COMPARISON AND STANDARDIZATION OF WHEAT PRE-HARVEST SPROUTING SCREENING METHODS, PRELIMINARY SCREENING OF GENOMIC PANEL LINES

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

MASTER OF SCIENCE

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ABSTRACT

Preharvest sprouting (PHS) in spring wheat (*Triticum aestivum*, L.) is a significant problem in the United States, with many ways to evaluate it. When unharvested wheat begins to sprout, the grain begins to germinate reducing functional quality. Screening methods for PHS can range from in-situ spike misting to seed wetting. Each method has multiple published protocols, each with differing results. This experiment sought to compare two common screening methods, in-situ spike misting and seed wetting, from both field grown, and greenhouse grown seed sources. The experiment was comprised of 528 wheat lines in 2020 and a 50-genotype subset in 2021. Results from the correlation of methods analysis yielded a high correlation (r=0.74). Results from the correlation of sourced material analysis yielded a high correlation also (r=0.87). A preliminary genome wide association study identified a significant QTL present on chromosome 4A. This work will serve as a foundation for future studies.

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INTRODUCTION

Pre-harvest sprouting (PHS) in wheat (*Triticum aestivum* L.) has impacted North Dakota for decades due to high moisture weather late in the growing season. Most recently, in 2019, the North Dakota State Climate Summary estimated that the state accumulated a record high annual rainfall of 619.76 mm that caused pre-harvest sprouting in wheat throughout the state (Frankson, 2022). Pre-harvest sprouting occurs when a physiologically mature grain imbibes enough water to start the germination process. Gibberellic acid is released at the start of germination which stimulates growth via the release of hydrolytic enzymes such as alpha amylase (Gupta, 2013). Abscisic acid (ABA), a plant hormone that assists in maintaining seed dormancy, begins to degrade when gibberellin acid increases (Kermode, 2005). Late season rain, heavy dew, and cooler temperatures can all trigger the germinating process, causing damage to the seed before the radicle is even visible. The radicle is the first observable growth after the germination process begins. PHS is a phenomenon that is genetically complex and consists of many different contributing factors (Wang et. al., 2019). For example: dormancy levels in the kernel, the waxiness of the glumes, and the shape of the spike are all examples of morphological characteristics of wheat that may delay germination (Pool & Patterson, 1958). When wheat is unable to prevent sprouting during inclement weather, it can devastate entire crops for farmers and end-users. For farmers trying to bring a sprouted crop to a grain elevator, they are usually met with a significant price discount or a full rejection of the crop altogether. When bakers use flour from grain that is sprouted, the cakes or loaves take on an unwanted sticky or crumbling texture (Olaerts, 2018). This loss of quality stems from the hydrolysis of starches into maltose, glucose, and oligosaccharide molecules in the endosperm by alpha-amylase (Baranzelli et al., 2018). Damage to the kernel from PHS can be detected using the Hagberg Falling Number test,

which is a destructive assay conducted using flour obtained from 250g of dry wheat. This estimate of PHS measures the level of alpha-amylase activity in a sample (AACC International Method 56-81.03). Each test takes approximately seven to eight minutes to complete. Given the substantial amount of seed needed and the length of time for each assay, this test is impractical for high volume screening of early-generation screening materials. Therefore, it is important that North Dakota State University (NDSU) wheat breeders have an effective and efficient early PHS screening method to evaluate current and future wheat lines.

To reach this goal, two different PHS screening methods were analyzed for their ability to provide consistent results on PHS and be used interchangeably to accommodate the resources available within different breeding programs.

An initial population of 528 spring wheat lines was screened for the first year of the study, with a 50-line subset being used in the second year. The population and subset consisted of both hard red spring (HRS) and hard white spring (HWS) wheat lines originating from multiple public and private institutions. Use of a large number of lines originating from multiple sources allowed a wide range of genetic material to be tested. Due to the low level of pre-existing information within the NDSU wheat breeding program, the original population tested in 2020 was used to create the focused subset of lines used in 2021. The smaller subset also enabled the study to be comprised of more replications of entries which could be evaluated in a shorter amount of time, which is critical for preventing confounding issues with stored samples.

The first screening method consisted of an in-situ test that involved misting the entire wheat spike. This method of testing has been widely used by several researchers but requires a large chamber specifically designed for effective misting (Patterson, 1989). This method allows the morphological traits of the spike (e.g., size of the spike, shape of the florets, presence of awns

and length, and waxiness of the glumes) to be considered (Thomason, 2009). These types of observations are lost when the seed is threshed for the second method.

The second method consisted of a seed wetting method that measured dormancy levels through germination in a petri dish. Since dormancy is a major component of sprouting (Nakamura, 2018), this method was hypothesized to be an alternate method for PHS screening. This method eliminates the need and cost of a misting chamber. However, as mentioned, the seed wetting method prevents any consideration of morphological characteristics of the wheat spike. Past literature suggests that both methods are applicable and produce useful results.

MATERIALS AND METHODS

Plant Materials

Data for this experiment were collected during 2020 and 2021. In 2020, experimental entries consisted of 528 spring wheat lines (see Table 1) that were grown at the Prosper field research site near Prosper, North Dakota (46.9630° N, 97.0198° W). After evaluation of the full set in 2020, a selected subset of fifty lines was characterized in 2021 (see Table 2). The 2021 subset was grown in Casselton, North Dakota (46.9005° N, 97.2112° W), Prosper, North Dakota (46.9630° N, 97.0198° W), and in a greenhouse located on North Dakota State University Campus (12-hour photoperiod at 21°C). The 2020 set was a panel selected from a historical population which consisted of every line used in crossing in the NDSU spring wheat breeding program from 2002 to present. A diversity analysis from 90k SNP (Illumina, Wang et.al, 2014) was conducted on those lines and 402 genotypes were selected to represent unique diversity and remove highly related individuals (e.g. full-siblings) across the breeding program. Additionally, an additional 126 lines were added originating from the genotypes from the two years of advanced yield trails from the hard red spring wheat (HRSW) and hard white spring wheat (HWSW) programs. A grand total of 528 genotypes were used in the 2020 evaluation.

