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DESCRIPTIONS OF PLANT GENETIC MATERIALS

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Exploring Fusarium head blight resistance in a winter triticale germplasm collection

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Abstract

Fusarium head blight (FHB; caused by *Fusarium graminearum*) is a destructive disease of wheat (*Triticum* spp.), barley (*Hordeum vulgare*), rye (*Secale cereale* L.), and triticale (×*Triticosecale* Wittmack) not only reducing their yield but also contaminating the grain with mycotoxins such as deoxynivalenol (DON). Developing varieties with genetic resistance is integral to successfully manage FHB. Triticale acreage worldwide is steadily increasing. However, the genetic diversity of triticale for FHB resistance is not well characterized. In the present study, a sequential screening of a set of winter triticale accessions from a global collection was done for their type-2 FHB resistance and DON accumulation. In the first-year screening, 298 triticale accessions were tested for FHB in an artificially inoculated, misted-field nursery with high inoculum density. Most of the triticale accessions were susceptible to FHB, and only 8% of the accessions showed resistance in the field nursery screening. Next, the 24 resistant accessions identified in the nursery screening were tested for 2 years in greenhouse and 17 accessions showed significantly lower FHB severity in Year 2 and/or Year 3. These 17 resistant accessions were further tested for their FHB severity and DON accumulation in Year 4 in greenhouse and for DON accumulation in Year 5 in the field FHB nursery. Eight accessions showed significantly lower FHB severity and nine accessions showed DON accumulation of less than 1 mg/kg in Year 4 greenhouse testing. Eleven accessions had significantly lower DON concentration than the susceptible check in the Year 5 field screening. The resistant accessions common across all years identified in the study can be used for enhancing FHB resistance and reducing DON accumulation in triticale breeding programs.

Abbreviations: DI, disease incidence; DON, deoxynivalenol; DS, disease severity; FHB, Fusarium head blight; NSGC, National Small Grains Collection; PDA, potato dextrose agar.

1 | INTRODUCTION

Triticale (×*Triticosecale* Wittmack) is a man-made cereal crop developed by hybridizing wheat (*Triticum* spp.) and rye (*Secale cereale* L.). It combines the superior grain quality and high yield potential of wheat with resistance to abiotic and biotic factors of rye (Mergoum et al., 2009; Oettler et al., 1991). European countries have spearheaded the development and breeding of triticale to adapt it to diverse environments and soil conditions (Randhawa et al., 2015). With more than 92% of world's triticale being grown in Europe, Poland, Germany, France, and Belarus are the top producer countries of triticale (FAOSTAT, 2024). The first improved commercial triticale cultivar was released in Hungary in 1968 (Blum, 2014). In North America, triticale breeding started at the University of Manitoba in 1954, and the first cultivar 'Rosner', was released in 1969 (Larter et al., 1970). According to the data from the Farm Service Agency, USDA, ~0.18 million ha was under triticale cultivation in the United States in 2012, whereas in 2015, this acreage increased to ~0.31 million ha. Further, in 2022, ~0.52 million ha of land in the United States was under triticale cultivation. This increased cultivation demonstrates the constantly increasing production and popularity of triticale in the United States. Triticale is being used as a superior forage crop because of its high biomass yield, high protein content, high digestibility coefficient, and good amino acid profile (Ayalew et al., 2018). Triticale has also gained popularity as a cover crop because of its high nitrogen use efficiency and biotic and abiotic stress tolerance, providing it an advantage in nutrient-poor soils over traditional cereal crops (Ayalew et al., 2018; Blum, 2014; Ketterings et al., 2015).

Core Ideas

- A set of 298 triticale accessions was sequentially tested for FHB severity and DON content over multiple years.
- Only 2% of the accessions were found to be resistant and with low DON content across all the years.
- The robustly characterized accessions with low FHB severity and DON content can be used as sources of resistance.

