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Reducing the generation time in winter wheat cultivars using speed breeding

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Abstract

Reducing generation time is critical to achieving the goals of genetic gain in important crops like wheat (*Triticum aestivum*). Speed breeding (SB) has been shown to considerably reduce generation times in crop plants. Unlike spring wheat cultivars, winter wheat varieties require typically 6–9 weeks of cold treatment, called vernalization, for flowering which extends the generation time for the development of improved winter wheat cultivars. Here, we optimized the SB method using a set of 48 diverse soft red winter wheat (SRWW) cultivars by testing vernalization duration, light and temperature requirements, and the viability of seeds harvested after different durations post-anthesis under extended daylight conditions. We have found that using a 22-h setting (22 h day/2 h night, 25˚C/22˚C) in high-density 50-cell trays results in rapid generation advancement. We used genotypic data for a panel of soft red winter wheat varieties from the regional programs to determine the impact of photoperiod and vernalization alleles on the efficiency of the SB approach. Using a set of 48 SRWW cultivars and germplasm from Maryland and four other public breeding programs, we establish that this protocol can allow for the advancement of four generations per year in controlled conditions for winter wheat varieties, experimental lines, or emerging cultivars. Our work shows the potential to reduce generation time

Abbreviations: DH, doubled haploids; DPF, days post flowering; HID, high intensity discharge; SB, speed breeding; SSD, single seed descent.

by ~30 days per generation faster than what had been reported in the SB strategies for winter wheat, thus allowing for a quicker turnaround time from original cross to genetically stable experimental genotypes that can be tested in field settings.

1 | INTRODUCTION

With the global human population expected to reach 10 billion by 2050, there is a subsequent requirement for an increase in food production; however, current trends show that progression toward higher yielding wheat (*Triticum aestivum*) cultivars will be insufficient to meet the impending demand (Hickey et al., 2019; Ray et al., 2013; Tilman et al., 2011). Breeding technologies have advanced significantly in recent years with the implementation of affordable high-throughput genotyping technologies that allow breeders to accurately pyramid genes that have the potential to result in high-yielding, disease-resistant cultivars through marker-assisted breeding (De La Fuente et al., 2013; Hickey et al., 2019; Rasheed et al., 2017). Yet, even with these advancements, a bottleneck exists in the length of the breeding cycle. In traditional breeding, after a cross is made, four to six generations of inbreeding and selection are required to produce a line with a stable genetic background before it can be considered for field testing. Moreover, in normal conditions, field crops can only be advanced one to two generations per year, making the breeding cycle a very slow process (Watson et al., 2018).

Doubled haploids (DH) have been used in breeding programs for several decades (Bhowmik & Bilichak, 2021; Węzdony et al., 2009). Doubled haploids provide a quick approach for the production of completely homozygous plants within one generation after the original cross. However, some significant drawbacks of this method are that there is no recombination after DH production, meaning larger DH populations are required in order to obtain homozygous plants containing all the desired genes from the two parents (Inagaki et al., 1998). Additionally, the costs associated with developing DH populations large enough to produce elite lines may not be economically feasible for all breeding programs. The single-seed descent (SSD) method for breeding allows for large segregating populations that can be screened for agronomically important traits in greenhouse and field settings, but as mentioned earlier, this requires several rounds of selfing in order to get completely homozygous plants. In order to accelerate this process, speed breeding (SB) protocols, which involve increasing the photoperiod and temperature conditions of developing plants, have been implemented on important crops such as pea (*Pisum sativum* L.), chickpea (*Cicer arietinum* L.), canola (*Brassica napus*), barley (*Hordeum vulgare*), and wheat resulting in a decrease in flowering time up to 50% (Cazzola et al., 2020; Cha et al., 2022; Ghosh et al., 2018; Watson et al., 2018).

Based on the seasonal growth pattern, wheat can be classified as either having "spring" or "winter" growth habits. Winter wheat (~75% of the wheat production in the United States) requires an extended cold treatment, or vernalization (*Vrn*), during the vegetative stage in order to reach reproductive maturity. In the Northern Hemisphere, it is traditionally planted in the autumn between October and December. Spring wheat does not require this cold treatment to flower and is planted between the months of March and May in the United States (Dubcovsky et al., 2006; USDA, 2019; Yan, Loukoianov et al., 2004). Diagnostic molecular markers have been developed in order to determine the *Vrn* and photoperiod requirements (*Ppd*) of wheat varieties (Chen et al., 2018; Dubcovsky et al., 2006; Fu et al., 2005; Shcherban et al., 2015; Xie et al., 2021; Yan, Loukoianov et al., 2004). In winter wheat, the gene *vrn-1* is expressed at low levels until vernalization, where the expression is upregulated, in contrast to spring wheat where a variation in the promoter region causes a dominant expression without the cold treatment (Fu et al., 2005; Yan, Helguera et al., 2004). Photoperiod genes, on the other hand, are related to the long-day sensitivity, the requirement of long days for flowering, or insensitivity of certain wheat varieties (Ortiz Ferrara et al., 1998; Shcherban et al., 2015). These genes and alleles, however, have not been evaluated critically to visualize their impact on reducing crop generation time using SB protocols in winter wheat.

