

Original Research Article

Studies on Variability on Isolates of *Neovossia Indica* causing Karnal Bunt of Wheat and Screening of Wheat Varieties

Comment [m91]: Which is more commonly used, *Neovossia* or *Tilletia*? I see the more common one is *Tilletia*

Comment [m92]: This title would be more precise if "resistance" were added.

ABSTRACT

A basic meal consumed by people all over the world, wheat is traded more globally than all other crops combined. *Tilletia indica* is the cause of Karnal bunt, also known as "Partial bunt," which affects wheat and is one of the most significant seed-borne diseases. It has significant effects on the wheat trade because most importing nations require that there be no trace of Karnal bunt in wheat imported. To assess responses to disease, ten different genotypes of wheat were sown in matched rows. We planted K1006 and PBW343, two susceptible checks, following each genotype. An athichmist was established for a duration of thirty days, and all suggested agricultural practices were adhered to. The genotypes that demonstrated resistance to the pathogen (below 10% disease intensity) were PBW 343 and K-1006 (2 genotypes). The genotypes with a modest response were K-9107 and K-9162 (two genotypes with a score below 15%). The reactivity of the K793 and K 9006 2 genotypes was somewhat sensitive (below 40% score). The genotypes K9465 HD 2824, K0307, and C306 all showed extremely sensitive reactivity (score exceeding 40%).

Key Words: Genotypes, Karnal bunt, *Neovossia indica*, Petridish

INTRODUCTION

When it comes to its employment in various types of food and feed, wheat is the most widely grown cereal worldwide, used for both grain production and feedstock in both developed and developing nations. The production of wheat in India is predicted to increase from 107.86 MT in the previous year (2019–20) to a record 109.24 MT in 2020–21. With a record average productivity of 337 kg/ha, India is the second-largest producer of wheat after China. The crop has been cultivated on about 30 million hectares, or 14% of the world's total area, to produce the highest output of 99.70 million tons of wheat, or 13.64% of world production, which has been consumed as various processed farms since prehistoric times. India now has an excess of wheat on hand and has the capacity to produce more. India produced 99.70 million tons of wheat from a cultivated area of 29.58 mha in 2017–18. *Tilletia indica*, also known as *Neovossia indica*, is a heterothallic fungus that can induce karnal bunt or partial bunt in wheat was initially documented by Mitra in Karnal, Haryana (Mitra, 1931). According to Nagarajan *et al.* (1997), *T. indica* is a heterothallic fungus that is a member of the family Ustilaginaceae and order Ustilaginales. Seventy-seven countries had imposed restrictions on import of wheat from the areas where the KB disease occurs (Bonde *et al.*, 2004). The disease cause losses in yield, seen vigor and deteriorates quality of the produce and appearance of grains. Quantitative losses ranged between 0.2-

0.6 percent of total production during the epiphytetic year. In 1969-70 in an estimated 1/3 of wheat producing area in North India, the loss in grain yield was 0.2 percent which was calculated equivalent to 40,000 metric tons of grains. The incidence was much more i.e. 5-20 percent in the year when disease appeared in epidemic form causing much higher losses (Aujla *et al.* 1989).

MATERIALS AND METHODS

Survey and collection of diseased material

Following the conventional technique, a regular and continuous observation was done on the occurrence and severity of Karnal bunt of wheat at Oilseed (Nawbganj), Vegetable Science (Kalyanpur), at the Crop Research Farm of the C.S.A. University of Agric. & Tech. Kanpur in the years 2020–2021. The wheat ears with bunt damage were gathered in poly bags and transported to the lab for analysis and segregation. The fungus was examined under a microscope and the infected kernels were teased in order to do a critical analysis. A few representative samples were chosen, dried, correctly pressed, and stored for additional research. For ascertaining the disease severity five diseased kernels of wheat were randomly selected from each during the survey. These kernels were arranged according to different categories.

Isolation and Purification of Fungus

Tap water was used to thoroughly wash the infected kernels in order to remove dust and other surface impurities. Young spore-containing kernels were chosen. Following the transfer of the small kernel pieces to sterilized petri dishes, the kernel pieces were incubated at room temperature (24–28 °C) in sterilized petri dishes that contained 2% glucose yeast extract agar media (GYEA) in the inoculation chamber. The hyphal tip from the edge of the young, developing colonies was transferred to 2% GYEA and left to grow for a week in order to purify the fungus. Using the single spore culture approach, various fungal isolates were further purified. 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.

Pathogenicity tests

As per Koch's postulates, the pathogenicity test of the fungus's fastest-growing isolate was conducted on the just emerging spikes of susceptible wheat "HD 2824" grown in a plot with sterilized soil. Using a hypodermic syringe, two ears from each plant and five plants per plot were used to inoculate newly developed spikes with a suspension of inoculums containing approximately 10,000 sporidia per milliliter. Regular water spraying helped to maintain a relative humidity of roughly 80%.

Screening of wheat genotypes for Karnal bunt reaction

A set of 10 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 paired rows of 5m large at division of RABI cereals from C.S.A. Univ. of Ag. & Tech. Kanpur 4-12-2020 (IInd year) for screening of genotypes against Karnal bunt. Five randomly selected plants of each genotype were 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) Resistant (ii) Moderate (iii) Moderately susceptible and (iv) Susceptible groups. The same steps were also adopted for

Comment [m93]: It would be better if at the end of this introduction, the objectives of the study and their importance for controlling Karnal bunt were added

Comment [m94]: It would be better if the abbreviation was written here because it will be used in subsequent writing.

Comment [m95]: What are the categories, can they be mentioned or explained?