Fusarium head blight (FHB), caused by *Fusarium graminearum* in the United States, is a major disease of wheat and barley (*Hordeum vulgare*) (McMullen et al., 2012). In addition to causing direct yield losses worth millions of dollars annually, FHB also contaminates grain with associated mycotoxins such as deoxynivalenol (DON) and nivalenol (Goswami & Kistler, 2004; McMullen et al., 2012). These mycotoxins are potent protein-synthesis inhibitors, and symptoms associated with their intake in humans include headache, fever, emesis, diarrhea, and loss of appetite (D'Mello et al., 1999; Pestka, 2010; Pestka & Smolinski, 2005). In animals, it leads to reduced feeding, poor growth, lower egg production, reduced carcass quality, poor fertility and hatchability of eggs, and immunosuppression (Bryden, 2012; Pestka, 2010). Genetic resistance is one of the major strategies of managing FHB and DON accumulation in wheat and barley (Bai & Shaner, 2004; McMullen et al., 2012).

Genetic resistance against FHB is quantitative, and more than 550 quantitative trait loci with varying effects on FHB severity and DON concentration have been reported in wheat (Steiner et al., 2017; Venske et al., 2019). Several wheat varieties with at least some level of effective resistance are available in all the wheat-growing regions of the world (Singh et al., 2023; Steiner et al., 2017; Venske et al., 2019). Triticale, being a synthetic crop developed by combining wheat and rye, is expected to be susceptible to FHB and DON accumulation (Arseniuk et al., 1999; Góral et al., 2016; Veitch et al., 2008; Yi et al., 2018). Veitch et al. (2008) analyzed seven winter-type and five spring-type triticale varieties in multi-year and multi-site tests and found them to have higher FHB susceptibility and DON concentration than the wheat checks. Similarly, Góral et al. (2016) analyzed 32 winter triticale and 34 winter wheat accessions and found that FHB severity and Fusarium damaged kernel percentages were lower for triticale, whereas DON concentration was higher in triticale than wheat. However, all these experiments have screened a relatively small number of local lines, not providing a clear information on the frequency of FHB genetic resistance among larger, more diverse collections of triticale.

In the present study, we performed a sequential screening for type-2 FHB resistance (resistance against fungal spread within spike) of a large set of diverse hexaploid winter-type triticale collection comprised of 298 accessions. This triticale panel has previously been shown to contain large genetic diversity using genotyping-by-sequencing and population structure analyses (Ayalew et al., 2021). First tier screening was done under high FHB pressure misted nursery field conditions for severity. Field studies for FHB evaluation involve considerable genotype × environment interaction, making it difficult to analyze the effectiveness of genetic resistance (Meidaner et al., 2001; Yi et al., 2018). Therefore, the lines showing a high level of type-2 resistance selected from the field screening were tested in greenhouse conditions for FHB severity over 2 years. Subsequently, an elite set of highly resistant triticale accessions were tested in greenhouse in Year 4 for their FHB severity and DON accumulation. Finally, in Year 5, the selected accessions were tested for their DON concentration in the artificially inoculated misted nursery. The accessions showing consistently low

FHB severity and DON concentration can be used as robust sources of FHB genetic resistance in triticale breeding. In addition, these sources could also be used for wheat and barley improvement.

2 | METHODS

2.1 | Field testing

2.1.1 | Plant material and experimental design for Year 1, and Year 5 field testing

Experiments were conducted at the Beltsville Research Farm Facility of the University of Maryland (−76.833195 long., 39.011599 lat.) during the planting season of 2017–2018 in Year 1 and 2021–2022 in Year 5. In Year 1, a set of 298 diverse winter-type hexaploid triticale accessions was obtained from the National Small Grains Collection (NSGC) Repository, Aberdeen, ID, USA. Fifty seeds of each accession were planted in 120 cm (4 feet) long single rows. Plant height and flowering times were recorded for each line. In Year 5, the selected accessions from greenhouse testing (from Years 2, 3, 4) were planted in three replications in a randomized complete block design. All field experiments were conducted on no-till plots with corn-stubble from previous growing cycles to ensure a high inoculum load for infection of the plants, as crop residues are known to enhance FHB in wheat (Dill-Macky & Jones, 2000).