The subset created in 2021 was used to focus solely on the two screening methods, to reduce the number of samples, and to ensure equal treatment of all samples throughout the harvest and testing process. Genotypes selected for the 2021 subset included both red and white spring wheat lines and were either released cultivars or experimental lines originating from both public and private breeding programs in the region. Seed source for the 2020 evaluation was source used to produce the plants which were genotyped using the Illumina 90k wheat SNP array (Wang, 2014). This source seed was then planted in a completely randomized design at the

Prosper, North Dakota location. The source seed for 2021 came from extra spikes acquired during the harvest in 2020. It was planted in a completely randomized design at Prosper and Casselton, North Dakota, and in the greenhouse on NDSU campus. Field plots in both years consisted of experimental units comprised of four rows, 1.524 meters long, on 3.66-meter row spacing. In 2020, the plots were treated with 437.6 ml/hectare of Prothioconazole (19%) and Tebuconazole (19%) in Prosper, ND. Due to lack of fungal disease in 2021, no fungicide was applied at either location. For the greenhouse evaluation, four seeds of each genotype were planted in one-liter pots (16 cm D x 16 cm W x 16 cm H) spaced 1 cm apart. A total of fifty pots were used in a configuration of ten rows and five pots per row.

Harvest and Storage

Spikes were harvested at physiological maturity, Feekes growth stage 11.3/Zadoks 91 with physiological maturity being characterized by the loss of green color in the glumes and the peduncle. No remarkable events of weather occurred prior to or during the two-week harvest window. Thirty to forty mature spikes from each genotype were harvested in both years to ensure a large quantity of remnant seed for future use. Spikes were cut approximately 10cmbelow the peduncle, placed in a 12.7 cm x18 cm paper envelope, and dried at 32°C for three days. After three days, spikes were removed and placed in a freezer at 0°C and stored until used for testing. Spikes were not kept frozen for more than three months to ensure viability during sprouting tests.

In-situ Spike Wetting

The misting chamber for this portion of the experiment consisted of a rotating carousel fitted with six platforms of wire mesh, misting nozzles on the ceiling, and a supplemental Hydrofogger™ humidifier (Hydrofogger, Greenville, South Carolina). Each mesh platform measured 22 cm by 70 cm and the holes measured 3 mm by 3 mm. The platforms also had a

double layer of mesh spaced 3 cm apart, so to ensure wheat stems remained upright and organized in a completely randomized experiment design (CRD). Misting nozzles were situated 40 cm above the platforms and dispersed one liter of city water per minute. A single rotation misted each mesh platform for 3.83 seconds and 23 seconds were required for a full rotation. Spikes were evenly spaced out among the platforms to maintain carousel balance and misting times. For each run in the misting chamber, three spikes from each genotype were inserted into the wire mesh, upright and spaced three cm apart. These three spikes were considered observational units to calculate the genotypic response to the sprouting variables that were recorded. The chamber produced a constant mist for 120 minutes then continued to maintain 99% RH (relative humidity) for six days. Ambient temperatures ranged from 68 degrees to 73 degrees. Spikes were treated with Folicur solution (0.5 mL tebuconazole/1L H_2O) after the initial 120-minute wetting. Any spike exhibiting mold, which would have compromised germination and potentially contaminated nearby spikes, was removed. Spikes were checked every 12 hours for the first three days and then every 24 hours for the following three days. Data recorded included number of days until the first sprout was observed, and percentage of spikes with a visible radicle on day six. A mean response of the three spikes per genotype was computed. A total of seven runs in the misting chamber were conducted (two each from Prosper 2020, Prosper 2021, and Casselton 2021; and one from Greenhouse 2021).

Seed Wetting

The germination chamber used for this portion of the experiment provided controlled temperature and humidity. This chamber was maintained at a constant 27°C and 99% RH. Spikes were removed from cold storage and mechanically threshed using a Seedburo Single Plant Belt Thresher. Twenty seeds from each genotype were placed crease down on germination paper in a

sterilized plastic petri dish of dimensions 150mm x 15mm. A total of 4 mL of distilled water and 1 mL of Folicur solution (0.5 mL tebuconazole/1L H_2O) were added to the petri dish. Petri dishes were then placed in the germination chamber for a total of six days in a randomized complete block design (RCBD). Data recorded were the number of days required for visual confirmation of sprouting via the presence of a radicle in the twenty seeds. If neither germination nor the presence of a radicle was observed by day six, the petri dish remained in the chamber until sprouting occurred. A special note was taken if seed did not sprout at all. A total of seven runs in the germination chamber were conducted (two each from Prosper 2020, Prosper 2021, and Casselton 202; and one from Greenhouse 2021).

Data Analysis

Data were analyzed using a variation of investigative approaches on JMP Pro 15 statistical software (SAS, Cary, NC). To create the subset of genotypes for Year 2 testing, fifty lines with a range of potential resistance and a consistent response, indicated by low variance, based on the 2020 experiment were selected. A Pearson Correlation analysis was the primary tool used to determine if the screening methods differed or could be used interchangeably.

A coincidence of selection analysis was done to further support the correlation analysis and to measure prediction accuracy between the two methods (Matias, 2022). To do this, each genotype was averaged across environments, by treatment, and the in-situ spike misting genotype averages were compared to the seed wetting genotype averages. To perform the coincidence of selection, the mean value for spike misting was presumed to be the true response.

To measure prediction accuracy of the genotypes between the combined average field sourced seed and combined average greenhouse sourced seed, a rank order comparison was used in a side-by-side table, as well as a supporting Pearson correlation and co-incidence of selection

analysis (Matias, 2022). Since breeding programs often make advancements based on a selection intensity, a rank order comparison and a co-incidence of selection provided different insights on the prediction accuracies. The rank order comparison is a simple indicator of prediction accuracy, and a co-incidence of selection stimulates prediction accuracy specifically for breeding programs.

