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Reaction of wheat germplasm to Karnal bunt artificial inoculation in the field during the 2017-2018 crop season

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Abstract

Fifteen advanced bread wheat lines (ABWL) candidates for release and commercial cultivars Borlaug 100 F2014, CIANO M2018, RSM Norman F2008, Onavas F2009, and Roelfs F2007; thirty two advanced durum wheat lines (ADWL) candidates for release and commercial cultivars Baroyeca Oro C2013, CIRNO C2008, and Quetchehueca Oro C2013; and fifty two elite advanced bread wheat lines (EABWL) were evaluated for their reaction to *Tilletia indica*, artificially inoculated in the field during the crop season 2017-2018, at the Norman E. Borlaug Experimental Station in Sonora, Mexico. Sowing dates were November 14 and 24, 2017, and December 1 as a third date for the EABWL. Inoculations were performed by injection during the boot stage. The percentage of infection was determined by counting healthy and infected grains. The range of infection of the ABWL and commercial cultivars in the first date was 1.0-53.6 % with a mean of 24.4 %, and 0-65.3 % with a mean of 33.6 % in the second date. The range of infection of the ADWL and commercial cultivars in the first date was 0-22.9 % with a mean of 2.3 %, while 0-21.9 % with a mean of 5.7 % in the second date. The range of infection of the EABWL in the first date was 0.37-42.6 % with a mean of 9.9 %, 0-39.0 % with a mean of 10.3 % in the second date, and 0.31-47.7 % with a mean of 13.3 % in the third date; 10 lines of this group showed an average lower than 5 % infection.

Keywords: Bread wheat; *Triticum aestivum*; Durum wheat; *Triticum durum*; Karnal bunt; Partial bunt; *Tilletia indica*

1. Introduction

Karnal bunt, also referred to as partial bunt of wheat, is caused by the fungus *Tilletia indica* Mitra (syn. *Neovossia indica* Mundkur). The disease affects bread wheat (*Triticum aestivum* L.), durum wheat (*T. turgidum* L.) and triticale (X *Triticosecale* Wittmack) [1,2]. Karnal bunt has been reported in several countries worldwide, including India, Mexico, Pakistan, Nepal, Brazil, the United States of America, Iran, the Republic of South Africa, and Afghanistan [1,3-10]. The pest risk analysis (PRA) conducted under the auspices of the European Commission (EC) identifies Karnal bunt as a serious disease and classifies *T. indica* as a 1/A1 harmful organism [11]; it represents a severe threat to global food security[12], and as a seed borne pathogen it threatens wheat trade between countries [13]. The fungus typically infects

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only a portion of the grain within a spike, and not all spikes on an infected plant are affected [14] (Figure 1). Sin some cases, however, grains may be completely destroyed. Although the pathogen is capable of penetrating the embryo, this does not always result in its death [15,16]. Partially infected grains can still produce healthy plants; nevertheless, several studies have demonstrated that germination rates decline with increasing levels of infection [17-19]. Severely infected grains may lose viability or display abnormal germination [18]. In contrast, Fuentes-Dávila *et al*. [20] reported that heavily infected grains with intact embryos produced the greatest number of tillers.

Figure 1 Characteristics symptoms of infected wheat grains with *Tilletia indica*

Management of *T. indica* is difficult due to the high resistance of its teliospores to physical and chemical factors [21-23]. However, fungicide applications at the wheat flowering stage can provide effective chemical control [24-31]. The generation of resistant wheat cultivars remains the most effective and sustainable control method in an integrated disease management strategy. By minimizing teliospore production and movement, the use of resistant germplasm significantly lowers the risk of introducing the disease into new, currently non-infested wheat-growing zones. The reaction to Karnal bunt inoculation of several species of *Triticum* have been evaluated since the 1940's [14,32,33]. *Triticum aestivum* is the wheat species most affected by the disease. Under artificial inoculation conditions, certain lines can exhibit infection levels exceeding 50 % infected grain [34-37], therefore, it is essential to continually assess newly developed advanced lines and commercial cultivars. Ongoing evaluation ensures the early identification of resistant materials, support breeding programs aimed at improving disease resilience, and helps maintain effective protection of wheat production in regions at risk of Karnal bunt. The objective of this work was to evaluate the reaction of an elite group of bread wheats as well as a group of advanced lines, and a group of durum wheat under artificial field inoculation with *Tilletia indica*.