2.1.2 | Fungal inoculation for field testing

Three *F. graminearum* isolates collected from Maryland (one each from Clarksville, Beltsville, and Wye farm locations in the state) were used for generating corn-spawn inoculum for field. Corn (*Zea mays*) kernels inoculated with fungal plugs from 50% glycerol stocks were cultured on potato dextrose agar (PDA) plates for each isolate and grown at room temperature. Maize kernels (6–7 kg) were rinsed with water and autoclaved twice for 30 min with each autoclave cycle. One week after starting fungal cultures, each corn tray was inoculated with cultures from the 3 PDA plates using 150 mL of autoclaved water containing 0.2 g of streptomycin sulfate to prevent bacterial contamination. Inoculated trays were covered with aluminum foil and incubated at room temperature for 2.5 weeks. Fungal growth was monitored at weekly intervals by observing pink pigmentation and white mycelial growth. After 2.5 weeks, the inoculum was transferred to autoclaved burlap sacks to half of their capacity and then dried for 1 week at 35˚C (Gilbert & Woods et al., 2006). At the tillering stage the corn–spawn inoculum was manually spread in the field at an application rate of 40 g inoculum per m^2. High humidity was maintained at the inoculated site by daily misting the field overnight for 5 min every hour from 9 p.m. to 6 a.m. until the latest flowering line reached anthesis, after which the misting was stopped (Chhabra et al., 2021a).

2.1.3 | Field FHB index data

Disease incidence (DI) and severity (DS) were evaluated as indicators of FHB spread. Readings were taken 25 days after anthesis for each line. Twenty spikes per row were randomly selected for phenotyping. DI was calculated by visually assessing the percentage of spikes infected in a whole row. DS was calculated by taking the average of number of diseased spikelets per spike. Diseased spikelets looked prematurely bleached whereas healthy spikes were still green. FHB index was calculated as a product of DI and DS divided by 100. Lines were classified into different FHB response groups based on their FHB index. On the basis of FHB index scores, a subset of 24 lines was selected for further testing for type-2 resistance in the greenhouse in 2018 and 2019. FHB index up to 10% was chosen as the criterion for selection.

2.2 | Greenhouse testing

2.2.1 | Greenhouse planting

Seeds of the selected 24 accessions plus a susceptible check from field testing were planted in the greenhouse in 2018 and 2019. In 2020, a smaller subset of selected 17 lines were planted to analyze FHB severity and DON accumulation. In each year, three plants per accession were grown in individual 15 cm pots (1 plant/pot) and vernalized for 6 weeks at 4˚C. Following vernalization, plants were transferred to a greenhouse with a day temperature of 23–25˚C, a night temperature of 16–18˚C, and 16 h of light and 8 h of darkness.

2.2.2 | Fungal inoculum preparation

F. graminearum isolate GZ3639, known for its strong virulence (Chhabra et al., 2021b; Rawat et al., 2016), was used for all the greenhouse experiments. For macroconidial production, 2 plugs of potato dextrose agar mycelial culture of the fungus were inoculated in Mung bean broth, which was shaken at 200 rpm at 28˚C for 7–10 days. Macroconidia were counted on a hemocytometer and inoculum was prepared by diluting the culture to a concentration of 1×10^5 spores/mL using sterile water.

2.2.3 | Inoculation and FHB severity measurement

Inoculations were performed at pre-anthesis stage on spikes, which was about 2 days prior to anthers emerging out of the spikes. The 10th and 11th spikelets (counted from the base of the spikes) were marked with a black Sharpie marker, and 10 μL macroconidial inoculum was injected between the lemma and palea of the florets (1 floret/spikelet), avoiding injury to any other part of the florets. Spikes were covered with moisture-saturated zip lock bags for 72 h to provide high humidity for optimal fungal growth (Chhabra et al., 2024). For each genotype, 8–10 spikes were tested in each experiment. Phenotyping was done 28 days after inoculation. FHB severity was calculated by dividing the number of bleached spikelets downward from the point of inoculation by 10 and multiplying by 100.