Furthermore, a genome wide association study (GWAS) was conducted on 2020 results. GWASpoly, a statistical package within R, along with the Bonferroni correction method were used (Maccaferri, 2015). A total of 24,235 markers were utilized from 402 variables (genotypes). To find the number of markers, a series of steps was taken to filter the alleles. GenomeStudio, a software by Illumina, called alleles into the program. Statistical software, R, then transcribed the AB alleles into numbers zero, one, and two. The major frequency allele was given the number two and the minor frequency allele was given zero. Heterozygous calls were transcribed to “n/a” and discarded. Minor frequency alleles were filtered out at a frequency of .05. Markers were removed which had an allele call rate with 10% or more of missing data. The SWEEP (Clevenger, 2015) filter rate was 0.8 and the k-nearest neighbor imputation was applied (Money, 2015). From these data a Manhattan plot was created (Gibson, 2010). The positions on the wheat genome of markers identified as having a high score and effect were then determined.

RESULTS

Comparison of Screening Methods

A Pearson Correlation was used to analyze data obtained from the 50-genotype subset to compare the two different screening methods for PHS (See Figure 1). The model showed a strong correlation ($r=.74$), $p<0.001$ (See Table 4). These results indicate that within the conditions of this experiment the two methods could be used interchangeably to screen for PHS.

The seed wetting method genotype mean was 4.75 days to sprout and ranged from a minimum of 1.29days to sprout and a maximum of 8.29 days to sprout. The variance of the seed wetting method was 3.99. The in-situ misting method overall mean was 4.18 days to sprout with a maximum of 6.29 days to sprout and a minimum of 1.43 days to sprout. The variance of the in-situ misting method was 1.2.

The co-incidence of selection for the comparison of methods, provided a percentage of accuracy of selected lines at various selection intensities. This resulted in similarity of classification percentages (e.g., accuracy percentages) that ranged from 80% to 90% (See Table 6). The lowest similarity was 80%, suggesting that 20% of selections were potentially misclassified. This infers that of the selected subset of 50, forty lines were correctly classified as advanced genotypes or as discarded genotypes and ten lines were incorrectly classified. The highest percentage of similarity was at the 90% selection intensity, which had 90% classified accurately, meaning approximately forty-five out of fifty lines within the subset were categorized accurately.

Comparison of Sourced Material

Genotypes grown in both field sourced kernels and greenhouse sourced kernels were ranked and compared to determine if rankings of genotype were similar using the two sources

(See Table 3). A Pearson Correlation analysis was calculated to compare ranks of the field seed sourced genotypes to the greenhouse seed sourced genotypes (See Figure 2). A Pearson correlation coefficient of r=0.87 and a probability value of <0.0001 was calculated from the data (See Table 5). Genotypes exhibiting the highest level of resistance to sprouting were: NDHRS16-16-678, NDHRS16-14-98 and NDHRS12-0065-0004, which had delayed sprouting average of 6.9 days. The genotypes exhibiting high sprouting susceptibility were NDHWS16-0007-H11, LNR2076, and NDHWS15-0209-C04, which had a combined sprouting average of 2.2 days.

The field sourced material genotypic mean was 4.5 days to sprout with a minimum of 1.67 days to sprout and a maximum of 7.08 days to sprout. The variance of the field sourced material was 2.04. The greenhouse sourced material overall mean was 4.24 days to sprout with a maximum of 7.5 days to sprout and a minimum of 1.5 days to sprout. The variance of the greenhouse material was 2.96.

The co-incidence of selection resulted in similar classification percentages that ranged from 86% to 90% (See Table 7). The lowest similarity was 86%, suggesting that 14% of selections were misclassified. This suggests that of the selected subset of 50, 43 were correctly classified as advanced genotypes or as discarded genotypes and seven were incorrectly classified. The overall range of the similarly classified percentages was 4%. A 2% decrease in accuracy results in one additional genotype being selected incorrectly. The highest percentage of similarity was at the 90% selection intensity, which had 90% similarly classified.

Preliminary Genome Wide Association Study

The genome wide association study was conducted using the Bonferroni Correction method and data obtained in 2020. A Manhattan plot shows a distinct perspective of the tight

grouping in chromosome 4a at position 603102885 (Figure 3). The marker associated with this position is wsnp_Ex_c13031_2062590 and showed an additive effect of -0.3. Dormancy is a known gene that maps to Chromosome 4A; however, there has been no validation between the marker listed and a dormancy gene (Torada, 2016). Further verification is needed to fully confirm the genetic marker and to investigate other nearby QTL.

DISCUSSION

Interpretation of Results

Results suggest that the two methods of screening can be used interchangeably due to the high r-value (.74) in the primary Pearson Correlation. The coincidence of selection also suggests that material can be sourced from either the field or greenhouse. Greenhouse sourced material allows this type of study to be conducted during any time of the year regardless of season. The high Pearson r-value (.87) in the correlation conducted between field material and greenhouse material strongly suggest that screening for PHS can be done effectively using both methods evaluated in this study.

Some limitations of this experiment must be noted. There is a significant workload when evaluating the larger sets of genotypes that are typical of plant breeding evaluations. In this study, the workload associated with the larger initial set of genotypes resulted in the creation of the smaller selected subset used in year 2021. While the smaller subset was more manageable, it limited the amount of information and data that could be gathered on the spring wheat program's collective germplasm and genotypes. There are many other considerations that determine why a research program may prefer one method over the other, among them are: cost and workload.

