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 row0.7 m long on a bed with two rows. Inoculations in the field were performed by injecting 1 mL of an allantoid sporidal 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/SUAUL/5/PBW343*2/KUKUNA*2//FRTL/PI FED/6/SUAUL/KRONSTADF2004,MEX94.27.1.20/3/SOKOLL//ATTILA/3*BCN/5/CHRZ//B OW/CROW/3/WBLL1/4/CROC_1/AE.SQUARROSA(213)//PGO,TACUPETOOF2001/6/CNDO/ R143//ENTE/MEXI_2/3/AEGILOPSSQUARROSA(TAUS)/4/WEAVER/5/PASTOR/7/ROLF0 7/8/KACHU#1//KIRITATT//KACHU, and BABAX/LR42//BABAX/3/ER2000/6/POTCH93/4/MILAN/KAUZ//PRINIA/3/BAV92/5/MILA N/KAUZ//PRINIA/3/BAV92. Lines with the highest percentage of infection were: TUKURU//BAV92/RA YON/3/WBLL1*2/BRAMBLING/8/TACUPETOOF2001/6/CNDO/R143/ /ENTE/MEXI_2/3/AEGILOPSSQUARROSA(TAUS)/4/WEAVER/5/PASTOR/7/ROLF07/9/T UKURU//BAV92/RA YON*2/3/JUCHI with 83.45 and TACUPETOOF2001/6/CNDO/R143//ENTE/MEXI_2/3/AEGILOPSSQUARROSA(TAUS)/4/WE AVER/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, Triticumaestivum, 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 (*Triticumaestivum*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 (Munjal,
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 seed 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.

Since teliospores of the fungus are resistant to physical and chemical factors (Krishna and Singh, 1982; Zhang et al., 1984; Smilanick et 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 (Bediet 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

**Figure 1.** Wheat spike with infected grains with *Tilletia indica*. The grain shows a characteristic lesion caused by the pathogen.
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 de primary and secondary sporidia, and inoculation by injection during the boot stage of the wheat plant.](image-url)
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.

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. (Warham et al., 1986; Rajaram et 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*), 2n=14, DD), followed by chromosome doubling of F₁ hybrids (Plamenov and Spetsov, 2011). They are genomically amphidiploids (2n=42, AABBDD), combining the genomes of their

![Infection categories (%)](image)

**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 F₁ 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 crop season 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/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   | TACUPETO2001/6/CNDO/R143//ENTE/MEXI_2/3/AEGILOPSSQUARROSA(TAUS) | |

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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/SUAL /KRONSTAFD2004, MEX94.27.1.20/3/SOKOLL//ATTILA/3*BCN/5/CHRZ//BOW/CROW /3/WBLL1/4/CROC_1/AE.SQUARROSA(213)//PGO, TACUPETO 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

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