



# Studies on Variability on Isolates of *Neovossia indica* Causing Karnal Bunt of Wheat and Screening of Wheat Varieties

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## Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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## 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%).

**Keywords:** Genotypes; karnal bunt; *Neovossia indica*; petridish.

## 1. 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" [1]. "This challenge will be further compounded by increased temperature due to global warming" [2] According to Nagarajan et al. [3], "*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" [4]. "Being a seed, soil and air borne pathogen, it mainly affects common wheat, durum wheat, triticale, and other related species making it the limiting factor in wheat export as the pathogen is regulated by most countries as a quarantine pest" [5]. "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 epiphyte tic 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" [6]. "Most wheat importing countries insist on an additional declaration from the exporting countries that the wheat consignment being traded is produced in a KB-free area" [7]. Therefore, it is of paramount importance that wheat breeding programs in the affected and vulnerable countries augment the efforts toward the identification and development of resistant sources [8].

## 2. MATERIALS AND METHODS

### 2.1 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, 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" [9].

## 2.2 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 so that we can identify which one is more virulent.

## 2.3 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%.

## 2.4 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 for screening of genotypes against Karnal bunt. Five randomly selected plants of each genotype were inoculated by the spore culture of *Neovossia indica* 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 another set of material of same genotypes in natural condition" [9].

## 3. RESULTS AND DISCUSSION

### 3.1 Morphological Study of Isolates Collected from Different Localities

#### 3.1.1 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 potato dextrose agar 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.

#### 3.1.2 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.

#### 3.1.3 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).

**Table 1. Locality of collection and growth rate of two isolate of *N. indica* day after inoculation on PDA plate**

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

**Table 2. Screening Of Wheat genotypes for comparison resistance percentage against pahogen in field after artificial inoculation condition**

S. NO	Entry	Replication		Replication II		Replication III		Mean	Mean	Score
		<i>In vitro</i>	<i>In vivo</i>	<i>In vitro</i>	<i>In vivo</i>	<i>In vitro</i>	<i>In vivo</i>	<i>In vitro</i>	<i>In vivo</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

### 3.1.4 Sporidia

The isolate from the vegetable farm had the least colony size, measuring 7.05 cm, while CRF Nawabganj, Kanpur isolate has 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 both somewhat puffy. In their colonies, each of these isolates had a smooth edge. There were minor variations among the isolates.

### 3.1.5 Varietal resistant 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 athichmist 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).

Multiple alleles at a single locus govern the bipolar incompatibility of *T. indica*, a heterothallic fungus [10,11]. Sharma et al. [12]. have observed that the dikaryotization takes place inside the host tissue. Grouping of isolates into various clusters was based on their response to host [13]. Purwar et al. [14]. employed an alternative methodology to examine the function of the cystatin gene family (WC1, WC2, and WC4) using wheat genotypes exhibiting immunity to KB.

## 4. CONCLUSION

Effective management of the KB illness requires an understanding of the pathogenicity, variety,

and aggressiveness of *N. indica*. Ten distinct genotypes of wheat were sown in matched rows in order to evaluate responses to disease. We planted two susceptible checks, K1006 and PBW343, after each genotype. Once the pathogenicity of a collection of KB isolates was established, it was discovered that K9465 HD 2824, K0307, and C306 were more sensitive to Karnal bunt whereas, PBW 343 and K-1006 were very less sensitive. K-9107 and K-9162, two genotypes with scores lower than 15%, were the genotypes with a moderate response. The K 9006 2 and K 793 genotypes had reactivity that was only moderately sensitive (below 40% score).

### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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