Received: 20 July 2023 | Accepted: 14 February 2024 | Published online: 20 March 2024

DOI: 10.1002/csc2.21226

Crop Science

ORIGINAL ARTICLE

Crop Breeding & Genetics

Evaluation of speed breeding conditions for accelerating Fusarium head blight and deoxynivalenol screening in wheat

Bhavit Chhabra[1] | **Saijagruti Thrasu**[1] | **Sydney Wallace**[1] | **Adam Schoen**[1] | **Fereshteh Shahoveisi**[1] | **Yanhong Dong**[2] | **Vijay Tiwari**[1] | **Nidhi Rawat**[1]

[1]Department of Plant Science and Landscape Architecture, University of Maryland, College Park, Maryland, USA

[2]Department of Plant Pathology, University of Minnesota, St. Paul, Minnesota, USA

Correspondence
Nidhi Rawat and Vijay Tiwari, Department of Plant Science and Landscape Architecture, University of Maryland College Park, 20742, Maryland, USA.
Email: nidhirwt@umd.edu and vktiwari@umd.edu

Assigned to Associate Editor Esten Mason.

Funding information
National Institute of Food and Agriculture Award, Grant/Award Numbers: 2020-67013-32558, 2020-67013-31460; US Wheat and Barley Scab Initiative Award, Grant/Award Numbers: 59-0206-2-130, 59-0206-2-163

Abstract

Feeding the world's ever-increasing population requires continuous development of high-yielding and disease-resistant cultivars of food crops such as wheat (*Triticum aestivum* L.). Speed breeding, which utilizes longer photoperiod times and higher temperatures, is a technique that accelerates plant development and is rapidly being adopted by wheat breeders across the globe to fast-track cultivar development. Plant diseases are a major threat to crop production, and breeding for disease resistance is a major goal of crop breeders. Fusarium head blight (FHB), caused by *Fusarium graminearum*, is a major disease of small grain cereals, affecting their yield and quality. The aim of present work was to assess if speed breeding conditions can be used to accelerate reliable assessment of FHB severity and mycotoxin deoxynivalenol (DON) accumulation in wheat varieties. We screened a set of six spring wheat genotypes with different levels of genetic resistance (two moderately susceptible, two highly susceptible, one moderately resistant, and one resistant) for their response to FHB at 14 days after inoculation (dai) and 21 dai and DON accumulation under normal versus speed breeding conditions. FHB severity and DON accumulation were found to be highly correlated at all time points under normal and speed breeding conditions. Robust differentiation between resistant and susceptible genotypes could be achieved at 14 dai rather than the normal period of 21 dai, saving at least a week in phenotyping. Combined with the accelerated growth, flowering, and maturity under these conditions, efficient FHB screening and DON evaluation under speed breeding conditions will fast-track development of resistant wheat varieties.

1 | INTRODUCTION

Fusarium head blight (FHB) is a serious fungal disease of small grain cereals, such as wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.), that reduces not only yield but also safety of the grain on a global scale (Goswami & Kistler, 2004; McMullen et al., 2012). In the United States, FHB is primarily caused by a hemibiotrophic, ascomycete fungus *Fusarium graminearum* (sexual stage: *Gibberella zeae*) (Goswami & Kistler, 2004; Parry et al., 1995; Xu & Nicholson, 2009). Typical symptoms of FHB are pre-mature bleaching of spikelets, resulting in production of sterile or shriveled "tombstone" kernels (Bai & Shaner, 1994; Parry et al., 1995). Additionally, *F. graminearum* produces

mycotoxins such as deoxynivalenol (DON) in the grain. DON is a virulence factor and protein synthesis inhibitor, and in the United States, it is regulated in grain to less than 1 ppm for human consumption by the Food and Drug Administration (Bai et al., 2002; Jansen et al., 2005; Rocha et al., 2005).

Genetic resistance is the most sustainable way of managing scab, however, availability of highly resistant cultivars is limited (McMullen et al., 2012). Therefore, breeding programs across the world strive to develop improved wheat cultivars with high FHB resistance (Fernando et al., 2021; Ma et al., 2020). Development of improved cultivars takes several years, with a typical breeding cycle of up to 12 years: starting with crossing the parents, multiple rounds of inbreeding, field testing, and then selections (Alahmad et al., 2018; Watson et al., 2018). Traditionally, only one generation per year can be achieved under field conditions for wheat (Watson et al., 2018). Therefore, the initial advancement efforts are generally performed under controlled conditions. Continuous efforts are being made to reduce the generational time. Breeders across the world are utilizing multiple strategies, such as incorporating shuttle breeding, doubled haploids, mutation breeding, speed breeding, genomic selections, and high-throughput phenotyping, to reduce generation times (Alahmad et al., 2018). Speed breeding is one of the most convenient ways to reduce generation time, as it is suitable for diverse germplasm, does not require in vitro culturing, or use of specialized equipment. Ghosh et al. (2018) developed glasshouse and growth chamber-based speed breeding approaches for small grain crops (such as wheat), legumes, *Brassica* spp., and *Brachypodium*.

Watson et al. (2018) screened a highly resistant wheat landrace Sumai 3 and a highly susceptible cultivar Timstein for FHB by growing the plants under speed breeding conditions (22-h light/2-h dark, 22˚C day/17˚C night temperatures). They found that plants showed response to FHB expected as per their genetic resistance, providing evidence that speed breeding protocols can be utilized to differentiate between highly susceptible and highly resistant genotypes. They did not, however, evaluate the response of varieties with subtly different levels of genetic resistance to FHB under speed breeding conditions. Furthermore, they did not study the impact of speed breeding on DON content accumulation in the grains. Zakieh et al. (2021) screened winter wheat genotypes against FHB, in which they grew plants under speed breeding conditions until heading stage but switched to normal conditions at the time of testing for FHB response. Though they showed the utilization of speed breeding to accelerate vegetative growth, they did not perform the evaluation of FHB severity and DON accumulation of the genotypes under speed breeding conditions. The applicability of testing for FHB within the framework of speed breeding conditions and the time-saving benefits associated with their implementation have yet to be determined.