2.3 | DON concentration measurement

DON concentration of seeds was measured by gas chromatography/mass spectrometry following Mirocha et al. (1998). Seeds from infected spikes from all the three plants of each accession were manually threshed, bulked, and divided into three technical replicates. Briefly, 1 g of ground samples were extracted with 12 mL of acetonitrile/water (84/16, v/v) in 15-mL centrifuge tubes. Each sample was placed on a shaker for 24 h, and then 4 mL of the extract was passed through a column packed with C18 and aluminum oxide (1/3, w/w). Two milliliter of the filtrate was evaporated to dryness under nitrogen at room temperature, and 70 μL of Trimethylsilyl (TMS) reagent (TMSI/TMCS, 100/1) was added to the vial, rotating the vial so that the reagent made contact with residue on the sides of the vial. *N*-trimethylsilylimidazole (TMSI, ≥ 98.0%) and trimethylchlorosilane (TMCS, ≥ 99.0%) were purchased from Sigma-Aldrich. The vial was placed on a shaker for 10 min, and then 700 μL of isooctane containing 0.5 μg/mL mirex was added and shaken gently. High-performance liquid chromatography water (700 μL) was added to quench the reaction, and the vial was vortexed so that the milky isooctane layer becomes transparent. The upper layer was transferred into a gas chromatography vial for gas chromatography/mass spectrometry analysis. All samples were analyzed on the Shimadzu GCMS-QP2020 gas chromatograph-mass spectrometer (Shimadzu Corp.). An Agilent J&W DB-5MS capillary column (0.25-μm film thickness, 0.25 mm i.d., and 30 m length) was used to separate compounds. A high-pressure injection method (300.0 kPa, 1.00 min) was used in the splitless injection system. Linear velocity of flow control mode (40.1 cm/s) was used with the following temperature program: 150˚C for 1 min and then 30˚C/min to 280˚C holding 4 min. The injection, ion source, and interface temperatures were kept at 240, 250, and 280˚C, respectively. Injection volume was 1 μL. Selected ion monitoring mode was used to detect the characteristic ions of DON with fragment ions of m/z 235.10 as target ion and 259.10 and 422.10 as reference ions.

2.4 | Statistical analysis

Analysis was done in R (version R x64 3.6.3) and R Studio, employing the *lme4*, *lmer*, *car*, and *ggplot2* packages. Experiments in Years 2, 3, and 4 were conducted in a completely randomized design, treating each spike as an individual replicate. In Year 5, the selected accessions from greenhouse testing (from Years 2, 3, 4) were planted in three replications in a randomized complete block design. Before analysis, assessments were conducted to ensure the data met the assumptions of normality and homogeneity of variance. A mixed-model ANOVA (Type-3) was conducted for the 2018 and 2019 FHB severity data, considering accessions as a fixed effect and year as a random effect. In contrast, one-way ANOVA (Type-3) analyses were performed for the 2020 FHB severity and DON concentration data, and two-way ANOVA was performed for 2022 DON concentration data from the field.

To address non-normalized FHB severity data, $\log_{10}$ transformation was applied, and square root transformation was used for the DON concentration data. Pair-wise comparisons between the susceptible check (PI 414968) and other selected accessions were done using Dunnett test at 95% confidence interval.

