GSC Biological and Pharmaceutical Sciences

eISSN: 2581-3250 CODEN (USA): GBPSC2

Cross Ref DOI: 10.30574/gscbps

Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

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Incidence of black point (*Alternaria* sp.) in elite advanced bread wheat (*Triticum aestivum*) lines

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GSC Biological and Pharmaceutical Sciences, 2023, 25(02), 222–229

Publication history: Received on 07 October 2023; revised on 14 November 2023; accepted on 16 November 2023

Article DOI: https://doi.org/10.30574/gscbps.2023.25.2.0487

Abstract

Eighty four elite advanced bread wheat lines were evaluated for resistance to black point during the crop season 2015-2016 at the Norman E. Borlaug Experimental Station in the Yaqui Valley, Mexico. Sowing dates were November 12, 19 and 26, 2015. Harvest and threshing were done manually in ten spikes per line, and the evaluation by visual inspection, counting healthy and infected grains to calculate the percentage of infection. The range of infection for the first sowing date was 0 to 19.8 %, with an average of 1.9; thirty nine lines did not show infected grains; for the second date it was 0 to 32.7 %, with an average of 4.8; twenty seven lines did not show infected grains; while for the third date, it was 0 to 44.9 % with an average of 6.8, and eighteen lines did not show infected grains. Eight lines did not show infected grains in the three dates. Lines with the highest average percentage of infection were: WHEAR/KUKUNA/3/C80.1/3* BATAVIA//2*WBLL1*2/4/WBLL1/KUKUNA//TACUPETOF2001/3/UP2338*2/VIVITSI with 20.8, and WAXWING*2/KRONSTADF2004/3/TRCH/SRTU//KACHU/4/SAUAL/YANAC//SAUAL with 20.5%. Within the infection categories based on the average of the three dates, thirty lines were within the 0.1-2.5 % infection category, fourteen within 2.6-5.0 %, twenty three within 5.1-10.0 %, and nine lines within 10.1-30.0 %. The infection average of the group was 4.5 % with a range of 0 to 20.8 %, but some lines showed high percentage of infection in the dates, like WHEAR/KUKUNA/3/C80.1/3*BATAVIA//2*WBLL1*2/4/WBLL1/KUKUNA//TACUPETOF2001/3/UP2338*2/VIVITSI, WAXWING*2/KRONSTADF2004/3/TRCH/SRTU//KACHU/4/SAUAL/YANAC//SAUAL, and BAV92//IRENA/KAUZ/3/HUITES/4/DOLL/5/SERI.1B//KAUZ/HEVO/3/AMAD*2/4/KIRITATI with 44.9, 44.7, and 37.1 %, respectively, in the third date.

Keywords: *Triticum aestivum*; Elite bread wheat; Black point; *Alternaria* spp.; Natural infection

1. Introduction

Black point (BP) is a disease that affects wheat (durum wheat *Triticum durum* Desf. and bread wheat *Triticum aestivum* L.), triticale (X *Triticosecale* Wittmack), and barley (*Hordeum vulgare* L.) [1,2,3]. Many fungal species, including *Alternaria* spp., *Bipolaris* spp., *Cladosporium* spp., *Curvularia* spp., *Fusarium* spp., and *Helminthosporium* spp. can be isolated from newly harvested wheat grain (*Triticum* spp.), particularly in humid field environments, where they may infect seed when the relative humidity exceeds 90% and seed moisture content 20% [1,4,5]. Kai-Ge *et al.* [6] reported that 21 strains representing 11 genera of fungi were isolated from wheat seed. BP or kernel smudge is favored by rainfall during seed maturation, as well as humid weather prevailing for a few days prior to harvest [7,8]; expanding green kernels are most susceptible. BP also promotes premature seed senescence because many of the fungi are saprophytic [1]. *Alternaria alternata* (Fries: Fries) von Keissler and *Bipolaris sorokiniana* (Sacc.) Shoemaker are generally considered the primary causal agents of the disease [9]. The disease is more noticeable around the embryo end of seed with a dark brown to black color (Figure 1). Severe infection causes discoloration and shriveling of the whole seed [9]; the

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discoloration may also occur near the brush, in the crease or any part of the seed. It may be light or dark or with a distinct margin.