Core Ideas

- Speed breeding is increasingly being adopted by crop breeding programs.
- We evaluated spring wheat genotypes with different levels of genetic resistance for Fusarium head blight (FHB) and deoxynivalenol (DON) levels under speed breeding versus regular conditions.
- Reliable distinction could be made in FHB severity at 14 days after inoculation (dai) under speed breeding as compared to 21 dai under normal growth conditions.
- Growing and testing the plants under speed breeding conditions saved a total of 21–23 days as compared to regular conditions.

The present study was conducted to (1) compare FHB severity in multiple wheat genotypes under normal and speed breeding conditions, (2) evaluate how much time can be saved with speed breeding to robustly phenotype subtly different levels of genetic resistance in wheat genotypes, and (3) study how speed breeding conditions impact DON content accumulation in FHB-inoculated wheat spikes.

2 | MATERIALS AND METHODS

2.1 | Plant material

For this study, we used three spring-type (Bobwhite, WL711, and HR58), a facultative winter-type (Fielder) hexaploid wheat genotypes, and two spring-type tetraploid durum wheat genotypes (Kronos and Altar). HR58 contains *Fhb1*, the most stable large effect quantitative trait locus against FHB and shows high resistance (Rawat et al., 2016). Fielder, however, is moderately resistant to FHB, while WL711 and Bobwhite are moderately susceptible (Han et al., 2012). Tetraploid genotypes Kronos and Altar are both susceptible to FHB (Gadaleta et al., 2019).

2.2 | Plant growth conditions

Ten seeds of each genotype were sown in 4.5-inch pots with one plant per pot in a randomized complete block design. Plants were then moved into speed breeding conditions (22 hours light/2 hours dark with temperatures of 25˚C in the day and 22˚C at night) as described by Schoen et al. (2023). This was done so that plants in each treatment would come to heading at the same time, reducing variables in the experiment.

At the time of heading, five plants for each genotype were moved to normal conditions (16 hours light/8 hours dark with a temperature of 23°C in the morning and 20°C in the night), while the other five plants remained in speed breeding conditions. FHB testing was performed on plants in both conditions three times independently (Spring 2021, Fall 2021, and Winter 2021) and will be referred to as Trial-1, Trial-2, and Trial-3, respectively henceforth. These experiments were conducted in greenhouses that contained supplementary high-intensity discharge lights to supplement light conditions after sunset.

2.3 | Fungal inoculations

Fusarium graminearum isolate GZ3639 (Fg), which is known for its aggressiveness, was used for inoculations (Rawat et al., 2016). Fungal plugs were taken from 50% glycerol stocks (stored at −80°C) and cultured on potato dextrose agar (PDA) media at room temperature for 1 week. After 7 days after inoculation (dai), two PDA plugs with actively growing fungus were inoculated on mung bean broth and shaken at 180 rpm. After 1 week of inoculations, macro-conidia were counted on a hemocytometer, and the working inoculum was prepared by diluting the primary culture to a concentration of 1×10^5 (Chhabra, Tiwari, et al., 2021). Plants were inoculated with Fg at Feeke's stage 10.5 (pre-anthesis stage). The 10th and 11th spikelets were counted from base of the spike and marked with a black permanent marker. Ten microliters of macro-conidial suspension was injected into each of the marked spikelets. Spikes were covered with moisture saturated Ziplock bags for 72 hours in order to give proper moisture for optimal fungal growth. Twelve to 15 spikes per genotype were inoculated for each of the conditions in all three trials. Inoculations were done on multiple days to capture spikes at pre-anthesis stage.

2.4 | Data collection

FHB severity values were taken at 14 and 21 dai. Severity was calculated by counting the number of bleached spikelets, including the inoculated 10th spikelet, downward from the point of inoculation. Infection downward from this point was counted as it is more consistent and reliable for assessing type-2 resistance response of the plant. For calculating the percentage of symptomatic spikelets, the number of bleached spikelets below the point of inoculation was divided by 10 and then converted into a percentage (Chhabra, Tiwari, et al., 2021).

Seeds from all the samples were harvested at maturity and hand threshed. For each trial, seeds from each genotype per growth condition were combined and ground to a fine powder. The powdered samples were divided into three technical replicates and measured for DON content at the USWBSI DON-testing laboratory, University of Minnesota, by GC/MS chromatography following the previously described protocols (Chhabra, Singh, et al., 2021; Mirocha et al., 1998).

2.5 | Data analysis

Data analysis was done for FHB severity and DON content in R version 3.6.3. using *lme4* package. Each spike was treated as an individual replicate, leading to 45–60 replicates per genotype in each trial. Homogeneity of variance (HOV) was calculated using Levene's test for each trial. If HOV was non-significant, a combined analysis was performed; otherwise, separate analysis was conducted for each trial. A two-way analysis of variance (ANOVA) with interaction was used with genotype and growth setting as fixed effects, and trials and replicates as random effects in case of non-significant HOV, or only replicates as random effects for significant HOV. Pairwise comparisons were made with Fisher's least significant difference (LSD) using emmeans package at 95% confidence interval (Lenth et al., 2018). Correlation analysis was performed by calculating Pearson correlation coefficient using R package "ggpubr" (Kassambara, 2020). Average values of genotypes per trial were used for calculating correlation values.

3 | RESULTS

3.1 | Effect of speed breeding conditions on FHB severity

FHB severity in plants phenotyped under speed breeding conditions was found to be significantly higher than the plants phenotyped under normal growing conditions. Phenotypic analysis at two different time points (14 dai and 21 dai) under both conditions implied that disease severity occurs more in speed breeding conditions (Tables S1 and S2). Furthermore, we observed that resistant and susceptible genotypes showed their expected phenotype for FHB under speed breeding conditions. HOV was non-significant at both 14 dai and 21 dai (Tables S3a,b), hence, analysis was done by combining severity values from all three trials. A two-way ANOVA showed significant genotype, growth conditions (normal vs. speed) effect, and significant interaction between two at $p < 0.05$ (Tables 1 and 2).

