Pathogenic and biochemical variations in *Neovossia indica*

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ABSTRACT: Host-pathogen responses on 10 differential hosts distinguished populations of six isolates into three pathotypes, pathotype I (KB-1,-2 and -4), pathotype II (KB-3 and -5) and pathotype III (KB-6). Isolate KB-2, which was the most virulent, showed the highest content of lipid (131.1 mg/g), nitrogen (24 mg/g), protein (15.3%), sugars (35 mg/g) and reducing sugar (0.131 mg/g). Comparatively, KB-6 which was less aggressive and slower growing isolate, had the minimum amount of these constituents. KB-2 could be distinguished from other isolates by the presence of linolenic acid and KB-6 by the absence of capric and pentadecenoic acids. Polyacrylamide gel electrophoretic analysis reflected variations in soluble protein fractions among the three differently virulent pathotypes of *N. indica*. In KB-6, 23 bands were resolved, of which 16 were common to all the representative isolates. Esterase isozyme detected in the mycelial extracts revealed the presence of four bands of different *Rf* values in KB-6. However, isolates KB-2 and KB-5 lacked bands with an *Rf* value of 0.76. Analysis of peroxidase isozyme showed the presence of six bands in KB-2 and KB-5, but in KB-6 a band of 0.87 *Rf* was missing, confirming variation within the isolates.

Key Words: Wheat, Karnal bunt, *Neovossia indica*, variability, pathogenic, biochemical

Karnal bunt [*Neovossia indica* (Mitra) Mundkur] of wheat causes reduction in yield as well as deteriorates the quality of grain (Mehdi et al., 1973; Bhat et al., 1980). The spread of the disease is restricted through International Quarantine regulations. Resultantly, the disease shadows the export of quality wheat form India in the International trade market. Use of resistant varieties is one of the important alternatives to overcome this problem and for breeding resistant varieties, knowledge of variability in the pathogen is essential. Very little information on pathogenic variability is available (Singh et al., 1995) and biochemical variability has not been studied. This paper presents results on pathogenic and biochemical variability existing in the pathogen.

MATERIALS AND METHODS

**Culturing of isolates**

Monoteliosporic cultures of *N. indica* were raised from Karnal bunt infected wheat seeds collected from different wheat growing areas of North-western India using technique described by Warham (1987).

**Pathogenic variability**

Pathogenic variability among these isolates was established by inoculating them on a set of differential hosts which included bread wheat, durum wheat and rye (Aujla et al., 1987). Inoculum of each isolate containing allantoid sporidia @ 1 x 10⁴ per ml were inoculated at boot leaf stage using hypodermic syringe. High humidity in the field was created by running perfospray. At the harvest, the diseased grains were categorised into four grades of infection and percent coefficient of infection was calculated (Aujla, et al., 1989).

**Biochemical variability**

Biochemical variability among the isolates with respect to lipids, fatty acids, nitrogen, protein contents and isozymic variations were studied.
**Estimation of lipid:** Lipid estimation was done taking teliospores of all isolates of *N. indica*. The techniques proposed by Folsch et al. (1957) and Bligh and Dyer (1959) with certain modifications were used. A sample of 500 mg of the dried teliospores from each isolate was crushed and used for the lipid estimation.

**Estimation of fatty acids:** Free and mixed fatty acids from teliospores were converted into their methyl esters according to the method of Morrison and Smith (1964) and detected by chromatography. The fatty acid methyl esters (FAME) were analysed using chemito 8510 gas chromatograph equipped with FID (681), fitted with a column packed with 15 percent FFAP (liquid phase) on 10 AW (solid support). The mesh size was 80/100, column length 8 feet and diameter 1/8 inch, and 1-2 μl sample of the methyl esters was injected by a 10 μl “Hamilton syringe”. The peaks were recorded and fatty acids in the chromatographs were identified by comparison of their retention time with those of standard FAME run separately.

**Nitrogen and Protein Contents:** Nitrogen and protein contents of the mycelial dry powder of six isolates of *N. indica* was estimated by a Technicon Auto Analyser following the method described by Mitcheson and Stowell (1971).

