Colegio de Postgraduados

Selection of Advanced Bread Wheat Lines for Their Response to Premature Ripening Caused by *Fusarium* sp.

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ABSTRACT
Objective: Screen a bread wheat trial for resistance to Crown root rot.
Design/methodology/approach: one hundred and fifty experimental wheat lines with different genetic and physiological characteristics, were inoculated under greenhouse conditions with a mixture of five species of Fusarium, which were isolated from wheat commercial fields. The response of the wheat germplasm to the disease was scored through a disease index.
Results: Sixty-three bread wheat experimental lines were identified with a disease severity of 1 to 9%, which are considered as resistant.
Limitations on study/implications: The evaluation of wheat germplasm for disease resistance in the field presents important variables, which cannot be controlled, mainly for the spatial distribution of pathogens; hence, a better option is to carry out the trials under a controlled environment.
Findings/conclusions: Eleven genotypes were selected based on their resistance to Crown rot and to a better grain yield per spike.

Keywords: Wheat, Crown root rot, *Fusarium* sp., Genetic resistance.

Citation: Ireta-Moreno, J., Hernández-González, M. I., Pérez-Domínguez, J. F., Bautista-Ramírez, E., Ramírez-Sánchez S.E., & Zacamo-Velázquez N.Y. (2024). Selection of Advanced Bread Wheat Lines for Their Response to Premature Ripening Caused by *Fusarium* sp. *Agro Productividad*. https://doi.org/10.32854/agrop.v17i9.3028

Academic Editor: Jorge Cadena Iñiguez
Guest Editor: Juan Francisco Aguirre Medina

Received: May 12, 2024.
Accepted: August 13, 2024.
Published on-line: September 20, 2024.

Agro Productividad, 17(9) supplement. September. 2024. pp: 59-67.

INTRODUCTION

Wheat is one of the most important cereals in human nutrition, along with maize, rice, and potatoes. One of the main diseases affecting wheat worldwide is caused by several species of the genus Fusarium, known as Wheat Premature Ripening (WPR), also referred to as Drying or Crown Rot Root. This disease has been reported in Australia, China, the USA, Argentina, India, Ukraine, Western Asia, Northern Africa, South America, and Mexico (Cook, 2010; Ôzdemir, 2022; Petronaitis *et al.*, 2024; Mariscal *et al.*, 2018; Wildermuth *et al.*, 1997). The complex of *Fusarium* species is of utmost importance in wheat cultivation, as it can cause yield losses of up to 89% (Klein *et al.*, 1991; Liu and Odbonnaya, 2015). These pathogens develop survival structures (chlamydospores) that can remain viable in the soil for several years. Currently, different *Fusarium* species causing root, crown, and stem rots have been identified. The Bajío region is the most important area for bread wheat production in Mexico, with an approximate cultivation area of 130,000 hectares (SIAP, 2024). The varieties cultivated in this region are of the bread and soft gluten type (Villaseñor-Mir *et al.*, 2022). Wheat Premature Ripening (WPR) or Drying is a disease that occurs in various parts of the Bajío, causing yield losses of 10-60% (Mariscal *et* *al.*, 2018). Root, stem, and crown rots in wheat can occur in all cereal-producing areas and are considered more aggressive in humid climates or environments (Gilchrist-Saavedra *et al.*, 2005). In various etiological studies of the disease conducted in the Bajío, the following species have been identified: *Fusarium proliferatum*, *F. graminearum*, *F. culmorum*, *F. equiseti*, *F. poae*, *F. nivale*, *F. longipes*, *F. subglutinans*, *F. dimerum*, *F. tricintum*, *F. verticillioides*, *F. oxysporum*, *F. thapsinum*, and *F. andiyazi* (Mariscal *et al.*, 2018; Rangel-Castillo *et al.*, 2017; Leyva-Mir *et al.*, 2017; Suaste-Franco *et al.*, 2020). In wheat seedlings, small brown lesions can be observed on the root, crown, and leaf sheaths or on the lower part of the stem. In the adult stage, a coppery wilting of the spikes can be observed in diseased plants. This increases when there is a period of water stress from anthesis to maturity (Liu and Ogbonnaya, 2015; Moya, 2013). In addition to the symptoms of WPR, reduced foliar growth, a decrease in the number of tillers, and lower quantity and quality of grains can also be observed. The best control of wheat diseases is through genetic resistance, which can be selected and derived from experimental germplasm. In the pre-improvement wheat program developed through the collaboration between the National Institute of Forestry, Agricultural, and Livestock Research (INIFAP) and the International Maize and Wheat Improvement Center (CIMMYT), advanced lines tolerant to WPR have been generated as one of the alternatives to reduce the damage caused by the *Fusarium* species complex (Ôzdemir, 2022; Liu and Ogbonnaya, 2015; Suaste-Franco, 2020). Therefore, it is necessary to generate germplasm banks that contain information on the response of experimental wheat lines to WPR, to integrate them into the breeding program with the aim of obtaining lines resistant to this disease that could later be released as commercial varieties. Thus, the objective of this work was to evaluate the reaction of four groups of advanced wheat lines to Premature Ripening caused by *Fusarium* spp.