Figure 1 Symptoms of black point on grain of bread wheat

Vertical sections of the endosperm may present brown to black spots [10]. Red smudge caused by *Pyrenophora tritici-repentis* (Died.) Drechs. may be present in seed affected by BP [11]. BP causes economic losses by degrading the quality of flour and semolina, and it also affects seed germination, and inhibits seedling growth [6]. In the southern region of the state of Sonora in Mexico, BP is an endemic disease of durum and bread wheat, and it also affects triticale [3,12,13]. Incidence of BP under natural conditions is caused primarily by *Alternaria* spp., and it varies each wheat season, therefore, there is no consistent data about the performance of experimental germplasm in order to carry out the best selection. Artificial inoculation with *Alternaria alternata* by injection or by vacuum infiltration have been reported as useful methods for the identification of resistant and susceptible germplasm [14]; Kai-Ge *et al*. [6] completed Koch´s postulates by inoculum spray on wheat plants in the greenhouse. The objective of this work was to determine the reaction of elite bread wheat advanced lines to BP during the wheat season 2015-2016 under natural conditions of infection.

2. Material and methods

Eighty four elite advanced bread wheat lines were evaluated for resistance to black point (BP) at the Norman E. Borlaug Experimental Station which belongs to the National Institute for Forestry, Agriculture, and Livestock Research (INIFAP) located in block 910 of the Yaqui Valley in the state of Sonora, Mexico (27º22´3.01" N, 105º55´40.22" W), during the crop season fall-winter 2015-2016. This region is characterized by a warm climate (BW (h)) and extreme heat according to Koppen's classification, modified by García [15]. Sowing dates were November 12, 19, and 26, 2015, in a clay soil with pH 7.8. Approximately 8 g of seed were used for a bed 0.7 m long in a single row, and without replications. Fertilization consisted of 150 kg ha^{-1} of urea before sowing. An irrigation for seed germination and three complementary ones were applied during the season; 100 kg ha^{-1} of urea were applied just prior to the first complementary irrigation. Thirty days after sowing, the herbicides Topik gold (clodinafop-propargyl) [16] and Situi® xl (metsulfuron methyl) [17] were applied at the rates of 750 mL and 25 g ha^{-1} of commercial product, respectively. The first product which is selective for wheat, was used for control of narrow leaf weeds and the second one for broad leaf weeds. Harvest was carried out manually and the evaluation by visual inspection, counting the number of infected and healthy grains in ten spikes per line in order to determine the percentage of infection; the lesion size was not taking into consideration. The temperature, relative humidity, and rainfall were obtained from the weather station CIANO-910, which belongs to the automated weather station network of Sonora [18]; data were recorded from March 15 to April 30, 2016. The bread wheat germplasm evaluated in this work, was produced by the collaborative breeding program between the International Maize and Wheat Improvement Center and the National Institute for Forestry, Agriculture, and Livestock Research.

3. Results and discussion

The range of infection for the first sowing date was 0 to 19.8 %, with an average of 1.9 (Figure 2); thirty nine lines did not show infected grains, 26 lines fell within the 0.1-2.5 % infection category, 8 lines within 2.6-5.0 %, 7 lines within 5.1-10 %, and 4 lines within 10.1-30 %. The range of infection for the second date was 0 to 32.7 %, with an average of 4.8 %; twenty seven lines did not have infected grains, 20 lines fell within the 0.1-2.5 % infection category, 6 within 2.6-5.0 %, 16 lines within 5.1-10 %, 14 lines within 10.1-30 %, and one line showed more than 30.1 % infected grains. The range of infection for the third sowing date was 0 to 44.9 % with an average of 6.8 %; eighteen lines did not have infected grains, 19 lines fell within the 0.1-2.5 % infection category, 13 lines within 2.6-5.0 %, 13 lines within 5.1-10 %, 18 lines within 10.1-30 %, and three lines showed more than 30.1 % infected grains. Some lines showed consistency in their reaction to BP: eight lines did not show any infected grains in the three dates, ten showed a range of infection between 0.1 and 2.5 %, five lines had no infection in two dates and a percentage of infection in the range already mentioned in one date, and four had no infection in one date and similar percentage of infection as the previous group, but in two dates. Those lines with high percentage of infection in two dates were MUNAL#1/3/TRCH/SRTU//KACHU with a