**Soluble proteins and isozyme patterns**

Polyacrylamide gel electrophoresis was used to separate soluble protein and isozymes. Soluble proteins were separated by SDS - discontinuous buffer system using 10% gel based on the method of Laemmli (1970). Isozymes of esterase and peroxidase were separated on 7.5% polyacrylamide gel using an anionic system (Ornstein, 1964; Davis, 1964). Representative isolates KB-2, 5 and 6 were grown in potato dextrose broth as stationary cultures at 18 ± 1°C for 15 days. The mycelial mats were harvested and washed with distilled water. Five gram of dried mycelial mat of each isolate was homogenised separately in mortar and pestle in liquid nitrogen and the flour was transferred to 50 ml capped Oakridge centrifuge tube. To each tube 1 ml of 0.01 M Tris-HCl buffer (pH 6.8) was added and centrifuged under refrigerated conditions (4°C) at 20,000 rpm for 20 minutes. The supernatant was taken and its protein concentration adjusted to 2000 μg/ml by extraction buffer following Lowry et al., (1951). An aliquot of extracted protein sample was mixed with equal amount of 2XSDS sample buffer and treated in boiling water bath for 3 minutes. These samples were centrifuged at 10,000 rpm for 5 minutes and applied to the wells in slab gel. The electrophoresis was carried out in Tris-glycine buffer at 30 mA current in stacking gel and 40 mA in resolving gel. Molecular weight markers were run along. The gel was stained following Wilson (1983) in 0.04% coomassie blue G 250 in 3.5% perchloric acid. After 90 minutes of staining, gel was placed in 5% acetic acid to change background to pale blue and R, value of each protein band was calculated.

**RESULTS**

**Pathogenic variability**

Based on coefficient of infection produced by different isolates on differentials, they were categorised into different reaction types. Average reactions of hosts to six isolates is presented in Table 1.

Isolate KB-2 collected from Karnal was the most virulent as it showed highly susceptible (HS) reaction on HD 2288, S 486, WL 711 and WL 1562; susceptible reactions in PBW 34. However, it produced moderately resistant (MR) reaction on HD 29. Differential host, OWL 5023 remained immune (I) to this isolate. Two other isolates, KB-1 from Gurdaspur and KB-4 from Saharanpur showed similar reaction types as KB-2 except a few differences, where HD 2255 produced MS reaction and S 485 and WL 1562 produced susceptible (S) type of reaction. Therefore, they were grouped with KB-2. On the other hand, isolates KB-3 and KB-5 showed similar aggressiveness to all the host genotypes except on HD 2255 and WL 711 where KB-3 produced MR and S reactions instead of R and HS reactions, therefore, these two isolates were clubbed together.

Isolate KB-6 from Faizabad was least aggressive as it produced MS reaction on WL 711.
and failed to attack DWL 5023, HD 29, PBW 34 and Sonalika, and reaction on other genotypes was also quite distinct. These observations grouped six isolates of Neovossia indica into three pathotypes: Pathotype I KB-1, KB-2 and KB-4; Pathotype II KB-3 and KB-5; Pathotype III KB-6

Biochemical variations

The quantitative analysis data on total lipids in teliospores of N. indica is enumerated in Table 2. It was noticed that fat contents in terms of mg/g dry teliospores was the highest (131.1) in isolate KB-2 followed by KB-1 (109.7), differing significantly from each other. Minimum fat content was quantified in KB-6 (74.1), which was at par with isolates KB-3, 4 and 5. Fatty acid profiles from all the six isolates of N. indica were obtained by gas chromatography. In case of isolate KB-1 presence of atleast 14 fatty acids was indicated. Like-wise, KB-2, KB-3, KB-4, KB-5 and KB-6 gave 16, 14, 17 and 11 peaks respectively representing different fatty acids (Table 3). Data on fatty acid composition revealed the presence of atleast 18 different fatty acids, ranging from heptanoic (C 7 :0), a saturated fatty acid to (C 18 :3), a saturated fatty acid to (C 18 :3). In all the isolates, 11 fatty acids were common; however, the quantitative differences existed. In general the relative percentage of palmitic acid (12.65 - 20.90%), oleic acid (37.24 - 53.58%) and linoleic acid (21.38 - 31.96%) were more than other fatty acids. Isolate KB-2 could be differentiated from all other isolates by the presence of an additional fatty acid, linolenic acid (C18:3). In case of KB-4 oleic acid was predominant and lacked undecanoic, myristic and linolenic acid. It was more or less identical with KB-3 except having steric acid. KB-5 proved to be different from rest of isolates due to presence of all the fatty acids except linolenic acid. Isolate KB-6 could be differentiated from other isolates by the absence of capric (C10:0) and pentadecanoic (C15:1) acids.