2. Materials and methods

Fifteen advanced bread wheat lines (ABWL) candidates for commercial release and commercial cultivars Borlaug 100 F2014 [38], CIANO M2018 [39], RSM Norman F2008 [40], Onavas F2009 [41], and Roelfs F2007 [42], thirty two advanced durum wheat lines (ADWL) candidates for commercial release and commercial cultivars Baroyeca Oro C2013 [43], CIRNO C2008 [44], and Quetchehueca Oro C2013 [45], and fifty two elite advanced bread wheat lines (EABWL) were evaluated for their reaction to *Tilletia indica*, artificially inoculated in the field during the crop season fall-winter 2017-2018, 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 [46]. Sowing dates were November 14 and 24, 2017 for ABWL and ADWL, and for the EABWL the same dates plus December 1, 2017, as a third sowing date. Eight g of seed were sown in a row 0.7 m long on a bed with two rows in a clay soil with pH 7.8. The agronomic management followed the technical recommendations by Figueroa-López *et al*. [47]. Inoculum was prepared as described by Fuentes-Bueno and Fuentes-Dávila [48]: Teliospores were isolated 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 allantoid sporidial suspension (10,000/mL) during the boot stage in five spikes from each line and cultivar (Figure 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. In order to protect the inoculated plants from bird damage, a plastic anti-bird net system was installed covering completely the experimental plots (Figure 3).

Figure 2 Inoculation by injection during the boot stage of the wheat plant

Figure 3 Mist-irrigation and anti-bird net in the experimental area

Inoculations started on January 2 and ended on April 2, 2018, for a total of 32 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. A known susceptible wheat line (KBSUS) was used as a check. The groups of lines 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).