Pairwise comparisons showed moderately susceptible and moderately resistant cultivars to have significantly higher FHB severity in speed breeding conditions over normal conditions at both time points. Kronos showed significantly higher FHB severity at 14 dai, whereas at 21 dai, FHB

TABLE 1 Analysis of variance of Fusarium head blight (FHB) severity at 14 days after inoculation (dai) under normal versus 14 dai under speed breeding conditions.

	Sum square	Mean square	Numerator df	Denominator df	*F* value	Pr (> *F*)
Genotype	29.9639	5.9928	5	338.42	113.7501	<2.2e-16*
Growth condition	1.6421	1.6421	1	340.85	31.1698	4.833e-08*
Genotype: Growth condition	0.9494	0.1899	5	340.49	3.6041	0.003438*

Numerator df stands for degrees of freedom for number of groups compared.
Denominator df stands for degrees of freedom for number of observations within the groups.
*Significance at $p < 0.05$.

TABLE 2 Analysis of variance of Fusarium head blight (FHB) severity at 21 days after inoculation (dai) under normal versus 21 dai under speed breeding conditions.

	Sum square	Mean square	Numerator df	Denominator df	*F* value	Pr (> *F*)
Genotype	36.595	7.3190	5	347.36	234.5464	<2.2e-16*
Growth condition	0.782	0.7824	1	347.40	25.0742	8.792e-07*
Genotype: Growth setting	0.565	0.1130	5	347.25	3.6199	0.00332*

Numerator df stands for degrees of freedom for number of groups compared.
Denominator df stands for degrees of freedom for number of observations within the groups.
*Significance at $p < 0.05$.

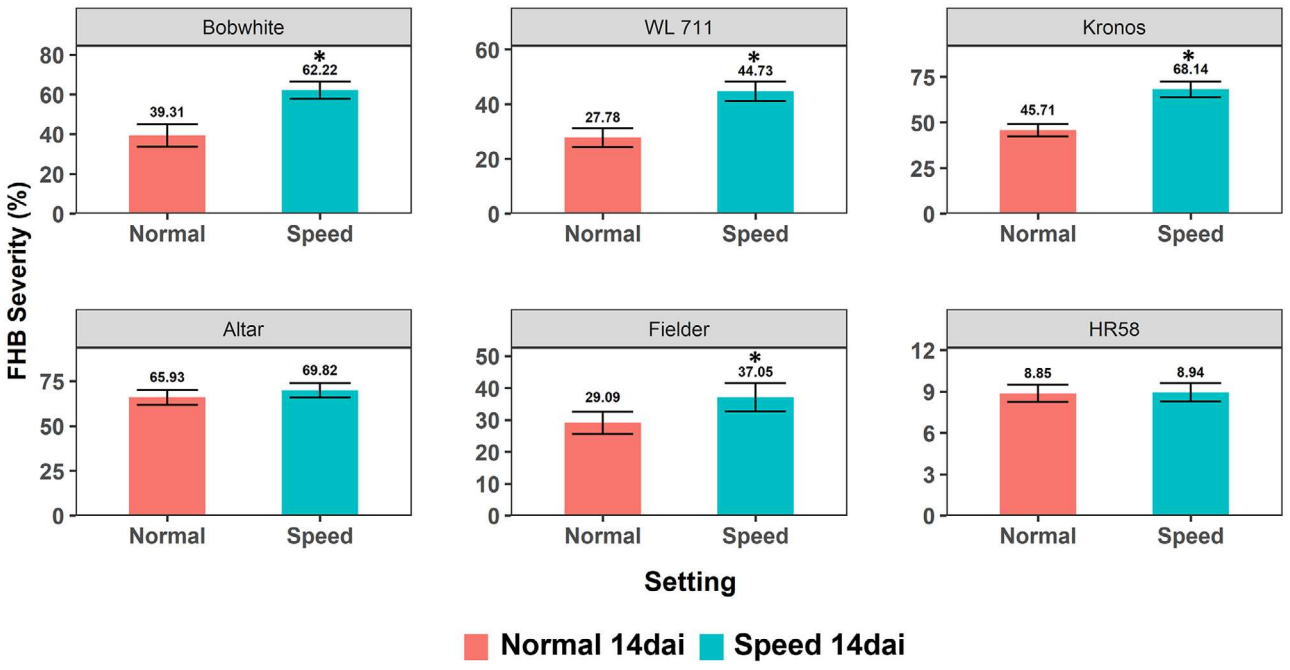

FIGURE 1 Combined Fusarium head blight (FHB) severity values of all tested genotypes over three trials at 14 days after inoculation (dai) under normal and speed breeding conditions. *X*-axis denotes growth conditions. *Y*-axis denotes FHB severity. Asterisk depicts significantly higher values at $p < 0.05$. Error bars depict standard error values.

severity was numerically higher but not found to be statistically significant. Highly susceptible cultivar Altar and highly resistant cultivar HR58 showed numerically similar FHB severity values at both 14 dai and 21 dai under both the conditions (Figures 1 and 2).