Coincidence of Selection

The co-incidence of selection analysis assesses the correct classifications (keep vs. discard) of line advancement for a breeding program at various levels of selection intensity. The various levels of selection intensity tested were 90%, 80%, 70%, 60% 50% and 40%. For example, if a selection intensity of 90% were used, we would discard the bottom 10% of genotypes from the set of fifty genotypes due to susceptibility to PHS. The coincidence of

selection compares the two datasets, where a higher percentage of ‘Similarly Classified’ means that the same individuals would be advanced or discarded using the identical criteria for the two datasets. It differs from prediction accuracy of ranks between datasets because it more accurately reflects situations in plant breeding, where exactly matching ranks (as would be used in a traditional measure of model prediction accuracy) are not as important as correctly classifying genotypes as “advance” vs. “discard.” This analysis was used to compare the percent similar when selection genotypes based on PHS screening method (seed wetting or in-situ spike misting) and source location (greenhouse material or field material).

Screening Factors

At first glance, the seed wetting method stands out as a simple and straightforward screening method, requiring little space. The extra steps that need to be considered for this method include time needed to gently thresh seeds to avoid breaking the germ, disinfecting the seeds with a diluted bleach solution, lining petri dishes with germination paper, counting seeds to be placed in the dishes, measuring specific amount of water/fungicide, and maintaining a set amount of water within the petri dish. These time-consuming steps along with the need to repeatedly remove the samples from the growth chamber to take notes add significant amounts of time and effort. It is also worth noting the number of single-use products required for the seed wetting method. All used labels, petri dishes, and germination paper were discarded after the project was completed. Not only is the use of single-use products potentially wasteful, but it collectively increases overall costs to the program.

A misting chamber that effectively screens for PHS requires an expensive up-front cost. However, these chambers can last many years. The misting chamber used in this study was custom built over 30 years ago and due to its age has been serviced for malfunctions multiple

times. During this study, the misting chamber had two mechanical failures but was quickly repaired. The extra steps needed to prepare for screening using the misting chamber were considerably less than those needed for the seed wetting method. After collecting and drying spikes, they were placed upright in a screen and a map was created to take notes. Following placement of the spikes in the chamber, the misting chamber was simply turned on and monitored. Notes were easily taken within the misting chamber without removing the screens from their carousel. Fungicide could also be applied quickly due to the open design of the chamber. Overall, the misting chamber, albeit expensive, saved on time and effort. If a program is repeatedly screening lines for PHS and has the means necessary to build or maintain a misting chamber, this method would be recommended.

Another option would be forgoing harvest all together by using greenhouse sourced material and placing a misting tent over experiments in the greenhouse to initiate germination. This method could be a useful alternative for breeding programs which already utilize a greenhouse space.

Methods Justification

Many different variations of screening methods for PHS exist among research groups. The methods used for this study were adapted from methods reported in published articles of Patterson et al. (1989), Burt (2008) and Nörnberg.et al. (2015). These methodologies, along with the available resources within the NDSU Wheat Breeding Research Program, shaped the structure of this study. Every reported experiment had slight methodology modification, presumably to fit their programs' abilities. There were three management factors that remained consistent across all reported experiments. Those factors were: (1) harvest at physiological

maturity, (2) drying for three days, and (3) freezer storage at zero degrees Celsius. All factors were incorporated into the framework of this study.

Noteworthy Genotypes

The breeding goal of the study was to characterize the level of resistance available in the breeding germplasm on the NDSU spring wheat breeding program. Further testing and evaluation are needed as the current study did not include known resistant and susceptible genotypes that could be used as consistent checks. As work continues, checks will be discovered and included in future evaluations. Preharvest sprouting had not been an evaluation criterion in the program for several years, so a broad scope study had to be initiated to characterize the germplasm within the program. Noteworthy resistant genotypes were NDHRS16-16-678 (Brennan (PI 658041)/Elgin-ND (PI668099)) and NDHRS16-14-98 (Granger (PI 636134)/ND804), which were the most resistant red wheat lines in the field. The presence of resistance in red wheat lines was to be expected though, since it is known that color genes are highly linked to dormancy and PHS (Singh, 2002)

Future Studies

This study's primary motivation was the desire to restart preharvest sprout screening in the NDSU wheat breeding program. Further studies are needed to gather information and data. Results of the study comparing screening methods could be bolstered by adding years and/or increasing number of environments. A study that relies on lab tests and genetic markers over visual scoring might also lend to more exact information. The preliminary genome wide association study used in this research would benefit from more years of data as well. Multiple markers were detected in this study but without more data these markers cannot be validated. There are also many extra lab tests such as Hagberg's Falling Number (AACC International

Method 56-81.03) that can be used to characterize the genotypes, thereby adding more information, and validating existing data (Hu, 2022).

It is widely accepted that wheat spike morphology can play a role in PHS. An examination of breeding program's diversity of spike characteristics may add to the understanding and characterization of preharvest sprouting. Spike morphology could be integral in a white wheat genotype if other genetic resistances are too difficult to incorporate due to linkage with the red color alleles.

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APPENDIX A: TABLES

Table A1: 2020 Genotype List for Preharvest Sprout Screening Methods: Seed Wetting and In-situ Spike Misting Planted in Prosper, ND