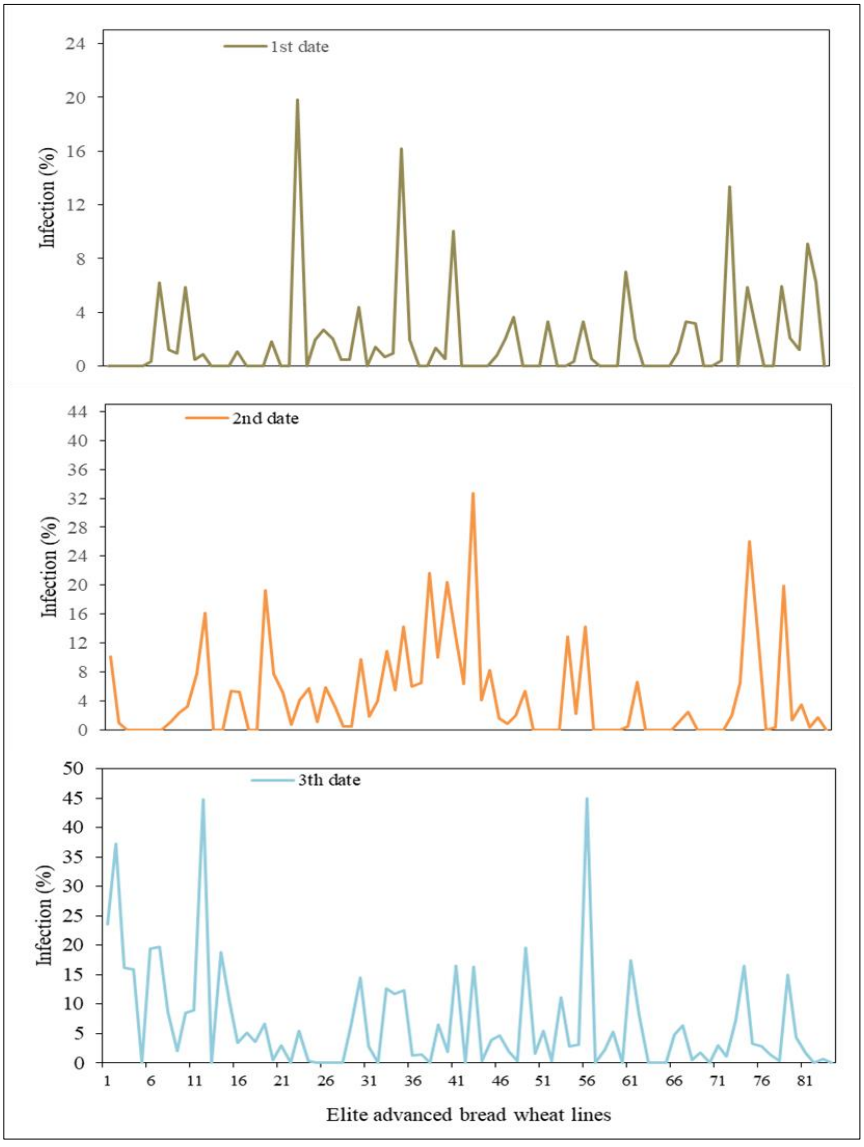

Figure 2 Percentage of infection with black point (Alternaria spp.) of 84 elite advanced bread (Triticum aestivum) lines, evaluated under natural conditions in three sowing dates at the Norman E. Borlaug Experimental Station in the Yaqui Valley, Sonora, Mexico, during the crop season 2015-2016

range of 10.1 to 23.5 %, WAXWING*2/KRONSTADF2004/3/TRCH /SRTU//KACHU/4/SAUAL/YANAC//SAUAL with a range of 16.0 to 44.7 %, BAJ#1*2/CIRNOC2008 with a range of 10.8 to 12.6 %, TUKURU//BAV92/RAYON/6/NG8201/

