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RESEARCH ARTICLE

ARTIFICIAL FIELD INOCULATION OFBREAD WHEAT ADVANCED LINES WITH TILLETIAINDICA, CAUSAL AGENT OF KARNAL BUNT

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Abstract

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The reaction to *Tilletiaindica* of one thousand and ninety twobread wheat advanced lines were evaluated in the field during the crop season 2016-2017. Sowing in beds with two rows was carried out on November 11 and 24, 2016, using 8 g of seed. Five spikes per line were inoculated by injection with 1 mL of an allantoidsporidial suspension (10,000/mL) during the boot stage, and at maturity the percentage of infection was determined by counting healthy and infected grains. The range of infection in the first date was 0-88.83 with a mean of 31.81%, while in the second date it was 0-82.65% with a mean of 24.44%.The range of infection of the two dates was 0.46-83.71% with a mean of 28.12%.Sixteenlines showed a percentage of infection equal or below 5.0% in both dates, and out of those lines, the following five showed less than 2.5%: two sister lines of MUNAL#1/FRANCOLIN#1*2/3/ATTILA*2/PBW65//MURGA(CMSS12Y00701T-099TOPM-099Y-099M-0SY-13M-0WGY), MUNAL#1/FRANCOLIN#1*2/3/ATTILA*2/PBW65//MURGA (CMSS12Y00701T-099TOPM-099Y-099M-0SY-17M-0WGY), BAJ#1/3/KIRITATI//ATTILA*2/PASTOR*2/4/MUTUS*2/TECUE#1, VILLAJUAREZF2009/6/ATTILA/3*BCN//BAV92/3/PASTOR/4/TACUPETOF2001*2/BRAMBLING/5/PAURAQ, and KACHU/BECARD//WBLL1*2/BRAMBLING/4/FRET2/TUKURU//FRET2/3/MUNAL#1. Lines with the highest percentage of infection were: BABAX/LR42//BABAX*2/3/KUKUNA/4/CROSBILL#1/5/BECARD/6/KSW/SAUAL//SAUAL/7/BABAX/LR42//BABAX*2/3/KUKUNA/4/CROSBILL#1/5/BECARD with 88.83 in the first date,MUU/KBIRD//2*KACHU/KIRITATIwith 84.77 and 82.65%in the first and second date, respectively, and TACUPETOF2001*2/BRAMBLING//WBLL1*2/BRAMBLING/6/WBLL1*2/KURUKU*2/5/REH/HARE//2*BCN/3/CROC_1/AE.SQUARROSA(213)//PGO/4/HUITES/7/BAV92//IRENA/KAUZ/3/HUITES/4/2*ROLF07 with 81.67% in the first date. The average of the three highest levels of infection of the susceptible checkwas99.7%.

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Introduction:-

Karnal bunt of wheat caused by the fungus*Tilletiaindica*Mitra, was first described from India in 1931 by Mitra. The fungus may infect bread wheat (*Triticumaestivum*L.), durum wheat (*T. turgidum*L.) and triticale (X *Triticosecale*Wittmack) (Mitra, 1931; Agarwal *et al*., 1977). In 1972, Duran reported the disease from Mexico, and from that time on it was reported from Pakistan, Nepal, Brazil, The United States of America, Iran, the Republic of South Africa, and Afghanistan. (Munjal, 1975; Singh *et al*., 1989; Da Luz *et al*., 1993; APHIS, 1996; Torarbi*et al*., 1996;Crous*et al*., 2001; CIMMYT, 2011).

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);sometimes, grains may be totally destroyed; although the fungus may penetrate the embryo, it may not belethal (Chona*et al*., 1961; Mitra, 1935); therefore, partially infected grains may produce healthy plants. It has been reported thatthe percentage of germination decreases basedon the 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);although it was reported that seed with the greatest infection, but with the embryo intact, produced the highest number of tillers (Fuentes-Dávila*et al*., 2013).

Figure 1:- Infectedgrains of wheat with*Tilletiaindica*, showing thecharacteristiclesion from the bottom towards the apex.

Teliosporesof the fungus are resistant to physical and chemical factors (Krishna and Singh, 1982; Zhang*et al*., 1984; Smilanick*et al*., 1985, 1988), therefore, control of this pathogen is cumbersome. Chemical control is highly effective by fungicide sprays during flowering (Fuentes-Dávila*et al*., 2005, 2016, 2018c; Salazar-Huerta *et al*., 1997), but this practice would not beeconomically feasible if phytosanitary regulations do not allow tolerance levels for seed production (SARH, 1987).

