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Pre-harvest Sprouting Tolerance of Triticale Genotypes in Brazil

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Abstract

Pre-harvest sprouting (PHS) represents one of the main factors, which causes yield, technological and physiological losses in triticale seeds (X *Triticosecale* Wittmack). This work aimed to rate the variability and identify potential pre-harvest sprouting tolerant sources in triticale genotypes. Based on that, 32 triticale and three wheat genotypes were sown in 2016, 2017 and 2018 growing seasons, in Londrina-PR, Brazil. After the ears harvesting, these were submitted to simulate raining, for sprouting induction, through nebulization in a greenhouse. After nebulization, ears were sun dried, later hand threshed to determain, grain germination percentage (GERM) and hectoliter weight (HW). Additionally, it was determined grains HW of ears, whom were not submitted to nebulization, as well as the whole meal flour falling number (FN). The experiment design was completely randomized design, with two replications, and the experimental unit was made of 20 ears. The data collected were subjected to analysis of variance (ANOVA) and Scott-Knott test. Frontana, ND 674 and Quartzo are a source of tolerance to PHS in wheat. In triticale genotypes, genetic variability was observed for GERM, FN, and HW prior and after nebulization. The triticale genotypes BRS Netuno, BRS Saturno, TCL 15116, X 092181, Tiguera 1 and Tiguera 8, where tolerant towards PHS.

Keywords: falling number, genotype × environment interaction, hectoliter weight, X *Triticosecale* Wittmack

1. Introduction

The triticale (X *Triticosecale* Wittmack), originated from the hybridization of wheat (*Triticum*) with rye (*Secale*), aiming to unite favorable traits of both these species. However, triticale cultivars still have deficiencies in some agronomic traits, like susceptibility to certain diseases (fusarium head blight, blast, leaf spots and viruses) (Mergoum et al., 2019).

In 2018, the harvest areas and production of triticale wordwide were 3,809,192 hectares and 12,802,592 tons, respectively (FAO, 2020). Poland, Germany, France, Belarus, China, and Spain are the leading triticale producing countries (FAO, 2020). Triticale can be used for several purposes. Triticale is used in animal feed, it is used especially for direct feeding as green forage or as silage of young or mature plants, moist or dry grains, and hay (Nascimento Junior et al., 2004). In human feeding the triticale grains, its flour has been used to compose mixtures with wheat flour for the manufacture of low fermentation products, such as cookies, pizza dough, meatballs, waffles, and cakes (Watanabe, 2016). Triticale has also aroused interest for biofuel production, and it has presented competitiveness when compared with other winter cereals (McGoverin et al., 2011).

Pre-harvest sprouting (PHS), is a premature grain sprouting in the ear, before the harvested. This problem has been observed over many years in several triticale and wheat growing areas, all over the world. This represents a limiting technological quality factor, to the processing industry and seed production physiological quality (Moś & Wójtowicz, 2004; Chapman, 2011). In general, dormant grain happens under cool growing conditions, whereas high temperatures during the later stages of grain growth, break embryo dormancy allowing grains to germinate in the ear, granted rain occurs around harvest time.

Pre-harvest sprouting occurs when the seed interacts with the water, thus the imbibition starts, triggering a slew of physiological processes, like the release of hormones and hydrolytic enzymes. Considered a simplified process, the gibberelic acid hormonal activity on the imbibed seed induce amylases synthesis; hydrolyzed carbohydrates reserves, induce the development of the embryo (Bassoi, 2004).

Many methods where described in scientific literature, assesing pre-harvest sprouting resistance (Nedel et al., 1983a; Humphreys & Noll, 2002; Gavazza et al., 2012). These methods use the visual assessment of seeds and the falling number, to quantify seed germination percentage and correlate it with seed dormancy.

The PHS genetic variability in triticale is lower in relation to wheat. However, the genetic variability available for triticale, is enough to obtain new cultivars through genetic improvement (Nedel et al., 1983b; Haesaert & De Baets, 1996; Oettler, 2005; Alaru et al., 2008; Bizova et al., 2011).

The identification of tolerant triticale genotypes, towards PHS, will grant its utilization as parents on breeding programs, thus, contributing to new cultivar development. This work aimed to rate the variability and identify potential pre-harvest sprouting tolerant sources in triticale genotypes.

2. Method

2.1 Material Studied

The experimental material was composed of 32 triticale genotypes, developed throught triticale genetic improvement programs, by different agencies in Brazil (IAPAR and EMBRAPA) and international institution (CIMMYT). The line ND 674 and the cultivars Quartzo and Frontana were used as controls towards tolerance to pre-harvest sprouting. Frontana cultivar is one of the best wheat genotypes in terms of resistance to pre-harvest germination (Bassoi et al., 2006; Franco et al., 2009; Nörnberg et al., 2015). Quartzo cultivar was classified as moderately tolerant to tolerant to PHS (Nörnberg et al., 2015), and the PHS tolerance of the wheat line ND 674 was reported by Dr. L. Okuyama (personal communication).

