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ASSOCIATION BETWEEN FALLING NUMBER AND GRAIN CHARACTERISTICS TO EVALUATE PREHARVEST SPROUTING IN WHEAT

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ABSTRACT

The falling number (FN) is a widely used parameter to evaluate sprouting damage in wheat. Despite its importance, many breeding programs lack facilities to determine the FN routinely. This study was conducted to elucidate the relationship of the falling number with germination percentage, visual sprouting score, hectoliter weight and the grain weight per spike, to improve the selection criteria in the pre-harvest sprouting (PHS) trials. Thirteen genotypes differing in PHS tolerance were evaluated in a rain simulator. The statistical analysis was conducted to understand the relationship of FN with grain characteristics. The path coefficient analysis revealed the highest association of the FN with the percentage of sprouted grains. Also, regression analysis determined that samples with more than 46% of sprouted grains had FN below 200s. The grains with 32 to 46% of germination resulted in FN values between 200 and 250s, and those with 19 to 32% of germination had FN between 250 and 300 s. Only those samples with less than 19% of germination resulted in FN values higher than 300s. The strong association between the germination percentage and the FN allows the selection of promising lines and the elimination of undesirable ones at an early stage of breeding.

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1 Introduction

The climatic conditions during wheat maturity are an important factor affecting pre-harvest sprouting (PHS) (Paterson et al., 1989). The PHS stimulated by the rain during the wheat harvest is associated with visible grain characteristics, such as swelling, wrinkles, discoloration, seed coat split, and the emergence of radicles (Thomason et al., 2019). On the other hand, the lower temperatures during the grain ripening induce a higher level of dormancy and, therefore, lower damage to the grains (Reddy et al., 1985). Under controlled conditions, PHS damage can be assessed by exposing spikes to artificial rain or through germination tests or by natural/ artificial weathering of the spike etc. It is also feasible to assess indirect damage caused by sprouting through Hagberg falling number (FN) parameter or molecular markers (DePauw et al., 2012), and the use of near-infrared (NIR) hyperspectral imaging (Barbedo et al., 2018). The falling numbers are influenced by the interaction between cultivar and environment and by the N management (Silva et al., 2019). The genetic expression of PHS resistance (low α -amylase activity, high FN values, maintenance of rheological and cooking properties) can be observed and measured reliably in wet pre-harvest conditions (Dencic et al., 2013). In other words, the level of α -amylase activity, which is affected by climatic variations, can be used as a practical parameter to estimate the industrial value of the grain and its bread-making quality (Rakita et al., 2015). It has been shown that higher α -amylase activity substantially lowers the quality of the final product resulting in lower price and loss of seed viability during the storage (Mares & Mrva, 2014). Given the negative relationship between α -amylase content and falling number values, this evaluation has been accepted in the international grain trade. Depending on the standard agreed by the wheat industry, FN values above 250 or 300 seconds are generally considered minimum quality grades (Mares & Mrva, 2008). In the Pacific Northwest region of the United States, a wheat lot with FN below 300 seconds is typically discounted, to promote higher values and compete well in the export market (Steber, 2016). Despite FN being an effective parameter, it is difficult to conduct it on a large number of preliminary genotypes in a wheat-breeding program, especially in developing countries. These difficulties can vary with the lack of equipment, a laboratory, or trained personnel etc., which limit the number of samples to be analyzed in one day. The alternative selection method *viz.* artificial weathering, allows a rapid evaluation of large number of genotypes and is used for the selection of PHS (MacMaster & Derera, 1976; Franco et al., 2009; Gavazza et al., 2012; Okuyama et al., 2018). In these studies, different parameters such as visual scoring (MacMaster & Derera, 1976; Franco et al., 2009); the percentage of sprouted grains (Basso et al., 2006; Franco et al., 2009; Gavazza et al., 2012; Zeeshan et al., 2018), and other characteristics such as grain weight per spike and hectoliter weight (Nörnberg et al., 2015b)

have been explored. Although such assessments have been successful to identify genotypes tolerant to PHS, they do not permit to establish their relationship with the FN value of the sample. Thus, the present study was carried out to determine an association between the FN and the grain germination percentage, the visual scores, the grain weight per spike, and the hectoliter weight. We believe that such information can be a useful criterion for the selection, early discard of unsuitable lines, and planning a new cycle of hybridization for resistance to PHS.