In a study performed by Ghosh et al. (2018), a protocol was established for SB in spring wheat. It was observed that viable seeds could be harvested ~60 days after sowing. They also reported that one complete generation of the winter wheat would require ~123 days after planting. Zakieh et al. (2021) optimized the SB protocol for winter wheat to accelerate growth for Fusarium head blight disease evaluation and were able to complete a full generation in 120 days. Though this is a slight reduction (2–3 days) in generation time, it is still not sufficient for breeding purposes. Further, a study by Cha et al. (2022) showed significant success in decreasing generation time in winter wheat by combining "Speed Vernalization", which involves an increased day/night cycle during cold treatment, with long day growing conditions post-vernalization. However, only 45 of 51 tested wheat varieties showed success with this method, with six cultivars not reaching flowering due to the modified vernalization. Additionally, their method utilized 18 Spring/facultative

verities to develop their protocol. A comprehensive SB method was also communicated by Song et al. (2022); however, their experiment only utilized one winter-type variety and required removal of tillers in order to achieve faster generation time. Due to the importance of winter wheat in the United States and worldwide, an optimized SB protocol is needed for winter wheat in order to increase the number of generations that can be advanced in a year, regardless genetic background.

In this study, we optimized a robust SB protocol for winter wheat cultivars with various combinations of photoperiod and vernalization alleles along with different vernalization timelines. Our SB protocol reduces the generation time in winter wheat to as low as 87 days, allowing for four generations in 365 days. To test and validate the effectiveness of this approach, we used a panel of 48 soft red winter wheat (SRWW) genotypes from varying environments from the eastern and southern regions of SRWW breeding programs.

2 | MATERIALS AND METHODS

2.1 | Plant materials

A set of five adapted SRWW cultivars, MDW315, MDW131, MDW133, Hilliard (Reg. No. CV-, PI 676271), and Shirley (Reg. No. CV-1039, PI 656753), were used to initially optimize our protocol. For the validation of our optimized approach, a panel of 43 diverse SRWW genotypes developed at the University of Maryland, Virginia Tech, University of Kentucky, North Carolina ARS, and Clemson University was included in the study. Table S1 explains the plant material used in this study. The last four entries were not genotyped and were only used for generation-time studies.

2.2 | Planting, germination, and vernalization

Fifty seeds per genotype were sown directly into 50-cell trays (Greenhouse Megastore: CN-PLG-050) ~2.5 cm deep using vermiculite/soil mixture. After sowing, trays were watered and placed into a greenhouse set up with misters for germination with 12-h day/night light cycles (150–280 µmol m^{-2} s^{-1}). This misted greenhouse was utilized in order to maintain adequate and uniform soil moisture during germination. After ~5 days, or at the one leaf stage, trays were placed in 4°C environmental growth chambers equipped with high-intensity discharge (HID) lights with 12-h day/night light cycles (150–280 µmol m^{-2} s^{-1}) for 6 weeks to satisfy vernalization requirements.

For the optimization of reduced cycle growing conditions, seeds (300/each) of the five cultivars were sown and germinated in the same way described above. At the one-leaf stage,

Core Ideas

- The pace of generating improved winter wheat cultivars is enhanced using the speed breeding (SB) approach.
- Genetic gain is enhanced using the SB approach.
- The optimization of the SB method for diverse soft red winter wheat cultivars is illustrated.

seedlings were vernalized at 4°C with 12-h day/night light cycles (150–280 µmol m^{-2} s^{-1}) period with light supplied by HID bulbs. Trays with the seedlings were taken out of vernalization after 5, 6, and 7 weeks, respectively.

For the diverse panel, 10 seeds per each of the 48 genotypes were planted randomly in rows of five between two trays and germinated using the methods described above. At the one-leaf stage, seedlings were again vernalized at 4°C with 12-h day/night light cycles (150–280 µmol m^{-2} s^{-1}) period with light supplied by HID bulbs; however, in order to avoid possible issues with uniform flowering, we chose 6 weeks of vernalization for the entire panel (Crofts, 1989; Li et al., 2013).

2.3 | Growing conditions and experimental design

Immediately after vernalization, plants were moved into either normal settings (12 h day/night; 23°C/20°C), 16 h settings (16 h day/8 h night; 23°C/20°C), or 22 h settings (22 h day/2 h night, 25°C/22°C).

Environmental growth chambers equipped with HID lights were used for normal, 16 h (150–280 µmol m^{-2} s^{-1}), and 22 h (315–350 µmol m^{-2} s^{-1}) treatments. This was done to remove any light or temperature variables that are more difficult to control under a greenhouse setting.

Under normal (12 h day/night; 23°C/20°C) settings, after 24-h of acclimation, for each genotype, all surviving seedlings (between 40 and 50 plants), were removed from their 50-cell trays and transplanted into 4-inch round pots (Greenhouse Megastore: CN-CXR-40) containing the same soil mixture used for sowing. Pots were arranged in two by five trays (Greenhouse Megastore: CN-TRS-1040), grouped by genotype, and placed uniformly throughout the growth chamber, roughly 1 m apart, to allow for equal light and temperature.

