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Pre-Harvest Sprouting Tolerance in 36 Bread Wheat Genotypes

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PHS selection

Pericarp rupture

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ABSTRACT

Pre-harvest sprouting (PHS), promoted by rainfall during crop maturity, is a high problem in many wheat-producing regions of the world. Considering its importance in Brazil, 36 national and international varieties and advanced lines of wheat were evaluated for their tolerance to PHS. For this purpose, two experiments were conducted over three years. Seed pericarp rupture was used as an indicator of the beginning of germination. The data were analyzed using analysis of variance, the Scott-Knott test, and the Lin and Binns method. The wide range of germination percentage values allowed the genotypes to be classified as tolerant (in experiment 1 - ND 674 and Grandin*2/RL 4137 and experiment 2 - Frontana and Grandin) and moderately tolerant (Alsen, CD 114, and Milan/3/Attila//Fang 69/CIMMYT 3 in Experiment 1; Avante, BRS 177, IAC 5-Maringá, Onix, OR 1, RL 4137, and Rubi in Experiment 2). Because tolerance to PHS is under genetic control and can be improved through breeding programs, the challenge for wheat breeders is to combine increased PHS tolerance with other requirements to meet market demands.

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1 Introduction

Rainfall at harvest time is one of the main factors affecting grain yield and end-use quality of wheat in many regions of the world. Pre-harvest sprouting (PHS) is a polygenic trait governed by genetic and environmental factors (Singh et al. 2021). In a genome-wide association study conducted on 298 bread wheat's, Rabieyan et al. (2022) found that the RRBLUP model is a useful tool for genomic selection.

Screening for PHS tolerance, facilitated by marker-assisted selection, is not a feasible practice in most wheat-breeding programs worldwide. Such screening must be based on easily visible grain characteristics stimulated by PHS, such as grain discoloration, swelling, wrinkling, splitting, and rootlet emergence, as listed by Thomason et al. (2019).

The falling number (FN) test has been considered a superior method for selecting PHS-tolerant genotypes in several studies (Barnard et al. 2005; Nörnberg et al. 2015a; Guarienti et al. 2017; Delwiche et al. 2018). However, the cost of equipment, the requirement of trained personnel, and a great number of samples in PHS selection limit its use as a screening tool. Alternative indirect methods, such as visual scoring (MacMaster and Derera 1976; Franco et al. 2009; Humphreys and Noll 2002) and/or calculation of the percentage of sprouted grains (Bassoi et al. 2006; Zeeshan et al. 2018), have been used in many breeding programs to evaluate PHS tolerance. Recently, Okuyama et al. (2020) found a high negative association between sprouting percentage and FN ($r = -0.9082^{**}$) and between visual germination score and FN ($r = -0.8956^{**}$); they concluded that in the absence of the FN test, calculating the germination percentage and/or grain germination score provided a valid option for PHS selection.

Studies have identified several PHS-tolerant genotypes, including Frontana, RL 4137, and BRS 177 (Andreoli et al. 2006); Frontana, Fundacep Cristalino, Fundacep Raízes, BRS Guamirim, TBIO Mestre, and TBIO Alvorada (Nörnberg et al. 2015b); Frontana, CD 1440, Quartzo, Jadeíte 11, LG Prisma, LG Oro, ORS Vintecinco, TBIO Iguaçu, TBIO Sinuelo, and TBIO Pioneiro (Guarienti et al. 2017); Frontana, CD 1440, and ORS Vintecinco (Rigatti et al. 2019); hard white winter wheat 'KS Venada' (Zhang et al. 2020); and the red-grained cultivar 'AAC Tenacious' (Dhariwal et al. 2021).

Given that the level of PHS tolerance of genotypes is not known in detail to plan the best combinations in a breeding program, the present study was undertaken to classify 36 progenitors to be used in crosses to increase tolerance to PHS.

2 Materials and Methods

Two experiments were conducted over two sowing dates in three years at the Paraná Rural Development Institute, IAPAR-EMATER, Londrina, Brazil. The plot size was 3 rows, 30 cm apart and 2 m long, with a sowing rate of 350 seeds m^{-2}. The following genotypes were evaluated in Experiment 1: Alsen, BRS 208, BRS 220, BRS 210, BRS Guabijú, CD 104, CD 108, CD 114, Chirya 7, Grandin*2/RL 4137, IPR 144, IPR 85, Milan/3/Attila//Fang 69/CIMMYT 3, ND 674, BRS Pardela, and SW 89-5124*2/Fasan. Experiment 2 consisted of the following genotypes: Alcover, Avante, BR 18-Terena, BRS 177, CD 105, CD 116, CEP 24, Frontana, Grandin, IAC 5-Maringá, IAPAR 17-Caeté, Ônix, OR 1, RL 4137, Rubi, and Supera.