2 Materials and Methods

Field experiments were carried out at the Instituto de Desenvolvimento Rural do Paraná - IAPAR – EMATER, situated in Londrina, Brazil. The plot size consisted of three rows, two meters long and 30 cm apart, at a seeding rate of 350 seeds m^{-2} . Thirteen genotypes of wheat, with wide variation for PHS tolerance (High: Frontana, CD 114 and Safira; Intermediate: OR1, CD 116, IPR 136, IPR 128, BRS 220, IPR 85 and CD108; Low: BR 18, CD 104 and CD 105), were evaluated over two sowing dates in four years. At maturity, approximately 100 spikes per cultivar were harvested manually by following the method given by Heft & Wych (1982) and kept at room temperature. Twenty spikes per cultivar were inserted in polystyrene trays spaced 5 cm in the row and 10 cm between the rows, in four replications. They were exposed to artificial rain (nebulizer) under controlled conditions using the previously described methodology (Okuyama et al., 2018). The nebulizer was scheduled to switch on/off, every 30 minutes, to produce around 280 mm of mist per day. The tests started around 9 AM and lasted 48 hours, after which, the spikes were sun-dried and threshed manually. The grain germination percentage (sprouting) was evaluated twice, each in a 50-grain sample, using a magnifying glass (10X), to observe the rupture of the pericarp, considered as the start of the germination process (Nyachiro et al., 2002; Basso et al., 2006; Flinham, 2005). The Falling Number tests were performed on a FN 1500 (Perten Instruments), according to the 56-81 B method approved by the American Association of Cereal Chemists (AACC, 2000). The hectoliter weight was determined by the ratio of grain weight to the volume of the container and reported as kg/hl. Grain germination was scored adopting the methodology proposed by Yanagisawa et al. (2005): 1: no visible germination; 2: low germination (1-20%); 3: moderate germination (21-40%); 4: high germination (41-60%); 5: extremely high germination (60-80%); 6: complete germination (> 81%). The grain weight measurement was based on the average of 10 spikes, at 13% moisture content. The data were transformed to satisfy the assumption of additivity in the following manner: Falling Number (logarithmic transformation); percent of germination (arc sine transformation), visual scoring (square root transformation) was estimated according to Snedecor & Cochran (1982). The Analysis of variance, the path coefficient procedure,

the Pearson's correlation coefficient, and the regression analysis was used to determine the relationship between the FN and the grain characteristics by the Genes software (Cruz, 2006a, Cruz, 2006b) and the SAS package (SAS, 2001).

3 Results and Discussion

The results demonstrated significant differences among genotypes for all the characteristics under study viz. the FN, germination percentage, visual sprouting score and the hectoliter weight. The combined analysis of variance (anova) revealed that the treatments, including year, block (year), and genotype were highly significant ($P \leq 0.01$), while the interaction hectoliter weight x block (year) was significant at $P \leq 0.05$. Given that the interaction year x genotype was not significant ($P > 0.05$) for the FN, the germination percentage, the visual sprouting score, the grain weight per spike, and the hectoliter weight, it can be surmised that all genotypes demonstrated a stable performance over the years (Table 1). The results of this study show a high negative association between the FN and the germination percentage ($r = -0.9082^{**}$) and between the FN and the visual sprouting score ($r = -0.8956^{**}$). A lower magnitude of association ($r = -0.6403^{*}$) was observed between the FN and the grain weight