3.2 | FHB severity at 14dai under speed breeding conditions

FHB severity at 21 dai under normal conditions and at 14 dai under speed breeding conditions showed statistically

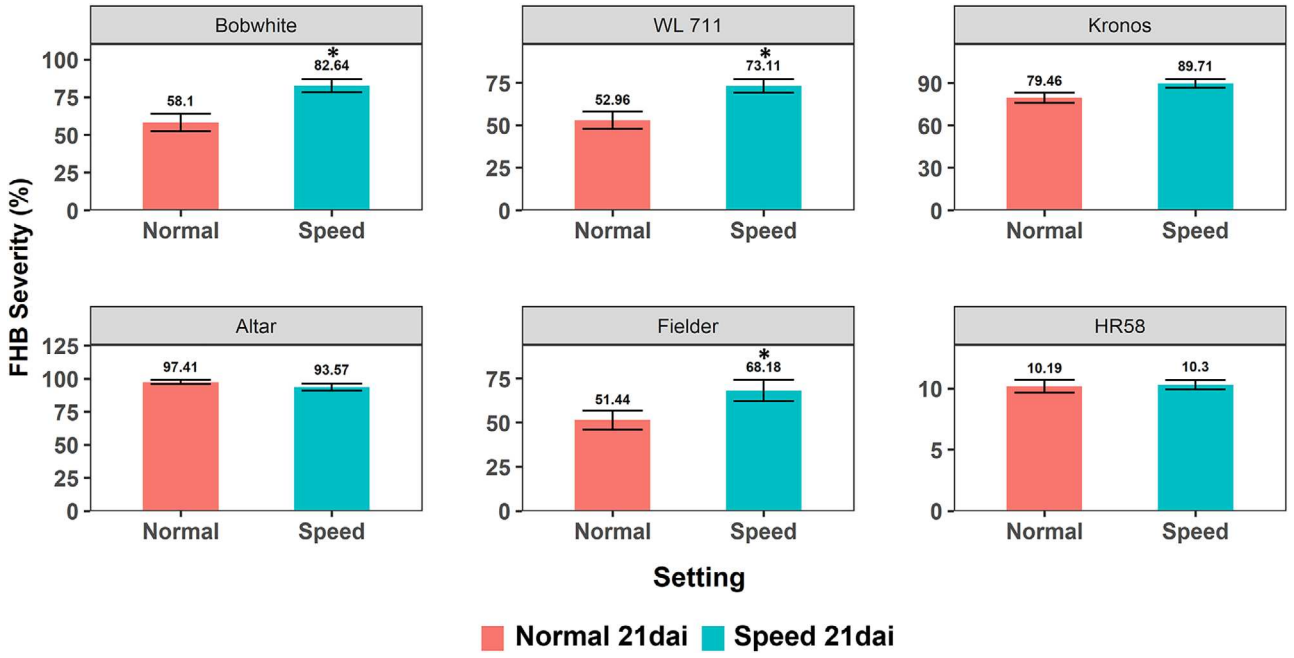

FIGURE 2 Combined Fusarium head blight (FHB) severity values of all tested genotypes over three trials at 21 days after inoculation (dai) under normal and speed breeding conditions. *X*-axis denotes growth conditions. *Y*-axis denotes FHB severity. Asterisk depicts significantly higher values at $p < 0.05$. Error bars depict standard error values.

TABLE 3 Analysis of variance of Fusarium head blight (FHB) severity at 21 days after inoculation (dai) under normal versus 14 dai under speed breeding conditions.

	Sum square	Mean square	Numerator df	Denominator df	*F* value	Pr (> *F*)
Genotype	35.073	7.0146	5	338.69	154.5033	<2.2e-16*
Growth condition	0.617	0.617	1	340.82	13.5965	0.000264*
Genotype: Growth condition	0.378	0.0757	5	340.81	1.6667	0.1420034

*Significance at $p < 0.05$.

similar values, implying that phenotyping for FHB under speed breeding conditions can be done at 14 dai instead of 21 dai. HOV was again found to be non-significant, and values were combined for all three trials (Table S3c). Significant variation was observed in the data set for both genotypes and growth conditions at $p < 0.05$, though their interaction was found to be non-significant (Table 3). Pairwise comparisons among FHB severity values of a particular genotype between two corresponding time points showed statistically similar severity values. An exception to this was observed in the highly susceptible genotype Altar, which showed significantly higher FHB severity at 21 dai under normal conditions in comparison with 14 dai under speed breeding conditions (Figure 3). At 14 dai, under speed breeding conditions, moderately susceptible and susceptible genotypes showed susceptible response, whereas moderately resistant and resistant genotypes showed resistant response, demonstrating that differentiation between resistance and susceptibility can be done at 14 dai under speed breeding conditions in comparison to 21 dai under normal conditions, allowing for a reduction of 7 days of phenotyping time (Tables S1 and S2).

Pearson's correlation coefficient between FHB severity values at 14 dai under normal versus 14 dai under speed breeding conditions was 0.895, and that for 21 dai under normal versus 21 dai under speed was also very high at 0.930, indicating that the FHB response of the genotypes was similar under both the conditions. FHB severity of the genotypes at 21 dai under normal versus 14 dai under speed breeding conditions was also highly correlated ($r = 0.921$), highlighting that the response of the genotype, although accelerated under speed breeding, was same as that under normal growth conditions at 21 dai.

3.3 | Effect of speed breeding conditions on DON content

DON content was found to be higher under speed breeding conditions for moderately susceptible, susceptible, and