At maturity, approximately 100 spikes per genotype were harvested by hand (Hanft and Wych 1982) and kept at room temperature of 20-25ºC. Styrofoam blocks, with pre-drilled holes of 5 cm in the line and 10 cm between the lines, were used to insert 20 spikes per genotype, with two replications. The spikes were kept under artificial rainfall (nebulizer) according to the procedure of Okuyama et al. (2018). The nebulizer was turned on/off in half-hour cycles to produce approximately 280 mm mist per day. After 48 h of nebulization, the spikes were dried in the sun and hand-threshed.

The percentage of grain germination (sprouting) was double-checked each time in a 50 grains sample. A magnifying glass (10×) was utilized to observe pericarp rupture, which was regarded as the beginning of the germination process (Nyachiro et al. 2002; Bassoi and Flintham 2005). The germination percentage data were transformed to arcsine values before analysis according to Snedecor and Cochran (1982). The analysis of variance (ANOVA), Scott-Knott test ($P \leq 0.05$), and the Lin and Binns (1988) methods were performed using Genes (Cruz 2006a; Cruz 2006b) and SAS software was used for statistical analysis (SAS 2001).

3 Results and Discussion

Artificial rainfall (nebulizer) was highly effective in obtaining genotypes with a wide range of germination percentages. Analysis using the Scott-Knott test and Lin and Binns method permitted clear categorization of the genotypes in both experiments. In Experiment 1, combined ANOVA for germination percentage showed that the effects of genotype and year were highly significant ($P \leq 0.01$), the effect of the block (year) was significant at $P \leq 0.05$, and the effect of the interaction genotype × year was not significant ($P > 0.05$) (Table 1). In Experiment 2, the effects of year, genotype, block (year), and the interaction year × genotype were all significant ($P \leq 0.01$) (Table 1).

The range of germinated grains in Experiment 1 varied from 13.83 to 78.83%, whereas it ranged from 8.83 to 85.33% in Experiment 2. Such a large variation in the evaluation of this characteristic allowed the classification of genotypes according to their degree of tolerance to PHS. In the absence of the availability of the FN test in many wheat-breeding programs, a viable option is to use a

Table 1 Variance analysis of the grain germination percentage in wheat genotypes

Source		Mean square Germination (%)	
	DF	Experiment 1	Experiment 2
Year	2	2.6473 **	0.5355 **
Block (year)	3	0.0879 *	0.0985 **
Genotype	15	0.4566 **	0.4414 **
Year x Genotype	30	0.0462 ns	0.0627 **
Error	45	0.0294	0.0189
R-Square		0.9125	0.9206
Coefficient of variation		19.7345	19.0772
Root MSE		0.1715	0.1375
Mean		56.67	44.43

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

Table 2 Percentage of germinated grains in genotypes exposed to artificial rain (nebulizer) in Experiment 1

Genotypes	Germinated grains (%)			
	Year 1	Year 2	Year 3	Mean
Alsen	11.50[b]	68.50[a]	16.50[c]	32.17[b]
BRS 208	56.00[a]	90.50[a]	45.00[b]	63.83[a]
BRS 210	47.50[a]	90.00[a]	80.00[a]	72.50[a]
BRS 220	35.00[a]	96.00[a]	91.00[a]	74.00[a]
BRS Guabijú	53.50[a]	95.50[a]	72.00[a]	73.67[a]
CD 104	41.50[a]	89.50[a]	84.00[a]	71.67[a]
CD 108	35.50[a]	96.50[a]	91.50[a]	74.50[a]
CD 114	7.50[b]	66.00[a]	13.00[c]	28.83[b]
Chirya 7	55.00[a]	97.00[a]	50.50[b]	67.50[a]
Grandin*2/RL 4137	14.50[b]	30.00[b]	8.50[c]	17.67[c]
IPR 144	37.50[a]	93.50[a]	78.00[a]	69.67[a]
IPR 85	12.00[b]	85.50[a]	73.00[a]	56.83[a]
Milan/3/Attilla//Fang 69/CIMMYT 3	22.00[b]	69.50[a]	29.00[c]	40.17[b]
ND 674	3.00[b]	23.00[b]	15.50[c]	13.83[c]
BRS Pardela	58.00[a]	95.00[a]	60.00[a]	71.00[a]
SW89-5124*2/Fasan	56.50[a]	98.00[a]	82.00[a]	78.83[a]
Mean	34.16	80.25	55.59	56.67