3 | RESULTS

3.1 | Evaluation of FHB index, plant height, and flowering times

A wide range of diversity for FHB indices was observed among the 298 winter-type accessions screened in *Fusarium*-inoculated corn-spawn misted nursery field conditions. Disease index among the accessions varied from 3% to 100%, showing maintenance of very high-disease pressure conditions in the nursery and a wide range of variation present in the triticale panel. The accessions were divided into four groups: resistant (group-1: 0–10% FHB index), moderately resistant (group-2: 10–40% FHB index), moderately susceptible (group-3: 40–70% FHB index), and susceptible (group-4: 70–100% FHB index). With 24 accessions classified in group-1, the resistant group was the smallest among all the four groups. Group-2, -3, and -4 contained 74, 122, and 78 accessions, respectively. The 24 lines from group-1 with the

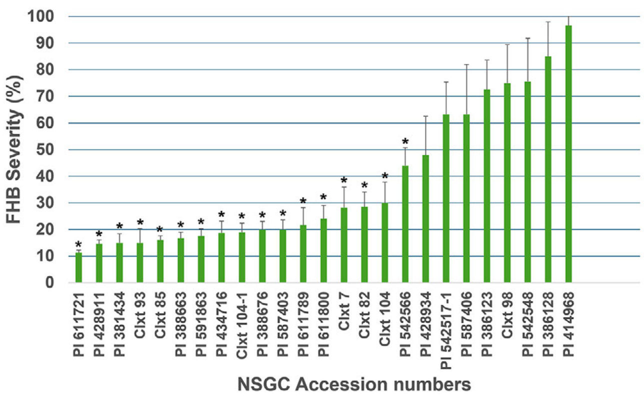

FIGURE 1 Fusarium head blight (FHB) severity (%) of selected 24 accessions and susceptible control PI 414968 tested in the greenhouse in 2018 and 2019. Error bars represent standard error. '*' indicates significant difference from control at $\alpha = 0.05$. NSGC, National Small Grains Collection.

lowest FHB index in field testing in 2017 were selected for further greenhouse testing in 2018 and 2019. Susceptible accession PI 414968 with a disease index of 100% was used as a susceptible check in all the greenhouse experiments. Plant height of the 298 accessions showed a wide distribution ranging from 54 cm to 174 cm, and flowering times ranged from 187 days to 220 days after planting (Supplementary Table 1). In the present work, FHB severity was not found to be correlated to plant height in the panel ($r = -0.1$). The height of the selected 24 accessions ranged from 75 to 142 cm, and flowering times of the selected set varied from 187 days to 210 days after planting, indicating the absence of association of FHB index with plant height or flowering times.

3.2 | Greenhouse evaluation of FHB severity in selected resistant lines in 2018 and 2019

In greenhouse conditions, the selected resistant accessions showed a wide range of FHB severity (Figure 1). A mixed-model ANOVA revealed a significant genotype effect at $p < 0.001$. Susceptible check triticale accession PI 414968 showed an average disease severity of 100%. Seventeen accessions (PI 611721, PI 428911, PI 381434, CIxt 93, CIxt 85, PI 388663, PI 591863, PI 434716, CIxt 104-1, PI 388676, PI 587403, PI 611789, PI 611800, Clxt 7, Clxt 82, Clxt 104, and PI 542566) showed significantly lower FHB severity than the susceptible check PI 414968. DON concentration accumulation was not measured in 2018 or 2019. A final set of 17 accessions and susceptible control PI 414968 was selected for final evaluation of FHB severity and DON concentration accumulation in 2020 in greenhouse and in 2022 in field inoculated nursery.

TABLE 1 Analysis of variance of Fusarium head blight (FHB) severity and deoxynivalenol (DON) concentration for the selected 17 accessions in 2020 greenhouse testing.

	FHB severity		DON concentration	
	F value	*p*-value	F value	*p*-value
NSGC Accession number	7.67	1.23e−13	5.63	6.75e−06

Abbreviation: NSGC, National Small Grains Collection.