3. Results and discussion

3.1. Advanced bread wheat lines (ABWL) candidates for commercial release.

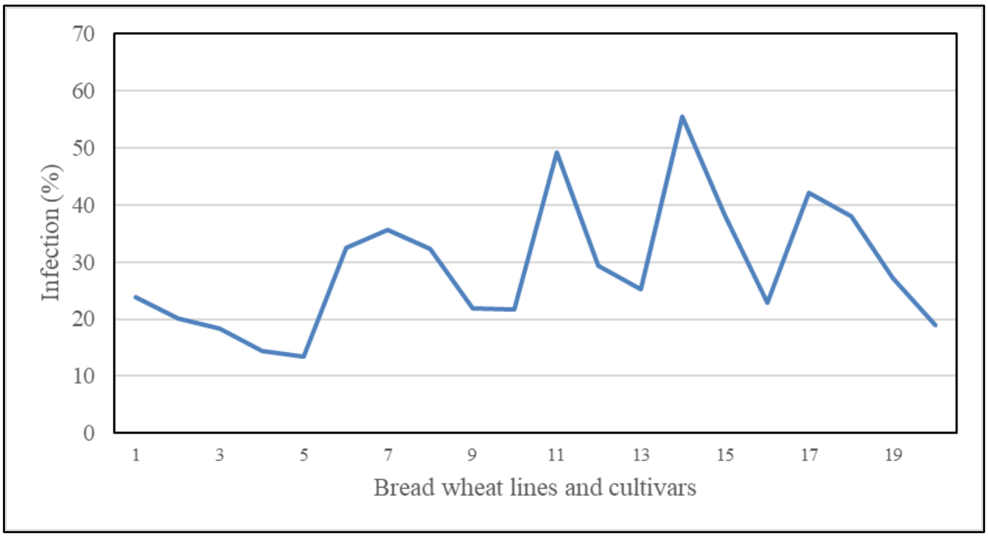

Figure 4 Average infection of 15 bread wheat advanced lines and 5 commercial cultivars, artificially inoculated in the field in two dates during the 2017-2018 crop season, at the Norman E. Borlaug Experimental Station in the Yaqui Valley, Sonora, Mexico. 1= Roelfs F2007, 2= RSM Norman F2008, 3= Onavas F2009, 6= Borlaug 100 F2014, 20= CIANO M2018

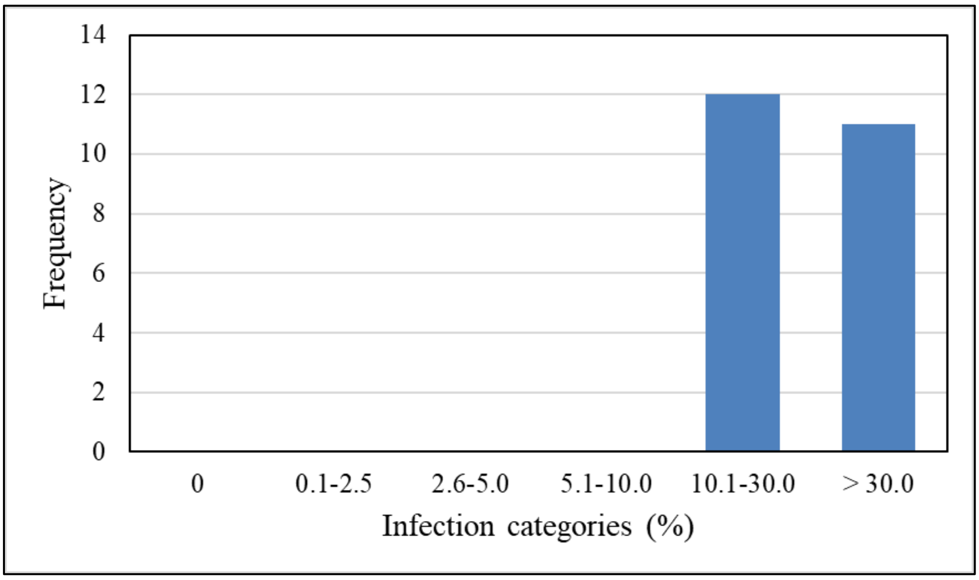

Figure 5 Karnal bunt infection categories (%) for 15 bread wheat advanced lines and 5 commercial cultivars, artificially inoculated in the field in two dates during the 2017-2018 crop season, at the Norman E. Borlaug Experimental Station in the Yaqui Valley, Sonora, Mexico

The range of the percentage of infection of the ABWL for commercial release in the first date was 1.0-53.6 % with a mean of 24.4 %; the infection categories in this date were: 2 lines and cultivar Onavas F2009 fell in the 0.1-2.5 % category, 3 lines and cultivar Roelfs F2007 fell in the 5.1-10.0 % category, 2 lines and cultivars CIANO M2018 and RSM Norman F2008 fell in the 10.1-30.0 % category, and 8 lines and cultivar Borlaug 100 F2014 fell in the > 30.1 % infection category. The range of infection in the second date was 0-65.3 % with a mean of 33.6 %. The infection categories in this date were: 5 lines and cultivars Borlaug 100 F2014, CIANO M2018, and RSM Norman F2008 fell in the 10.1-30.0 % category, and 10 lines and cultivars Onavas F2009 and Roelfs F2007 fell in the > 30.1 % infection category. The average infection of lines and cultivars in both dates is shown in Figure 4. The overall infection categories of this group was as follows: 8 lines and cultivars CIANO M2018, RSM Norman F2008, Onavas F2009, and Roelfs F2007 fell in the 10.1-30.0 % category, and 7 lines and cultivar Borlaug 100 F2014 fell in the > 30.1 % infection category. In this group, none of the lines showed less than 10 % infection, therefore, they would not offer an acceptable reaction to the inoculation with *Tilletia indica*, the causal agent of the disease (Figure 5).