For plants grown under 16 h or 22 h conditions, seedlings were not removed from their 50-cell trays and allowed to grow to maturity in the tray's cells. For the optimization of reduced cycle growing conditions, 50 seedlings per genotype were placed uniformly throughout growth chambers set with either 16 h or 22 h conditions as mentioned above (50 reps per

genotype per condition). Plants were grouped by their vernalization times (either 5, 6, or 7 weeks), and spread at least 1 m apart to allow for sufficient growth. Similarly, for the diverse panel, trays containing seedlings (five reps per genotype per condition) were placed uniformly throughout their respective growth chambers, roughly 50 cm apart, to allow for equal light conditions.

Plants were placed in trays that are meant to retain water (Greenhouse Megastore: CN-FLHD-X2), and watering was done by filling these trays, avoiding watering the aboveground portions, every other day or when needed. This was done intentionally to not reduce the internal temperature of the plants by foliar water spray. Liquid fertilizer, with a composition of NPK 15-5-17 + Ca and Mg at a rate of 100 ppm, was applied once a week to the water-retaining trays. Watering of the plant trays was stopped at the ripening stage (Z91) to allow maturing.

2.4 | Phenotyping and seed harvest

Days to heading (Z57) and flowering/anthesis (Z61) were recorded for all combinations of vernalization and growth conditions. As to not disturb vegetative growth, no-tillers were removed before flowering. In order to determine the earliest time at which seeds could be harvested, spikes were removed from two random plants per treatment 10, 15, and 20 days post-flowering (DPF), respectively. Harvested spikes were immediately placed in coin envelopes (ULINE S-6285) and placed into 50°C drying ovens for at least 3 days (McNeill & Overhults, 2019). Dried spikes were then threshed by hand and stored in separate coin envelopes. Seeds from 15 random spikes per treatment were chosen to determine seed set and for photographical representation of the effect of early harvest. Seeds were then stored at room temperature until planting.

2.5 | Genotyping

After taking the trays out of the vernalization room, the leaf tissue was collected in 96-well racked 1.1-mL microtubes and these tubes were covered with cheesecloth and secured. Tissue was desiccated by placing tissue in MicroTubes in airtight containers filled with silica gel for roughly 2 days. DNA was extracted and sent to AgriPlex Genomics for targeted sequencing of amplicons for *Vrn1* and *Ppd1* gene-based markers (Table S2).

2.6 | Data analysis

Statistical analyses were performed using R statistical analysis software version 4.1.1 Utilizing packages rstatix v.0.7 (Kassambara, 2023; R Core Team, 2021). Tests of normality of phenotypic data were conducted prior to conducting *t*-test using the Shapiro-Wilk test (Shapiro & Wilk, 1965). For genotypic group significance, a fixed effect linear model where phenotypic value was considered as a function of genotype for each marker tested while setting was the fixed effect was used using the lm() function in a modified protocol similar to Whittal et al. (2018). *F*-values were extracted from the analysis to evaluate significance of the relationship between genotype and phenotype (Table S3).

3 | RESULTS

3.1 | Optimization of SB settings in the SRWW cultivars

Under normal settings, across all genotypes, plants flowered (Z61) in an average of 111.8 days. MDW315 showed the fastest maturation time with an average of 97 days until flowering (Z61), and 125 days to full maturation (Z91), while MDW133 showed the longest maturation time with an average 120 days until flowering and 138 days until maturation (Table 1).

In the optimization panel, all plants grown in 22 h settings flowered earlier than those grown in 16 h settings (Figure 1). On average, regardless of vernalization treatment, plants flowered 11.6 days earlier when grown in 22 h settings compared to those grown in 16 h conditions. With 5 weeks of vernalization, the average time from sowing to flowering was 83.4 days. This was consistent over two cycles of optimization (Figure S1). This difference in flowering under 22 h versus 16 h settings time ranged from 6.8 days earlier in the case of MDW133 with 5 weeks of vernalization to 21.6 days earlier in the case of ''Hilliard'' with 5 weeks of vernalization. Seven weeks vernalization increased the generation time in all the genotypes. In all cases, under 22 h settings with 5 weeks of vernalization, our optimized protocol showed a reduction of ∼28 days to flowering in comparison to normal settings and ∼20 days in comparison with established winter wheat SB methods (Ghosh et al., 2018).

Further, 16 h settings, most plants matured faster (5–15 days) than plants grown under the previously established SB settings (Ghosh et al., 2018; Zakieh et al., 2021). Plants grown in 22 h settings as well as in 16 h settings showed a significant decrease in flowering time when comparing vernalization times, except in the case of Hilliard 22 h settings, where no significant difference in flowering time was observed between 5 and 6 weeks of vernalization. The seed set in all conditions yielded more than 15 seeds per spike on average (Figure 2). Seeds bulked according to their variety, harvest time (DPF), and growing conditions showed germination of >75% when harvested after 10 days, >90% after 15 days, and ∼100%

TABLE 1   General characteristics of the control genotypes used for the optimization of the speed breeding (SB) approach. A total of 50 plants per genotype were sown and transplanted after vernalization. The ± denotes the standard deviation in days.