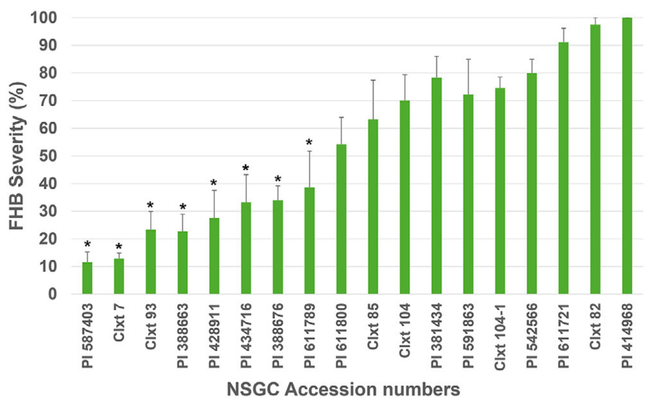

FIGURE 2 Fusarium head blight (FHB) severity of the set of selected 17 accessions and susceptible control PI 414968 tested in the greenhouse in 2020. '*' indicates significant difference from control for FHB severity at $\alpha = 0.05$. Error bars indicate standard error. NSGC, National Small Grains Collection.

3.3 | Greenhouse evaluation of FHB severity and DON concentration of final selected set in 2020

A one-way ANOVA for FHB severity of the selected 17 accessions and susceptible check PI 414968 tested under greenhouse conditions revealed a significant genotypic effect at $p < 0.001$ (Table 1). Eight accessions (PI 587403, CIxt 7, CIxt 93, PI 388663, PI 428911, PI 434716, PI 388676, PI 611789) showed significantly lower mean FHB severity than the susceptible check which showed a 100% average FHB severity (Figure 2). Other accessions had numerically lower, but statistically similar mean FHB severity to control.

ANOVA for DON concentration in the 2020 greenhouse test showed a significant genotypic effect at $p < 0.001$ (Table 1). Since PI 414968 was highly susceptible, sufficient seed sample weight could not be arranged for DON testing of the check from this set. DON concentration in the analyzed samples ranged from 0 mg/kg to 9.2 mg/kg. Nine accessions (PI 611800: 0 mg/kg, CIxt 93: 0 mg/kg, PI 611721: 0.1 mg/kg, PI 591863: 0.1 mg/kg, CIxt 104: 0.1 mg/kg, CIxt 7: 0.1 mg/kg, CIxt 85: 0.1 mg/kg, PI 587403: 0.5 mg/kg, and CIxt 104-1: 0.8 mg/kg) were found to have less than 1 mg/kg DON and were selected as low DON concentration lines. CIxt 82, with

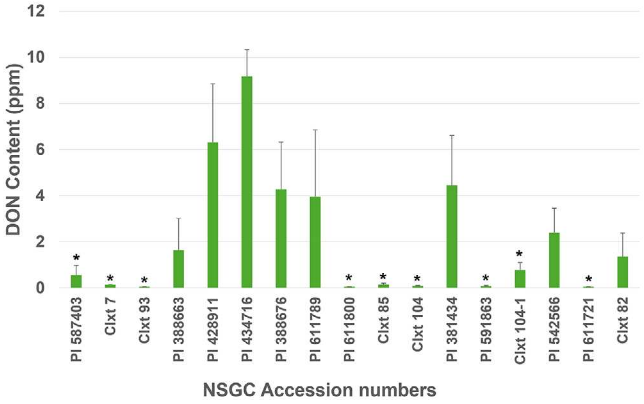

FIGURE 3 Deoxynivalenol (DON) concentration of the set of selected 17 accessions tested in the greenhouse in 2020. DON concentration could not be measured for control PI 414968 because of very high susceptibility and poor seed set in it. '*' indicate DON levels less than 1 mg/kg. Error bars indicate standard error. NSGC, National Small Grains Collection.

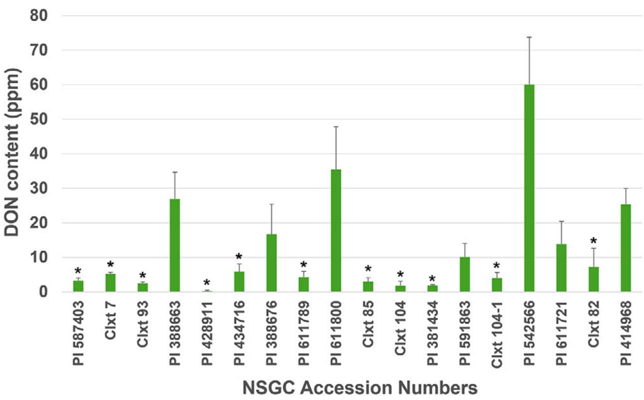

FIGURE 4 Deoxynivalenol (DON) concentration of the set of selected 17 accessions and control PI 414968 tested in the field nursery in 2022. '*' indicates significant difference from control at $\alpha = 0.05$. Error bars indicate standard error. NSGC, National Small Grains Collection.