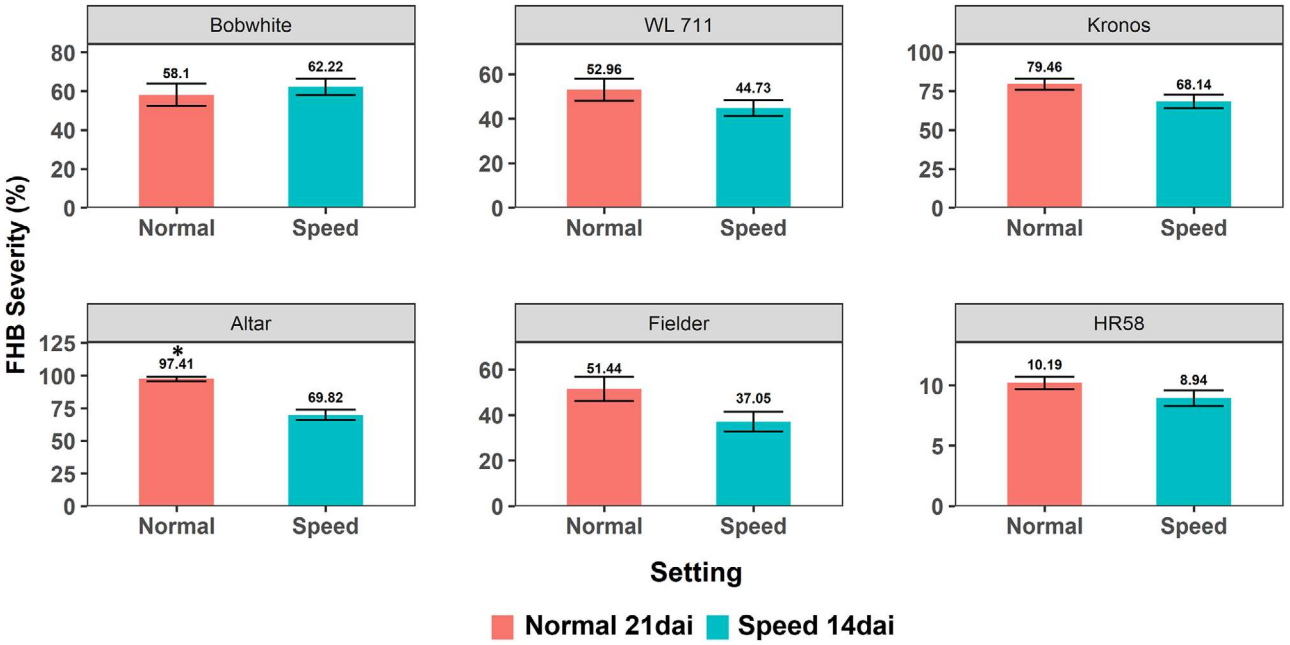

FIGURE 3 Combined Fusarium head blight (FHB) severity values of all tested genotypes over three trials at 21 days after inoculation (dai) under normal conditions versus 14 (dai) under speed breeding conditions. *X*-axis denotes growth conditions. *Y*-axis denotes FHB severity. Asterisk depicts significantly higher values at $p < 0.05$. Error bars depict standard error values.

TABLE 4 Analysis of variance of deoxynivalenol (DON) content under normal versus speed breeding conditions.

Trial-1						
	Sum square	**Mean square**	**Numerator df**	**Denominator df**	***F* value**	**Pr (> *F*)**
Genotype	199.01	66.34	3	5.09	86.45	8.66e-05*
Growth condition	118.10	118.10	1	5.00	153.91	6.03e-05*
Genotype: Growth condition	112.42	37.47	3	5.00	48.84	0.0003*
Trial-2						
Genotype	17073.5	3414.7	5	16	33.87	5.77e-08*
Growth condition	3574.2	3574.2	1	16	35.46	2.01e-05*
Genotype: Growth condition	6277.0	1255.4	5	16	12.45	4.77e-05*
Trial-3						
Genotype	50627	10125.4	5	16	65.50	4.39e-10*
Growth condition	2715	2715.2	1	16	17.56	0.0006*
Genotype: Growth condition	2114	422.8	5	16	2.73	0.04*

*Significance at $p < 0.05$.

moderately resistant cultivars (Table S2). HOV was found to be significant in this experiment (Table S3d), so separate analysis was performed for each trial. A two-way ANOVA showed significant genotype and growth conditions (normal vs. speed breeding) effect, as well as a significant interaction between the two for all three trials at $p < 0.05$ (Table 4). Moderately susceptible genotypes, Bobwhite and WL711, showed higher DON content under speed breeding conditions in all three trials when compared to normal conditions. For highly susceptible tetraploid genotypes (Kronos and Altar), DON content could only be measured for Trial-2 and Trial-3. Kronos showed significantly higher DON content under speed breeding conditions for both the trials, whereas DON content values observed for Altar were not significantly different. Fielder showed higher DON content in speed breeding over normal conditions in all three trials, but significant differences were found in only Trial-2 and Trial-3. HR58 had the lowest DON content among all the genotypes (<0.25 ppm) under both speed breeding and normal conditions. which was expected because of presence of the *Fhb1*-locus (Figure 4).