3.2. Advanced durum wheat lines (ADWL) candidates for commercial release

The range of the percentage of infection of the ADWL in the first date was 0-22.9 % with a mean of 2.3 %; the infection categories in this date were: 13 lines and cultivars Baroyeca Oro C2013 and CIRNO C2008 did not show any infected grains, 11 lines fell in the 0.1-2.5 % category, 4 lines fell in the 2.6-5.0 %, 3 lines in the 5.1-10.0 %, and 1 line and cultivar Quetchehueca Oro C2008 in the 10.1-30.0 % infection category. The range of infection in the second date was 0-21.9 % with a mean of 5.7 %. The infection categories in this date were: 1 line did not show any infected grains, 11 lines and cultivar Baroyeca Oro C2013 fell in the 0.1-2.5 % category, 4 lines and cultivar CIRNO C2008 fell in the 2.6-5.0 %, 10 lines and cultivar Quetchehueca Oro C2008 in the 5.1-10.0 %, and 6 lines in the 10.1-30.0 % infection category. The average infection of lines and cultivars in both dates is shown in Figure 6.

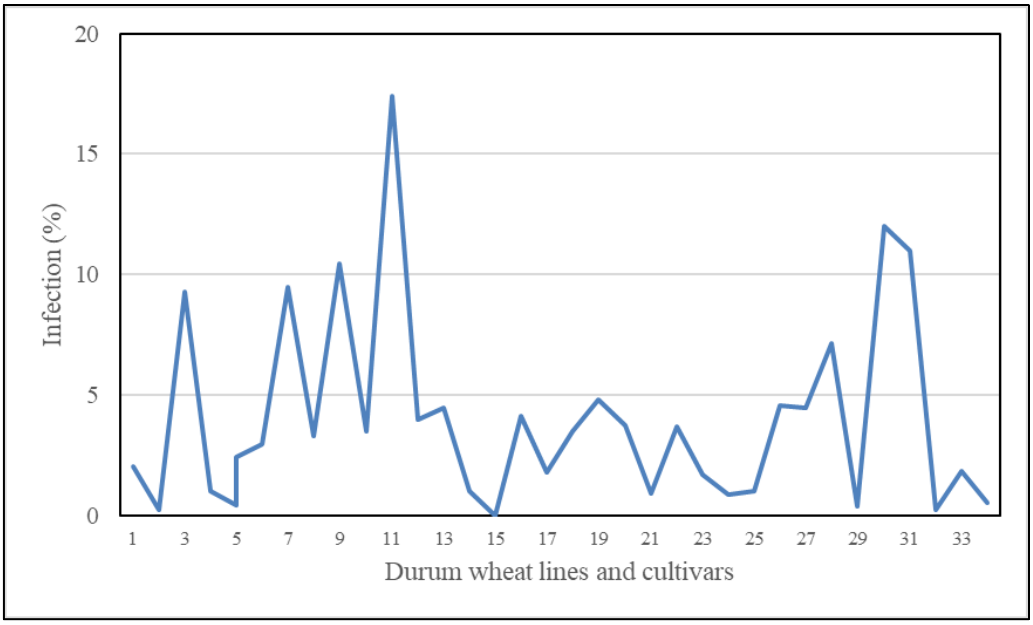

Figure 6 Average infection of 32 durum wheat advanced lines and 3 commercial cultivars, artificially inoculated in the field in two dates during the 2017-2018 crop season, at the Norman E. Borlaug Experimental Station in the Yaqui Valley, Sonora, Mexico. 1= CIRNO C2008, 2= Baroyeca Oro C2013 3= Quetchehueca Oro C2013