Plot	Name	Plot	Name
1	GLENN	**267**	NDHRS16-12-16
2	LCS REBEL	**268**	NDHRS16-12-18
3	AC SNOWBIRD	**269**	NDHRS16-12-25
4	NDSW0601	**270**	NDHRS16-12-28
5	NDHWS14-0099-A02	**271**	NDHRS16-12-33
6	NDHWS16-0099-H16	**272**	NDHRS16-12-34
7	NDHWS14-0088-A17	**273**	NDHRS16-12-37
8	NDHWS16-0040-H13	**274**	NDHRS16-12-44
9	NDHWS14-0029-A05	**275**	NDHRS16-13-11
10	NDHWS14-0099-A07	**276**	NDHRS16-13-18
11	NDHWS16-0065-H04	**277**	NDHRS16-13-20
12	NDHWS16-0069-H01	**278**	NDHRS16-13-32
13	NDFROHBERG	**279**	NDHRS16-13-51
14	NDHWS14-0066-A24	**280**	NDHRS16-13-67
15	NDHWS15-0003-H14	**281**	NDHRS16-13-80
16	NDHWS16-0040-H10	**282**	NDHRS16-13-97
17	NDHWS16-0092-H01	**283**	MN09157
18	NDHWS14-0010-C13	**284**	MN10055
19	NDHWS14-0092-A05	**285**	MN10285
20	NDHWS15-0211-C04	**286**	MN10362
21	NDHWS15-0209-A07	**287**	MN10368
22	NDHWS15-0209-A08	**288**	SD4536
23	NDHWS14-0116-A12	**289**	MN10021
24	NDHWS15-0209-C21	**290**	MN10388
25	NDHWS15-0209-A04	**291**	MN11397-1
26	NDHWS14-0060-H20	**292**	SD4510
27	NDHWS14-0006-A18	**293**	SD4514
28	NDHWS14-0114-A06	**294**	08S0036-19
29	NDHWS14-0118-A05	**295**	LNR10-0177RS6
30	NDHWS14-0118-A02	**296**	SD4559
31	NDHWS15-0209-A10	**297**	SD4607
32	NDHWS15-0209-A06	**298**	MN12307-3
33	NDHWS14-0099-A13	**299**	BW485
34	NDHWS16-0037-A13	**300**	BW961
35	NDHWS14-0066-A22	**301**	07S0068-11
36	NDHWS14-0006-C10	**302**	08S0303-16

Table A1: 2020 Genotype List for Preharvest Sprout Screening Methods: Seed Wetting and In-situ Spike Misting Planted in Prosper, ND (continued)

Plot	Name	Plot	Name
37	NDHWS16-0040-H02	**303**	08S0339-23
38	NDHWS14-0010-H03	**304**	MN13056-7
39	NDHWS15-0209-C07	**305**	MN13515-8
40	NDHWS16-0042-A32	**306**	SD4587
41	NDHWS14-0118-A10	**307**	SD4675
42	NDHWS16-0007-H06	**308**	09S0018-2
43	NDHWS16-0099-H17	**309**	09S0084-14
44	NDHWS15-0209-A03	**310**	LNR14-0677
45	NDHWS14-0060-C17	**311**	LNR14-0747
46	NDHWS14-0023-H05	**312**	LNR14-1868
47	NDHWS16-0098-C15	**313**	FURANO
48	NDHWS14-0066-H11	**314**	MAJOR
49	NDHWS15-0209-A05	**315**	ORLEANS
50	NDHWS14-0088-A20	**316**	TOPAZE
51	NDHWS15-0016-H07	**317**	HRS3419
52	NDHWS14-0116-A16	**318**	LNR150026
53	NDHWS15-0076-A03	**319**	LNR15-1990
54	NDHWS15-0209-A09	**320**	MN13304-5
55	NDHWS15-0209-C04	**321**	MN14470-5
56	NDHWS14-0114-A10	**322**	NDHRS16-12-19
57	NDHWS14-0066-H01	**323**	NDHRS16-12-51
58	NDHWS14-0029-A02	**324**	NDHRS16-13-13
59	NDHWS16-0007-H11	**325**	NDHRS16-13-29
60	NDHWS14-0010-H09	**326**	NDHRS16-13-69
61	NDHWS14-0029-A12	**327**	NDHRS16-13-86
62	NDHWS15-0029-H07	**328**	NDHRS16-13-98
63	NDHWS14-0029-A14	**329**	NDSW1312
64	NDHWS16-0070-C05	**330**	SD4724
65	SYINGMAR	**331**	LANNING
66	ELGIN-ND	**332**	CROMWELL
67	NDHWS15-0209-C03	**333**	NDSW0987
68	NDHWS14-0010-A17	**334**	LCSREBEL
69	NDHWS14-0118-A12	**335**	SYLONGMIRE
70	NDHWS14-0113-A01	**336**	SYMCCLOUD
71	NDHWS14-0075-A09	**337**	NDHRS11-0612-0001
72	NDHWS15-0209-A02	**338**	NDHRS16-12-31
73	NDHWS14-0118-C16	**339**	NDHRS16-13-63
74	NDHWS14-0088-A16	**340**	KWSCOCHISE
75	NDHWS14-0060-A32	**341**	KWSCHILHAM

Table A1: 2020 Genotype List for Preharvest Sprout Screening Methods: Seed Wetting and In-situ Spike Misting Planted in Prosper, ND (continued)

Plot	Name	Plot	Name
76	NDHWS15-0209-A01	**342**	KWSWILLOW
77	NDHWS14-0113-A02	**343**	KWSALDERON
78	MAX	**344**	MN15029-8
79	NDHWS16-0007-H04	**345**	MN15219-2
80	NDHWS14-0118-A08	**346**	MN15501-4
81	NDHWS14-0029-A10	**347**	NDHRS16-14-36
82	NDHWS16-0042-A28	**348**	NDHRS16-14-41
83	NDHWS16-0098-A16	**349**	NDHRS16-14-168
84	NDHWS14-0060-C03	**350**	NDHRS16-14-12
85	NDHWS14-0099-A10	**351**	AGAWAM
86	LANGMN	**352**	WAIKEA
87	NDHWS15-0209-C23	**353**	NDSW0450
88	NDHWS13-0016-0003	**354**	NDSW0612
89	NDHWS14RIL-0060-057	**355**	NDSW0703
90	NDHWS14-0120-H02	**356**	NDSW0802
91	NDHWS14RIL-0060-007	**357**	NDSW0803
92	NDHWS15-0200-C37	**358**	03S0182-4-5WL
93	NDHWS13RIL-0016-058	**359**	99S0155-14
94	NDHWS13-0076-H05	**360**	00S0219-10
95	NDHWS14RIL-0088-050	**361**	ARGENT
96	NDHWS14RIL-0060-078	**362**	WL711
97	NDHWS14RIL-0064-014	**363**	HY682
98	NDHWS13-0044-Y08-5	**364**	ND899
99	TCG-SPITFIRE	**365**	NDSW14087&8
100	MSCHEVELLE	**366**	DUCLAIR
101	CP3903	**367**	SX600
102	AMBUSH	**368**	NDSW0932
103	MSBARRACUDA	**369**	NDSW10044
104	SY ROCKFORD	**370**	NDSW0701
105	BALLISTIC	**371**	NDSW0849
106	DAGMAR	**372**	NDSW0805’S’
107	MN15005-4	**373**	NDSW0914
108	APMURDOCK	**374**	NDSW1370
109	MNWASHBURN	**375**	EXPLORER
110	LNR2076	**376**	NDSW10043
111	TCG-WILDCAT	**377**	NDSW1309
112	CP3530	**378**	NDSW10084
113	VELOCITY	**379**	NDSW10118
114	DRIVER	**380**	B110-125