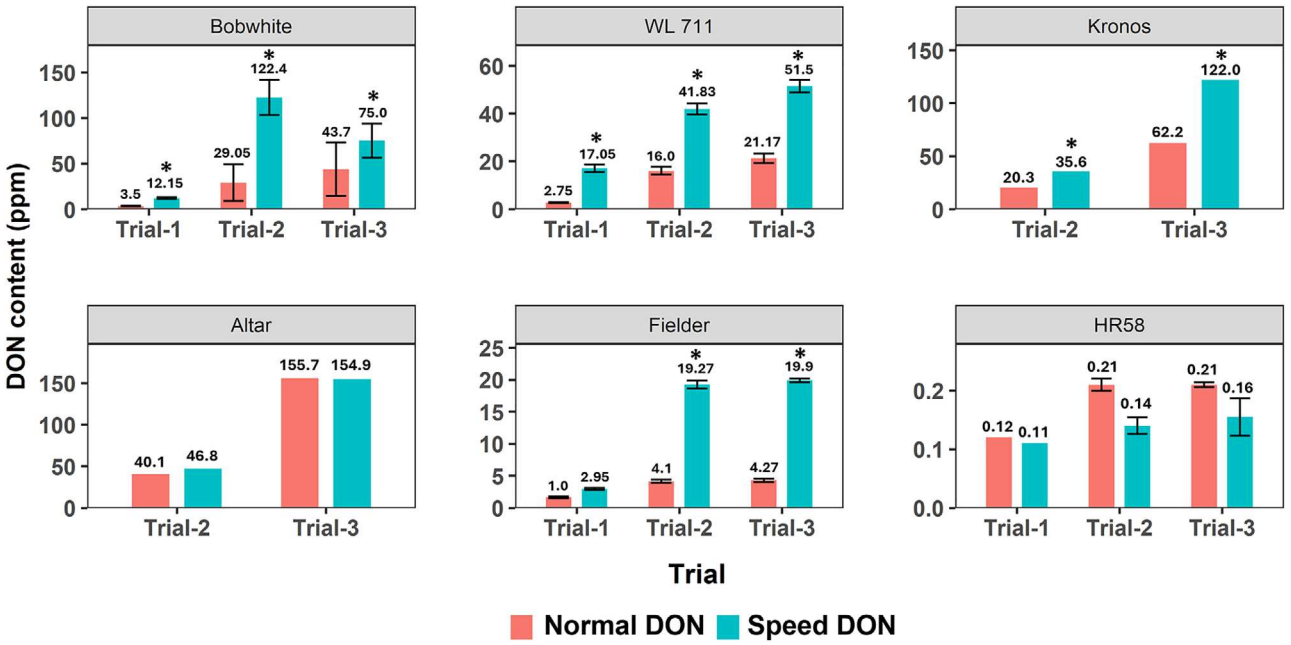

FIGURE 4 Deoxynivalenol (don) values of all tested genotypes over three trials under normal and speed breeding conditions. *x* axis denotes trial. *y* axis denotes DON content. Asterisk depicts significantly higher values at $p < 0.05$. Error bars depict standard error values.

The correlation coefficient for DON values of genotypes under normal growth conditions versus speed breeding conditions was 0.871, indicating that the latter did not cause a low DON accumulating genotype to behave as a high DON content genotype. DON content under both the conditions was similar, showing that speed breeding condition does not enhance DON accumulation in the resistant genotype, and thus these accelerated growth conditions can be successfully used to evaluate DON content accumulation.

4 | DISCUSSION

Speed breeding is a technique that manipulates environment under controlled conditions to accelerate plant growth and development. This technique is gaining popularity to accelerate wheat research and breeding. Speed breeding can help to achieve three to nine generations of a crop per year compared to one to two generations achieved per year under normal conditions, allowing for rapid development of stable genotypes and fast tracking of the development and release of new cultivars (Ghosh et al., 2018; Ochatt et al., 2002). In wheat, a major focus has been on developing speed breeding protocols for expediting generation advancement (Ghosh et al., 2018; Hickey et al., 2019; Watson et al., 2018). Evaluation of leaf rust, stripe rust, stem rust, yellow spot, and crown rot diseases has been successfully performed under speed breeding conditions, accelerating phenotyping for these important diseases (Alahmad et al., 2018; Dinglasan et al., 2016; Hickey et al., 2012; Pretorius et al., 2007; Riaz & Hickey, 2017). However, detailed analysis of response of varying levels of genetic resistance for different FHB parameters under speed breeding conditions has not yet been done.

In the present study, we show that speed breeding conditions can be used to not only grow wheat but also test the plants for FHB response and distinguish different levels of genetic resistance faster under these conditions. Furthermore, the mycotoxin, DON, accumulates to a higher level under speed breeding conditions in susceptible genotypes, though the resistant genotypes still maintain low DON content as expected.

Our panel comprised moderately susceptible (Bobwhite and WL711), susceptible (Kronos and Altar), moderately resistant (Fielder), and resistant (HR58) genotypes. All the genotypes were characterized for their response to FHB under greenhouse conditions and then divided into different categories. Significant interaction for FHB severity at same data points and DON content under normal versus speed breeding conditions depicts variability for FHB parameters between genotypes. Most genotypes showed higher FHB severity under speed breeding conditions than normal conditions, with the exception of Altar. No significant interaction was found for FHB severity between genotypes and growth conditions for 21 dai under normal and 14 dai under speed breeding conditions, indicating that severity under these conditions was similar. Resistant genotype (HR58) showed a resistance response in speed breeding conditions even after 21 dai. This indicates that testing for FHB under speed breeding conditions can help reduce phenotyping time by 7 days. Additionally, the

differentiation of susceptibility can be done at 14 dai under speed breeding conditions as compared to 21 dai under normal conditions. DON content showed a similar trend, where genotypes grown under accelerated growth conditions had higher DON content. These results were also further supported by correlation analysis of the genotypes for FHB severity as well as DON content under normal growth conditions versus speed breeding conditions. The very high positive values of correlation coefficients for FHB severity ($r = 0.894$ for 14 dai normal and 14 dai speed; $r = 0.930$ for 21 dai normal and 21 dai speed; and $r = 0.921$ for 21 dai normal and 14 dai speed) imply that the genotypes behave according to their genetic resistance levels under both the conditions. Likewise, the high level of correlation ($r = 0.871$) in DON content between spikes tested under speed breeding and normal growth conditions means that the DON accumulation can also be correctly determined under speed breeding conditions.

Ghosh et al. (2018) optimized a speed breeding protocol for winter wheat, where they were able to grow one generation in ~120–123 days (from seed to flowering in 100–105 days and from flowering to seed set in ~20–22 days). In the same way, spring wheat takes ~84–89 days to get one generation under normal conditions, whereas under speed breeding conditions, one generation can be completed in ~63–67 days, saving ~21–22 days per generation (Ghosh et al., 2018; Watson et al., 2018). Zakieh et al. (2021) grew their winter wheat genotypes under speed breeding conditions until flowering and were able to reach that stage by 100–105 days, which were similar to reported by Ghosh et al. (2018). However, to test for FHB, they shifted the plants to normal conditions, as no protocol was established to test for FHB under speed breeding conditions. Under normal growing conditions, it took ~33–35 days from flowering to maturity. Utilizing our protocol, plants can be scored for FHB under speed breeding conditions within 2 weeks of anthesis, which also reduces the overall time from flowering to maturity by ~11–15 days. So overall, growing the plants in speed breeding followed by their testing for FHB also in the same conditions saves a total of 21–23 days.