The overall infection categories of this group was as follows: 1 line did not show any infected grains, 13 lines and cultivars Baroyeca Oro C2013 and CIRNO C2008 fell in the 0.1-2.5 % infection category, 12 lines in the 2.6-5.0 %, 2 lines and cultivar Quetchehueca Oro C2013 in the 5.1-10.0 %, and 4 lines in the 10.1-30.0 % infection category. In this group, 28 lines with less than 10 % infection comprise 87.5 % of the group of lines which would offer an acceptable reaction to the inoculation with *Tilletia indica*. Even better, 81.2 % offer genotypes with resistance to the pathogen (Figure 7).

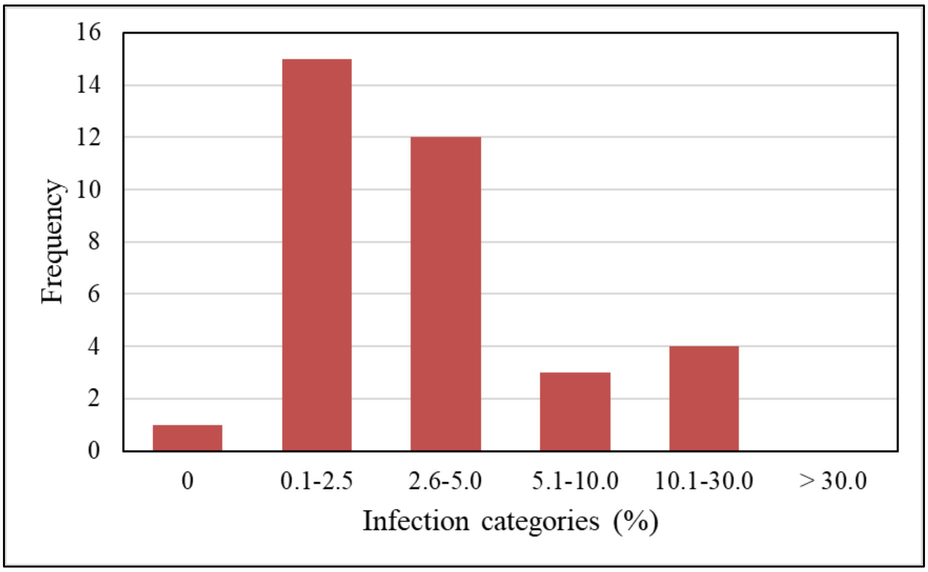

Figure 7 Karnal bunt infection categories (%) for 32 durum wheat advanced lines and 3 commercial cultivars artificially inoculated in the field in two dates during the 2017-2018 crop season, at the Norman E. Borlaug Experimental Station in the Yaqui Valley, Sonora, Mexico

3.3. Elite advanced bread wheat lines (EABWL).

The range of the percentage of infection of the EABWL in the first date was 0.37-42.6 % with a mean of 9.9 %; the infection categories in this date were: 7 lines fell in the 0.1-2.5 % infection category, 5 lines in the 2.6-5.0 % category, 17 lines in the 5.1-10.0 % category, 21 lines in the 10.1-30.0 % category, and 2 lines fell in the > 30.1 % infection category. The range of infection in the second date was 0-39.0 % with a mean of 10.3 %. The infection categories in this date were: 3 lines did not show any infected grains, 2 lines fell in the 0.1-2.5 % category, 6 lines in the 2.6-5.0 % category, 20 lines in the 5.1-10.0 % category, 19 lines in the 10.1-30.0 % category, and 2 lines fell in the > 30.1 % infection category. The range of infection in the third date was 0.31-47.7 % with a mean of 13.3 %. The infection categories in this date were: 4 lines fell in the 0.1-2.5 % category, 1 line fell in the 2.6-5.0 % category, 17 lines in the 5.1-10.0 % category, 27 lines in the 10.1-30.0 % category, and 3 lines fell in the > 30.1 % infection category. The average infection of lines and cultivars in the three dates is shown in Figure 8.

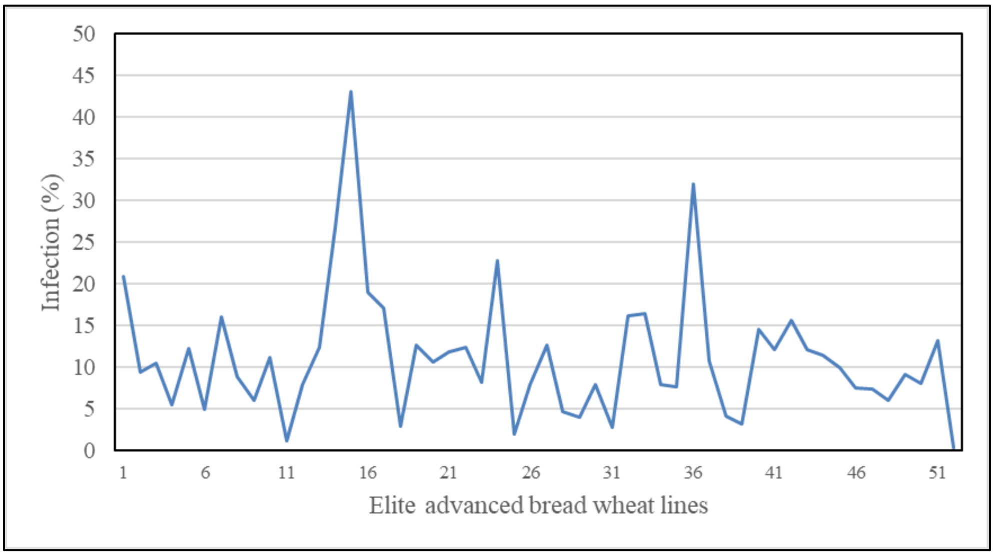

Figure 8 Average infection of 52 elite advanced bread wheat lines, artificially inoculated in the field in three dates during the 2017-2018 crop season, at the Norman E. Borlaug Experimental Station in the Yaqui Valley, Sonora, Mexico

The overall infection categories of this group was as follows: 3 lines fell in the 0.1-2.5 % category, 7 lines fell in the 2.6-5.0 % category, 16 lines fell in the 5.1-10.0 % category, 24 lines in the 10.1-30.0 %, and 2 lines in the > 30.1 % infection category. In this group, 26 lines with less than 10 % infection comprise 50 % of the group which would offer an acceptable reaction to the inoculation with *Tilletia indica*. Those 26 lines should further tested for grain yield and quality to determine their suitability for commercial cultivars (Figure 9).

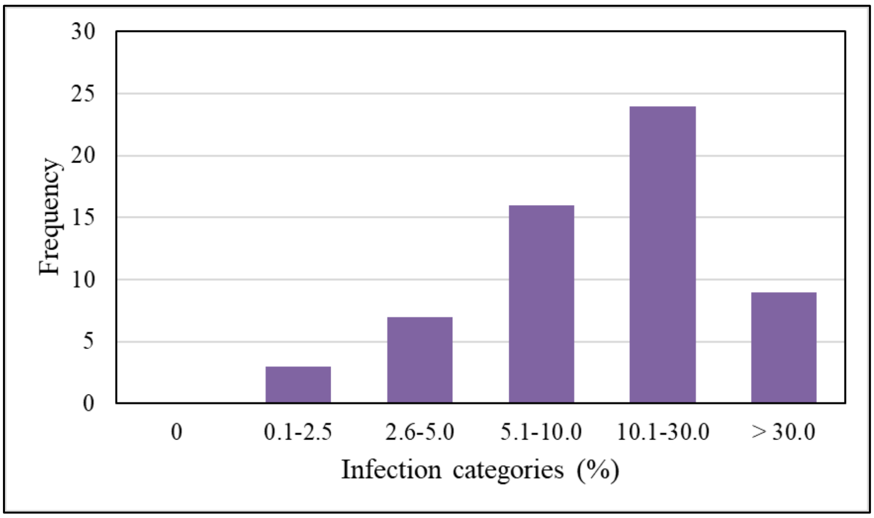

Figure 9 Karnal bunt infection categories (%) for 52 elite advanced bread wheat lines, artificially inoculated in the field in three dates during the 2017-2018 crop season, at the Norman E. Borlaug Experimental Station in the Yaqui Valley, Sonora, Mexico