Table A1: 2020 Genotype List for Preharvest Sprout Screening Methods: Seed Wetting and In-situ Spike Misting Planted in Prosper, ND (continued)

Plot	Name	Plot	Name
115	COMMANDER	**381**	NDSW10090
116	CP3915	**382**	NDSW1356
117	CP3910	**383**	NDSW1379
118	MN15119-2	**384**	NDSW14056
119	NP12100559-16	**385**	NDSW15128
120	MSRANCHERO	**386**	NDSW16-12W-8
121	TCG-HEARTLAND	**387**	NDHWS13-0012-0028
122	NP1100135-1CL2	**388**	NDHWS13-0023-0006
123	LCSTRIGGER	**389**	NDHWS13-0041-0008
124	SY611CL2	**390**	NDHRS16-14-126
125	LCSCANNON	**391**	NDHRS16-15-166
126	MNTORGY	**392**	NDHRS16-15-225
127	ND VITPRO	**393**	NDHRS16-15-228
128	BOOST	**394**	NDHRS16-15-252
129	PARSHALL	**395**	NDHRS16-15-320
130	REEDER	**396**	NDHRS16-15-329
131	ALSEN	**397**	NDHRS16-15-362
132	KNUDSON	**398**	NDHRS16-15-378
133	ND740	**399**	NDHRS16-15-66
134	ND756	**400**	NDHRS16-16-429
135	DAPPS	**401**	NDHRS16-16-482
136	ND739	**402**	NDHRS16-16-487
137	ND749	**403**	NDHRS16-16-488
138	ND750	**404**	NDHRS16-16-499
139	ND3085	**405**	NDHRS16-16-507
140	ND3077	**406**	NDHRS16-16-550
141	ND3084	**407**	NDHRS16-16-615
142	BRIGGS	**408**	NDHRS16-16-654
143	GRANITE	**409**	NDHRS16-16-678
144	ND801	**410**	NDHRS16-16-679
145	ND802	**411**	NDHRS16-16-697
146	ND803	**412**	NDHRS06-14-162
147	ND819	**413**	NDHRS06-14-45
148	ND805	**414**	NDHRS11-0244-0001
149	ND806	**415**	NDHRS11-0765-0002
150	ND807	**416**	NDHRS12-0065-0004
151	PROSPER	**417**	NDHRS12-0145-0002
152	2375	**418**	NDHRS12-0146-0002
153	NORPRO	**419**	NDHRS12-0185-0003

Table A1: 2020 Genotype List for Preharvest Sprout Screening Methods: Seed Wetting and In-situ Spike Misting Planted in Prosper, ND (continued)

Plot	Name	Plot	Name
154	OKLEE	**420**	NDHRS12-0195-0006
155	FRYER	**421**	NDHRS12-0498-0006
156	ND804	**422**	NDHRS12-0646-0006
157	EXPRESS	**423**	NDHRS12-0788-0001
158	TRAVERSE	**424**	NDHRS12-0821-0004
159	KUNTZ	**425**	NDHRS12-0821-0006
160	ND810	**426**	NDHRS12-0822-0003
161	ND811	**427**	NDHRS12-0979-0007
162	SD3635	**428**	NDHRS12-1005-0004
163	ND816	**429**	NDHRS12-1031-0007
164	BUCKPRONTO	**430**	NDHRS12-1120-0008
165	BRICK	**431**	NDHRS12-1148-0002
166	RB07	**432**	NDHRS13-0144-0001
167	KELBY	**433**	NDHRS13-0165-0003
168	KEENE	**434**	NDHRS13-0177-0001
169	ERNEST	**435**	NDHRS13-0177-0006
170	ND706	**436**	NDHRS13-0181-0002
171	ND812	**437**	NDHRS13-0205-0009
172	ND813	**438**	NDHRS13-0210-0026
173	ND814	**439**	NDHRS13-0247-0032
174	ALPINE	**440**	NDHRS13-0248-0009
175	00S0219-10W	**441**	NDHRS13-0273-0036
176	S/WCOMPW	**442**	NDHRS13-0310-0010
177	MOTT	**443**	NDHRS13-0314-0027
178	CHOTEAU	**444**	NDHRS13-0318-0003
179	SD3942	**445**	AACBRANDON
180	ALBANY	**446**	AACPENHOLD
181	ND817	**447**	WB9479
182	SABIN	**448**	NDHRS16-13-89
183	SELECT	**449**	NDHRS16-14-28
184	SD4011	**450**	NDHRS16-14-108
185	ND822	**451**	NDHRS16-14-26
186	ND823	**452**	NDHRS16-14-205
187	BRENNAN	**453**	NDHRS16-14-258
188	SYSOREN	**454**	NDHRS16-14-64
189	BROGAN	**455**	NDHRS16-14-94
190	PIVOT	**456**	NDHRS16-14-119
191	WBDIGGER	**457**	NDHRS16-14-305
192	WBMAYVILLE	**458**	NDHRS16-15-172

Table A1: 2020 Genotype List for Preharvest Sprout Screening Methods: Seed Wetting and In-situ Spike Misting Planted in Prosper, ND (continued)