Line ID	Days to flower under normal conditions	Days to complete a full generation	PPD alleles	Vrn alleles
MDW315	97 ± 6	125 ± 5	*Ppd-D1a Insensitive*	*vrn-A1 Short, vrn-B1, vrn-D1*
MDW131	112 ± 10	135 ± 7	*Ppd-A1a.1 Insensitive*	*vrn-A1, vrn-B1, vrn-D1*
MDW133	120 ± 6	138 ± 6	*Ppd-A1a.1 Insensitive*	*vrn-A1, vrn-B1, vrn-D1*
Hilliard	113 ± 5	135 ± 8	*Ppd-A1a.1 Insensitive, Ppd-D1a Insensitive*	*vrn-A1, vrn-B1, vrn-D1*
Shirley	117 ± 12	135 ± 10	*Ppd-A1a.1 Insensitive, Ppd-D1a Insensitive*	*vrn-A1, vrn-B1, vrn-D1*

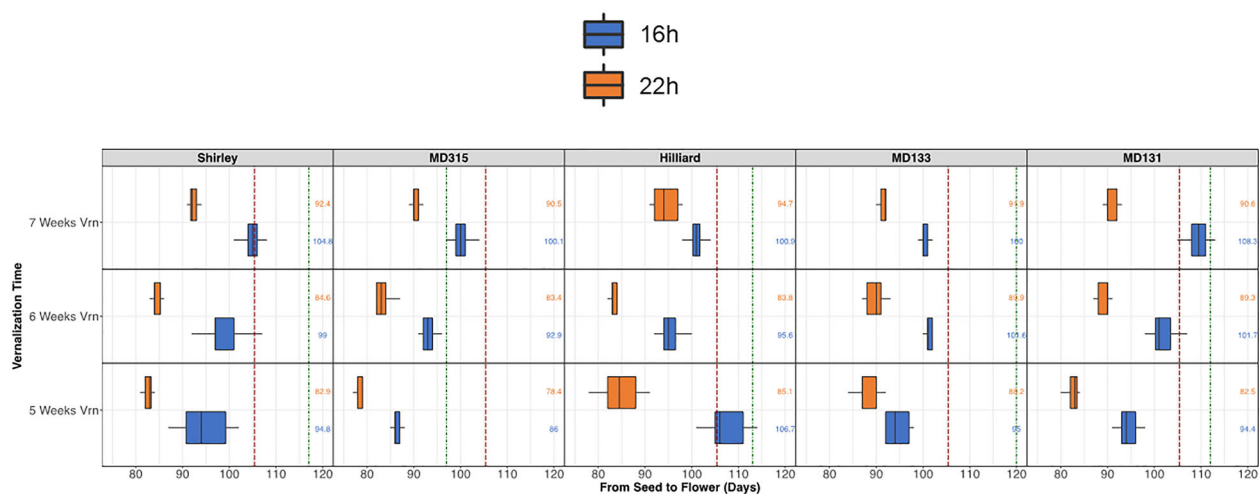Abbreviations: PPD, photoperiod; Vrn, vernalization.

FIGURE 1   Comparison of days from seed to flower between 22 and 16 h settings in optimization panel separated by time in vernalization. The dashed red line indicates the days from seed to flower in the protocol communicated by Ghosh et al. (2018). The dotted green line indicates the flowering time under 12 h photoperiod settings. Days to flowering are to the right of the box plots corresponding to their respective treatment.

germination after 20 days (data not shown). Seeds harvested 10 and 15 DPF showed differential maturation rates, with seeds harvested from plants grown in 22 h settings showing fatter, more mature seeds than those grown in 16 h settings (Figure 3).

3.2 | Validation of optimized approach on a diverse set of winter wheat cultivars

After optimization of the approach using five diverse SRWW cultivars, we then tested this approach on a set of 43 new wheat breeding germplasm developed at five different SRWW breeding programs (ST-1). In the diverse panel, with 6 weeks of vernalization used for all varieties, an average of 83.6 ± 4.3 days to flower was seen with 22 h settings, which was significantly ($p < 0.0001$) less than the average, 95.3 ± 4.4 days to flower, seen in plants grown in 16 h settings. This is consis-

tent with what was observed in the optimization panel, and more importantly, this was consistent with the reduction in time in comparison with the previously established protocol (Ghosh et al., 2018; Zakieh et al., 2021). The days from seed to flower ranged from 76.4 to 99 days in plants grown in 22 h settings, which contrasts with 16 h settings where a range of 88.5–104 days was observed. Further, plants grown in 22 h settings flowered an average of 11.9 days earlier, with the highest difference being 21 days earlier between 22 and 16 h settings.