per spike, while no association was observed between the FN and the hectoliter weight ($r = 0.5326^{ns}$) (Table 2). Additionally, the direct and indirect effects of the grain characteristics that influence the falling number were determined by the path coefficient analysis (Table 3). It was observed that the germination percentage was the single most important source of variation in the FN ($r = -0.9047^{**}$), with a medium negative direct effect (-0.5156). On the other hand, despite a highly significant correlation coefficient ($r = -0.8957^{**}$), the relationship between the visual sprouting score and the FN was not so clear when the path analysis was performed and showed a lower direct effect (-0.1518). It is believed that the high correlation resulted primarily from the high indirect effect of the germination percentage (-0.4569). Both hectoliter weight and grain weight per spike showed a low correlation (0.5308 and -0.6432 respectively), as well as a low direct effect (0.2181 and -0.3439 respectively) with the FN. Thus, it is safe to conclude that the FN was more affected by the germination percentage than any other grain character studied. Our findings support the results reported by Nörnberg et al. (2015a), in that the percentage of sprouted grains affected negatively the FN in adverse crop environments. It is interesting to observe that the relationship between the FN and the grain characteristics behaved differently in the path coefficient analysis compared to the simple

Table 1 Analysis of variance for the Falling Number and the grain characteristics of wheat genotypes

Source	df	Falling number (s)	Germination (%)	Mean square Visual scoring (1 to 6)	Hectoliter weight (Kg/hl)	Grain spike ¹ (g)
Year	3	0.4514 **	0.5513 **	0.3309 **	70.2685 **	0.2411 **
Block(year)	4	0.1810 **	0.1537 **	0.1470 **	9.9852 *	0.2014 **
Genotype	12	0.2335 **	0.4310 **	0.4574 **	16.0577 **	0.3899 **
Year x Genotype	36	0.0153 ns	0.0332 ns	0.0460 ns	5.9201 ns	0.0383 ns
Error	48	0.0178	0.0315	0.029	3.5696	0.0279
R-Square		0.8644	0.8510	0.8626	0.7930	0.8500
Coef. Var		5.9499	24.1512	9.4306	2.5438	12.0833
Root MSE		0.1333	0.1775	0.1701	1.8893	0.1670
Mean		202.05	45.69	3.36	74.27	1.38

*, ** Significant at $P < 0.05$ and $P < 0.01$, respectively; ns: Not significant ($P > 0.05$).

Table 2 Pearson's correlation between Falling Numbers and grain characteristics

Traits	Germination	Visual scoring	Hectoliter weight	Grain spike ¹ (g)
Falling Number (s)	-0.9082**	-0.8956**	0.5326 ^{ns}	-0.6403**
Germination (%)		0.8899**	-0.4583 ^{ns}	0.4529 ^{ns}
Visual scoring			-0.5910*	0.4554 ^{ns}
Hectoliter weight (kg/hl)				0.0259 ^{ns}

* Significant at $P \leq 0.05$; **significant at ≤ 0.01 ; ns: Not significant.

correlation analysis. While the correlation analysis established a negative relationship between the FN and the germination percentage, the visual score, and the grain weight per spike, the path analysis assigned importance only to the first character (germination percentage). Such a discrepancy can be explained by the differences in the two analytical approaches. While the correlation simply identifies the mutual association among the parameters, the path coefficient analysis allows the study of the magnitude of each effect (Dewey & Lu, 1959), thereby providing a better understanding of the complexities of factors involved in the selection process (Scheiner et al., 2000). The FN test has proven to

be the most reliable for determining PHS and α -amylase activity (Barnard et al., 2005; Nörnberg et al., 2015a). Yet, the lack of equipment/trained personnel or the difficulty and cost involved to analyze a large number of samples in a breeding program, make it prohibitive as a screening tool. Therefore, many programs use sprouting scores to discard the most susceptible lines (Humphreys & Noll, 2002) or the germination test to select for PHS resistance (Zeeshan et al., 2018). Artificial weathering tests can also be very useful when high throughput is required, such as in mid-generation selection, where the objective is to screen rather than calibrate (Shorter et al., 2005).