*Means followed vertically by the same letters represent statistical homogeneity by the Scott-Knott test (P ≤ 0.05).

germination test (Franco et al. 2009; Gavazza et al. 2012) or sprouting scores to exclude the most susceptible lines (MacMaster and Derera 1976; Humphreys and Noll 2002; Franco et al. 2009). The effectiveness of the percentage of germinated grains was recently confirmed by Okuyama et al. (2020), who reported its high negative correlation with FN (r = −0.91).

Considering the lack of significance in the year × genotype interaction (P > 0.05) for germination percentage in Experiment 1, it is fair to assume that all genotypes showed stable behavior over the years. The mean germination percentage of the genotypes was compared by the Scott-Knott test (P ≤ 0.05). The genotypes ND 674 and Grandin*2/RL4137 showed the lowest percentage of grain

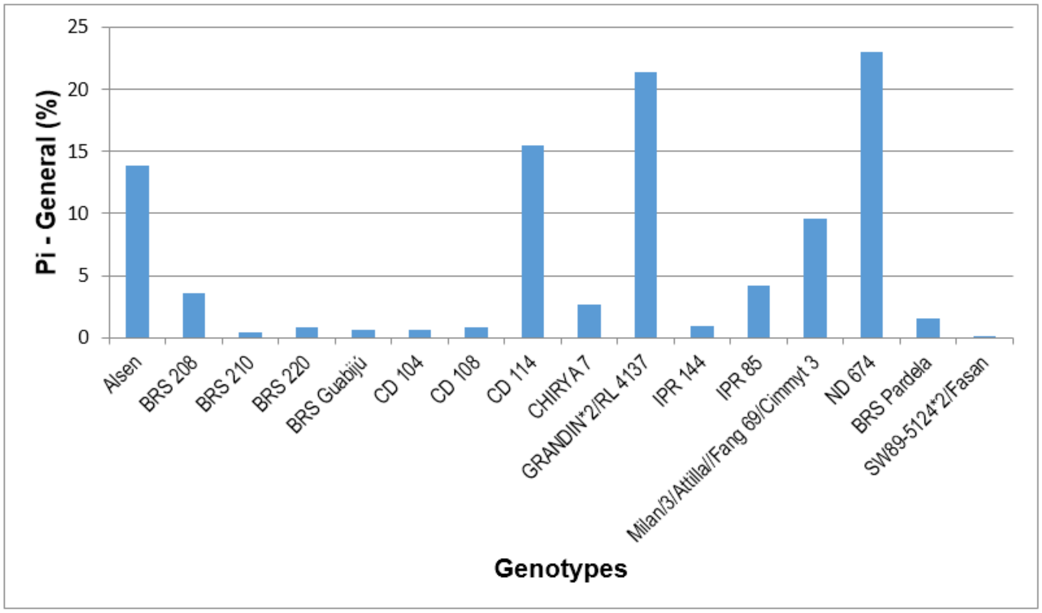

Figure 1 Pi values (estimate of adaptability and stability) of genotypes tested in Experiment 1. The Lowest Pi values correspond to genotypes with the highest percentage of germinated grains (Lin and Binns 1988).

germination and thus can be considered the most tolerant to PHS. The genotypes CD 114, Alsen, and Milan/3/Attila//Fang 69/ CIMMYT 3 were classified as moderately tolerant, whereas IPR 85, BRS 208, Chirya 7, IPR 144, BRS Pardela, CD 104, BRS 220, BRS Guabiju, BRS 220, CD 108, and SW89-5124*2/Fasan were classified as susceptible (Table 2).