2.2 Site, Weather and Agronomic Management and Data Colletion

The experiments were carried out in the 2016, 2017 and 2018 growing seasons, in Londrina, state of Paraná, Brazil (latitude 23°22′S; longitude 51°10′W; 585 m altitude). Sowing was carried out in three dates, with intervals of at least one week. Intercalated sowing was used to allow ear harvesting with physiological ripening at the same date. Each field plot was made of 3 lines, 2 m of length each, spaced 0.3 m. Minimum and maximum temperature (°C), and rainfall (mm) data, were registered and are shown in Figure 1.

Ears were harvested at physiological ripening stage, which is, when they lose their green coloring, but stem nodes are still green. After harvesting, the ears were stored in a protected environment, until grain drying finished (approximately 13% moisture). After, a fraction of ears were submitted to sprouting induction; the rest were threshed to quantify its hectoliter weight (HW), and after, whole meal flour falling number (FN).

The ears were taken to a greenhouse for nebulization. This method allowed an artificial rain simulation, similar to what occurs in the field. Ears were exposed to nebulization for 42 hours, 68 hours and 42 hours in 2016, 2017 and 2018 growing seasons, respectively. This method has been utilized successfully in the main wheat breeding programs in Brazil, for pre-harvest sprouting resistant genotypes selection (Franco et al., 2009; Okuyama et al., 2017). The experiment design was completely randomized, with two replications, being the experimental unit made of 20 ears.

Grain percentage sprouting (GERM), was determined from ears submitted to nebulization. After nebulization, ears were sun dried and later, hand threshed. GERM was determined based on assessment of two random 50 grain samples on each plot. Germinated grain are those with visible vegetative structure or even visible pericarp breaking.

The FN (also known as Hagberg Number) was determined in samples (7 g) of ground wheat, which were mixed with 25 mL of distilled water in a FN tube with a stirrer and shaken to form slurry. This slurry was heated in a boiling water bath at 100 °C and stirred constantly. The time it took the stirrer to drop through the paste was recorded as the FN value in a Perten FN 1500 (Perten Instruments), according to the 56-81 B method of the American Association of Cereal Chemists (American Association of Cereal Chemists [AACC], 2000).

Hectoliter weight was determined from two sources, from grains which were not submitted to nebulization (HW_s) and from grains originated from ears following nebulization (HW_n).