Resistant wheat cultivars is the best control measure and itcontributes tominimize the possibilities of introduction of the disease into karnal buntfree-areas. Species of *Triticum* have been tested fortheir reaction to karnal bunt inoculation (Bedi*et al*., 1949; Singh *et al*., 1986; 1988), being*Triticumaestivum*the most affected by the disease; some lines may show more than 50% infected grain (Fuentes-Dávila*et al*., 1992; 1993; 2018a; 2019; 2020).

According to theFood and Agriculture Organization of the United Nations (FAOSTAT), in the year 2019 world wheat production was 765,769,635 million tons, being the second most widely cultivated cereal, after maize; therefore, the identification of new sources of resistance to karnal bunt, as well as the generation of information regarding the advanced lines derived from the breeding programs must be carried out in order to have new cultivars for the areas affected by the disease. The objective of this work was to evaluate the reaction of one thousand and ninety twoadvanced bread wheat lines to inoculation with*Tilletiaindica* in the field.

Materials and Methods:-

The experimental germplasm (1,092 lines) originated from the collaborative 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). The evaluation was carried out during the crop season fall-winter 2016-2017, 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 11 and 24,2016, 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.The agronomic management followed the recommendations by (Figueroa-López*et al*., 2011).

The inoculum was prepared by isolatingteliospores 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 germination, fungal colonies were transferred and multiplied on potato-dextrose-agar. Inoculations were carried out by injecting 1 mL of an allantoidsporidial suspension (10,000/mL) during the boot stage in five spikes from each line (Fig. 2). An automated mist spray-irrigation system and anti-bird net were installed covering completely the experimental area (Fuentes-Dávila*et al*., 2021).Inoculations initiated on January 4 and ended on March 16, 2017, for a total of 35 inoculation dates. Inoculated spikes were collected in paper bags and threshed by hand, then, healthy and infected grains were counted in order to determine the percentage of infection of each line.

Figure 2:- Artificial inoculation of wheatplantsduringthebootstage, byinjection.

Results and Discussion:-

The percentage of infection of the advanced bread wheat lines for the first date had a range of 0-88.83% with a mean of 31.81%; the infection categories in this date were: 2 lines did not show any infected grains, 13 fell in the 0.1-2.5% category, 20 in the 2.6-5.0%, 72 in the 5.1-10.0%, 434 in the 10.1-30.0% category, and 551 showed an infection percentage greater than 30.0%. In this date, lines with less than 10% comprise 9.7% of the group which would offer an acceptable reaction to the inoculation with *Tilletiaindica*, the causal agent of the disease.

The range of the percentage of infection in the second date was 0-82.65% with a mean of 24.44%; the infection categories in this date were: 7 lines did not show any infected grains, 10 fell in the 0.1-2.5% category, 22 in the 2.6-5.0%, 92 in the 5.1-10.0%, 609 in the 10.1-30.0% category, and 352 showed an infection percentage greater than 30.0%. In this date, lines with less than 10% comprise 11.99%, very similar to the first date.

In the overall results (mean of the two dates),the range of the percentage of infection was 0.46-83.71% with a mean of 28.12%; 12lines fell into the 0.1-2.5% infection category, 18 within 2.6-5.0% (16 lines had less than 5.0% infection in both dates), 83 within 5.1-10.0%, 548 within 10.1-30%, and 431with more than 30% infection (Fig. 3).Out of the 16 lines that had less than 5.0% infection in both dates, there were 5 lines that had less than 2.5% infection also in both dates (Table 1).Lines with the highest percentage of infection were: BABAX/LR42//BABAX*2/3/KUKUNA/4/CROSBILL#1/5/BECARD/6/KSW/SAUAL//SAUAL/7/BABAX/LR42//BABAX*2/3/KUKUNA/4/CROSBILL#1/5/BECARD with 88.83 in the first date, MUU/KBIRD//2*KACHU/KIRITATI with 84.77 and 82.65% in the first and second date, respectively, and TACUPETOF2001*2/BRAMBLING//WBLL1*2/BRAMBLING/6/WBLL1*2/KURUKU*2/5/REH/HARE//2*BCN/3/CROC_1/AE.SQUARROSA(213)//PGO/4/HUITES/7/BAV92//IRENA/KAUZ/3/HUITES/4/2*ROLF07 with 81.67% in the first date.The range of infection of the susceptible check KBSUS was 68.5% onFebruary 23, 2017, to 100% on February 8, with a mean of 90.2%.The mean of the three highest percentage of infection of the susceptible check KBSUS was 99.7%.

