

**REACTION OF BREAD WHEAT ADVANCED LINES TO KARNAL BUNT  
ARTIFICIAL INOCULATION DURING THE 2015-2016 SEASON**

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**ABSTRACT**

One thousand and ninety two bread wheat advanced lines were evaluated for their reaction to *Tilletia indica* during the crop season 2015-2016. Sowing dates were November 12 and 19, 2015, using 8 g of seed for a row 0.7 m long on a bed with two rows. Inoculations in the field were performed by injecting 1 mL of an allantoid sporidial suspension (10,000/mL) during the boot stage in 5 spikes per line. The percentage of infection was determined by counting healthy and infected grains. The range of infection in the first date was 0-75.18 with a mean of 17.89%, while in the second date it was 0-83.45% with a mean of 21.45%. The range of infection of the two dates was 0.18-70.64% with a mean of 19.67%. Thirty seven lines showed a percentage of infection equal or below 2.5% in both dates, and out of those lines, the following four showed less than

0.5%: SAUAL/3/SW89.3064//CMH82.17/SERI/4/SAUAL/5/PBW343\*2/KUKUNA\*2//FRTL/PI FED/6/SAUAL/KRONSTADF2004,MEX94.27.1.20/3/SOKOLL//ATTILA/3\*BCN/5/CHRZ//B OW/CROW/3/WBLL1/4/CROC\_1/AE.SQUARROSA(213)//PGO,TACUPETOF2001/6/CNDO/R143//ENTE/MEXI\_2/3/AEGILOPSSQUARROSA(TAUS)/4/WEAVER/5/PASTOR/7/ROLF07/8/KACHU#1/KIRITATI/KACHU, and BABAX/LR42//BABAX/3/ER2000/6/POTCH93/4/MILAN/KAUZ//PRINIA/3/BAV92/5/MILAN/KAUZ//PRINIA/3/BAV92. Lines with the highest percentage of infection were: TUKURU//BAV92/RAYON/3/WBLL1\*2/BRAMBLING/8/TACUPETOF2001/6/CNDO/R143//ENTE/MEXI\_2/3/AEGILOPSSQUARROSA(TAUS)/4/WEAVER/5/PASTOR/7/ROLF07/9/TUKURU//BAV92/RAYON\*2/3/JUCHI with 83.45 and TACUPETOF2001/6/CNDO/R143//ENTE/MEXI\_2/3/AEGILOPSSQUARROSA(TAUS)/4/WEAVER/5/PASTOR/7/ROLF07\*2/8/SUP152/MUU with 83.10% in the second date. The average of the three highest levels of infection of the susceptible check was 100%..

**Keywords:** Bread wheat, *Triticum aestivum*, bread wheat, karnal bunt partial bunt, *Tilletia indica*.

**1. INTRODUCTION**

Karnal bunt or partial bunt of wheat caused by the fungus *Tilletia indica* Mitra (syn. *Neovossia indica* Mundkur), affects bread wheat (*Triticum aestivum* L.) (Mitra, 1931), durum wheat (*T. turgidum* L.) and triticale (X *Triticosecale* Wittmack) (Agarwal *et al.*, 1977). The disease has been reported from India (Mitra, 1931), Mexico (Duran, 1972), Pakistan (Munjil,

1975), Nepal (Singh *et al.*, 1989), Brazil (Da Luz *et al.*, 1993), The United States of America (APHIS, 1996), Iran (Torarbi *et al.*, 1996), the Republic of South Africa (Crouset *et al.*, 2001), and Afghanistan (CIMMYT, 2011). It is common that the fungus affects partially some grains in a spike, and not all the spikes are affected in a plant (Bedi *et al.*, 1949) (Fig. 1), but in some occasions, grains may be totally destroyed; although the fungus may penetrate the embryo, it is not necessarily lethal (Chona *et al.*, 1961; Mitra, 1935). Partially infected grains may produce healthy plants, although there are reports that indicate that the percentage of germination decreases depending on the level of infection of the grain (Bansal *et al.*, 1984; Rai and Singh, 1978; Singh, 1980), and that severely affected seeds lose viability or show abnormal germination (Rai and Singh, 1978); Fuentes-Dávila *et al.*, 2013 reported that seed with the greatest infection, but with the embryo intact, produced the highest number of tillers.



**Figure 1.** Wheat spike with infected grains with *Tilletia indica*. The grain shows a characteristic lesion caused by the pathogen.

Since teliospores of the fungus are resistant to physical and chemical factors (Krishna and Singh, 1982; Zhanget *al.*, 1984; Smilanicket *al.*, 1985, 1988), control of this pathogen is difficult; however, chemical control can be accomplished by spraying fungicides during flowering (Fuentes-Dávila *et al.*, 2005, 2016, 2018c; Salazar-Huerta *et al.*, 1997). This measure would not be economically feasible where quarantine of phytosanitary regulations do not allow tolerance levels for seed production (SARH, 1987). Within a disease management scheme, the use of resistant wheat cultivars is the best control option, which would also contribute to reduce the possibilities of introduction of the disease into other areas where karnal bunt has not been established. The reaction to karnal bunt inoculation of several species of *Triticum* have been evaluated since the 1940's (Bedi *et al.*, 1949; Singh *et al.*, 1986; 1988). *Triticum aestivum* is the species most affected by the disease; under artificial inoculation some lines may show more than 50% infected grain (Fuentes-Dávila *et al.*, 1992; 1993; 2018a; 2019; 2020); therefore, it is important to keep evaluating the new advanced lines and wheat cultivars. The objective of this

work was to evaluate the reaction of one thousand and ninety two advanced bread wheat lines to inoculation with *Tilletia indica* in the field.

## 2. MATERIALS AND METHODS

One thousand and ninety two advanced bread wheat lines were evaluated for their reaction to *Tilletia indica*, artificially inoculated in the field during the crop season fall-winter 2015-2016, at the Norman E. Borlaug Experimental Station in the Yaqui Valley, Sonora, Mexico, located in block 910 (27° 22'04.64" latitude north and 109° 55'28.26" longitude west, 37 masl). The region has a warm climate [BW (h)] and extreme heat according to Koppen's classification modified by García (1988). Sowing dates were November 12 and 19, 2015, using 8 g of seed for a row 0.7 m long on a bed with two rows in a clay soil with pH 7.8. For the agronomic management, INIFAP's technical recommendations were followed (Figueroa-López *et al.*, 2011). The inoculum was prepared by the isolation of teliospores from infected grains after shaking them in a water+tween solution, followed by centrifugation in 0.5% sodium hypochlorite, and plating on 2% water-agar Petri plates. After teliospore germination, fungal colonies were transferred and multiplied on potato-dextrose-agar. Inoculations were performed by injecting 1 mL of an allantoidsporidial suspension (10,000/mL) during the boot stage in five spikes from each line (Fig. 2). The use of an automatic mist spray-irrigation system five times a day for 20 min each time, provided high humidity in the area (Fig. 3). In order to protect the inoculated plants from bird damage, a plastic anti-bird net system was installed covering completely the experimental plots. Inoculations started on January 4 and ended on March 23, 2016, for a total of 33 inoculation dates. Inoculated spikes were collected in paper bags and threshed by hand, then, the counting of healthy and infected grains was done visually to determine the percentage of infection. The experimental germplasm originated from the joint project between the Global Wheat Program of the International Maize and Wheat Improvement Center (CIMMYT) and the National Institute for Forestry, Agriculture and Livestock Research in Mexico (INIFAP).



**Figure 2.** Teliospore germination, producción of primary and secondary sporidia, and inoculation by injection during the boots tage of the wheat plant.

### 3. RESULTS AND DISCUSSION

The range of the percentage of infection of the advanced lines in the first date was 0-75.18% with a mean of 17.89%; the infection categories in this date were: 25 lines did not show any infected grains, 66 fell in the 0.1-2.5% category, 63 in the 2.6-5.0%, 165 in the 5.1-10.0%, 592 in the 10.1-30.0% category, and 181 showed an infection percentage greater than 30.0%. In this date, lines with less than 10% comprise 29.21% of the group which would offer an acceptable reaction to the inoculation with *Tilletia indica*, the causal agent of the disease.

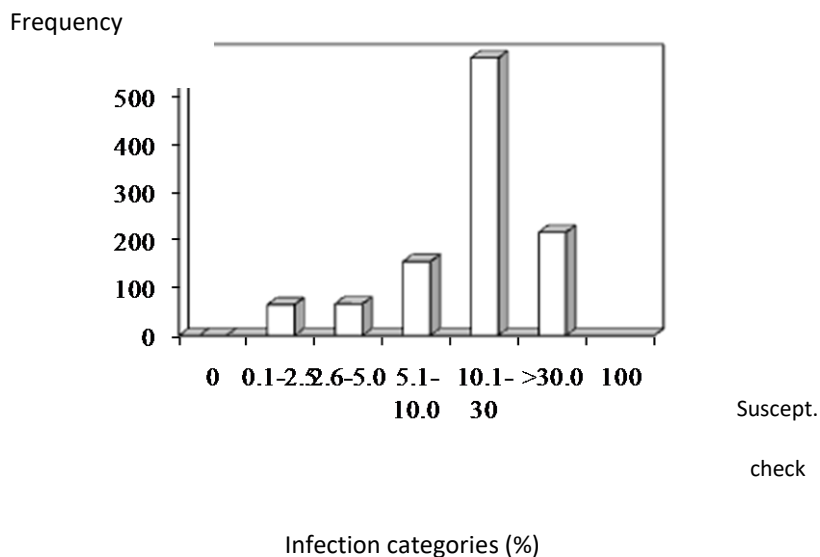


**Figure 3.** Mist-irrigation and anti-bird net in the experimental area, where bread wheat germplasmise valuated for their reaction to artificial inoculation with *Tilletia indica*.

The range of the percentage of infection of the advanced lines in the second date was 0-83.45% with a mean of 21.45%; the infection categories in this date were: 23 lines did not show any infected grains, 37 fell in the 0.1-2.5% category, 74 in the 2.6-5.0%, 147 in the 5.1-10.0%, 532 in the 10.1-30.0% category, and 279 showed an infection percentage greater than 30.0%. In this date, lines with less than 10% comprise 25.73% very similar to the first date. In the overall results (mean of the two dates), 65 lines fell into the 0.1-2.5% infection category (37 lines had less than 2.5% infection in both dates), 67 within 2.6-5.0%, 156 within 5.1-10.0%, 586 within 10.1-30%, and 218 with more than 30% infection (Fig. 4). Out of the 37 lines that had less than 2.5%

infection in both dates, there were 10 lines that had less than 1.0% infection in both dates and 4 with less than 0.5% (Table 1). The range of infection of the susceptible check KBSUS was 69.81% on January 27, 2016, to 100% on February 4, 8, 15, 16, and 29, and on March 2, 4, 7, 11, and 23, with a mean of 92.53%. The mean of the three highest percentage of infection of the susceptible check KBSUS was 100%.

Since the late 80's and beginning of the 90's, in the Wheat Program of CIMMYT, the testing of experimental germplasm, introductions and *Triticum* species, high levels of resistance were detected not only in durum and triticale, but also in several *Aegilops* spp. (Warhamet *al.*, 1986; Rajaramet *al.*, 1991). Therefore, interspecific hybrids between *Triticumturgidum* x *Triticumtauschii*(Coss.) Schmalh. (syn. *Aegilopssquarrosa*L.) were made since 1989 with the objective to incorporate resistance into bread wheat from the best synthetic hexaploids with good agronomic characteristics and cytogenetic stability (Villarealet *al.*, 1995; Mujeeb-Kaziet *al.*, 2006). Synthetic hexaploid wheats (SHW) are products of crosses involving tetraploid cultivars (*Triticumturgidum*, 2n=28, AABB) and diploid goat grass [*Aegilopstauschii*Coss. (syn. *Aegilopssquarrosa*, *Triticum*



**Figure 4.** Karnal bunt infection categories (%) for 1,092 bread wheat advanced lines artificially inoculated in the field in two dates during the 2015-2016 crop season, at the Norman E. Borlaug Experimental Station in the Yaqui Valley, Sonora, Mexico. The average of the three highest scores of infection of the susceptible check KBSUS was 100%.

*tauschii*], 2n=14, DD), followed by chromosome doubling of F<sub>1</sub> hybrids (Plamenov and Spetsov, 2011). They are genomically amphidiploids (2n=42, AABBDD), combining the genomes of their

parents. SHW exhibit resistance to some biotic factors, mainly fungi and insects, and may serve as valuable resources in wheat breeding. They can be involved in backcrosses with elite bread wheat cultivars to produce lines with superior quality, disease resistance and yield. Out of the 1,092 lines subject of the present evaluation, 22.06% carry in their pedigree a synthetic with the potential to be a contributor for the resistance to karnal bunt of a particular bread wheat line. In the case of CROC\_1/AEGILOPS SQUARROSA(224) with 39 lines, only 12.8% of those lines were in the resistant category 0.1-5.0% (Fuentes-Dávila and Rajaram, 1994); for CROC\_1/AE.SQUARROSA(213) with 39 lines, 15.4% were resistant; for CROC\_1/AE.SQUARROSA(205) with 23 lines, 4.3% were resistant; for ALTAR84/AE.SQUARROSA(221) with 8 lines, 12.5% were resistant; for ALTAR84/AE.SQUARROSA(205) with 9 lines, 22.2% were resistant; for AE.SQUARROSA(TAUS) with 106 lines, 15.1% were resistant; for CHEN/AE.SQUARROSA(TAUS) with 4 lines, 25% were resistant. There were no resistant lines for AE.SQUARROSA(372), AE.SQUARROSA(409), and AE.SQUARROSA(498.). Other evaluations of elite bread wheat lines and synthetic hexaploid wheat derivatives like CROC\_1/Ae. tauschii (205)//KAUZ/3/Attila have shown excellent resistance where the line did not show any infected grains; other lines with similar pedigree had between 0.1 to 2.5% (Fuentes-Dávila and Singh, 2006). In the case of the resistant line to karnal bunt MUNAL#1(Fuentes-Dávila *et al.*, 2014) which is used routinely as check as well as in combination with other lines (Fuentes-Dávila *et al.*, 2018b), out of 37 lines with MUNAL#1 in their pedigree, 8.1% showed resistance to the disease. In this group of 1,092 advanced bread wheat lines evaluated during the crop season 2015-2016, there were 132 which must be evaluated in the following season in order to verify their resistance shown to *Tilletia indica*, since they may be prospects for commercial release, or at least be part of the progenitors used in bread wheat breeding programs.

**Table 1. Bread wheat advanced lines that were artificially inoculated in the field with *Tilletia indica*, and showed less than 1% infection in two dates, during the cropseason 20215-2016, at the Norman E. Borlaug Experimental Station in the Yaqui Valley, Sonora, Mexico.**

No.	Pedigree and selection history
Lines with less than 0.5%	
1	SAUAL/3/SW89.3064//CMH82.17/SERI/4/SAUAL/5/PBW343*2/KUKUNA*2//FRTL/PIFED/6/SAUAL/KRONSTAD F2004  CMSS11Y01091T-099TOPM-099Y-099M-099NJ-099NJ-16WGY-0B
2	MEX94.27.1.20/3/SOKOLL//ATTILA/3*BCN/5/CHRZ//BOW/CROW/3/WBLL1/4/CROC_1/AE.SQUARROSA (213)//PGO  CMSA11Y00402S-099Y-099M-099NJ-099NJ-18WGY-0B
3	TACUPETOF2001/6/CNDO/R143//ENTE/MEXI_2/3/AEGILOPSSQUARROSA(TAUS)

/4/WEAVER/5/PASTOR/7/ROLF07/8/KACHU #1/KIRITATI//KACHU

CMSS11B00310S-099M-099NJ-099NJ-9RGY-0B

- 4 BABAX/LR42//BABAX/3/ER2000/6/POTCH  
93/4/MILAN/KAUZ//PRINIA/3/BAV92/5/MILAN/KAUZ//PRINIA/3/BAV92

CMSA11Y00305S-099Y-099M-099NJ-099NJ-1RGY-0B

Lines with less than 1%

- 1 SEHER 06/3/PBW343\*2/KUKUNA//TECUE #1

CMSS11Y00430S-099Y-099M-099NJ-099NJ-10WGY-0B

- 2 WBLL1\*2/4/YACO/PBW65/3/KAUZ\*2/TRAP//KAUZ/5/SAUAL/6/2\*KINGBIRD  
#1//INQALAB 91\*2/TUKURU

CMSS11Y00822T-099TOPM-099Y-099M-099NJ-099NJ-8WGY-0B

- 3 UP2338\*2/4/SNI/TRAP#1/3/KAUZ\*2/TRAP//KAUZ/5/MILAN/KAUZ//CHIL/CHUM18/6/UP23  
38\*2/4/SNI/TRAP#1/3/KAUZ\*2/TRAP//KAUZ\*2/7/PBW343\*2/KUKUNA\*2//FRTL/PIFED

CMSS11Y00993T-099TOPM-099Y-099M-099NJ-099NJ-25WGY-0B

- 4 SAUAL/3/SW89.3064//CMH82.17/SERI/4/SAUAL/5/PBW343\*2/KUKUNA\*2//FRTL/PIFED/6/  
SAUAL/KRONSTAD F2004

CMSS11Y01091T-099TOPM-099Y-099M-099NJ-099NJ-25WGY-0B

- 5 MUNAL #1/3/ATTILA\*2/PBW65//MURGA

CMSS11B00006S-099M-0SY-9M-0WGY

- 6 WBLL1/3/STAR//KAUZ/STAR/4/BAV92/RAYON/5/TRAP#1/BOW/3/VEE/PJN//2\*TUI/4/BAV  
92/RAYON/6/WAXWING/KIRITATI\*2/3/C80.1/3\*BATAVIA//2\*WBLL1/7/PBW65/2\*PASTO  
R//TACUPETOF2001\*2/BRAMBLING/3/TACUPETO F2001\*2/

BRAMBLING

CMSS11B00716T-099TOPY-099M-099NJ-099NJ-35WGY-0B

- 7 ATTILA\*2/PBW65//MUU#1/3/FRANCOLIN#1/4/ATTILA\*2/PBW65\*2//KACHU/5/  
ATTILA\*2/PBW65//MUU #1/3/FRANCOLIN#1

CMSS11B00899T-099TOPY-099M-099NJ-099NJ-13WGY-0B

- 8 KACHU#1/6/NG8201/KAUZ/4/SHA7//PRL/VEE#6/3/FASAN/5/MILAN/KAUZ/7/KACHU/8/KZ  
A//WH 542/2\*PASTOR/3/BACEU #1/9/KACHU #1/KIRITATI//KACHU

CMSS11B00916T-099TOPY-099M-099NJ-099NJ-2WGY-0B

- 9 PASTOR/3/URES/JUN//KAUZ/4/WBLL1/5/GKARON/AGSECO7846//2180/4/2\*  
MILAN/KAUZ//PRINIA/3/BAV92

CMSA11Y00276S-099Y-099M-099NJ-099NJ-13WGY-0B

- 10 BAV92//IRENA/KAUZ/3/HUITES/4/FN/2\*PASTOR/5/BAV92//IRENA/KAUZ/3/HUITES/8/TA  
CUPETO F2001/6/CNDO/R143//ENTE/MEXI\_2/3/AEGILOPS SQUARROSA  
(TAUS)/4/WEAVER/5/PASTOR/7/ROLF07/9/BAV92//IRENA/KAUZ/3/HUITES\*2/4/GONDO/  
TNMU

CMSS11Y01014T-099TOPM-099Y-099M-099NJ-099NJ-3RGY-0B

#### 4. CONCLUSION

The range of the mean percentage of infection of one thousand and ninety two advanced bread wheat lines evaluated for their reaction to karnal bunt artificial inoculation in two sowing dates, during the crop season fall-winter 2015-2016, was 0.18-70.64% with a mean of 19.67%. There were 4 lines that in both dates consistently showed a percentage of infection below 0.5%: SAUAL

/3/SW89.3064//CMH82.17/SERI/4/SAUAL/5/PBW343\*2/KUKUNA\*2//FRTL/PIFED/6/SAUAL/KRONSTADF2004, MEX94.27.1.20/3/SOKOLL//ATTILA/3\*BCN/5/CHRZ//BOW/CROW/3/WBLL1/4/CROC\_1/AE.SQUARROSA(213)//PGO, TACUPETOF2001/6/CNDO/R143//ENTE/MEXI\_2/3/AEGILOPSSQUARROSA(TAUS)/4/WEAVER/5/PASTOR/7/ROLF07/8/KACHU#1/KIRITATI//KACHU, and BABAX/LR42//BABAX/3/ER2000/6/POTCH93/4/MILAN/KAUZ//PRINIA/3/BAV92/5/MILAN/KAUZ//PRINIA/3/BAV92; and 33 below 2.5%. Lines with the highest percentage of infection were: TUKURU//BAV92/RAYON/3/WBLL1\*2/BRAMBLING/8/TACUPETOF2001/6/CNDO/R143//ENTE/MEXI\_2/3/AEGILOPS SQUARROSA(TAUS)/4/WEAVER/5/PASTOR/7/ROLF07/9/TUKURU//BAV92/RAYON\*2/3/JUCHI with 83.45 and TACUPETOF2001/6/CNDO/R143//ENTE/MEXI\_2/3/AEGILOPS SQUARROSA(TAUS)/4/WEAVER/5/PASTOR/7/ROLF07\*2/8/SUP152/MUU with 83.10% in the second date. The average of the three highest levels of infection of the susceptible check was 100%.

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