Plot	Name	Plot	Name
193	RWG10	**459**	NDHRS16-15-221
194	PI277012	**460**	NDHRS16-15-233
195	RWG20	**461**	NDHRS16-15-239
196	RWG21	**462**	NDHRS16-15-243
197	RWG22	**463**	NDHRS16-15-247
198	RWG24	**464**	NDHRS16-15-248
199	RWG25	**465**	NDHRS16-15-283
200	RWG28	**466**	NDHRS16-15-287
201	ND824	**467**	NDHRS16-15-293
202	BW931	**468**	NDHRS16-15-296
203	BW932	**469**	NDHRS16-15-315
204	NORDEN	**470**	NDHRS16-15-349
205	FOREFRONT	**471**	NDHRS16-15-64
206	LINKERT	**472**	NDHRS16-15-65
207	MN07098-6	**473**	NDHRS16-16-413
208	SYROWYN	**474**	NDHRS16-16-454
209	SD4189	**475**	NDHRS16-16-465
210	SD4215	**476**	NDHRS16-16-491
211	SD4299	**477**	NDHRS16-16-515
212	BW483	**478**	NDHRS16-16-551
213	05S0157-4	**479**	NDHRS16-16-554
214	05S0242-6	**480**	NDHRS16-16-624
215	06S0157-1	**481**	NDHRS16-16-627
216	LCSPRO	**482**	NDHRS16-16-666
217	ND826	**483**	NDHRS16-16-672
218	FA9S10-0008R	**484**	NDHRS16-16-676
219	F9N12-0153	**485**	NDHRS16-16-696
220	F9N12-0168	**486**	NDHRS16-16-700
221	BOLLES	**487**	NDHRS16-16-732
222	LCSNITRO	**488**	NDHRS16-16-733
223	11YUYR1	**489**	NDHRS16-13-4
224	11YUYR5	**490**	NDHRS16-13-24
225	WB9507	**491**	NDHRS16-13-26
226	ND829	**492**	NDHRS16-13-27
227	FA9S10-022R	**493**	NDHRS16-13-28
228	FA9S10-0038R	**494**	NDHRS16-13-50
229	FA9S10-0048R	**495**	NDHRS16-13-25
230	F9N12-0151	**496**	NDHRS16-13-5
231	F9N12-0152	**497**	NDHRS16-13-82

Table A1: 2020 Genotype List for Preharvest Sprout Screening Methods: Seed Wetting and In-situ Spike Misting Planted in Prosper, ND (continued)

Plot	Name	Plot	Name
232	F9N12-0162	**498**	NDHRS16-13-84
233	F9N12-0172	**499**	NDHRS16-13-94
234	SHELLY	**500**	NDHRS16-14-29
235	ND643	**501**	NDHRS16-14-24
236	NDHRS15-12-6	**502**	NDHRS16-14-27
237	SD4393	**503**	NDHRS16-14-31
238	MN11394-6	**504**	NDHRS16-14-104
239	BW499	**505**	NDHRS16-14-110
240	NDHRS16-12-22	**506**	NDHRS16-14-113
241	NDHRS15-12-74	**507**	NDHRS16-14-116
242	R16109	**508**	NDHRS16-14-189
243	R16110	**509**	NDHRS16-14-204
244	R16111	**510**	NDHRS16-14-217
245	R16112	**511**	NDHRS16-14-252
246	ND830	**512**	NDHRS16-14-257
247	ND831	**513**	NDHRS16-14-260
248	ND832	**514**	NDHRS16-14-265
249	ND833	**515**	NDHRS16-14-58
250	ND834	**516**	NDHRS16-14-59
251	SYVALDA	**517**	NDHRS16-14-71
252	NDSW0976-51	**518**	NDHRS16-14-73
253	NDSW10004	**519**	NDHRS16-14-90
254	NDHRS16-26-0470	**520**	NDHRS16-14-98
255	NDHRS16-26-0584	**521**	NDHRS16-14-124
256	NDHRS16-26-0593	**522**	NDHRS16-14-127
257	NDHRS16-26-0723	**523**	NDHRS16-14-141
258	NDHRS16-26-0765	**524**	NDHRS16-14-143
259	NDHRS16-26-0791	**525**	NDHRS16-14-149
260	NDHRS16-26-0858	**526**	NDHRS16-14-153
261	NDHRS16-26-0864	**527**	NDHRS16-14-156
262	NDHRS16-26-0890	**528**	FALLER
263	NDHRS16-26-0895	**529**	BARLOW
264	NDHRS16-26-0902	**530**	NDVITPRO
265	NDHRS16-26-1197	**531**	LANG-MN
266	NDHRS16-26-1233		

Table A2: 2021 Genotype Subset List for 2021 Preharvest Sprout Screening Methods: Seed Wetting and In-situ Spike Misting Planted in Prosper, ND, and Casselton, ND

Plot	Name	Plot	Name
1	NDHWS16-0040-H13	26	MN13304-5
2	NDHWS14-0099-A07	27	NDSW1312
3	NDHWS15-0209-A05	28	CROMWELL
4	NDHWS14-0088-A20	29	SYLONGMIRE
5	NDHWS15-0209-C04	30	NDSW0612
6	NDHWS16-0007-H11	31	NDSW0703
7	NDHWS14-0029-A14	32	99S0155-14
8	ELGIN-ND	33	ARGENT
9	NDHWS14-0113-A01	34	ND899
10	NDHWS16-0007-H04	35	NDSW14087&8
11	NDHWS14RIL-0060-078	36	NDHRS16-16-678
12	LNR2076	37	NDHRS12-0065-0004
13	COMMANDER	38	NDHRS12-1120-0008
14	BRENNAN	39	NDHRS12-1148-0002
15	WBDIGGER	40	NDHRS13-0177-0001
16	SD4299	41	NDHRS13-0177-0006
17	ND826	42	NDHRS13-0318-0003
18	FA9S10-0048R	43	NDHRS16-16-627
19	SHELLY	44	NDHRS16-13-50
20	NDHRS15-12-6	45	NDHRS16-13-25
21	NDHRS16-26-0858	46	NDHRS16-13-82
22	NDHRS16-13-20	47	NDHRS16-14-29
23	SD4675	48	NDHRS16-14-252
24	TOPAZE	49	NDHRS16-14-73
25	HRS3419	50	NDHRS16-14-98