KAUZ/4/SHA7//PRL/VEE#6/3/FASAN/5/ MILAN/KAUZ/7/CIRNOC2008/8/SAUAL/KRONSTAD F2004 with a range of 14.2 to 16.2 %, MEX94.27.1.20/3/SOKOLL//ATTILA/3*BCN*2/6/OASIS/5*BORL95/5/CNDO/R143//ENTE/MEXI75/3/AE.SQ/4/2*OCI with a range of 13.1 to 16.4 %, MUNAL #1*2//SOKOLL/WBLL1 with a range of 16.3 to 32.7 %, WHEAR/KUKUNA/3/C80.1/3*BATAVIA//2*WBLL1*2/4/WBLL1/KUKUNA//TACUPETOF2001/3/UP2338*2/VIVITSI with a range of 14.2 to 44.9 %, and C80.1/3*BATAVIA//2*WBLL1/5/REH/HARE//2*BCN/3/CROC_1/AE.SQUARROSA(213)//PGO/4/HUITES*2/6/PICAFLOR #1 with a range of 14.9 to 19.8 %. Lines with the highest average percentage of infection were: WHEAR/KUKUNA/3/C80.1/3*BATAVIA//2*WBLL1*2/4/WBLL1/KUKUNA//TACUPETOF2001/3/UP2338*2/VIVITSI with 20.8 %, WAXWING*2/KRONSTADF2004/3/TRCH/SRTU//KACHU/4/SAUAL/YANAC//SAUAL with 20.5 %, MUNAL#1*2//SOKOLL/WBLL1 with 16.3 %, and TUKURU//BAV92/RAYON/6/NG8201/KAUZ/4/SHA7//PRL/VEE#6/3/FASAN/5/MILAN/KAUZ/7/CIRNOC2008/8/SAUAL/KRONSTADF2004 with 14.2 %. The average infection of each line in the three dates is shown in Figure 3, and lines that did not have any infected grain in the three dates are shown in Table 1.

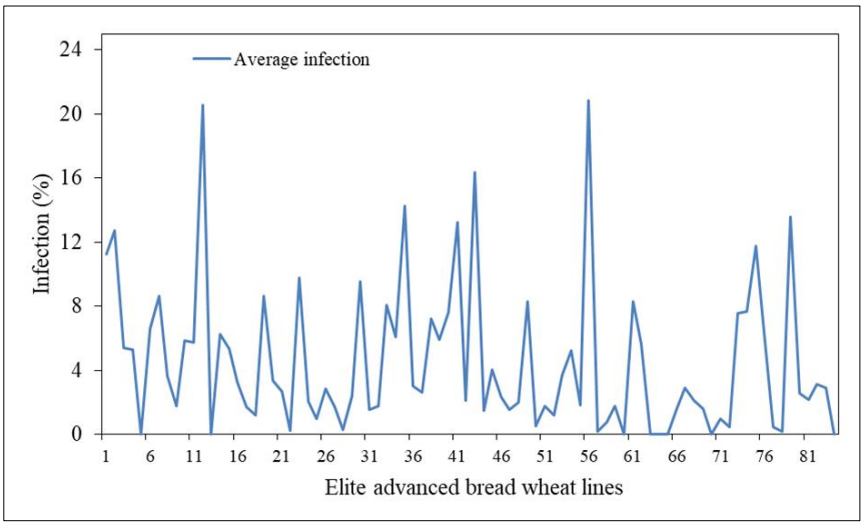

Figure 3 Average percentage of infection with black point (*Alternaria* spp.) of 84 elite advanced bread (*Triticum aestivum*) lines, evaluated under natural conditions in three sowing dates at the Norman E. Borlaug Experimental Station in the Yaqui Valley, Sonora, Mexico, during the crop season 2015-2016

Within the infection categories of the group based on the average of the three dates, eight lines did not show any infected grains, 30 lines fell in the 0.1-2.5 % category, 14 lines in 2.6-5.0 %, 23 lines in 5.1-10.0, and 9 in the 10.1-30.0 infection category. The overall average of the group was 4.5 % with a range of 0 to 20.8 % (Figure 4).

Table 1 Elite advanced bread wheat (*Triticum aestivum*) lines that did not have any infected grains with black point (*Alternaria* sp.), and that were evaluated under natural conditions in three sowing dates, during the crop season 2015-2016, in the Yaqui Valley, Sonora, México

No.	Pedigree	Selection history
1	KRONSTAD F2004/3/TRCH/SRTU//KACHU/4/TRCH/SRTU//KACHU	CMSS10Y00992T-099TOPM-099Y-099M-099NJ-099NJ-10WGY-0B
2	PBW343*2/KUKUNA//PBW343*2/KUKUNA/3/TRCH/SRTU//KACHU/4/TRCH/SRTU//KACHU	CMSS10B00819T-099TOPY-099M-099NJ-099NJ-24WGY-0B
3	FRANCOLIN#1/3/PBW343*2/KUKUNA*2//YANAC/4/KINGBIRD#1//INQALAB91*2/ TUKURU	CMSS10B00779T-099TOPY-099M-099NJ-099NJ-6WGY-0B
4	SUP152*2/3/TRCH/SRTU//KACHU	CMSS10B00797T-099TOPY-099M-0SY-2M-0WGY
5	SUP152*2/3/TRCH/SRTU//KACHU	CMSS10B00797T-099TOPY-099M-099NJ-099NJ-5WGY-0B

6	NELOKI/4/MELON//FILIN/MILAN/3/FILIN/5/WBLL1/ KUKUNA//TACUPETO F2001/3/BAJ #1	CMSS10B00848T-099TOPY-099M-0SY-26M-0WGY
7	WBLL1*2/4/YACO/PBW65/3/KAUZ*2/TRAP//KAUZ/5/ KACHU#1/6/MARCHOUCH*4/SAADA/3/2*FRET2/ KUKUNA//FRET2/7/WBLL1*2/4/YACO/PBW65/3/KAUZ*2/TRAP//KAUZ/5/KACHU #1	CMSS10B00974T-099TOPY-099M-099NJ-099NJ-11WGY-0B
8	BAVIS/8/BOW/VEE/5/ND/VG9144//KAL/BB/3/YACO/ 4/CHIL/6/CASKOR/3/CROC_1/AE.SQUARROSA(224)// OPATA/7/PASTOR//MILAN/ KAUZ/3/BAV92	CMSA10M00484S-050ZTM-0SY-31M-0WGY

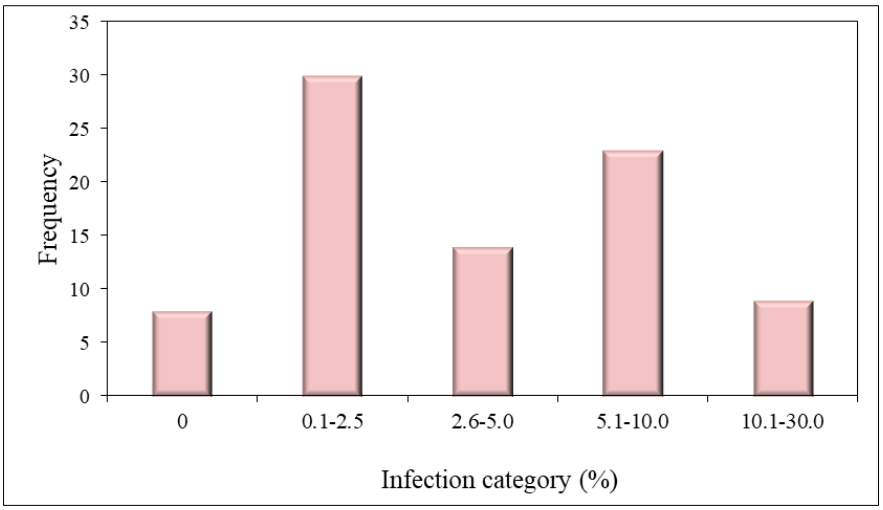

Figure 4 Black point (*Alternaria* spp.) infection category (%) of 84 elite advanced bread (*Triticum aestivum*) lines, evaluated under natural conditions in three sowing dates at the Norman E. Borlaug Experimental Station in the Yaqui Valley, Sonora, Mexico, during the crop season 2015-2016