DON concentration of 1.3 mg/kg, was also close to this range (Figure 3). It is important to note that accessions PI 428911, PI 434716, and PI 388676 had low average FHB severity, but had high mean DON concentration, indicating different regulation of these two parameters of FHB resistance.

3.4 | DON concentration accumulation in the final field testing in 2022

Since PI 414968, the control accession, could not be tested for DON concentration in the 2020 greenhouse test, the major emphasis of this trial was to collect sufficient samples to perform comparative DON concentration data of all selected 17 accessions from the field (Figure 4). The maximum DON concentration was higher in the field samples of this set than the greenhouse set of 2020. The DON concentrations ranged from 0 mg/kg to 60 mg/kg in this set, including PI 414968, the control accession, with a DON concentration of 25.2 mg/kg. Eleven accessions (PI 587403: 3.3 mg/kg; CIxt 7: 5.2 mg/kg; CIxt 93: 2.6 mg/kg; PI 428911: 0 mg/kg; PI 434716: 5.9 mg/kg; PI 611789: 4.3 mg/kg, CIxt 85: 3.0 mg/kg; CIxt 104: 1.8 mg/kg; PI 381434: 1.8 mg/kg; CIxt 104-1: 4.0 mg/kg; and CIxt 82: 7.2 mg/kg) had significantly lower DON concentration than that of the control. PI 611721 had numerically lower DON concentration (13.9 mg/kg) than control but not significantly different.

It is important to note that not all the accessions with low DON concentrations in the field testing were common to the greenhouse set. The variation may be coming from differences in the isolates used for inoculations, differential moisture settings in the field versus greenhouse conditions, as well as the different flowering times providing different exposure lengths to the conducive conditions for infection. Nevertheless, the six accessions (PI 587403, CIxt 7, CIxt 93, CIxt 85, CIxt 104, and CIxt 104-1) which showed consistently low DON concentration and FHB severity in all the tests could be used as reliable sources of genetic resistance to FHB and DON accumulation in triticale breeding and beyond.

4 | DISCUSSION

We systematically screened a large collection of triticale accessions for identification and confirmation of accessions with high levels of FHB resistance and low DON concentration accumulation. In the field testing in Year 1 of the study, 298 accessions were screened in a corn-kernel inoculated, FHB-misted nursery. A relatively small number of lines (8%) showed a high level of FHB resistance in the field screening, whereas the majority of the accessions (67%) were moderately to highly susceptible.

Since field evaluation of FHB response of the plants is subject to genotype × environment interaction, the lines selected for their low FHB index from the field were subsequently tested for three more seasons in greenhouse conditions with point inoculations. Finally, the resistant accessions were tested for one last time in field conditions. Compiling all the datasets, six accessions were found to be significantly resistant to FHB and had low DON concentration relative to the control. Consistent sifting of the lines removed several accessions with every round of testing, which might have shown misleading phenotypes earlier on account of the disease escape. As a consequence, the final selected resistant lines represent only 2% of the original set. This very small percentage of lines with robust resistance is not surprising, considering that most of the resistant sources in wheat have been reported from Asian sources (Bai & Shaner, 2004;

Steiner et al., 2017), yet most of the triticale lines are of European origin. Even in wheat, a previously reported analysis of 34,571 wheat landraces and other germplasm from China and Japan identified 1765 lines with high levels of FHB resistance (Bai et al., 2018), which accounted for 5.1% of the lines. The small set of lines identified in the present study with low FHB severity and DON concentration constitute useful sources of FHB resistance for use not only in triticale breeding programs, but also for wheat improvement.

