was collected and stored under freezing condition (-35^{0}C) for further analysis. For starch gel electrophoresis, gel plates were prepared with the hydrolyzed starch and degassed with the help of a suction pump.

Culture extracts were loaded on gel plates with whatmann filter paper no. 3 cut into 1 cm x 0.5 cms wicks. Gel plates were run on horizontal electrophoresis chambers filled with tray buffer (pH 8.65) at 15 mA and 225-240 V for esterase and for 16 hrs for peroxidase. The gel plates were run at low temperatures in the refrigerator. The plates were monitored from time to time so as to avoid run off of the samples loaded on gel plates. The coloured band of the isozymes appeared on the anodal side. After passing through electric current, the gels were put in glass trays containing respective stains for each enzyme and incubated at 37^0± 1^0 C for 3-4 hrs for esterase and overnight for acid phosphatase and peroxidase (Shielder *et al.* 1983).

Bands for peroxidase were not observed with any of the cultures (i.e. fresh as well as old). However, in 45 days old cultures, on the basis of number of coloured bands observed both for esterase and acid phosphatase, the isolates could be categorized into two distinct types. The isolates 2, 3 and 12 showed two bands for esterase (Fig. 1) and isolates 2 and 3 (Fig. 1) showed two bands for acid phosphatase. The bands for isolate no. 6 were of light colour intensity both for esterase and acid phosphatase. For the rest of the isolates, the bands were of almost similar intensity.

To study the aging effect of the cultures on the isozymes; 7, 14 and 21 days old cultures of five isolates (nos. 3, 5, 8, 11 and 13) were analyzed for the banding pattern for esterase (Fig. 2) and acid phosphatase (Fig. 2). The band of isolate no. 12 resolved into two for esterase and only one coloured band for acid phosphatase, run as control treatment of 45 days old cultures. Bands for isolate no. 8 did not appear. There was no difference in the isolates after 7 days, whereas after 14 days, the intensity of the bands for isolates 5, 8, 11 and 13 were dark. The band of isolate no. 3 was very dark after 21 days which distinctly got differentiated into two bands (A1 and A2) as the mycelium matured. Differences in the staining intensity in the bands and development of two bands of different colour intensity might have been produced due to crossing of monosporidial lines with different alleles. Bonde and associates (1988; 1989) have shown that the crossing of two monosporidial lines with same alleles always produced teliospores with expected single band. To

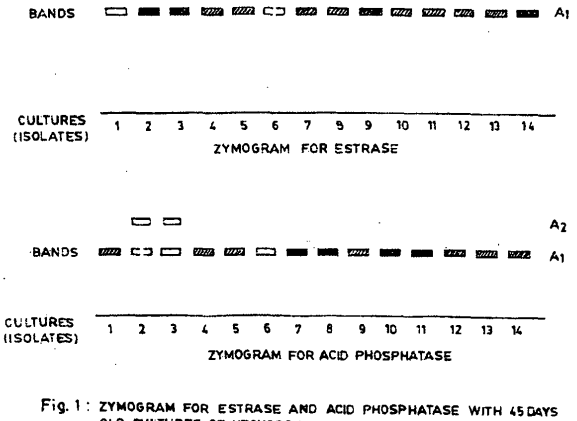

Fig. 1 : ZYMOGRAM FOR ESTRASE AND ACID PHOSPHATASE WITH 45 DAYS OLD CULTURES OF NEOVOSSIA INDICA

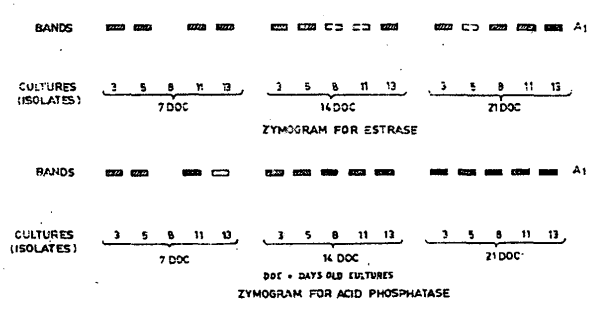

Fig. 2 : ZYMOGRAM FOR ESTRASE AND ACID PHOSPHATASE WITH 7, 14 AND 21 DAYS OLD CULTURES OF NEOVOSSIA INDICA

distinguish between different species of *Tilletia*, out of 36 presumed isozyme loci identified from 66 monoteliospore cultures of *T. indica* and 12 of *T. barclayana*, 15 were polymorphic indicating high degree of variability in the cultures (Bonde *et al.* 1989). The greater frequency of out crossing facilitated sexual recombination and greater genetic variability (Micales *et al.* 1986). Double stranded RNA has been detected in some of the isolates (Beck *et al.* 1994). Differences in the karyotypes in different isolates has also pointed towards variability in the pathogen (Tooly *et al.* 1995).

On the basis of pathogenecity, the isolates collected from different areas of Punjab were categorized into 5 groups. Depending upon banding pattern for esterase and acid phosphatase, the isolates distinctly fell in two groups i.e. (i) isolates 2, 3 and 12 and isolates 2 and 3 respectively, which produced two bands of varying colour intensities (ii) rest of the isolates falling in a particular group based on isozymes, having varied pathogenecity (aggressiveness) on wheat varieties tested. The genes coding for these enzymes appear to be different than the ones controlling the pathogenic potential of the isolates.

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