Table 3 Direct and indirect effects of the factors that influence the Falling Number in wheat genotypes submitted to the pre-harvest sprouting test

Association	Genotype path coefficient		Correlation
	Direct effect	Indirect effect	
Falling Number vs. Germination percentage			
Direct effect	-0.5156		
Indirect effect via Visual scoring		-0.1345	
Indirect effect via Hectoliter weight		-0.0979	
Indirect effect via Grain weight per spike		-0.1567	
TOTAL			-0.9047
Falling Number vs. Visual scoring			
Direct effect	-0.1518		
Indirect effect via Germination percentage		-0.4569	
Indirect effect via Hectoliter weight		-0.1289	
Indirect effect via Grain weight per spike		-0.1581	
TOTAL			-0.8957
Falling Number vs. Hectoliter weight			
Direct effect	0.2181		
Indirect effect via Germination percentage		0.2314	
Indirect effect via Visual scoring		0.0898	
Indirect effect via Grain weight per spike		-0.0085	
TOTAL			0.5308
Falling Number vs. Grain weight per spike			
Direct effect	-0.3439		
Indirect effect via Germination percentage		-0.2349	
Indirect effect via Visual scoring		-0.0698	
Indirect effect via Hectoliter weight		0.0054	
TOTAL			-0.6432
Coefficient of determination			0.9394
Residual			0.2462

Figure 1 Negative linear regression between the germination percentage and the Falling Number of the wheat genotypes submitted to the pre-harvest sprouting.

The linear regression analysis conducted to study the grain characteristics elucidated a negative association between the percentage of germinated grains and the FN (Figure 1). Genotypes with a higher percentage of sprouted grains resulted in lower FN values. These results permit us to suggest that in those cases where the FN test cannot be performed, the germination percentage can be used as a selection tool for PHS. Even if the FN values and the grain germination percentage cannot be obtained, it is still possible to use the grain germination score, which has a high correlation ($r = 0.8899^{**}$) with the grain germination percentage. Our result revealed no association between the grain germination percentage and the hectoliter weight or the grain weight per spike (Table 2).

As per Brazilian norms for milling and other uses, a minimum value of FN (250 seconds) is required for the top wheat category (strong or high quality), 220 (seconds) for bread and domestic class, and 200 (seconds) for basic wheat (Brasil, 2010). Through linear regression analysis, it was possible to show that any germination percent or sprouting above 46% will lead to FN value below 200s. Sprouting scores between 32 and 46% were associated with FN values between 200 and 250s and all wheat samples with sprouting from 19 to 32% reached FN values between 250 and 300s. To reach FN values higher than 300 s, the samples must not contain more than 19% of sprouted grains (Figure 1). These data suggest that the germination percentage accounts for 84% of the variation found in the falling number ($R^2 = 0.8382$). It must be asserted that these values can only be used as a guideline to develop a selection criterion to discard the most

susceptible lines representing lower FN, which can vary among locations and different crop cycles (Silva et al., 2019). The genotypes with a percent of germination above 46 % (FN less than 200 s), can be discarded at any stage of the selection. The lines with a percent of germination higher than 84 % (corresponding to FN <62 s), even though with other useful characteristics, should be discarded at the beginning of the selection process or used in a hybridization program to improve their tolerance to PHS.

Conclusions

The grain germination percentage (sprouting) was more associated with the Falling Number (FN) than the visual sprouting score, the hectoliter weight and the grain weight per spike. Based on the grain germination percentage, the corresponding values of the FN (s) were calculated. A sample above 46% of the germinated grains would not reach the FN value of 200s. Values between 32 to 46% of germinated grains were associated with FN values between 200 and 250s and from 19 to 32% of germinated grains with FN values of 250 to 300s. Samples with less than 19% germinated grains achieved FN values above 300s. This association between the grain germination percentage and the FN allows the wheat breeding programs to have a useful criterion for the selection, early discard of unsuitable lines and planning a new cycle of hybridization for resistance to PHS.