The range of infection of the susceptible check KBSUS was 53.4 to 95.0 % with an average of 86.1 %. From the ABWL group, the line with the best performance for the inoculation with *Tilletia indica* was PBW343//CAR422/ANA/3/ELVIRA with an average percentage of infection of 14.42. In the case of the ADWL group, the line WBDTBO/10/PLATA_10/6/MQUE/4/USDA573//QFN/AA_7/3/ALBA-D/5/AVO/HUI/7/PLATA_13/8/THKNEE_11/9/CHEN/ALTAR84/3/HUI/POC//BUB/RUFO/4/FNFOOT/11/MÂALI/10/ALTAR84/CMH82A.1062//ALTAR84/3/YAZI_10/4/SNITAN/9/USDA595/3/D67.3/RABI//CRA/4/ALO/5/HUI/YAV_1/6/ARDENTE did not show any infected grains in both dates, 13 lines and cultivars CIRNO C2008 and Baroyeca Oro C2013 fell within the 0.1-2.5 % infection category, and 12 lines fell within the 2.6-5.0 % infection category. In the case of the EABWL group, 3 lines fell within the 0.1-2.5 % infection category, and 7 within the 2.6-5.0 % category (Table 1).

Table 1 Elite advanced bread wheat lines that were artificially inoculated in the field with *Tilletia indica*, and showed less than 5 % average infection in three dates, during the crop season 2017-2018, at the Norman E. Borlaug Experimental Station in the Yaqui Valley, Sonora, Mexico

No.	Pedigree and selection history
	Lines with less than 2.5 %
11	MUNAL #1/FRANCOLIN #1*2/3/ATTILA*2/PBW65//MURGA CMSS12Y00701T-099TOPM-099Y-099M-0SY-13M-0WGY
25	BAJ #1/3/SUP152//WBLL1*2/BRAMBLING CMSS12B00004S-099M-0SY-9M-0WGY
52	PREMIO//PI 610750/PIFED*2/3/KSW/SAUAL//SAUAL
	CMSS12B01153T-099TOPY-099M-099NJ-099NJ-3RGY-0B
Lines within 2.51-5.0 % infection	
6	FITIS/3/KACHU #1/KIRITATI//KACHU CMSS12Y00107S-099Y-099M-0SY-50M-0WGY
18	BAJ #1/3/KIRITATI//ATTILA*2/PASTOR*2/4/MUTUS*2/TECUE #1

	CMSS12Y00928T-099TOPM-099Y-099M-0SY-3M-0WGY
28	VILLAJUAREZF2009/6/ATTILA/3*BCN//BAV92/3/PASTOR/4/TACUPETO F2001*2/BRAMBLING/5/PAURAQ CMSS12B00006S-099M-0SY-31M-0WGY
29	MUNAL #1/FRANCOLIN #1/4/KIRITATI//ATTILA*2/PASTOR/3/AKURI CMSS12B00079S-099M-0SY-6M-0WGY
31	KACHU/BECARD//WBLL1*2/BRAMBLING/4/FRET2/TUKURU//FRET2/3/MUNAL #1
	CMSS12B00312S-099M-0SY-5M-0WGY
38	FITIS*2/3/ATTILA*2/PBW65*2//MURGA
	CMSS12B00671T-099TOPY-099M-0SY-17M-0WGY
39	FITIS*2/3/ATTILA*2/PBW65*2//MURGA
	CMSS12B00671T-099TOPY-099M-0SY-18M-0WGY