Through field evaluation of experimental wheat germplasm, introductions, *Triticum* species, and other grass species, resistance was detected in durum wheat, triticale, and *Aegilops* spp. (Warham*et al*., 1986; Rajaram*et al*., 1991). Since 1989, interspecific hybrids between *Triticumturgidum* x*Triticumtauschii*(Coss.) Schmalh. (syn. *Aegilopssquarrosa*L.) were made in an attempt to incorporate resistance into bread wheat from the best synthetic hexaploids with good agronomic characteristics and cytogenetic stability (Villareal*et al*., 1995; Mujeeb-Kazi*et al*., 2006).

The synthetic hexaploid wheats (SHW) are generated by crosses involving tetraploid cultivars (*Triticumturgidum*, 2n=28, AABB) and diploid goat grass [*Aegilopstauschii*Coss. (syn. *Aegilopssquarrosa*, *Triticumtauschii*), 2n=14, DD], followed by chromosome doubling of F_1 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 total number of lines subject of the present evaluation, 17.85% carry in their pedigree a synthetic with the potential to be a contributor for the resistance to karnal bunt by a particular bread wheat line. However, in the case of CROC_1/AEGILOPS SQUARROSA(213) with 23 lines, ALTAR84/AE.SQUARROSA(221) with 27 lines, AE.SQUARROSA(TAUS) with 8 lines, T.DICOCCON PI94624/AE.SQUARROSA (409) with 6 lines, AE.SQUARROSA(372) with 5 lines, AE.SQUARROSA(408) with 2 lines, MEXI75/AE.SQUARROSA with 2 lines, CROC_1/AE.SQUARROSA(444) with 1 line, and the species T.SPELTA PI348599 with 2 lines, none of those lines were resistant to the disease.

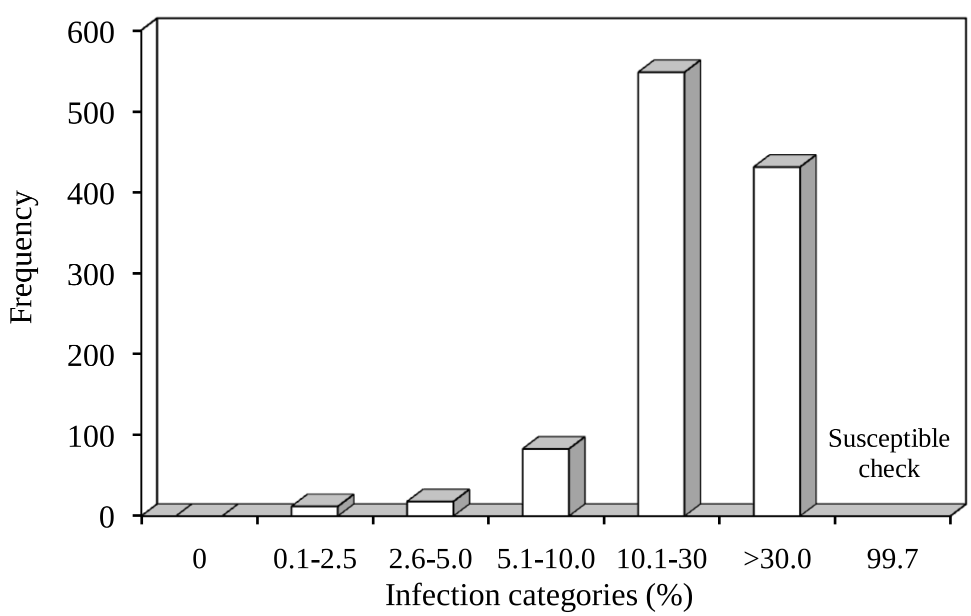

Figure 3:- Karnal bunt infection categories (%) in 1,092 bread wheat advanced lines artificially inoculated in the field in two dates during the 2016-2017 crop season, at the Norman E. Borlaug Experimental Station in the Yaqui Valley, Sonora, Mexico. The average of the three highest scores of infection of the susceptible check KBSUS was 99.7%.