Conflict of interest

The authors declare that they have no conflict of interest

References

- AACC (2000) Approved methods of the American Association of Cereal Chemists, 10th ed, American Association of Cereal Chemists, St Paul, MN, USA.
- Barbedo JGA, Guarienti EM, Tibola CS (2018) Detection of sprout damage in wheat kernels using NIR hyperspectral imaging. *Biosystems Engineering* 175: 124-132.
- Barnard A, Van Deventer CS, Maartens H (2005) Comparison between methods for estimating sprout damage in wheat. *South African Journal of Plant and Soil* 22(1):44-48.
- Bassoi M, Flintham J (2005) Relationship between grain colour and preharvest sprouting-resistance in wheat. *Pesquisa Agropecuária Brasileira* 40:981-988.
- Bassoi MC, Flintham J, Riede CR (2006) Analysis of pre harvest sprouting in three Brazilian wheat populations. *Pesquisa Agropecuária Brasileira* 41:583-590.
- Brasil (2010) Ministério da Agricultura, Pecuária e Abastecimento. Instrução Normativa nº 38, de 30 de novembro de 2010. Regulamento técnico do trigo. Diário Oficial da República Federativa do Brasil, Brasília, DF, n 229, Seção 1. Available at <http://sistemasweb.agricultura.gov.br/sislegis/action/detalhaAto.do?method=visualizarAtoPortalMapa&chave=358389789>, accessed on 14 February 2019.
- Cruz CD (2006a) Programa Genes: análise multivariada e simulação. Cosme Damião Cruz. Viçosa: Ed. UFV, Pp.175.
- Cruz CD (2006b) Programa Genes: estatística experimental e matrizes / Cosme Damião Cruz. Viçosa: Ed. UFV, Pp. 285.
- Dencic S, Depauw R, Kobiljski B, Momcilovic V (2013) Hagberg Falling Number and Rheological Properties of Wheat Cultivars in Wet and Dry Pre harvest Periods. *Plant Production Science* 16(4):342- 351.
- DePauw RM, Knox RE, Singh AK, Fox SL, Humphreys DG, Hucl P (2012) Developing standardized methods for breeding pre-harvest sprouting resistant wheat, challenges and successes in Canadian wheat. *Euphytica* 188:7–14.
- Dewey DR, Lu KH (1959) A correlation and path-coefficient analysis of components of crested wheatgrass seed production. *Agronomy Journal* 51(8):515-518.
- Franco FA, Pinto RB, Scapim CA, Schuster I, Predebon CT, Marchioro VS (2009) Tolerância à germinação na espiga em cultivares de trigo colhido na maturação fisiológica. *Ciência Rural* 39:2396-2401.
- Gavazza MIA, Bassoi MC, Carvalho TC, Bessalho Filho JC, Panobianco M (2012) Methods for assessment of pre-harvest sprouting in wheat cultivars. *Pesquisa Agropecuária Brasileira* 7:928-933
- Hanft JM, Wych RD (1982) Visual indicators of physiological maturity in hard red spring wheat. *Crop Science* 22:584-587.
- Humphreys DG, Noll J (2002) Methods for characterization of preharvest sprouting resistance in a wheat breeding program. *Euphytica* 126: 61–65.
- MacMaster GJ, Derera NF (1976) Methodology and sample preparation when screening for sprouting damage in cereals. *Cereal Research Communications* 4:251-254.
- Mares DJ, Mrva K (2008) Late-maturity alpha-amylase: low Falling Number in wheat in the absence of preharvest sprouting. *Journal of Cereal Science* 47:6–17.
- Mares DJ, Mrva K (2014) Wheat grain preharvest sprouting and late maturity alpha amylase. *Planta* 240(6):1167-1178.
- Nörnberg R, Luche H S, Grolí EL, Danielowski R, Santos RL, Figueiredo RG, Silva JAG, Elias, MC, Maia LC, Oliveira AC (2015a) The search for productivity and pre-harvest sprouting tolerance in wheat. *African Journal of Agricultural Research* 10:1719-1727.
- Nörnberg R, Silva JAG, Luche HS, Tessmann EW, Kavalco SAF, Zimmer CM (2015b) Tolerance to preharvest sprouting and yield of wheat genotypes from different breeding programs. *Pesquisa Agropecuária Brasileira* 50(8):698-706.
- Nyachiro J M, Clarke F R, DePauw RE, Knox RE, Armstrong KC (2002) Temperature effects on seed germination and expression of seed dormancy in wheat. *Euphytica* 126:123-127.
- Okuyama LA, Fonseca Junior NS, Caramori PH, Kohli MM (2018) Preharvest sprouting assessment in wheat genotypes influenced by temperature and degree days. *Experimental Agriculture* 54(4):483-490.
- Paterson AH, Sorrells ME, Obendorf RL (1989) Methods of evaluation for preharvest sprouting resistance in wheat breeding programs. *Canadian Journal of Plant Science* 69:681-689.
- Rakita SM, Torbica AM, Dokić LP, Tomić JM, Pojić MM, Hadnadev MS, Hadnadev TRD (2015) Alpha-amylase activity in wheat flour and breadmaking properties in relation to different climatic conditions. *Food & Feed Research* 42(2):91-100.
- Reddy LV, Metzger RJ, Ching M (1985) Effect of temperature on seed dormancy of wheat. *Crop Science* 25:455-259.