The adaptability and stability parameter (Pi) of cultivars was also estimated in Experiment 1 using the Lin and Binns (1988) model, where lower values of Pi represent better model performance. In other words, the genotype with the highest percentage of sprouted grains was the most susceptible to PHS. In addition, the genotypes ND 674 and Grandin*2/RL 4137 exhibited the highest tolerance to PHS, as indicated by the lower percentage of germinated grains. In another group, the genotypes CD 114, Alsen, and Milan/3/Attila//Fang69/CIMMYT3were considered moderately tolerant, and IPR 85, BRS 208, and Chirya 7 were moderately susceptible. The genotypes with the lowest Pi values were IPR 144, BRS Pardela, CD 104, BRS 210, BRS Guabijú, BRS 220, CD 108, and SW 89-5124*2/Fasan; these genotypes resulted in the highest values of sprouted grains and were, therefore, most susceptible to PHS (Figure 1).

In Experiment 2, three years of evaluation by the Scott-Knott test ($P \leq 0.05$) showed that Frontana and Grandin were the most tolerant to PHS, whereas BRS 177, Rubi, and IAC 5-Maringá were moderately tolerant. The cultivars OR 1, Onix, Avante, CD 105, CEP 24, CD 116, and IAPAR 17-Caeté were moderately susceptible, and Supera, Alcover, and BR 18-Terena were classified as susceptible (Table 3).

The Lin and Binns (1988) model in Experiment 2 showed that the Frontana and Grandin genotypes had the highest Pi values, thereby representing the highest tolerance to PHS. In this group, genotypes RL 4137, BRS 177, Rubi, IAC 5-Maringá, OR 1, Onix, and Avantewere considered moderately tolerant. Other genotypes, such as CD 105, CEP 24, CD 116, and IAPAR 17-Caeté, were classified as moderately susceptible, whereas Supera, Alcover, and BR 18-Terena, with the lowest Pi values, were susceptible to PHS (Figure 2).

Considering both experiments together, the most tolerant genotypes to PHS were ND 674 and Grandin*2/RL 4137 (Experiment 1), with 13.8 and 17.7% of germinated grains, and Grandin and Frontana (Experiment 2), with 8.8 and 19.8% of germinated grains, respectively. Comparing the PHS data from these experiments with an earlier study by Okuyama et al. (2020), it can be surmised that all genotypes with seed germination above 46% would have FN values of less than 200s, between 32 and 46% would associate with FN values in the range of 200 to 250s, and between 19 and 32% would have FN values in the range of 250 to 300 s. The most tolerant genotypes, representing seed germination below 19%, would all be associated with high FN values over 300s. Based on this comparison, we can infer that even after 48 h under artificial rain conditions, the genotypes ND 674, Grandin*2/RL 4137, and Grandin had FN values higher than 300 s and Frontanahad FN values between 250 and 300s.

The genealogy and characteristics of PHS-tolerant genotypes (ND 674 and Grandin*2/RL 4137) and moderately tolerant genotypes (CD 114, Alsen, and Milan/3/Attila//Fang 69/CIMMYT 3) in

Table 3 Percentage of germinated grains of genotypes exposed to artificial rain (nebulizer) in Experiment 2

Genotypes	Germinated grains (%)			
	Year 1	Year 2	Year 3	Mean
Alcover	83.00Aa*	95.00Aa	58.50Ba	78.83
Avante	38.50Ac	49.50Ab	36.00Ab	41.33
BR 18-Terena	87.00Aa	94.50Aa	74.50Aa	85.33
BRS 177	5.00Bc	18.50Bc	53.50Aa	25.67
CD 105	18.50Cc	83.00Aa	54.50Ba	52.00
CD 116	73.00Aa	83.00Aa	33.50Bb	63.17
CE P24	47.50Ab	54.50Ab	66.50Aa	56.17
Frontana	29.00Ac	23.50Ac	7.00Ac	19.83
Grandin	3.00Ac	20.50Ac	3.00Ac	8.83
IAC 5-Maringá	14.50Ac	30.50Ac	43.50Aa	29.50
IAPAR 17-Caeté	33.50Cc	93.00Aa	68.50Ba	65.00
Ônix	19.50Ac	42.00Ab	34.50Ab	32.00
OR 1	12.00Bc	58.50Ab	25.00Bb	31.83
RL 4137	9.50Bc	42.00Ab	20.50Bb	24.00
Rubi	25.50Ac	27.50Ac	25.00Ab	26.00
Supera	68.00Aa	82.00Aa	64.00Aa	71.33
Mean	35.44	56.09	41.75	44.43

*Means followed by the same uppercase letters horizontally or lowercase letters vertically indicate a statistically homogeneous group by the Scott-Knott test ($P \leq 0.05$).

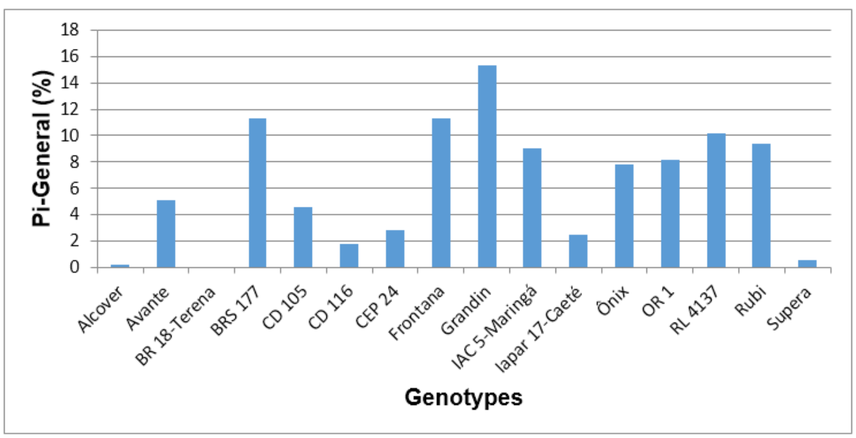

Figure 2 Pi values (estimate of adaptability and genotype stability) of genotypes tested in Experiment 2. The Lowest Pi values were those with the highest percentage of germinated grains (Lin and Binns 1988).

Experiment 1 are presented in Table 4. RL 4137, which is considered a source of PHS resistance (DePauw et al. 2009; Rasul et al. 2012; Liton et al. 2021), probably derives its resistance from multiple sources in its pedigree, including Frontana, Thatcher, Kenya farmer, and RL 2265 (Exchange/McMurachy).

The genotypes Frontana and Grandin were also classified as tolerant to PHS in Experiment 2, whereas RL 4137, Avante, BRS 177, IAC 5-Maringá, Ônix, and Rubi were classified as moderately tolerant. The genealogies and characteristics of the genotypes are presented in Table 5.

Table 4 Genealogy and characteristics of PHS superior genotypes in Experiment 1

Genotype	PHS in Experiment 1	Genealogy	Characteristics
ND 674	Tolerant	Grandin (PI 531005)*2/Glupro (Frohberg et al. 2006)	High and stable bread quality characteristics (Andrade et al. 2001; Singh et al. 2006; Mergoum et al. 2008)
Grandin*2/RL 4137	Tolerant	Grandin*2/RL 4137 (Frontana//RL2265/2*Redman/3/Thatcher *6/ Kenya Farmer) (Martynov and Dobrotvorskaya 2016)	The parent line RL 4137, in the cross, is considered a source of PHS resistance (DePauw et al. 2009; Rasul et al. 2012; Liton et al. 2021)
CD 114	Moderately tolerant	PF 89232 / OC 938 (Souza and Caierão 2014)	Classified as moderately susceptible to PHS (Reunião 2007)
Alsen	Moderately tolerant	ND674//ND2710 (PI 633976)/ND688 (Frohberg et al. 2006).	Resistance to *Fusarium* Head Blight and leaf rust caused by *Puccinia triticina* (Oelke and Kolmer 2005)
Milan/3/Attila//Fang 69/CIMMYT 3	Moderately tolerant	CIMMYT advanced line	Resistance to several isolates of Pyriculariaoryzae (Marangoni et al. 2013)

Table 5 Genealogy and characteristics of PHS superior genotypes in Experiment 2

Genotype	PHS in Experiment 2	Genealogy	Characteristics
Frontana	Tolerant	Fronteira/Mentana (Souza and Caierão 2014)	Tolerant to PHS (Andreoli et al. 2006; Czarnecki 1986; Souza 2001; Nörnberg et al. 2015a), source of resistance against leaf and stripe rust (Chaves et al. 2020) and Fusarium Head Blight (Zhu et al. 2019)
Grandin	Tolerant	Len/Butte*2/ND507/3/ND593 (Dagou and Richard 2016)	High grinding requirements and cooking quality (Mergoum et al. 2006), adult plant resistance to leaf rust disease based on Lr13 and Lr34 genes (Liu and Kolmer 1997) as well as used in multiple crosses (Mergoum et al. 2005; Dagou and Richard 2016; Zhao et al. 2018; Thambugala et al. 2021)
RL 4137	Moderately tolerant	Frontana//RL2265/2*Redman/3/Thatcher *6/ Kenya Farmer (Martynov and Dobrotvorskaya 2016)	Consistently high levels of PHS resistance across years and environments (DePauw et al. 2009; Martynov and Dobrotvorskaya 2016)
Avante	Moderately tolerant	PF89232/2*OR1 (Souza and Caierão 2014)	Moderately resistant to PHS and a moderate level of resistance to foliar blights (Reunião 2007)
BRS 177	Moderately tolerant	PF83899/PF813//F27141 (Caierão et al. 2014)	Superior performance to PHS (Andreoli et al. 2006), moderate level of resistance to foliar leaf blights, and soil-borne mosaic virus (Reunião 2007)
IAC 5-Maringá	Moderately tolerant	Frontana/Kenya 58//Ponta Grossa 1 (Souza and Caierão 2014)	Adapted to the warmer regions of Brazil (Souza and Caierão 2014)
Ônix	Moderately tolerant	CEP 24/Rubi "S" (Souza and Caierão 2014)	Classified as resistant/moderately tolerant for PHS (Reunião 2007). Widely adopted in Brazil and Argentina (Souza and Caierão 2014)
Rubi	Moderately tolerant	Embrapa 27/Klein H3450 C3131 (Souza and Caierão 2014)	Moderate level of tolerance to PHS (Reunião 2007) and source of resistance against Soil Borne Mosaic Virus in Brazil

Some genotypes such as ‘AC Majestic', 'AC Domain', and 'Red RL 4137' (Rasul et al. 2012), have the potential to be used as a parent to incorporate PHS resistance in a breeding program. However, in the absence of locally adapted or high-yielding parents with tolerance to PHS, another strategy suggested by Andreoli et al. (2006) emphasizes on the pre-breeding effort to transfer major genes from Frontana or obtained lines, such as BRS 177 or RL 4137, into modern breeding lines. Our results confirm the findings of these authors that the genotypes Frontana, RL 4137, and BRS 177 are good options for combining PHS resistance with locally or regionally adapted modern breeding lines.

Additional studies (data not shown) have demonstrated an increased level of PHS tolerance in newer lines derived from ND 674, Grandin*2/RL 4137, Grandin, and Frontana. Beyond PHS, Nörnberg et al. (2015b) reported that TBIO Mestre and TBIO Alvorada combination with Fundacep Cristalino led to the identification of many superior genotypes exhibiting PHS tolerance and high grain yield.

According to Singh et al. (2021), back-cross breeding can be effectively applied for the introgression of identified major quantitative trait loci (QTLs) for PHS tolerance. The importance of identifying QTLs with the potential to enhance PHS resistance in spring wheat has been highlighted by Liton et al. (2021). In their study, a combination of RL 4137 carrying three QTLs on chromosomes 4A, 6B, and 6D and 'Roblin' carrying a new QTL on 1D increased resistance to PHS in the Roblin/RL 4137 population.

Despite advances in the molecular marker-assisted selection, many breeding programs may not be able to use these tools to develop PHS-tolerant genotypes. On the other hand, the identification and transfer of PHS-related traits from genotypes such as Frontana, Grandin, ND 674, and RL 4137 into locally adapted and high-yielding germplasms are successful. Therefore, we believe that the utilization of tolerant and moderately tolerant germplasms identified from this study and previous studies is key to global wheat-breeding programs affected by PHS. Besides improving sprouting tolerance, these progenitors will help enhance end-use quality, disease resistance, and other traits required by the market.

Conclusions

This study confirms that grain germination percentage under controlled spike wetting allows the classification of genotypes for PHS tolerance. Genotypes such as Frontana, Grandin, ND 674, and Grandin*2/RL 4137 were classified as tolerant to PHS, while Alsen, Avante, BRS 177, CD 114, IAC 5-Maringá, OR 1, Onix, RL 4137, Rubi, and Milan/3/Attila//Fang 69/ CIMMYT 3 were classified as moderately tolerant. Tolerance to PHS can be further enhanced by combining tolerant and moderately tolerant genotypes and/or incorporating the tolerance characteristic of these genotypes into other agronomically desirable germplasms. PHS tolerance should be further combined with high grain yield, disease resistance, good end-use quality, and other traits of interest in a region.

Conflict of interest

The authors declare that there is no conflict of interest.

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