Nitrogen and Protein Contents: Nitrogen and protein contents estimated in dry mycelial powder from 15, 25 and 35 days growth indicated that mycelia from 15 days growth contained significantly higher amounts than 25 and 35 days. The isolate KB-2 showed maximum amount of nitrogen (24.4 mg/g) and protein (15.3%). The isolates KB-1, 3 and 4 incorporated 21.5 - 22.2 mg nitrogen per g dry mycelia, and 13.4 - 13.9 per cent protein and did not differ significantly from each other. In comparison to these isolates, mycelia from KB-6 and KB-5 incorporated minimum amount of both nitrogen and protein (Table 4).
Table 3. Relative fatty acid composition of fat synthesized by six isolates of *Neovossia indica*

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>Carbon skeleton</th>
<th>Average retention</th>
<th>Mass Percentage of fat in Teliospore</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Time (min)</td>
<td>KB-1</td>
<td>KB-2</td>
</tr>
<tr>
<td>Heptanoic</td>
<td>C7:0</td>
<td>0.93</td>
<td>0.58</td>
</tr>
<tr>
<td>Caprylic</td>
<td>C8:0</td>
<td>1.26</td>
<td>0.35</td>
</tr>
<tr>
<td>Nonanoic</td>
<td>C9:0</td>
<td>1.42</td>
<td>1.59</td>
</tr>
<tr>
<td>Capric</td>
<td>C10:0</td>
<td>1.59</td>
<td>0.73</td>
</tr>
<tr>
<td>Undecanoic</td>
<td>C11:0</td>
<td>1.87</td>
<td>0.30</td>
</tr>
<tr>
<td>Lauric</td>
<td>C12:0</td>
<td>2.06</td>
<td>1.17</td>
</tr>
<tr>
<td>Dodecanoic</td>
<td>C12:1</td>
<td>2.34</td>
<td>1.15</td>
</tr>
<tr>
<td>Tridecanoic</td>
<td>C13:0</td>
<td>2.69</td>
<td>1.03</td>
</tr>
<tr>
<td>Myristic</td>
<td>C14:0</td>
<td>3.10</td>
<td>0.00</td>
</tr>
<tr>
<td>Mristoleic</td>
<td>C14:1</td>
<td>3.53</td>
<td>1.35</td>
</tr>
<tr>
<td>Pentadecanoic</td>
<td>C15:1</td>
<td>4.02</td>
<td>0.62</td>
</tr>
<tr>
<td>Palmitic</td>
<td>C16:0</td>
<td>6.32</td>
<td>13.36</td>
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<tr>
<td>Palmitoleic</td>
<td>C16:1</td>
<td>6.92</td>
<td>Tr</td>
</tr>
<tr>
<td>Stearic</td>
<td>C18:0</td>
<td>8.15</td>
<td>0.00</td>
</tr>
<tr>
<td>Oleic</td>
<td>C18:1</td>
<td>12.01</td>
<td>53.08</td>
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<tr>
<td>Linoleic</td>
<td>C18:2</td>
<td>13.72</td>
<td>23.76</td>
</tr>
<tr>
<td>Linolenic</td>
<td>C18:3</td>
<td>16.52</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Tr : Traces

Table 4. Estimation of nitrogen and total protein contents in mycelia of six isolates of *Neovossia indica*

<table>
<thead>
<tr>
<th>Isolate</th>
<th>15 DAI* Nitrogen (mg/g) and per cent protein</th>
<th>25 DAI Nitrogen (mg/g) and per cent protein</th>
<th>35 DAI Nitrogen (mg/g) and per cent protein</th>
<th>Mean Nitrogen Protein</th>
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<tbody>
<tr>
<td></td>
<td>Nitrogen</td>
<td>Protein</td>
<td>Nitrogen</td>
<td>Protein</td>
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<tr>
<td>KB-1</td>
<td>30.7</td>
<td>19.2</td>
<td>19.7</td>
<td>12.3</td>
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<tr>
<td>KB-2</td>
<td>33.3</td>
<td>20.8</td>
<td>21.7</td>
<td>13.5</td>
</tr>
<tr>
<td>KB-3</td>
<td>29.0</td>
<td>18.1</td>
<td>19.6</td>
<td>12.1</td>
</tr>
<tr>
<td>KB-4</td>
<td>30.7</td>
<td>19.2</td>
<td>19.7</td>
<td>12.3</td>
</tr>
<tr>
<td>KB-5</td>
<td>28.3</td>
<td>17.7</td>
<td>18.0</td>
<td>11.3</td>
</tr>
<tr>
<td>KB-6</td>
<td>27.7</td>
<td>17.3</td>
<td>17.0</td>
<td>10.6</td>
</tr>
</tbody>
</table>

* Days after incubation
CD at 5%
Isolate 1.3
Protein 0.9
Growth period 0.9
Isolate x Growth period Non significant
Non significant

Polyacrylamide gel electrophoresis was carried out to study variability in the soluble protein profiles and esterase and peroxidase isozymes of three representative isolates, (KB-2, 5 and 6) of *N. indica*.