Our study provides evidence that testing for FHB and DON accumulation can be efficiently done under speed breeding conditions. Speed breeding techniques allow six generations of spring wheat and four generations of winter wheat to be grown in a year, accelerating plant growth and development (Hickey et al., 2019). Using our technique, accurate phenotyping for FHB can be done just after 14 dai as compared to 21 dai or 28 dai under normal conditions, saving around 3 to 3.5 weeks from start to harvest under speed breeding. This phenotyping approach will allow rapid testing of genotypes and will accelerate the development of FHB-resistant cultivars. However, because this research was done on spring wheat and durum genotypes, the exact time that can be saved in winter wheat genotypes for FHB evaluation under speed breeding conditions still needs to be evaluated. More research needs to be done to determine the explanation of increased severity under speed breeding conditions. The *F. graminearum* isolate used in this study: FgGZ3639 is known to be very aggressive, it will be interesting to see how less aggressive strains respond to speed breeding conditions. More genotypes with moderate resistance and susceptibility need to be evaluated under speed breeding conditions to ensure that differentiation occurs correctly and genotypes with unknown resistance can be tested reliably.

AUTHOR CONTRIBUTIONS

Bhavit Chhabra: Formal analysis; investigation; methodology; writing—original draft. **Saijagruti Thrasu**: Methodology. **Sydney Wallace**: Formal analysis; methodology. **Adam Schoen**: Investigation; methodology. **Fereshteh Shahoveisi**: Formal analysis. **Yanhong Dong**: Investigation; methodology; resources. **Vijay Tiwari**: Conceptualization; supervision; writing—review and editing. **Nidhi Rawat**: Conceptualization; resources; supervision; writing—original draft; writing—review and editing.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

ORCID

Vijay Tiwari https://orcid.org/0000-0001-7297-4048
Nidhi Rawat https://orcid.org/0000-0003-4820-0020

REFERENCES

Alahmad, S., Dinglasan, E., Leung, K. M., Riaz, A., Derbal, N., Voss-Fels, K. P., Able, J. A., Bassi, F. M., Christopher, J., & Hickey, L. T. (2018). Speed breeding for multiple quantitative traits in durum wheat. *Plant Methods*, *14*(1), Article 36. https://doi.org/10.1186/s13007-018-0302-y

Bai, G., & Shaner, G. (1994). Scab of wheat: Prospects of control. *Plant Disease*, *78*(8), 760–766. https://doi.org/10.1094/PD-78-0760

Bai, G.-H., Desjardins, A. E., & Plattner, R. D. (2002). Deoxynivalenol-nonproducing *Fusarium graminearum* causes initial infection, but does not cause disease spread in wheat spikes. *Mycopathologia*, *153*, 91–98. https://doi.org/10.1023/A:1014419323550

Chhabra, B., Singh, L., Wallace, S., Schoen, A., Dong, Y., Tiwari, V., & Rawat, N. (2021). Screening of an EMS mutagenized population of a wheat cultivar susceptible to Fusarium head blight identifies resistant variants. *Plant Disease*, *105*(11), 3669–3676. https://doi.org/10.1094/PDIS-03-21-0670-RE

Chhabra, B., Tiwari, V., Gill, B. S., Dong, Y., & Rawat, N. (2021). Discovery of a susceptibility factor for Fusarium head blight on chromosome 7A of wheat. *Theoretical and Applied Genetics*, *134*(7), 2273–2289. https://doi.org/10.1007/s00122-021-03825-y

Dinglasan, E., Godwin, I. D., Mortlock, M. Y., & Hickey, L. T. (2016). Resistance to yellow spot in wheat grown under accelerated growth conditions. *Euphytica*, *209*(3), 693–707. https://doi.org/10.1007/s10681-016-1660-z

Fernando, W. D., Oghenekaro, A. O., Tucker, J. R., & Badea, A. (2021). Building on a foundation: Advances in epidemiology, resistance

breeding, and forecasting research for reducing the impact of fusarium head blight in wheat and barley. *Canadian Journal of Plant Pathology*, *43*(4), 495–526. https://doi.org/10.1080/07060661.2020.1861102

Gadaleta, A., Colasuonno, P., Giove, S. L., Blanco, A., & Giancaspro, A. (2019). Map-based cloning of QFhb.mgb-2A identifies a WAK2 gene responsible for Fusarium head blight resistance in wheat. *Scientific Reports*, *9*, Article 6929. https://doi.org/10.1038/s41598-019-43334-z

Ghosh, S., Watson, A., Gonzalez-Navarro, O. E., Ramirez-Gonzalez, R. H., Yanes, L., Mendoza-Suárez, M., Simmonds, J., Wells, R., Rayner, T., Green, P., Hafeez, A., Hayta, S., Melton, R. E., Steed, A., Sarkar, A., Carter, J., Perkins, L., Lord, J., Tester, M., … Hickey, L. T. (2018). Speed breeding in growth chambers and glasshouses for crop breeding and model plant research. *Nature Protocols*, *13*(12), 2944–2963. https://doi.org/10.1038/s41596-018-0072-z

Goswami, R. S., & Kistler, H. C. (2004). Heading for disaster: *Fusarium graminearum* on cereal crops. *Molecular Plant Pathology*, *5*(6), 515–525. https://doi.org/10.1111/j.1364-3703.2004.00252.x

Han, J., Lakshman, D. K., Galvez, L. C., Mitra, S., Baenziger, P. S., & Mitra, A. (2012). Transgenic expression of lactoferrin imparts enhanced resistance to head blight of wheat caused by *Fusarium graminearum*. *BMC Plant Biology*, *12*(1), Article 33. https://doi.org/10.1186/1471-2229-12-33

Hickey, L. T., N Hafeez, A., Robinson, H., Jackson, S. A., Leal-Bertioli, S. C. M., Tester, M., Gao, C., Godwin, I. D., Hayes, B. J., & Wulff, B. B. H. (2019). Breeding crops to feed 10 billion. *Nature Biotechnology*, *37*(7), 744–754. https://doi.org/10.1038/s41587-019-0152-9

Hickey, L. T., Wilkinson, P. M., Knight, C. R., Godwin, I. D., Kravchuk, O. Y., Aitken, E. A. B., Bansal, U. K., Bariana, H. S., De Lacy, I. H., & Dieters, M. J. (2012). Rapid phenotyping for adult-plant resistance to stripe rust in wheat. *Plant Breeding*, *131*(1), 54–61. https://doi.org/10.1111/j.1439-0523.2011.01925.x

Jansen, C., von Wettstein, D., Schäfer, W., Kogel, K.-H., Felk, A., & Maier, F. J. (2005). Infection patterns in barley and wheat spikes inoculated with wild-type and trichodiene synthase gene disrupted *Fusarium graminearum*. *Proceedings of the National Academy of Sciences*, *102*(46), 16892–16897. https://doi.org/10.1073/pnas.0508467102

Kassambara, M. A. (2020). *ggpubr: 'ggplot2' based publication ready plots* (R package version 0.1.6). https://cran.r-project.org/package=ggpubr

Lenth, R., Singmann, H., Love, J., Buerkner, P., & Herve, M. (2018). *Package "Emmeans"* (R package version 4.0-3). https://cran.r-project.org/web/packages/emmeans/index.html

Ma, Z., Xie, Q., Li, G., Jia, H., Zhou, J., Kong, Z., Li, N., & Yuan, Y. (2020). Germplasms, genetics and genomics for better control of disastrous wheat Fusarium head blight. *Theoretical and Applied Genetics*, *133*(5), 1541–1568. https://doi.org/10.1007/s00122-019-03525-8

McMullen, M., Bergstrom, G., De Wolf, E., Dill-Macky, R., Hershman, D., Shaner, G., & Van Sanford, D. (2012). A unified effort to fight an enemy of wheat and barley: Fusarium head blight. *Plant Disease*, *96*(12), 1712–1728. https://doi.org/10.1094/PDIS-03-12-0291-FE

Mirocha, C. J., Kolaczkowski, E., Xie, W., Yu, H., & Jelen, H. (1998). Analysis of deoxynivalenol and its derivatives (batch and single kernel) using gas chromatography/mass spectrometry. *Journal of Agricultural and Food Chemistry*, *46*(4), 1414–1418. https://doi.org/10.1021/jf970857o

Ochatt, S. J., Sangwan, R. S., Marget, P., Ndong, Y. A., Rancillac, M., Perney, P., & Röbbelen, G. (2002). New approaches towards the shortening of generation cycles for faster breeding of protein legumes. *Plant Breeding*, *121*(5), 436–440. https://doi.org/10.1046/j.1439-0523.2002.746803.x

Parry, D. W., Jenkinson, P., & McLEOD, L. (1995). Fusarium ear blight (scab) in small grain cereals—A review. *Plant Pathology*, *44*(2), 207–238. https://doi.org/10.1111/j.1365-3059.1995.tb02773.x

Pretorius, Z. A., PienaarA, L., & Prins, R. (2007). Greenhouse and field assessment of adult plant resistance in wheat to *Puccinia striiformis f. sp. Tritici*. *Australasian Plant Pathology*, *36*(6), 552–559. https://doi.org/10.1071/AP07058

Rawat, N., Pumphrey, M. O., Liu, S., Zhang, X., Tiwari, V. K., Ando, K., Trick, H. N., Bockus, W. W., Akhunov, E., Anderson, J. A., & Gill, B. S. (2016). Wheat Fhb1 encodes a chimeric lectin with agglutinin domains and a pore-forming toxin-like domain conferring resistance to Fusarium head blight. *Nature Genetics*, *48*(12), 1576–1580. https://doi.org/10.1038/ng.3706

Riaz, A., & T Hickey, L. (2017). Rapid phenotyping adult plant resistance to stem rust in wheat grown under controlled conditions. *Wheat Rust Diseases: Methods and Protocols*, *1659*, 183–196. https://doi.org/10.1007/978-1-4939-7249-4_16

Rocha, O., Ansari, K., & Doohan, F. M. (2005). Effects of trichothecene mycotoxins on eukaryotic cells: A review. *Food Additives & Contaminants*, *22*(4), 369–378. https://doi.org/10.1080/02652030500058403

Schoen, A., Wallace, S., Holbert, M. F., Brown-Guidera, G., Harrison, S., Murphy, P., Sanantonio, N., Van Sanford, D., Boyles, R., Mergoum, M., Rawat, N., & Tiwari, V. (2023). Reducing the generation time in winter wheat cultivars using speed breeding. *Crop Science*, *63*(4), 2079–2090. https://doi.org/10.1002/csc2.20989

Watson, A., Ghosh, S., Williams, M. J., Cuddy, W. S., Simmonds, J., Rey, M.-D., Asyraf Md Hatta, M., Hinchliffe, A., Steed, A., Reynolds, D., Adamski, N. M., Breakspear, A., Korolev, A., Rayner, T., Dixon, L. E., Riaz, A., Martin, W., Ryan, M., Edwards, D., … Hickey, L. T. (2018). Speed breeding is a powerful tool to accelerate crop research and breeding. *Nature Plants*, *4*(1), 23–29. https://doi.org/10.1038/s41477-017-0083-8

Xu, X., & Nicholson, P. (2009). Community ecology of fungal pathogens causing wheat head blight. *Annual Review of Phytopathology*, *47*, 83–103. https://doi.org/10.1146/annurev-phyto-080508-081737

Zakieh, M., Gaikpa, D. S., Leiva Sandoval, F., Alamrani, M., Henriksson, T., Odilbekov, F., & Chawade, A. (2021). Characterizing winter wheat germplasm for fusarium head blight resistance under accelerated growth conditions. *Frontiers in Plant Science*, *12*, Article 705006. https://www.frontiersin.org/articles/10.3389/fpls.2021.705006

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Chhabra, B., Thrasu, S., Wallace, S., Schoen, A., Shahoveisi, F., Dong, Y., Tiwari, V., & Rawat, N. (2024). Evaluation of speed breeding conditions for accelerating Fusarium head blight and deoxynivalenol screening in wheat. *Crop Science*, *64*, 1586–1594. https://doi.org/10.1002/csc2.21226