MATERIALS AND METHODS

Isolation and Production of *Fusarium* sp. Inoculum

Samples of diseased wheat plants were obtained from the localities of La Laja de Cervantes and La Maltaraña, both in the municipality of Jamay, Jalisco. Twenty-two isolations were made from roots, crowns, and stems with infection symptoms. The isolated and purified species were identified based on their morphology and physiology, including type of mycelium, growth rate, colony color and appearance, conidia morphology, and chlamydospores. For inoculum production, the "oat grain colonization" method (Erginbas-Orakci *et al.*, 2016) was used, with only the five most frequent and identified *Fusarium* spp. species. Plastic bags of 15×10×35 cm were employed. A mixture of peat moss, sand, and perlite in a 1:1:1 ratio was used as the substrate.

The inoculum was placed at the midpoint of each pot, where 10 seeds of each experimental wheat line were sown. The trials were conducted in a greenhouse at the Centro Altos de Jalisco Experimental Station of INIFAP, in Tepatitlán, Jalisco. An experiment was designed with four groups of wheat lines and two repetitions (healthy and inoculated). The four groups of wheat lines were: 1) Trial 1R, with 36 entries of bread wheat, sourced from the INIFAP national wheat program and specifically adapted to the Bajío region; 2) Trial LTP-Fusarium, consisting of 45 wheat lines with different agronomic characteristics, from the Seed Discovery initiative of CIMMYT; 3) Trial 10° SATYN (Stress Adapted Trait Yield Nurseries), with 36 advanced bread wheat lines, from a nursery evaluated and selected under extreme heat and/or drought conditions, also from CIMMYT; and 4) Trial 8° WYCYT (Wheat Yield Consortium Yield Trial), with 33 bread wheat lines, from a high-yielding grain nursery of CIMMYT.

Germplasm was assessed for symptom presence at 14 and 70 days after sowing (DAS) using a severity scale from 1 to 5, proposed by Wildemuth *et al.* (1997) and modified by Ôzdemir (2022). The scale was as follows: 1=1-9% as resistant, 2=10-29% moderately resistant, 3=30-69% moderately susceptible, 4=70-89% susceptible, and 5=50-99% highly susceptible. The scale data were transformed using the formula $\sqrt{x+1}$, where x=scale value (Gómez & Gómez, 1984). Grain yield per spike was obtained for each wheat line, and a regression analysis was performed between the incidence of WPR and grain yield.

RESULTS AND DISCUSSION

Twenty strains were isolated, of which 5 species were identified as the most frequent: *Fusarium verticillioides*, *F. andiyazi*, *F. graminearum*, *F. chlamydosporum*, and *F. globosum*, according to Leslie's classification system (2006). The identified species partially match those reported in the Bajío (Mariscal *et al.*, 2018; Rangel-Castillo *et al.*, 2017; Leyva-Mir *et al.*, 2017), although they differ from reports in Australia, Argentina, or the USA, as *F. pseudograminearum* was not found.