3.3 | *Vrn* and *Ppd* alleles show an effect on flowering time in short-day settings

In many cases, the *Vrn* and *Ppd* alleles did show to have an effect on flowering time in 22 h settings (Figure 4). Varieties that contained A-copy *vrn_short* allele showed significantly
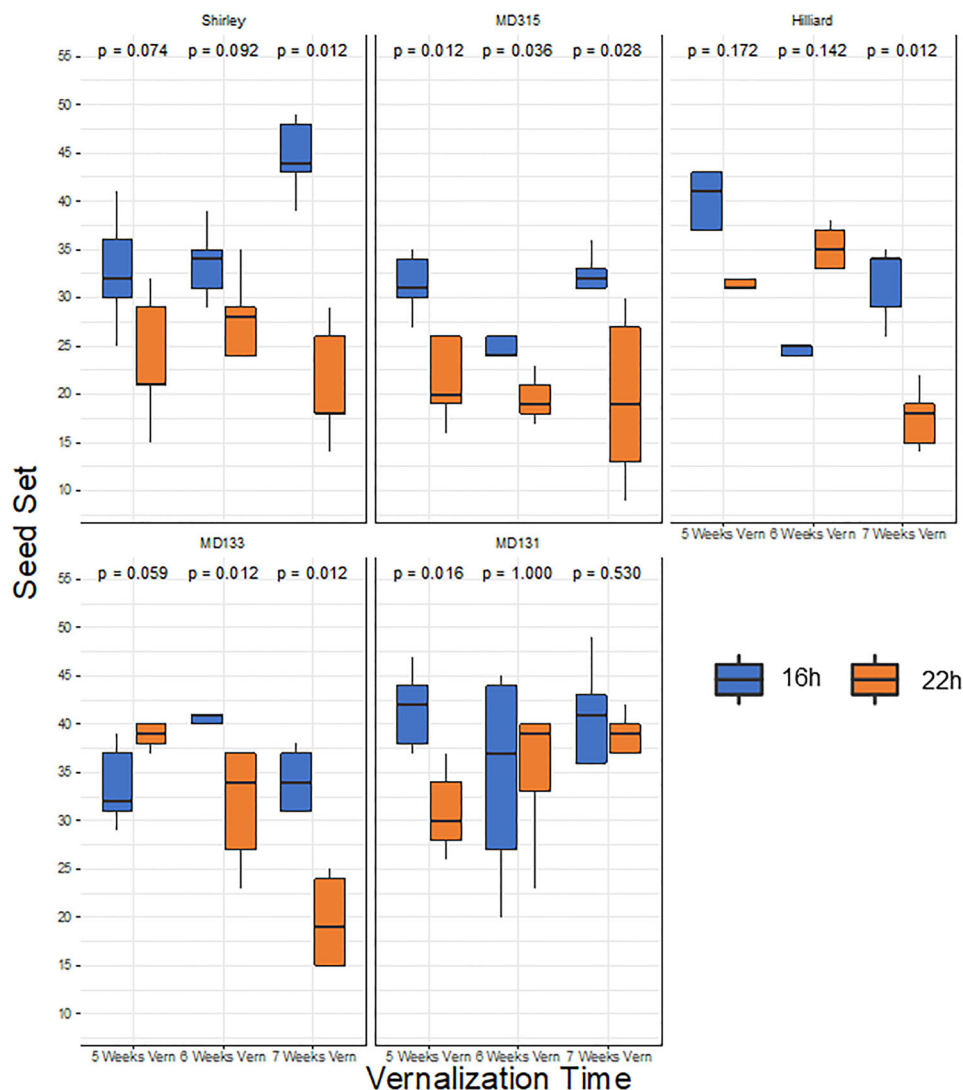
FIGURE 2 Number of seed set per spike in plants grown in different vernalization times and light conditions in set 2. Wilcoxon signed-rank test *p*-values are located above each comparison.

($p < 0.001$) reduced flowering time in comparison with those not containing this allele. Plants that were found to contain *Ppd_A1a* also showed an increase in flowering time, while *Ppd_B1_CS* showed a trend to decrease flowering time. The other *Ppd* alleles showed no significant effect on flowering time under 22 h settings.

4 | DISCUSSION

The ideas that lay the foundation for SB date back to the early 20th century, when researchers looked at how manipulating artificial illumination affected the growth and development of plants (Arthur et al., 1930; Garner & Allard, 1927; Pfeiffer, 1926). Since then, specific protocols have been developed for different systems to take advantage of these founding ideas in order to increase the generational cycle of important crops

in order to accelerate genetic gain (Fang et al., 2021; Hickey et al., 2017, 2019; Mobini et al., 2020; Saxena et al., 2019). Ghosh et al. (2018) optimized a protocol that allows winter wheat to flower in 105.4 days and to harvest the seeds in 123 days. Seeds in this study that were considered "early harvest" were collected 18 days after heading, bringing the total days from seed to viable seed to be ~123 days. Another study helped in reducing this time slightly (3–5 days) by implementing long day, high-intensity lights on a population meant for disease scoring. These studies only allow two generations in a calendar year, or three in a breeding year, which goes from mid-July, when variety trial results become available, and early to mid-October, after the Hessian fly-free date, which equates to roughly 450 days (Zakieh et al., 2021). This is not sufficient to allow for the advancement of fixed experimental lines to be planted in the field for evaluation. This time was reduced further by reducing the vernalization time to 2 weeks
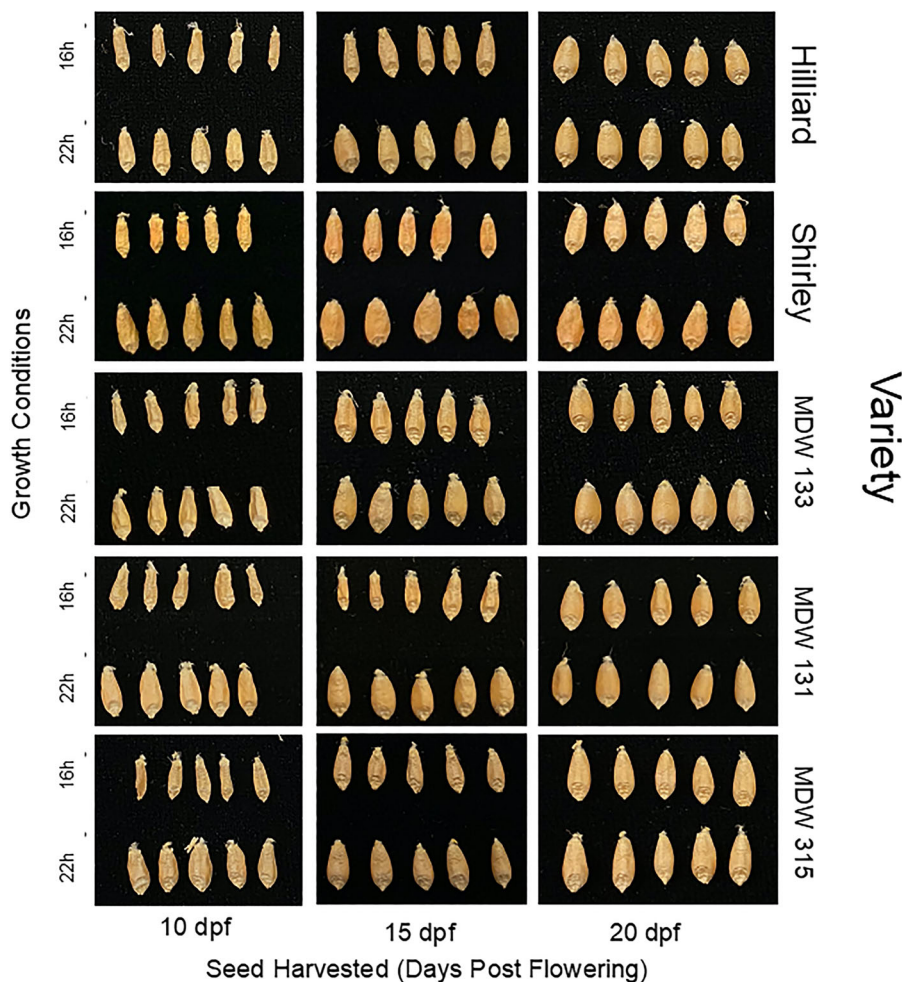
FIGURE 3 Seeds of different varieties, shown on the right, grown in either 16 h settings (top) or 22 h settings (bottom), harvested 10 days post-flowering (s) (left column), 15 DPF (middle column) or 20 DPF (right column). Seeds shown were harvested at their respective times and allowed to dry in 50°C oven for 3 days and left at room temperature in coin envelopes for ~1 month before pictures were taken.

with extended day and higher (10° C) temperatures; however, this method only was effective in a portion of the total number of lines used (Cha et al., 2022). Another study showed that by removing tillers from growing plants before anthesis can show further reduction in generation time, and while this could be beneficial when dealing with small populations, when considering the size of winter breeding programs, it is not feasible with large populations.

In this study, we establish a SB protocol for winter wheat breeding programs that can increase generations in greenhouse settings from only two to between four and five generations in a breeding year depending on the genetic background of the experimental line. By utilizing long day and short night cycles with increased temperature and light intensity, we were able to decrease the time from seed to flower by ~11 days in comparison with our 16 h settings, and over 20 days faster than what has been reported in other protocols that did not modify vernalization or harvest seeds early (Ghosh et al., 2018). Our experiments were done in high-

density format, with plants growing to maturity in 50-well trays, in order to make efficient use of space in these environmentally controlled chambers, giving rise to the opportunity for SSD methods of breeding, where one seed per plant is selected to advance the generation following the original cross (Chahal & Gosal, 2002).

In order to further determine efficacy of this protocol, we investigated five *Ppd* and *vrn-A1_short* alleles and their effect on 22 h settings in a panel of diverse genotypes. It was found that within our 22 h settings, the inclusion of the *vrn_A1_short*, allele significantly ($p < 0.001$) reduced the time to flowering in 22 h settings, whereas *Ppd_A1.a.1* actually increased the time significantly ($p < 0.01$) to flowering in 22 h settings. The sample size for this study was relatively low to make concrete conclusions for the role of these alleles in SB, but this does provide evidence that the *Vrn/Ppd* allele composition may have an impact on this breeding strategy.