Table A3. 2021-2022 Order Rank Comparison for Sourced Field and Greenhouse Material in Preharvest Sprout Screening Study

Name	Field Rank	Greenhouse Rank
NDHRS16-16-678	1	9
NDHRS16-14-98	2	14
NDHRS12-0065-0004	3	2
NDHRS13-0177-0001	4	10
NDHRS16-14-252	5	11
NDHRS13-0177-0006	6	6
NDSW14087&8	7	3
NDHRS13-0318-0003	8	7
NDHRS12-1120-0008	9	4
BRENNAN	10	23
NDHRS12-1148-0002	11	1
SHELLY	12	5
NDHRS16-14-73	13	8
TOPAZE	14	15
NDHRS15-12-6	15	16
COMMANDER	16	24
NDHRS16-13-25	17	17
HRS3419	18	13
NDHWS14RIL-0060-078	19	12
ND899	20	18
NDSW0703	21	26
NDHRS16-13-20	22	19
NDSW1312	23	20
MN13304-5	24	27
CROMWELL	25	28
NDHRS16-14-29	26	30
NDHRS16-26-0858	27	29
99S0155-14	28	31
NDHRS16-16-627	29	35
FA9S10-0048R	30	32
SD4299	31	36
NDHRS16-13-50	32	45
ND826	33	21
NDHRS16-13-82	34	25
SD4675	35	37
ARGENT	36	34
NDHWS14-0029-A14	37	22
NDHWS14-0099-A07	38	46
SYLONGMIRE	39	38
WBDIGGER	40	39
NDHWS14-0088-A20	41	40
NDHWS14-0113-A01	42	49
ELGIN-ND	43	41
NDSW0612	44	42
NDHWS16-0007-H04	45	33
NDHWS15-0209-A05	46	43
NDHWS16-0040-H13	47	47
NDHWS15-0209-C04	48	48
LNR2076	49	44
NDHWS16-0007-H11	50	50

Table A4. 2021 Pearson Correlation Test for Preharvest Sprout Screen Method Comparison: Mist Method vs. Seed Method

Variable	by Variable	Pearson's r	Prob>|ρ|
Mist Method	Seed Method	0.7392	<.0001

Table A5. 2021 Pearson Correlation Test for Preharvest Sprout Material Source Comparison: Greenhouse Source vs. Field Source

Variable	by Variable	Pearson's r	Prob>|ρ|
Greenhouse	Field	0.8635	<.0001

Table A6. 2021 Coincidence of Selection Given Varying Levels of Selection Intensity for Preharvest Sprout Screening Method Comparison: Mist Method vs Seed Method

Selection Intensity	90%	80%	70%	60%	50%	40%	30%
Similarly Classified	90%	84%	82%	86%	80%	84%	84%

Table A7. 2021 Coincidence of Selection Given Varying Degrees of Selection Intensity for Preharvest Sprout Material Source Comparison: Greenhouse Source vs. Field Source

Selection Intensity	90%	80%	70%	60%	50%	40%	30%
Similarly Classified	90%	86%	88%	86%	86%	86%	88%

APPENDIX B: FIGURES

Figure B1. 2021 Pearson Correlation for Wheat Preharvest Sprout Screening Method Comparison: Seed Method vs. Misting Method

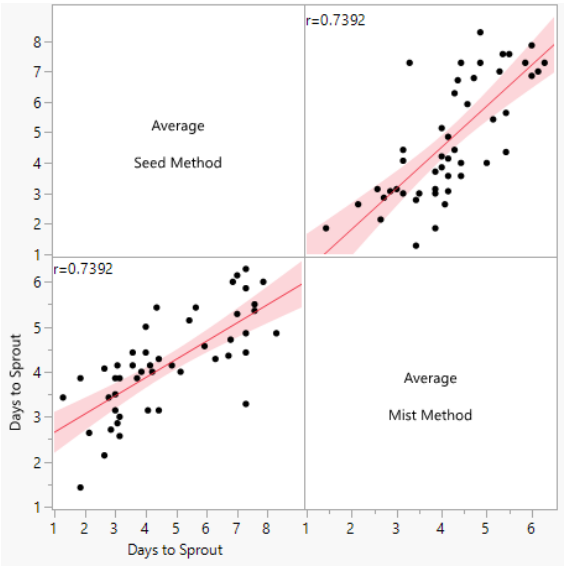

Figure B2. 2021 Pearson Correlation for Wheat Preharvest Sprout Screening Method Comparison: Field Source vs. Greenhouse Source

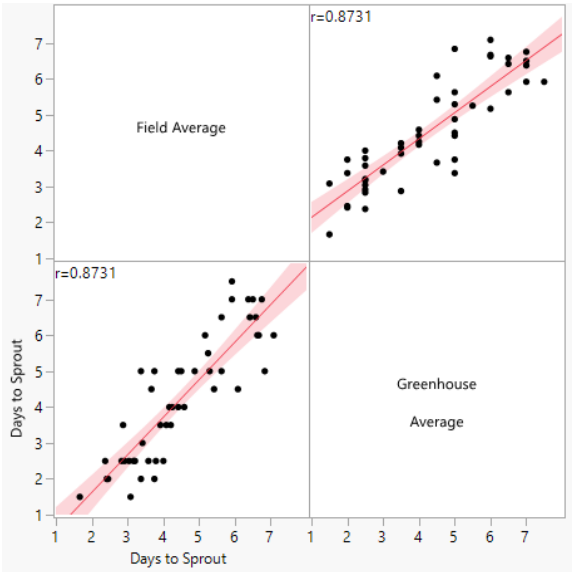

Figure B3. 2021 Manhattan Plot GWAS Graph for Preliminary Preharvest Sprout Screening of NDSU Wheat Breeding Program Lines: