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Morpho-physiological studies of *Neovossia indica* (Mitra) Mundkur causing Karnal bunt of wheat

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Abstract
Wheat (*Triticum aestivum*) is an important food crop of India ranks next to rice contributing about 27 percent of total food production. Karnal bunt of wheat caused by *Neovossia indica,* has become a potential threat to wheat production in our country. Hence, for minimizing the losses caused by Karnal Bunt need inexpensive and environmentally safe management practices. It was, therefore considered desirable to identify resistant to Karnal bunt for the new bread wheat strains. Colony characters of different isolates from diseased wheat plants were collected from different localities of Kanpur as well as adjoint areas and were observed that the isolates obtained from CRF Nawabganj, CRF Araul, CRF Deegh Chaubepur and Mandhana were fast growing in nature as their colony size ranged from 8.6 to 8.2 cm. with partially fluffy mycelium while isolates from NDF Kalyanpur, CRF Saini, Vegetable farm, Kalyanpur and CRF Manimau were slightly slow in nature, colony size 7.2 to 7.4 with fluffy dark brown mycelium. Mycelium septate, profusely branched, septa are more abundant in older mycelium than younger ones. The teliospore is darkening brown, spore measure 22 to 49 micron. The spores germination from a start, stout mycelium primary sporidia had mean length and with ranging from 64.4 to 78.8 µm and the secondary sporidia had mean length and width ranging from 11.9 to 1.30 µm and 2.00 to 2.03 µm Forty wheat genotype planted at Nawabganj Research From C.S.A. University of Agriculture & Technology Kanpur in which none of the genotype showed immune reaction or 0% disease under artificial condition, 22genotype below 10% disease intensity, 11genotype below 15% disease intensity, 4 genotype below 20% disease intensity namely HD 3043, C306, HD 3133 and K 1213 and only HD 3090 below 40% disease intensity.

Keywords: Morpho-physiological, *Neovossia indica*, Karnal bunt

Introduction
Wheat (*Triticum aestivum*) is an important food crop of India ranks next to rice contributing about 27 percent of total food production. India is the second largest wheat growing county in the world after China. It contributes ¼ of the global wheat production and covers 1/5 of the total cropped area of the world. There has been tremendous increase in both production and productivity in India after independence which has gone up from 5.6 million metric tonnes to 98.61 million metric tonnes (D.W.R. Report) 2017-18. Consequently the productivity of wheat per hectare was gone up from 8.87 quintals in the 1966-67 to 33.18 quintal in 2017-18. Among 8 major wheat growing states (Uttar Pradesh, Punjab, Haryana, Rajasthan, Delhi, Madhya Pradesh, Bihar and Gujarat) of the country, Uttar Pradesh ranked first both in area (9.67 million hectare) and production (27.52 million metric tonnes) with a productivity of 36.50 q/ha (D.W.R. Report) 2017-18. India has made an excellent progress in wheat production during the past three decades making the country to become self sufficient in food grains. Wheat also plays a major role in the grain pool of the country and it is the main source of food security in India. Karnal bunt of wheat caused by *Neovossia indica,* which was first reported by Mitra in 1931, has become a potential threat to wheat production in our country. This disease was not considered important till 1969-70; The high incidence of this disease in the crop seasons 1974-75, 1975-76 and 1978-79 the consecutive seasons in the *tarai* regions of Uttar Pradesh and Punjab has made it a major problem of wheat in India. Karnal bunt was responsible for an annual loss of 40,000 metric tonnes of grain per year during late sixties (Munjal, 1975a) [1]. The disease affects both the quality and quantity of wheat grains which, seriously reduces its market value and creates problem of the wheat growers. Wheat grains having 0.1 per cent infection were unacceptable for human consumption (Mehdi *et al.,* 1973) [2]. Hence, for minimizing the losses caused by Karnal Bunt need inexpensive and environmentally safe management practices. It was, therefore considered desirable to identify resistant to Karnal bunt for the new bread wheat strains will be made with the following objectives:

i. Survey and collection of diseased materials.
ii. To study the symptomatology of Karnal bunt morphological and cultural variation of Karnal bunt *(Neovossia indica).*
iii. To screen wheat germplams/varieties *in vivo* for Karnal Bunt (*Neovossia indica).*

Material & Methods

Survey and collection of diseased material

A regular and constant observation on the occurrence and severity of Karnal bunt of wheat was made at different farmer's field and also at Crop Research Farm of the C.S.A. University of Agric. & Tech., Kanpur following the standard methodology. The bunt affected ears of wheat were collected in poly bags and brought to the laboratory for examination and isolation. The critical examination was made by testing the diseased Kernels and observing the fungus under the microscope. Some typical specimens were selected, dried, pressed properly and kept for further studies.

Severity of the diseases

For ascertaining the disease severity fifty diseased Kernels of wheat were randomly selected from each field during the survey. These Kernels were arranged according to different categories.