Additionally, we explored how shortening vernalization can potentially decrease generation time without necessarily
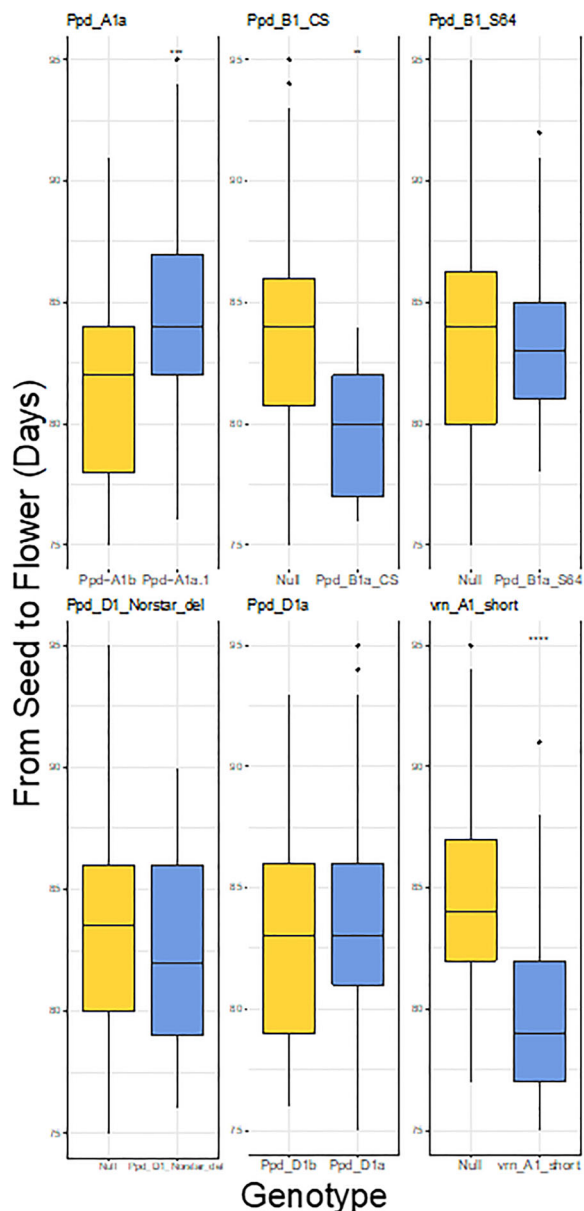
FIGURE 4 Days from seed to flower in either speed breeding settings among different *Ppd* and *Vrn* alleles in set 3. Significance was calculated by comparing means of days from seed to flower between allelic composition at each loci, separated by growing conditions. *, **, ***, and **** indicate significance at 0.05, 0.01, 0.001, and 0.0001 probability levels, respectively. Those without significance symbols were found not to be significant.

reducing the productivity of the plants. Plants grown with only 5 weeks of vernalization all flowered efficiently under 22 h settings, and in all cases, average flowering time was reduced in comparison with 16 h settings. The time from vernalization to flowering did not change much in many of the conditions, though in Hilliard and Shirley, 5 weeks of vernalization did seem to have an undesirable increase in flowering time (Table S4). The number of seeds set superficially increased or decreased depending on the cultivar,

setting, and vernalization time. Seven weeks of vernalization did not provide any advantage in reducing the flowering time. 22 h settings reduced flowering times numerically and/or statistically in all the vernalization duration treatments. In comparison with Cha et al. (2022), where 2 weeks of vernalization was used on uncovered seeds, and quick generation times were observed, but 12% of lines did not reach reproductive maturity, all of our tested lines reached flowering effectively.

To further reduce the generation time, seeds were harvested after 10, 15, and 20 DPF to determine if early harvest influences germination. Seed sizes and maturity varied depending on photoperiod settings as well as the time seeds were harvested. In three genotypes, germination of seeds harvested 10 DPF showed germination rates > 75%. Including the drying time of 3 days in a 50°C oven, means that viable seeds can be planted 13 days after flowering, bringing the total average days from seed sowing to seed sowing to ∼96 days. This is ∼27 days shorter than what had been reported in earlier studies (Ghosh et al., 2018; Zakieh et al., 2021). Moreover, we also report that even under our 16 h, growing conditions protocol, we found that the average flowering time was between 90 and 95 days after sowing, which is ∼20 days shorter than what has been reported by Ghosh et al. (2018). This could be in part due to the baseline light intensities and temperatures, as well as the pot sizes that we use in our greenhouses and growth chambers (Poorter et al., 2012; Wheeldon et al., 2021).