- SAS (Statistical Analysis System) (2001). Version 8.2. SAS Institute Inc., Cary, NC, USA.
- Scheiner SM, Mitchell RJ, Callahan HS (2000) Using path analysis to measure natural selection. *Journal of Evolutionary Biology* 13:423-433.
- Shorter SC, Munro CA, Hodgkinson J (2005) Predicting pre-harvest sprouting susceptibility in New Zealand wheat cultivars. *Euphytica* 143: 309–312
- Silva RR, Zucareli C, Fonseca ICB, Riede CR, Gazola D (2019) Nitrogen management, cultivars and growing environments on wheat grain quality. *Revista Brasileira de Engenharia Agrícola e Ambiental*, 23(11): 826-832.
- Snedecor GW, Cochran WG (1982) *Statistical methods*. 7th edition, Ames: Iowa State University Press, Pp.507.
- Steber CM (2016) Managing the Risk of Low Falling Numbers in Wheat. USDA-ARS, and adjunct faculty, Dept. of Crop and Soil Science, Washington State University Pullman WA, 6p. Available at <https://research.wsulibs.wsu.edu/xmlui/handle/2376/6373> accessed on 14 September 2020.
- Thomason W, Hughes KR, Griffey CA, Parrish DJ, Barbeau WE (2019) Understanding pre-harvest sprouting of wheat. Virginia Tech, Virginia State University, Publication 424-060, available at <https://www.pubs.ext.vt.edu/424/424-060/424-060.html>, accessed on 15 September 2020
- Yanagisawa A, Nishimura T, Amano Y, Torada A, Shibata S (2005) Development of winter wheat with excellent resistance to pre-harvest sprouting and rain damage. *Euphytica* 143:313–318.
- Zeeshan M, Arshad W, Khan MI, Ali S, Nawaz A, Batool A, Tariq M, Akram MI, Ali MA (2018) Breeding for pre-harvest sprouting resistance in bread wheat under rainfed conditions. *Frontiers of Agricultural Science and Engineering* 5(2): 253–261.