Regression analyses of the incidence of Drying at 70 DAS in relation to grain yield per spike revealed that only the lines from the 10° SATYN trial showed a negative effect on yield.

Based on the transformed and graphed values of WPR severity, it is observed that the lines from the Trial 1R are moderately resistant to moderately susceptible, while the other three trials fall within the resistant range (Figure 1).

From each trial, lines with the lowest incidence of WPR and the best grain yield per spike were selected (Table 4).

The group of lines with the highest tolerance was the 8° WYCYT, with 18 lines (54%) in the resistant category and 12 lines in the moderately resistant category, followed by the 10° SATYN trial with 17 lines (47%) in the resistant category; the LTP-Fusarium trial with 16 lines (35%) resistant, and finally, the 1R trial with 12 lines (33%) resistant and 5 lines as moderately susceptible. When selecting by yield, it was observed that lines rated in scales 1 and 2 showed the best grain yields per spike (Table 4). In the 8° WYCYT trial, the lines are characterized by having a higher yield capacity under irrigation conditions. However, for the Bajío conditions, they fall outside the production system due to their longer vegetative cycle, up to 20 days longer (124 days to maturity) compared to the 1R lines, which are the earliest, with an average of 113 days.

The effect of WPR was from slight to moderate, as most of the wheat lines were rated between values 1 and 3 (Table 2), and the regression equations showed little effect. Only in the 10° SATYN trial was a low negative effect on yield observed (Figure 1c).

These results are preliminary and indicate that, while there are lines with tolerance genes, these need to be identified in detail. Field selection of wheat germplasm for

Table 1. Morphological characteristics of *Fusarium* species used in the inoculation of wheat lines.

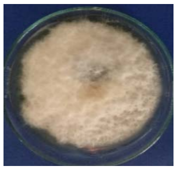
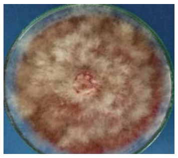
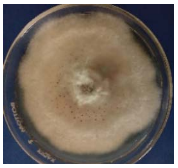
Species	Growth ratio colony	Morphology colony	Pigmentation
F. verticillioides	14.7	Mycelium dowmy White color turning grayish-violet.	
F. andiyazi	16.2	Powdery mycelium, White color turning grayist salmon color	
F. graminearum	20.3	Abundant mycelium, downy White-yellow color, turning red-brown color.	
F. chlamydosporum	10.8	Downy mycelium, White color, turning yellow.	
F. globosum	12.1	Downy mycelium, White color turning violet.	

Figure 1. Characteristic symptoms of WPR under field conditions, from which the *Fusarium* spp. species were isolated.

Table 2. Wheat lines classified by their response to Wheat Premature Ripening at 70 days after sowing, artificially inoculated in pots and under greenhouse conditions.

SCALE (1-5)	Trials of wheat experimental lines			
	LTP-Fusarium	8°WYCYT	1R	10°SATYN
1	16*	18	12	17
2	9	12	0	14
3	14	3	19	3
4	6	0	5	2
5	0	0	0	0
Total:	45	33	36	36

*Number of wheat lines selected in each severity scale.

Table 3. Regression equations and parameters of disease incidence at 70 DAS versus grain yield per spike for each of the wheat trials.

Trials	Equations	R^2	MSE	C.V.
LTP	$Y=4.444+12.592\ X$	0.168	74.51	32.29
8°WYCYT	$Y=30.028+1.257\ X$	0.0009	71.16	26.34
IR	$Y=19.393+7.580\ X$	0.118	43.26	19.73
10°SATYN	$Y=27.406-1.084\ X$	0.0018	38.86	24.31