Table 1:- Bread wheatadvancedlinesthatwereartificiallyinoculated in thefieldwith*Tilletia indica*, and showedlessthan 2.5% infection in two dates, duringthecropseason 2016-2017, at the Norman E. Borlaug Experimental Station in the Yaqui Valley, Sonora, Mexico.

No.	Pedigree and selectionhistory
1	MUNAL#1/FRANCOLIN#1*2/3/ATTILA*2/PBW65//MURGA CMSS12Y00701T-099TOPM-099Y-099M-0SY-13M-0WGY
2	MUNAL#1/FRANCOLIN#1*2/3/ATTILA*2/PBW65//MURGA CMSS12Y00701T-099TOPM-099Y-099M-0SY-17M-0WGY
3	BAJ#1/3/KIRITATI//ATTILA *2/PASTOR*2/4/MUTUS*2/TECUE#1 CMSS12Y00928T-099TOPM-099Y-099M-0SY-3M-0WGY
4	VILLAJUAREZF2009/6/ATTILA/3*BCN//BAV92/3/ PASTOR/4/TACUPETOF2001*2/BRAMBLING/5/PAURAQ CMSS12B00006S-099M-0SY-22M-0WGY
5	KACHU/BECARD//WBLL1*2/BRAMBLING/4/FRET2/TUKURU//FRET2/3/MUNAL #1 CMSS12B00312S-099M-0SY-5M-0WGY

Unlike these results, Fuentes-Dávila*et al*. (2021) reported that the bread wheat nursery evaluated in the crop season 2015-2016 contained 22.06% of lines that carried in their pedigree a synthetic; for CROC_1/AEGILOPS SQUARROSA(224) with 39 lines, 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 as a check as well as in combination with other lines (Fuentes-Dávila*et al*., 2018b), out of the 119 lines with MUNAL#1 in their pedigree in 2016-2017, 5.8% showed resistance to the disease.

In this group of 1,092 advanced bread wheat lines evaluated during the crop season 2016-2017, there were 7 which must be evaluated in the following season in order to verify their resistance shown to *Tilletiaindica*. These lines may be prospects for commercial release, or they may be part of the progenitors used in bread wheat breeding programs.

Conclusion:-

The range of the mean percentage of infection of one thousand and ninety two advanced bread wheat linesevaluated for their reaction to karnal bunt artificial inoculation in two sowing dates, during the crop season fall-winter 2016-2017, was 0.46-83.71% with a mean of 28.12%. There were 5 lines that in both dates consistently showed a percentage of infection below 2.50%: two sister lines of MUNAL#1/FRANCOLIN#1*2/3/ATTILA*2/PBW65//MURGA(CMSS12Y00701T-099TOPM-099Y-099M-0SY-13M-0WGY), MUNAL#1/FRANCOLIN#1*2/3/ATTILA*2/PBW65//MURGA (CMSS12Y00701T-099TOPM-099Y-099M-0SY-17M-0WGY), BAJ#1/3/KIRITATI//ATTILA*2/PASTOR*2/4/MUTUS*2/TECUE#1, VILLAJUAREZF2009/6/ATTILA/3*BCN//BAV92/3/PASTOR/4/TACUPETOF2001*2/BRAMBLING/5/PAURAQ, and KACHU/ BECARD//WBLL1*2/BRAMBLING/4/FRET2/TUKURU//FRET2/3/MUNAL#1. Lines with the highest percentage of infection were: BABAX/LR42//BABAX*2/3/KUKUNA/4/CROSBILL#1/5/BECARD/6/KSW/SAUAL//SAUAL/7/BABAX/LR42//BABAX*2/3/KUKUNA/4/CROSBILL#1/5/BECARD with 88.83 in the first date, MUU/KBIRD//2*KACHU/KIRITATI with 84.77 and 82.65% in the first and second date, respectively, and TACUPETOF2001*2/BRAMBLING//WBLL1*2/BRAMBLING/6/WBLL1*2/KURUKU*2/5/REH/HARE//2*BCN/3/CROC_1/AE.SQUARROSA(213)//PGO/4/HUITES/7/BAV92//IRENA/KAUZ/3/HUITES/4/2*ROLF07 with 81.67% in the first date.The average of the three highest levels of infection of the susceptible check was 99.7%.

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