Comment [m96]: Maybe it will be clearer if it is explained why you should use mycelium which grows the fastest.

Comment [m97]: What does this word mean? If you mean time, you can write it in a separate sentence to make it clearly.

Comment [m98]: Karnal bunt spores or *Tilletia indica* spores?

Comment [m99]: Typing error?

Comment [m910]: typing error?

another set of material of same genotypes in natural condition.

RESULTS AND DISCUSSION

Morphological study of isolates collected from different localities

Colony Characters:

This study used colony characteristics of various *N. indica* isolates that were obtained from damaged wheat plants that were gathered from Kanpur localities as well as adjacent areas. Colony characteristics of isolates on solid PDA medium served as the foundation for isolate differentiation.

Table 1 shows that the isolates from the Rabi cereals Crop, Kanpur's CRF Nawabganj division were slower growing than the isolates from Vegetable Science Kalyanpur, Kanpur, with colony sizes ranging from 8.75 to 8.24 cm were slightly slow growing in nature with colony size between 7.05 to 7.25 cm. The isolate from the vegetable farm had the smallest colony size of 7.05 cm, while the largest colony size of 8.75 cm was discovered in CRF Nawabganj, Kanpur. Kalyanpur. Both the Nawabganj isolate from CRF and the Kalyanpur isolate from CRF's Vegetable Farms were partially puffy dark brown mycelium. Each of these isolates had a smooth edge in its colony. The isolates differed slightly from one another.

Table 1: Locality of collection and growth rate of two 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.	Vegetable Science Kalyanpur	0.5	1.5	2.8	3.7	5.6	7.0	8.7	Partially fluffy mycelium
2.	CRF Nawabganj	0.5	1.6	2.9	3.6	5.6	6.3	7.5	Fluffy dark Brown mycelium

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 *N. 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 35 μ).

Sporidia:

The isolate from the vegetable farm had the least colony size, measuring 7.05 cm, while CRF Nawabganj, Kanpur, had the maximum colony size, measuring 8.75 cm. The dark brown mycelium in the Nawabganj isolation from CRF and the Kalyanpur isolate from CRF's Vegetable Farms were

Comment [m911]: Please write down the full name of the medium first (potato dextrose agar).

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Comment [m913]: how many days after inoculation?

both somewhat puffy. In their colonies, each of these isolates had a smooth edge. There were minor variations among the isolates.

Comment [m914]: This paragraph repeats the previous section. The subtitle of the paragraph is about sporidia, but the content is about mycelium/colony.

VARIETAL SCREENING EXPERIMENT

Ten wheat genotypes were planted in paired rows to test disease reactions. Susceptible checks PBW343 and K1006 were planted after each genotype. All recommended agricultural operations were followed, and an artificial inoculum was created for 30 days. The data in Table 2 makes it evident that, in artificial conditions, the genotype names indicated immunological reaction or 0% illness. However, PBW 343 and K-1006 (2 genotypes) were the genotypes that shown resistance to the pathogen (below 10% disease intensity). K-9107 and K-9162 were the genotypes that had a moderate response (2 genotypes below 15% score). K793 and K 9006 2 genotypes had a somewhat sensitive reactivity (below 40% score). The K9465 HD 2824, K0307, and C306 genotypes all displayed the very sensitive reactivity (4 above 40% score).

Comment [m915]: It would be better if it were titled according to its contents, namely "varietal resistance screening/experiment".

Table 2: Screening Of Wheat genotypes in field after artificial inoculation condition.

Comment [m916]: The table title should be clearer about what it wants to show? Is the result of the screening for resistance to the pathogen or what?

S. NO	Entry	I year		II Year		III Year		Mean	Mean	Score
		<i>Invitro</i>	<i>Invivo</i>	<i>Invitro</i>	<i>Invivo</i>	<i>Invitro</i>	<i>Invivo</i>	<i>Invitro</i>	<i>Invivo</i>	
1.	PBW343	3.2	2.1	4.8	3.6	2.7	8.6	3.5	4.7	R
2.	K1006	3.0	2.1	3.4	2.4	3.6	2.3	3.3	2.2	R
3.	K9107	28.6	24.6	29.9	26.3	58.0	49.6	38.3	33.5	S
4.	K9162	28.8	25.3	23.8	19.4	24.6	19.9	25.7	21.5	S
5.	K7903	12.2	10.6	22.6	18.6	29.8	24.6	21.5	17.9	MS
6.	K9006	4.2	3.2	21.8	20.6	22.0	18.7	16.0	14.1	MS
7.	C306	54.2	50.2	56.6	49.8	54.8	49.2	55.2	49.7	HS
8.	K9465	42.0	38.2	42.6	38.1	44.2	39.1	42.9	38.4	HS
9.	HD2824	48.6	41.6	51.8	46.7	44.8	40.9	48.4	43.0	HS
10.	K0307	46.8	41.0	50.6	43.6	48.2	42.1	48.5	42.2	HS

Comment [m917]: In the previous method there was no explanation about the existence of a multi-year trial. Where did these results come from? If there are other experiments, they should be described in the methods.

Multiple alleles at a single locus govern the bipolar incompatibility of *T. indica*, a heterothallic fungus (Krishna and Singh, 1983; Aggarwal *et al.*, 2010). Sharma *et al.*, (2008) have observed that the dikaryotization takes place inside the host tissue.

Comment [m918]: What do the numbers in this table indicate? what is percentage? or score? It is best to provide clear information.

Comment [m919]: This section should contain results and discussion. however, here only the results are presented and have not been discussed properly. Research results should be discussed with reference to the latest references.

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