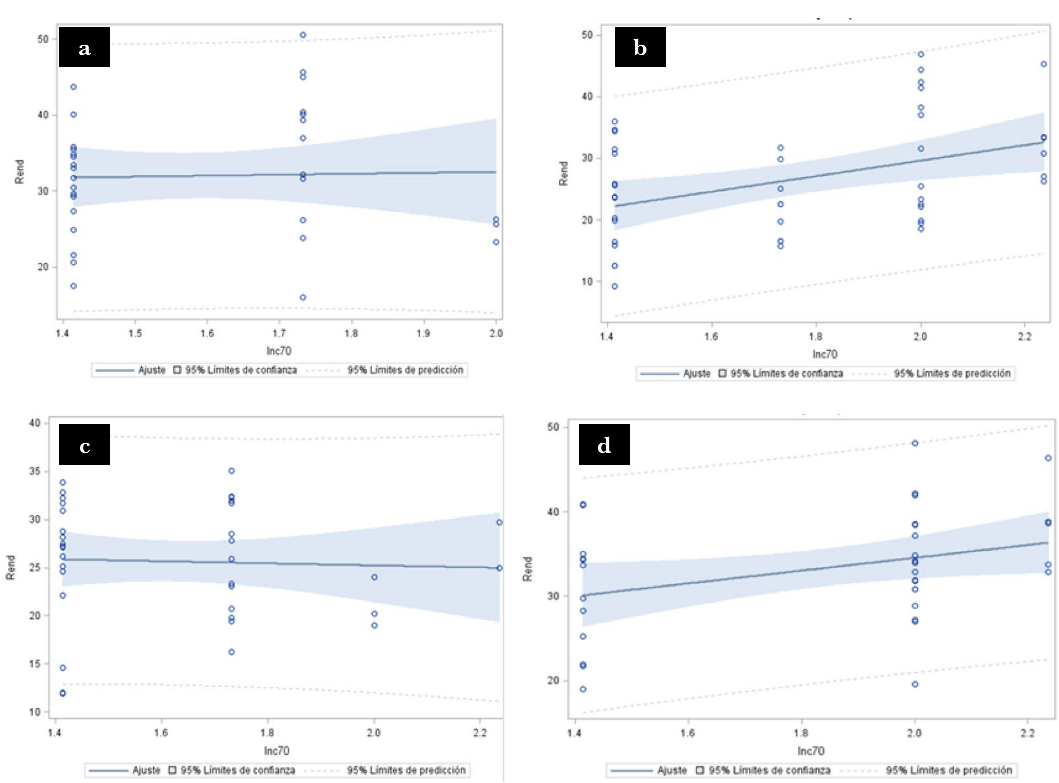


Figure 2. Regression graphs for the trials: a) 8° WYCYT, b) LTP-Fusarium, c) 10° SATYN, and d) Trial 1R.