In order to keep up with the food demand and security, elite wheat varieties need to be developed that improve yield by 1.5%–1.7% annually (Dixon et al., 2009; Shiferaw et al., 2013). Though cultural practices are an important aspect of increasing wheat productivity, genetic gain stands to be a major player in remedying an impending issue (Hawkesford et al., 2013; Mueller et al., 2012). Breeders have a toolbox in front of them, that is, growing as the cost of sequencing goes down, and the availability of genetic resources increases (Chhabra, Singh et al., 2021; Chhabra, Tiwari et al., 2021; Dong et al., 2020; Liang et al., 2017; Sánchez-Martín et al., 2016; Walkowiak et al., 2020). We have the ability now to make accurate crosses using genetic markers or predict the breeding value of new varieties using genomic selection, technologies that did not exist during the Green Revolution (Bassi et al., 2016; Gupta et al., 2010; Pingali, 2012). These genetic and genomic technologies still are not enough unless we can reduce the time it takes to make an elite cultivar available to the public. The equation for genetic gain (ΔG), or the "breeders equation" is $\Delta G = i r \sigma_a / L$, where σ_a is the additive genetic variation in a population, i is the selection intensity, r is the selection accuracy, and finally, L is the generation time. Because the genetic gain is inversely related to the length of the generation cycle, by reducing the time it takes to advance the generation in a breeding population, we are increasing the genetic gain (Cobb et al., 2019; Falconer & Mackay,
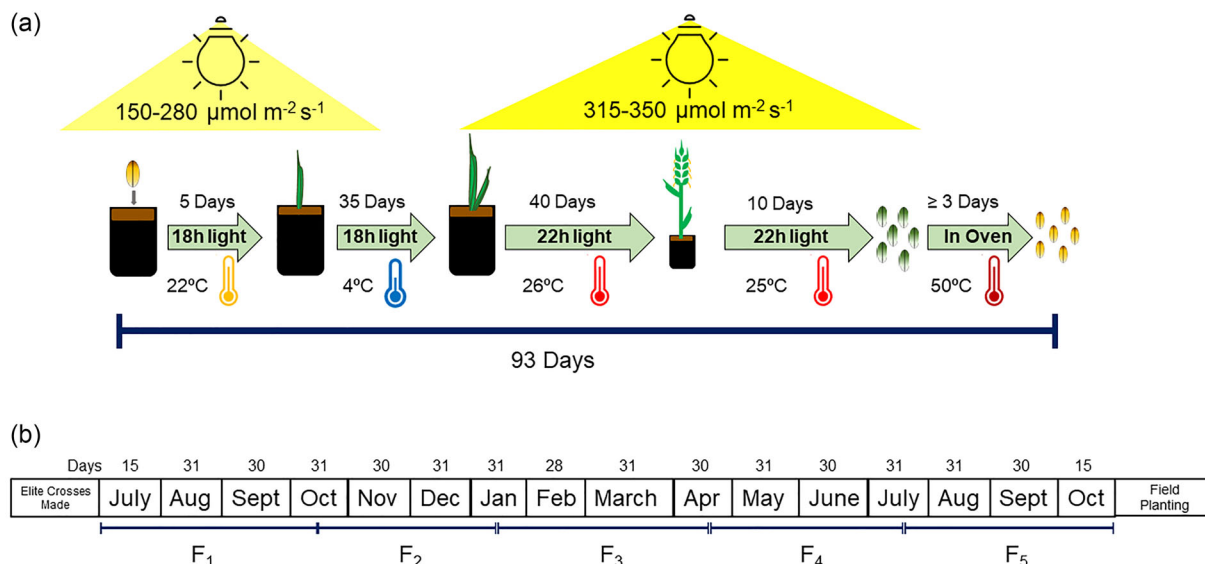
FIGURE 5 Schematic for our enhanced speed breeding method for winter wheat. (a), showing the optimal conditions and timing in order to get a generation from seed to seed in ~93 days. (b), the breeding year based on original elite cross to the subsequent year's field planting. Each bracket represents the ~93 days.

1996; Fehr, 1987; Lozadaid et al., 2020). Though technologies such as generating doubled haploid populations exist which can reduce the time it takes to get a genetically stable variety, the genetic recombination in these populations is lower than in SSD methods, meaning larger DH populations would be needed to produce varieties with desirable genotypes, which may not make it a feasible option (Cobb et al., 2019; Guzy-Wró belska et al., 2007; Inagaki et al., 1998; Santra et al., 2017; Wędzony et al., 2009).

Our protocol establishes a method to achieve a reduction in generation time of winter wheat such that four to five generations can be achieved within a breeder's year (Figure 5). By utilizing reduced vernalization, long days, high-intensity lighting, and increased temperature conditions, we were able to achieve flowering, on average, in under 90 days, and with seed harvest after 10 days, a full generation in under 100 days. This protocol, though shown here in environmental growth chambers, can be achieved in greenhouse settings as well with the use of supplementary HID lighting to increase the light cycle to 22 h for those breeders without access to high-quality growth chambers. Our avenue for efficient breeding in winter wheat has the potential to reduce the time it takes from original elite cross to elite variety release with minimal labor and a reduced cost than other methods that aim to do the same. Furthermore, this method is compatible with genotypes that are adapted to various climactic conditions. With this method, we now have the ability to increase the genetic gain in winter wheat breeding programs not only in the United States but also across the globe.

AUTHOR CONTRIBUTIONS

Adam Schoen: Conceptualization; investigation; data curation; data analysis; visualization; writing—original draft; writing—review and editing. **Sydney Wallace**: Conceptualization; investigation; data curation; methodology; care and upkeep of material; resources. **Meghan Fisher Holbert**: Investigation; data curation; methodology; resources. **Gina Brown-Guidera**: Writing—review and editing; resources; supervision. **Stephen Harrison**, **Paul Murphy**, **David Van Sanford**, **Richard Boyles**, **Mohamed Mergoum**: Resources; conceptualization; writing—review and editing. **Nicholoas Sanantonio**: Resources; and editing. **Nidhi Rawat**: Supervision, resources, writing—original draft; writing—review and editing. **Vijay Tiwari**: Conceptualization; investigation; data curation; project administration; resources; funding acquisition; visualization; writing—original draft; writing—review and editing.

CONFLICT OF INTEREST STATEMENT
The authors declare no conflicts of interest.

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