Sources of resistance to Karnal bunt have been identified in bread wheat and durum wheats, triticale, *Aegilops* spp., synthetics and derivatives of synthetic hexaploids [49-55]. The interspecific hybrids of *Triticum turgidum* x *Triticum tauschii* (Coss.) Schmalh. (syn. *Aegilops squarrosa* L.) were developed with the objective of transferring resistance into bread wheat from the best synthetic hexaploids, those combining strong agronomic traits with cytogenetic stability [52,53]. Synthetic hexaploid wheats (SHW) are produced by crossing tetraploid cultivars (*Triticum turgidum*, 2n = 28, AABB) and diploid goat grass [*Aegilops tauschii* Coss. (syn. *Aegilops squarrosa*, *Triticum tauschii*], 2n = 14, DD], followed by chromosome doubling of the resulting F_1 hybrids [56]. The resulting plants are amphidiploids (2n = 42, AABBDD) that combine the genomes of both parents. In the groups evaluated in the present study, only one EABWL carry a SHW (PVN/5/2*REH/HARE//2*BCN/3/CROC_1/AE.SQUARROSA(213)//PGO/4/HUITES*2/6/KFA/2* KACHU) with an overall percentage of infection of 10 %. As for the resistant bread wheat line to Karnal bunt MUNAL#1 [57], in one evaluation out of 37 lines carrying it in their pedigree, 8.1 % showed resistance to the disease, while in another evaluation, lines carrying MUNAL#1 in their pedigree, showed an average infection of 12.35 % [58]. In the present study, 3 lines in the ABWL group carry MUNAL#1 in their pedigree: BORL14*2/MUNAL#1, BORL14*2//MUNAL#1/FRANCOLIN#1, and STLN/MUNAL#1//2*BORL14, however, their average percentage of infection were 38.1, 22.9, and 19.0 %, respectively. In the EABWL group, 13 lines carry MUNAL#1 in their pedigree and exhibited a range of infection between 1.2 to 31.9 %. Eight lines exhibited less than 10 % infection and out of those 8, three showed less than 5 % (Table 1). Several molecular studies have mapped several QTLs associated with resistance to its causal agent [59-62], which provide valuable tools for wheat improvement. Integrating resistant germplasm with well-characterized QTLs into wheat breeding pipelines, facilitates the development of agronomically sound advanced lines and cultivars that combine yield potential with durable resistance, thereby benefiting farmers in regions where Karnal bunt continues to pose a significant production challenge.

4. Conclusion

The range of infection in the advanced bread wheat lines candidates for commercial release, and commercial cultivars in the first date was 1.0-53.6 % with a mean of 24.4 %, and 0-65.3 % with a mean of 33.6 % in the second date. In this group, none of the lines showed less than 10 % infection, therefore, they would not be suitable to become candidates for commercial release as cultivars.

The range of infection in the advanced durum wheat lines candidates for commercial release, and commercial cultivars in the first date was 0-22.9 % with a mean of 2.3 %, while 0-21.9 % with a mean of 5.7 % in the second date. In this group, 28 lines with less than 10 % infection comprise 87.5 % of the group of lines which would offer an acceptable reaction to the inoculation with *Tilletia indica*. Even better, 81.2 % offer genotypes with resistance to the pathogen.

The range of infection in the elite advanced bread wheat lines in the first date was 0.37-42.6 % with a mean of 9.9 %, 0-39.0 % with a mean of 10.3 % in the second date, and 0.31-47.7 % with a mean of 13.3 % in the third date. In this group, 26 lines with less than 10 % infection comprise 50 % of the group which would offer an acceptable reaction to the inoculation with *Tilletia indica*. Those 26 lines should further tested for grain yield and quality to determine their suitability for commercial release as cultivars.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare that No conflict of interest.

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