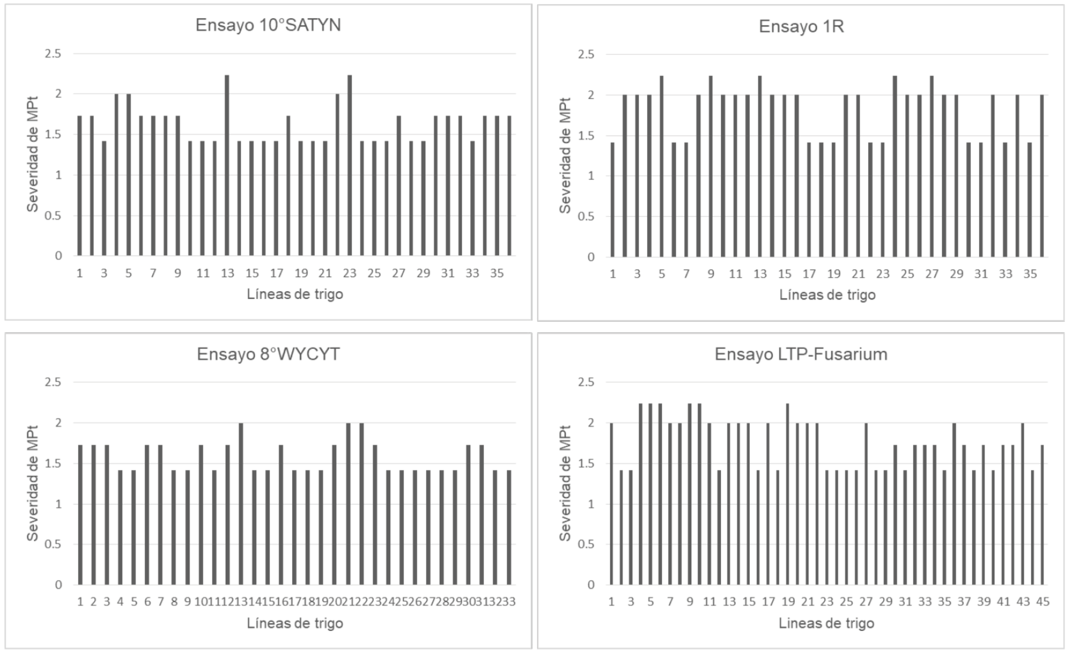


Figure 4. Severity of Wheat Premature Ripening in 150 experimental wheat lines, artificially inoculated with 5 *Fusarium* species under greenhouse conditions.

resistance to *Fusarium* spp. is inefficient due to the irregular distribution of the pathogen. In contrast, selection under greenhouse conditions increases efficiency, although it is not 100%. Therefore, different inoculation methods and disease assessment techniques should be combined (Petronaitis *et al.*, 2024). In the Ciénega de Chapala region, wheat is rotated with maize, and this system has led to an increase in *Fusarium* spp. infections in both wheat and maize. This rotation system may influence the prevalence of certain *Fusarium* spp. species in both crops (Ireta *et al.*, 2023).

CONCLUSIONS

Sixty-three experimental wheat lines were identified as resistant (1 to 9%), 35 lines as moderately resistant (10-29%), 39 lines as moderately susceptible (30-69%), and 13 lines as susceptible (70-89%) to the severity of Wheat Premature Ripening, after being inoculated with the *Fusarium* complex including *F. verticillioides*, *F. andiyazi*, *F. graminearum*, *F. chlamydosporum*, and *F. globosum* under greenhouse conditions.

The best lines based on their tolerance to WPR and grain yield per spike were: H-1539/BORL14/3/FRET22/SHAMA//KACHU in the LTP-Fusarium trial; SOKOLL/5/W15.92/4/PASTOR//HXL7573/2BAU/3/WBLL1/6/SOKOLL/3/PASTOR//HXL7573/2BAU in the 10° SATYN trial; TUR.180085//QUAIU2/KINDE in the 8° WYCYT trial; and COLI-BRI/ACACIA in the 1R trial.

In selecting wheat germplasm for disease resistance, a combination of disease response and grain yield should be considered. Under this concept, as shown in Table 4, the LTP-Fusarium, 8° WYCYT, and 1R trials had better grain yields compared to the 10° SATYN trial, although all selected lines remained within the 1 to 2 range on the severity scale.

Table 4. Crossing and selection history of the lines selected for their response to WPR incidence and grain yield.