The incidence of BP in both wheat species is quite variable in each fall-winter season in the regions where this cereal is cultivated in southern Sonora; this is partly due not only to weather conditions during the season, but also, to the different climatic zones [19,20]. The primary inoculum is an important aspect which will be influenced by weather, as well as the capacity of acting as saprophytes by some fungal species like *Alternaria triticina*, *Helminthosporium sativum*, and *Fusarium* spp. which may survive in plant debris [1]. In some seasons, the infection levels might be high in some bread wheat lines, like in 2009-2010, BABAX/LR42//BABAX/3/ER2000, BABAX/LR42//BABAX*2/4/SNI/TRAP#1/3/ KAUZ*2/TRAP//KAUZ, and TC870344/GUI//TEMPORALERAM87/AGR/3/2*WBLL1 had 30.8, 27.3, and 20.53 % infected grains, respectively [21]; in 2012-2013, NL1048/4/CHIBIA//PRLII/CM65531/3/SKAUZ/BAV92 showed a maximum of 12.3 % [22]; in 2013-2014, SOKOLL*2/3/ BABAX/LR42//BABAX and KISKADEE#1/CHYAK both had 31.4 % [23]; in 2014-2015, the same line SOKOLL*2/3/BABAX/LR42//BABAX had a maximum of 22.6 % [24], and PFAU/MILAN/5/CHEN/AEGILOPSSQUARROSA(TAUS)//BCN/3/VEE#7/BOW/4/PASTOR/6/WHEAR/4/SIN/TRAP #1/3/KAUZ*2/TRAP//KAUZ/5/C80.1/3*BATAVIA//2*WBLL1/7/C80.1/3*BATAVIA//2*WBLL1/5/REH/HARE//2 *BCN/3/CROC_1/AE.SQUARROSA(213)//PGO/4/HUITES showed 43.6 %, and WAXWING/KRONSTADF2004//2* FRNCLN 38.3 % [25]. BP has an adverse effect on seed weight, germination, and seedling emergence [5]. Increased seedling mortality, reduced seedling vigour and grain yield may also occur when affected seed is used for sowing. It also affects the lustre and plumpness of the grain, and so the quality with the consequent reduction in market value of the products. Therefore, besides aiming to the use of resistant cultivars [2], BP can be partially controlled by reducing the frequency of irrigation after heading and by reducing nitrogen rates, without affecting the grain yield or its quality. Harvested grain should be maintained at a moisture level of 12 % in well ventilated storage, and grain from affected fields should not be used as seed for the following season, since seedling rots and blight may occur, otherwise, seed must be treated with a systemic fungicide before sowing [4]. The evaluation of bread wheat germplasm for their reaction to BP should be a continuous effort in order to secure tolerant and resistant genotypes that could be candidates for commercial release.

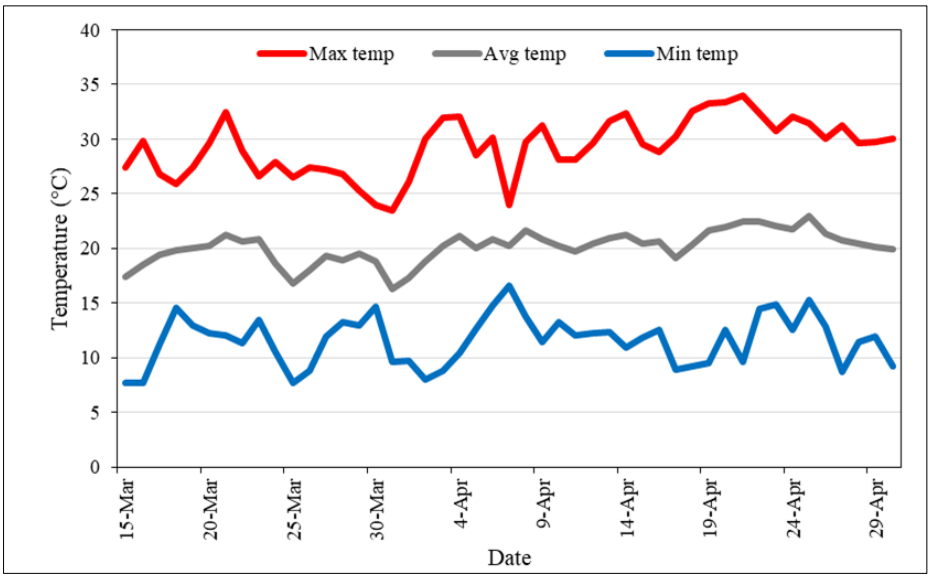

Figure 5 Average temperature, maximum and minimum recorded by the weather station CIANO-910 from March 15 to April 30, 2016, installed at the Norman E. Borlaug Experimental Station, during the fall-winter crop season 2015-2016