Isolation the fungus (Pathogen)

Infected Kernels were thoroughly washed with tap water for removing dust and other surface contaminants Kernel having young spores were selected. Thus Kernels were dipped in 0.1% mercuric chloride for 30 seconds and transferred in pertridishes with the help of sterilized forceps and washed thrice with sterilized water to remove the traces of mercuric chloride. The small pieces of Kernels were then transferred to sterilized pertridishes containing 2@ glucose yeast extract agar media (GYEA) in the inoculation chamber and then incubated at room temperature (24-28^{0}C).

Purification of the fungus

Purification of the fungus was done by transferring the hyphal tip taken from margin of the young growing colonies on 2% GYEA and allowed to grow for a week. Different isolates of the fungus were further purified by single spore culture method.

The growth of the isolates of the pathogen obtained from the samples of different places was studied critically for finding out the fastest growing one. These isolates were then stored on GYEA medium at 6-9^{0}C for further studies. The cultures were renewed after two months.

Pathogenicity of fungus

The pathogenicity test of the fastest growing isolate of the fungus was carried out following Koch's postulates on the newly emerging spikes of the susceptible wheat variety HD2009 raised in pots containing sterilized soil. Newly emerged wheat were inoculated by 2 ml of inoculums suspension containing about 10,000 sporidia per ml were injected into the boot preceding own emergence with the help of hypodermic syringe in two ears in each plant and five plants in each pot. Relative humidity was maintained about 80% by spraying of water regularly.

Screening of wheat genotypes against Karnal bunt disease

A set of 85 diverse wheat genotypes collected from the gene bank maintained at Section of Economic Botanist (Rabi cereals) of C.S.A. Univ. of Ag. & Tech., Kanpur were sown in two rows of 5 m large at Nawabganj Farm on 15-11-2012 (1st year) and 10-11-2013 (IInd year). for screening of genotypes against Karnal bunt. Five randomly selected plants of each genotype was inoculated by the spore culture of Karnal bunt at the beginning of ear emergence and the number of infected seeds per ear was counted at maturity which was deducted from total seeds of ear and transformed in percent, on the basis of infection the genotypes were categorized into (I) Immune, (ii) Resistant (iii) Moderately susceptible and (iv) Susceptible groups. The same steps were also adopted for another set of material of same genotypes in natural condition. Experiment conducted constituently three years.

Results and Discussion

Karnal bunt is a major disease of wheat growing states of India. The disease is particularly serious in sub-mountainous areas of Uttar Pradesh and Punjab. To find out suitable management practices to control like Karnal bunt disease is an important research challenge. Most of the present day popular varieties are highly susceptible to the disease and a good source of resistance is yet to be recognized for se in future crop breeding programe. Since Karnal bunt is soil-borne (Mitra, 1937) [4], its management through soil amendment particularly with organic substances and manipulation of agronomic practices needs investigation. Studies were conducted on incidence of Karnal bunt of wheat caused by *Neovossia indica* (Mitra & Mundkur).

Comparative morphological studies of nine isolates collected from different localities

Various morphological characters of the fungus *viz*; Colony character, mycelium, Teliospore, Primary sporidia and Secondary sporidia were also studied in nature (host) on the basis of visual and microscopic examination of the fungus, Different isolates of *Neovossia indica* collected the disease wheat plant from nine locations of Kanpur district and adjoining area were used in this investigation.

Table 1: Locality of collection and growth rate of nine isolates of N. *indica* on PDA medium.

S. No.	Localities	Diameter of colony (cm)							Nature of Mycelium
		1	2	3	4	5	6	7	
1.	CRF Nawabgang	0.5	1.6	2.8	3.8	5.9	7.0	8.6	Partially fluffy Mycelium
2.	CRF Araul	0.5	1.5	2.7	3.6	4.9	6.9	8.5	
3.	CRF Deegh	0.5	1.6	2.8	3.6	6.1	7.1	8.3	
4.	Chaubepur	0.5	1.6	3.0	3.5	5.8	7.1	8.3	
5.	Mandhana	0.5	1.5	2.9	4.0	5.1	6.1	8.2	
6.	NDF Kalyanpur	0.5	1.5	2.9	4.0	5.4	6.1	7.2	Fluffy dark Brown Mycelium
7.	CRF Saini	0.5	1.6	2.7	3.9	6.9	7.1	7.0	
8.	Vegetable Farm, Kalyanpur	0.5	1.5	2.7	3.1	5.6	6.2	7.0	
9.	CRF Manimau	0.5	1.5	2.9	3.9	5.9	6.2	7.4	

Colony Characters

Colony characters of different isolates of *Neovossia indica* isolated from diseased wheat plants which were collected from localities of Kanpur as well as adjoint areas were used in this investigation. The basis of differentiation among isolates was colony characters of isolates on solid PDA medium.