Crossing and Selection	Incidence (Scale 1-5)	Yield (gr spike)
Trial: LTP-Fusarium		
H-1539/BORL14/3/FRET2*2/SHAMA//KACHU SDSS14Y00156T-0M-0Y-0B-0Y-0B-20Y	2	46.9
CETA/AE.SQUARROSA (299)//BORL14/3/FRET2*2/SHAMA//KACHU SDSS14Y00144T-0M-0Y-0B-0Y-0B-28Y	2	44.4
D67.2/PARANA 66.270//AE.SQUARROSA (741)/3/BORL14/4/FRET2*2/SHAMA//KACHU SDSS14Y00123T-0M-0Y-0B-0Y-0B-58Y	2	42.4
IG 41643/BORL14/3/FRET2*2/SHAMA//KACHU SDSS14Y00128T-0M-0Y-0B-0Y-0B-52Y	2	41.5
Trial: 10° Satyn		
SOKOLL/5/W15.92/4/PASTOR//HXL7573/2*BAU/3/WBLL1/6/SOKOLL/3/PASTOR//HXL7573/2*BAU PTSS12SHB00020T-0TOPB-099Y-099B-6Y-020Y-0B	2	35.1
SOKOLL/3/PASTOR//HXL7573/2*BAU/4/WBLL4//OAX93.24.35/WBLL1/5/D67.2/PARANA 66.270//AE.SQUARROSA (320)/3/CUNNINGHAM/4/VORB PTSS15Y00138S-099B-099Y-099M-5Y-020Y-0B	1	33.9
KS940935.7.1.2/2*PASTOR/4/FRAME//MILAN/KAUZ/3/PASTOR/5/D67.2/PARANA 66.270//AE.SQUARROSA (320)/3/CUNNINGHAM/4/VORB PTSS14Y00103S-0B-099Y-099B-8Y-020Y-0B	1	32.8
SOKOLL/3/PASTOR//HXL7573/2*BAU/4/WBLL4//OAX93.24.35/WBLL1/5/D67.2/PARANA 66.270//AE.SQUARROSA (320)/3/CUNNINGHAM/4/VORB PTSS15Y00138S-099B-099Y-099M-9Y-020Y-0B	2	32.4
MEX94.27.1.20/3/SOKOLL//ATTILA/3*BCN/4/PUB94.15.1.12/WBLL1/5/MUCUY PTSS14Y00328S-0B-099Y-099B-19Y-020Y	2	32.3
SOKOLL/WBLL1/4/PIHA//WORRAKATTA/2*PASTOR/3/PRL/2*PASTOR PTSS15Y00152S-099B-099Y-099M-24Y-020Y-0B	1	32.2
BAJ #1 CGSS01Y00134S-099Y-099M-099M-13Y-0B	2	32
SOKOLL/WBLL1/5/D67.2/PARANA 66.270//AE.SQUARROSA (320)/3/CUNNINGHAM/4/VORB PTSS15Y00111S-099B-099Y-099M-2Y-020Y-0B	1	31.7
SOKOLL/5/W15.92/4/PASTOR//HXL7573/2*BAU/3/WBLL1/6/SOKOLL/3/PASTOR//HXL7573/2*BAU PTSS12SHB00020T-0TOPB-099Y-099B-25Y-020Y-0B	2	31.7
Trial: 8° Wycyt		
TUR.180085//QUAIU*2/KINDE PTSS16Y00047S-0B-099Y-099M-20Y-0B-0Y	2	45.6
CMH79A.955/4/AGA/3/4*SN64/CNO67//INIA66/5/NAC/6/RIALTO/7/SOKOLL/WBLL1 PTSS16Y00074S-0B-099Y-099M-22Y-0Y	2	40.6
ALTAR 84/AE.SQUARROSA (237)//2*KUTZ PTSS16B00017T-099Y-099M-16Y-0B-0Y	2	40.1
SOKOLL/5/W15.92/4/PASTOR//HXL7573/2*BAU/3/WBLL1/6/SOKOLL/3/PASTOR//HXL7573/2*BAU PTSS12SHB00020T-0TOPB-099Y-099B-6Y-020Y-0B	1	40.1
Trial:1R		
COLIBRI/ACACIA TR13CS148-100R-100C-0C-5C-0C-1R	3	42.2
COLIBRI/3/WBLL1/FRET2//PASTOR TR07CS225-12R-0C-0R-5C-0R-0C	3	42
ENE/ZITA/3/WBLL4/KASOS//PASTOR/8/TACUPETO F2001/6/CNDO/R143//ENTE/ TR11CS152-0R-0C-0R-0C-9R-0C-0R	1	40.9
HALITA/COLIBRI//THELIN/2*WBLL1 TR13CS226-100C-100C-0R-4C-0R-0C	1	40.8

ACKNOWLEDGMENTS

The Research Station 'Altos de Jalisco', now known as 'Campo Experimental Centro-Altos de Jalisco', was established in 1974. We acknowledge the institution and its dedicated personnel for their unwavering support and valuable contributions that have benefitted the people of Mexico, marking a significant milestone over decades. This manuscript stands as our tribute, commemorating 50 years of their remarkable achievements.

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