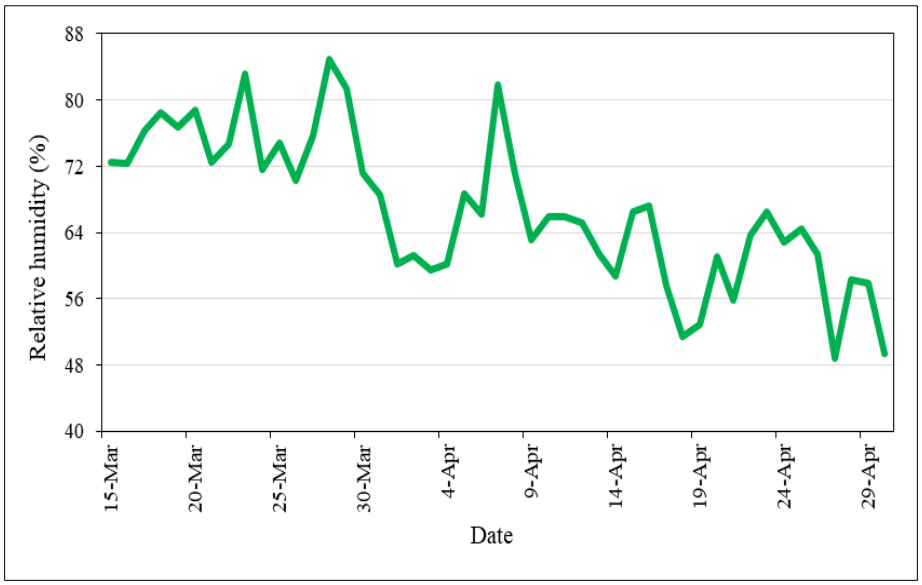

Figure 6 Average relative humidity recorded by the weather station CIANO-910 from March 15 to April 30, 2016, installed at the Norman E. Borlaug Experimental Station, during the fall-winter crop season 2015-2016

The period of grain formation for most of the lines which would fall after March 15 onwards, had and average temperature of 20.1 °C with a range of 16.3 to 22.9 °C (Figure 5), while the average maximum temperature was 29.2 with a range of 23.4 to 33.9 °C, and the minimum average temperature was 11.5 with a range of 7.6 to 16.6 °C. During that period, the total rainfall was 1.3 mm: 0.10 were recorded on March 23, 0.2 on March 28, and 1.0 mm on April 7. The periods of March 15 to 20, March 21 to 25, and March 26 to 31, had averages of relative humidity of 75.8, 75.3, and 75.3%, respectively (Figure 6), which might have contributed to levels of BP in some lines that were above 10 % and even some that were above 40 %. The following five day periods in April reduced somewhat gradually the percentage of relative humidity: 61.9, 69.5, 63.5, 58.0, 62.6, and 55.1% for April 1-5, 6-10, 11-15, 16-20, 21-25, and 26-30, respectively.

4. Conclusion

Lines that did not have any infected grains with black point in the three dates were KRONSTAD F2004/3/TRCH/SRTU//KACHU/4/TRCH/SRTU//KACHU, PBW343*2/KUKUNA//PBW343*2/KUKUNA/3/TRCH/SRTU//KACHU/4/TRCH/SRTU//KACHU, FRANCOLIN#1/3/PBW343*2/KUKUNA*2//YANAC/4/KINGBIRD#1//INQALAB91*2/TUKURU, SUP152*2/3/TRCH/SRTU//KACHU (CMSS10B00797T-099TOPY-099M-0SY-2M-0WGY), SUP152*2/3/TRCH/SRTU//KACHU (CMSS10B00797T-099TOPY-099M-099NJ-099NJ-5WGY-0B), NELOKI/4/MELON//FILIN/MILAN/3/FILIN/5/WBLL1/KUKUNA//TACUPETOF2001/3/BAJ#1, WBLL1*2/4/YACO/PBW65/3/KAUZ*2/TRAP//KAUZ/5/KACHU#1/6/MARCHOUCH*4/SAADA/3/2*FRET2/KUKUNA//FRET2/7/WBLL1*2/4/YACO/PBW65/3/KAUZ*2/TRAP//KAUZ/5/KACHU#1, and BAVIS/8/BOW/VEE/5/ND/VG9144//KAL/BB/3/YACO/4/CHIL/6/CASKOR/3/CROC_1/AE.SQUARROSA(224)// OPATA/7/PASTOR//MILAN/KAUZ/3/BAV92.

Lines with the highest percentage of infection were WHEAR/KUKUNA/3/C80.1/3*BATAVIA//2*WBLL1*2/4/WBLL1/KUKUNA//TACUPETOF2001/3/UP2338*2/VIVITSI, WAXWING*2/KRONSTADF2004/3/TRCH/SRTU//KACHU/4/SAUAL/YANAC//SAUAL, and BAV92//IRENA/KAUZ/3/HUITES/4/DOLL/5/SERI.1B//KAUZ/HEVO/3/AMAD*2/4/KIRITATI with 44.9, 44.7, and 37.1 %, respectively, in the third date

Compliance with ethical standards

Acknowledgments

This research was financially supported by the Mexican National Institute for Forestry, Agriculture, and Livestock Research (INIFAP).

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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