Plant height has been reported to be associated with FHB severity, with shorter genotypes developing more severe disease (Hilton et al., 1999; Kalih et al., 2015; Mesterházy, 1995; Miedaner & Voss, 2008; Yan et al., 2011). This might be specifically applicable for field-based screening, as chances of splash dispersal of *Fusarium* spores to the spikes are higher at levels closer to the soil (Hilton et al., 1999; Yan et al., 2011). The final set of resistant accessions obtained after three rounds of greenhouse testing displayed a wide range of plant height (75–142 cm), including three accessions with average heights of less than 100 cm, further confirming the lack of association between plant height and FHB severity. Although Miedaner and Voss (2008) reported increased FHB severity in reduced-height mutants from a near-isogenic line set, they concluded that the contribution of reduced height to FHB severity can be counteracted by a more resistant genetic background. This supports the idea that genetic resistance is independent of plant height and is consistent with our observations.

Correlations between heading and flowering times with FHB severity have previously been reported in wheat, rye, and triticale (Börner et al., 2000; Kalih et al., 2014; Miedaner, 1997; Miedaner & Voss, 2008). Early flowering lines are considered predisposed to higher FHB intensity (Mesterházy, 1995). However, FHB severity and heading time were not found to be correlated in our study ($r = 0.02$). The heading times of the final six resistant lines in our study varied considerably, where four of the lines had the shortest heading times. This discrepancy might be related to the fact most of these previous studies were conducted in fields where environmental factors may favor earlier development of disease. All of these traits are pleiotropic and multigenic, and association among them becomes complicated in field conditions. In our final set of lines, which were confirmed multiple times in controlled environmental conditions, we did not find any particular association between FHB severity and heading times.

It is important to note that the DON concentration of the finally selected triticale accessions did not show a correlation with the FHB severity in the greenhouse test ($r = 0.1$). Only four of the nine low DON concentration lines also had significantly lower FHB severity in the 2020 set than the susceptible control (Figures 2, 3). Miedaner et al. (2016) also reported a poor correlation between DON concentration and FHB severity in a doubled-haploid population of 146 individuals of triticale and concluded that prediction of DON concentration from FHB severity was not possible. Such poor correlation has also been reported for wheat (He et al., 2019; Paul et al., 2005). Paul et al. (2005) performed a meta-analysis of 163 studies in wheat reporting FHB visual symptoms and DON concentration and found a low level of correlation between FHB incidence and DON concentration. In fact, the correlation between FHB severity and DON concentration in triticale has been reported to be even lower than in wheat (Miedaner et al., 2016; 2004). All these studies, including the current study, indicate that there are different genetic controls or mechanisms of FHB severity and DON accumulation that appear to be independent of each other. This indicates that the breeding efforts must focus on both traits separately. The triticale lines robustly confirmed to contain low FHB severity and DON concentration in multiple years of greenhouse testing, and field testing can be used as sources of genetic resistance for breeding improved triticale and wheat varieties. The NSGC accession numbers of the six most consistently resistant lines are: PI 587403, CIxt 7, CIxt 93, CIxt 85, CIxt 104, and CIxt 104-1. Genetic mapping for the underlying genes will help in performing markers-assisted selection and pyramiding for their usage in breeding programs.

AUTHOR CONTRIBUTIONS

Sydney Wallace: Investigation; methodology; writing—original draft. **Bhavit Chhabra**: Formal analysis; methodology; software; writing—review and editing. **Yanhong Dong**: Formal analysis; writing—review and editing. **Xuefeng Ma**: Resources; writing—review & editing. **Gary Coleman**: Resources; writing—review and editing. **Vijay Tiwari**: Conceptualization; funding acquisition; resources; supervision; writing—review & editing. **Nidhi Rawat**: Conceptualization; formal analysis; funding acquisition; methodology; project administration; resources; supervision; writing—original draft.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

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