It was observed in Table 1 that the isolates obtained from CRF, Nawabganj, CRF Araul, CRF Chaubepur and Mandhana were fast growing in nature as their colony ranged from 8.6 to 8.2 cm. while isolates from NDF Kalyanpur, CRF

Dibiapur, Vegatable from Kalyanpur and Manimau were slightly slow growing in nature with colony size between 7.2 to 7.4 cm. Among all the maximum colony size 8.6 cm. was found in CRF Nawabganj, Kanpur and minimum colony size 7.0 cm was found in the isolate from CRF Saini and Vegetable Farm. The isolate from CRF, Nawabganj, CRF Araul, CRF Deegh, Chaubepur and Mandhana were partially fluffy mycelium as well as NDF Kalyanpur, CRF Saini, Vegatable farm Kalyanpur and Manimau were fluffy dark brown mycelium. Colony of all these isolates had smooth margin. There were minor differences among the isolates.

Mycelium Characters

Mycelium initially cottony white becoming brown to black with age, septate, profusely branched. Septa are more abundant in older mycelium than younger ones.

Teliospores

The teliospores of *Nevossia indica* are darker brown than those of *T.caries* and *T.foetida*. They are spherical and oval, with reticulations on the epispore which appear as curved spines. These spores measure 22 to 49 micron (average 35µ)

Primary sporidia

The spores germinate from a start, stout basidium. The sporidia are produced in large numbers (60-120) at the apex of the basidium. Primary sporidia had mean length and with ranging from 64.4 to 78.8 µm respectively.

Secondary sporidia

The secondary sporidia had mean length and width ranging from 11.9 to 1.30 µm and 2.00 to 2.03 µm, respectively

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Varietal screening experiment

Fourty wheat genotypes/varieties were planted in paired rows of 5 m long spaced at 30 cm apart to test for disease reaction of genotypes. Susceptible checks Infector were planted after each 20 genotypes. The experimental field was also surrounded by these two checks. All the recommended agricultural operations were adopted to raise a good crop. In each genotypes ten randomly chosen plants were inoculate with the help syringe. The inoculums (10000-15000 allantoids/ml water) were injected in the hypodermis of ear. Athichmist was created over the canopy for 30 days just after day to inoculation.

Table 2: Screening of wheat genotypes against Karnal bunt disease

S.No.	Varieties	2012-13	2013-14	Mean (%)
1.	HD 2967	9.2	10.5	9.85
2.	HD 3043	15	16	15.5
3.	HD 3059	10.4	11	10.7
4.	HD 3086	13	13.2	13.1
5.	BRW 3723	3.1	4.2	3.65
6.	DBW 107	14.4	15.1	14.75
7.	HD 3118	10.8	11.4	11.1
8.	K 1114	11.6	12.9	12.25
9.	C 306	15.2	16.6	15.9
10.	DBW 14	3.6	5.2	4.4
11.	DBW 39	12.9	14.4	13.65
12.	HD 2733	5.1	6.3	5.7
13.	HD 2888	9.1	10.9	10
14.	HD 2985	14.5	15.4	14.95
15.	HI 1563	7.2	8.3	7.75
16.	K 0307	1.8	2.5	2.15
17.	K 1006	4.1	5.2	4.65
18.	K 8027	9.1	10	9.55
19.	NW 2036	3.5	4.1	3.8
20.	NW 5054	13.9	15	14.45
21.	INFECTOR	18.3	19.6	18.95
22.	HD 2864	2.4	3.8	3.1
23.	HD 2932	3.7	5	4.35
24.	HD 3090	29.9	31	30.45
25.	HD 3091	2.3	3.6	2.95
26.	HD 3093	10	11	10.5
27.	HD 3128	14.1	15.2	14.65
28.	HD 3132	7.3	8.8	8.05
29.	HD 3133	19.1	20.2	19.65
30.	HD 3139	6.4	7.9	7.15
31.	HD 4730	0.2	0.4	0.3
32.	K 1204	4.9	6.1	5.5
33.	HD 3127	8.1	9.2	8.65
34.	HD 3146	7.4	9.8	8.6
35.	HD 4728	1.3	2.4	1.85
36.	K 1215	6.4	7.9	7.15
37.	K 1217	4.8	6.4	5.6
38.	K 1213	16.2	17.3	16.75
39.	K 9006	11.3	12	11.65
40.	INFECTOR	23.1	24.4	23.75

It is clear from the data that name of the genotypes showed immune reaction or 0% disease under artificial condition. However, the genotypes which showed resistance reaction to the pathogen (below 10% disease intensity) were HD2967, BRW3723, DBW14, HD2733, HI1563, K0307, K1006, K8027, NW2036, HD2864, HD2932, HD3091, HD3132, HD3139, HD4730, K1204, HD 3127, HD3146, HD4728, K1215 and K1217 (21 genotypes).

The genotypes showed moderate reaction were HD 3059, HD 3086, DBW 107, HD 3118, K 1114, DBW 39, HD 2985, NW 5054, HD 3093, HD 3128, HD 2288 and K 9006(12 genotypes below 15% score). Moderately susceptible reaction was showed by HD 3043, C 306, HD 3133 and K 1213 (4 genotypes below 20% score). The susceptible reaction was shown by the genotypes namely HD 3090 (1 genotypes below 40% score).

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