SDS-Polyacrylamide gel electrophoretic profiles of soluble proteins of mycelial extracts (Fig.1) revealed that the number of total protein fractions varied from a minimum of 17 in isolate KB-2 to a maximum of 23 in isolate KB-6 and isolate KB-5 showed 19 bands. Out of 24 fractions 16 were common to all the isolates. Isolate KB-2 lacked three bands with R, 0.01, 0.02 and 0.05 in the range of 669,000 to 232,000 daltons, one between...
232,000 and 140,000 dalton (R, 0.07) and two bands with R, 0.86 and 0.91, below 14,300 daltons.

Isolate KB-5 lacked four protein fractions viz, R, 0.01 (approximate molecular weight, 669,000 dalton), R, 0.99 between 55,400 and 45,000 daltons and two fractions with R, 0.86 and 0.91 below 14,300 daltons. In comparison to isolates KB-2, and 5, isolate KB-6 had 23 protein fractions. Thus, it was apparent that all three isolates were different from each other in relation to soluble protein patterns.

Isozyme pattern

Esterase and peroxidase isozyme bands of isolates KB-2, 5 and 6 in healthy and infected caryopsis of wheat cv. WL 711 were resolved on polyacrylamide gel. A total of 4 esterase bands were resolved from mycelia of three isolates. KB-2 and 5 showed three bands but lacked a band of R, 0.76, which was present in case of isolate KB-6. Two bands with R, 0.65 and 0.71, of little more intensity appeared in isolate KB-6, while these bands were very light in isolate KB-2 and 5 (Fig.2).

Peroxidase isozyme of these isolates revealed the presence of six bands of R, values 0.04, 0.09, 0.16, 0.20, 0.25 and 0.87. First five bands were common to all the isolates, only band with R, 0.87 was lacking in isolate KB-6. The diseased caryopsis and healthy caryopsis also showed these bands except that the band with R, 0.04 was lacking in healthy caryopsis indicating that it was of fungal origin.

The wheat caryopsis showed four additional bands over fungal mycelia out of which one with R, 0.45 was not present in healthy caryopsis. Similarly, the band with R, 0.98 was absent in caryopsis infected with isolate KB-2.

DISCUSSION

In the present studies, six isolates of *N. indica* showed pathogenic variation amongst themselves on 10 host differentials. Isolate KB-2 was the most virulent and KB-1 and KB-4 matched in their aggressiveness to isolate KB-2 and were grouped as pathotype I. Singh *et al.* (1995) categorised 8 isolates of *N. indica* into 5 populations i.e., KBAg-1, KBAg-2, KBAg-3, KBAg-4 and KBAg-5 based on aggressiveness.

Biochemical comparison in terms of lipids, fatty acids, nitrogen and protein contents in
teliosporic material and mycelial growth of six isolates clearly showed that isolate KB-2 and KB-6 are significantly different, KB-2 being the most aggressive and KB-6 the least aggressive. The studies have indicated that more aggressive isolates contained more lipids, nitrogen and protein. Mohanty and Gangopadhyay (1983) also observed differences in the biochemical constituents of three different isolates of *Pyricularia oryzae*. Six races of *Ascochyta rabei* and four races of *Fusarium oxysporum* were distinguished on the basis of variation in sugar, protein and nitrogen contents (Sangwan et al., 1990; Desai et al., 1992a,b).

Polyacrylamide gel electrophoresis has been used to differentiate isolates of *Pseudocercosporella herpotrichoides* (Jullian and Lucas, 1990); isolates of *Phakopsora pachyrhizi* from Asia, Australia and New World (Bonde et al., 1988); and species of *Phytophthora* (Gill and Powell, 1986).

During the studies variations in banding pattern of isozymes of esterase and peroxidase was observed in isolate KB-2, KB-5 and KB-6, collected from different geographic locations. Bonde et al. (1985) did not find any difference among various isolates of *N.indica*, however, higher number of alleles were found to be common among isolates of *N.indica* which facilitated their differentiation from these of *N. barclayana* isolates.

Biochemically, highly virulent and fast growing KB-2 isolate incorporated highest content of lipid, nitrogen and protein. Comparatively, KB-6 which was less aggressive and slow growing isolate had minimum amount of these constituents.

REFERENCES


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