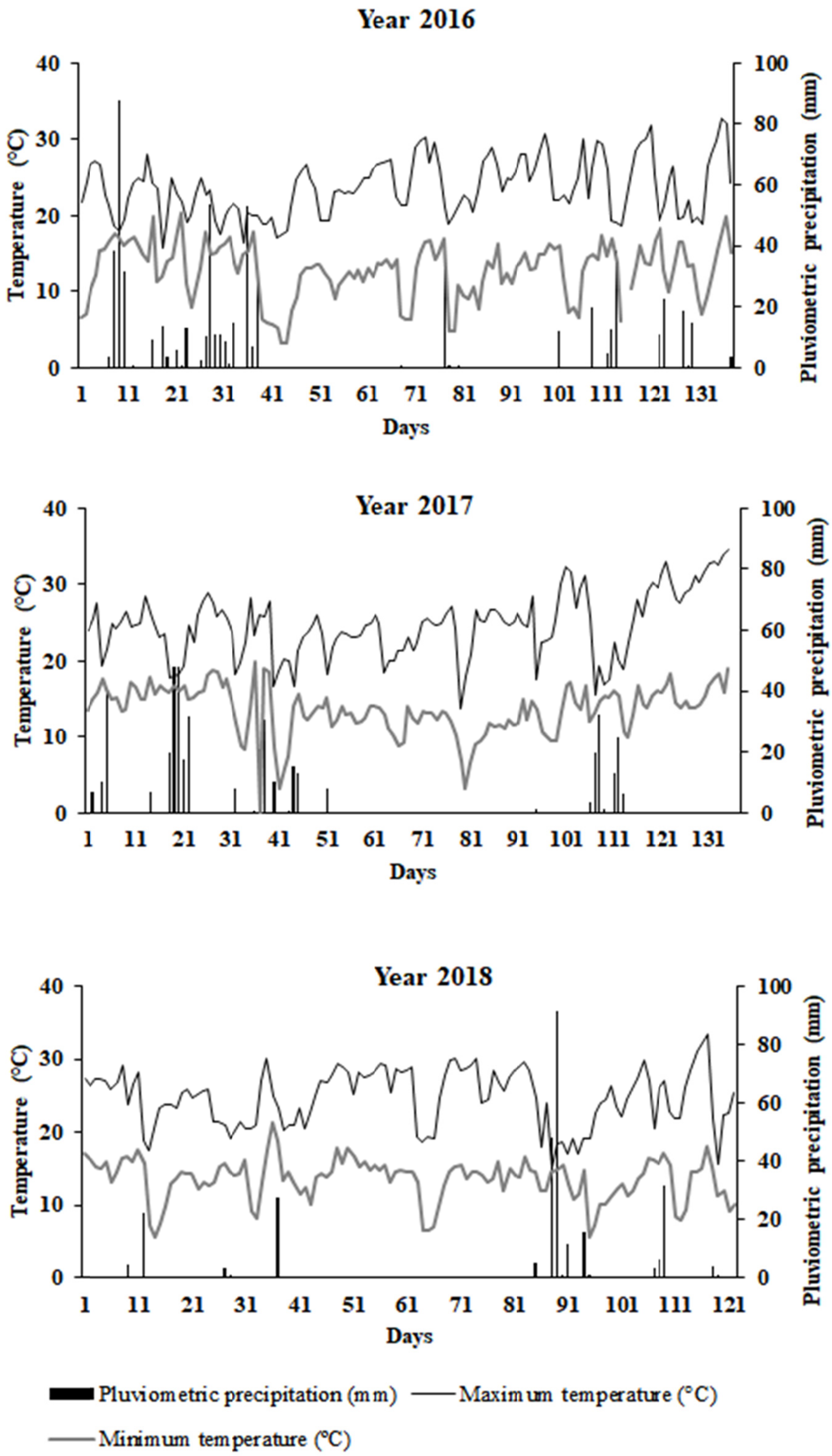

Figure 1. Rainfall (mm), maximum and minimum temperatures (°C) data during the field experiment course during 2016, 2017 and 2018 (years) growing seasons. Data acquired from the IAPAR Weather Station

2.3 Statistical Analysis

The gathered data from sprouting percentage (GERM), falling number (FN), hectoliter weight without nebulization (HW_s) and with nebulization (HW_n) were submitted to individual analysis of variance. Then, residuals variance homogeneity was verified through F maximum test. Once residuals variance homogeneity was verified, joint variance analysis was perfomed, considering genotypes and years effects as fixed. Scott Knott test at 5% probability was carried out for all traits.

Pearson correlation coefficients were estimated to observe the magnitude and direction between trait pairs. Correlations significance was done through t test with two freedom degrees. Statistical analyses were done with the Genes software aid (Cruz, 2016).

3. Results and Discussion

Table 1 demonstrates the variance analyses results for every assessed trait. Significant effect for genotype ($p < 0.05$) was observed for all assessed traits. This result indicates the existence if the differences between the evaluated genotypes for hectoliter weight prior and following nebulization, falling number and ear with germinated grains. Considering unfolding within the genotype effect, triticale's significant effect ($p < 0.05$) was observed, indicating performance differences among the genotypes, which allow a selection of superior genotypes. The control effect was not significant ($p > 0.05$), for ear sprouting percentage (GERM), an anticipated fact, as Frontana, Quartzo and ND 674 wheat genotypes, utilized as controls, are rated as tolerants to pre-harvest sprouting. The group significant effect ($p < 0.05$) reveals that there is a difference among triticale vs wheat groups towards the assessed traits.

Table 1. Joint variance analysis for hectoliter weight prior (HW_s) and following nebulization (HW_n), falling number (FN) and ear with germinated grains (GERM) assessed on the triticales and wheats genotypes. Londrina, State of Paraná, Brazil. 2016, 2017 and 2018 growing seasons

Sources of variance	Means Square				
	DF	HW_s	HW_n	FN	GERM
Genotype (G)	34	17.42**	30.25**	35.29**	1.320**
Triticale	31	11.21**	18.65**	14.68**	1.296**
Control	2	1.82**	3.84**	7.276**	5.39^{ns}
Group	1	241.24**	442.55**	730.22**	4.702**
Year	2	17.23**	597.70**	198.31**	6.883**
Genotype × Year	68	7.21**	7.61**	5.10**	323.5**
Triticale × Year	62	6.64**	6.67**	3 821**	339.3**
Control × Year	4	5.52**	19.54**	4 215**	10.5^{ns}
Group × Year	2	28.25**	13.06**	46.65**	460.07**
Residual	102	0.05	0.50	18.42	19.82
General average		77.7	70.6	143.5	18.4
Triticales' Average		77.4	70.1	125.4	19.9
Controls' Average		81.2	75.3	336.1	2.9
CV (%)		0.29	1.00	2.99	24.2

Note. HW_s = hectoliter weight prior nebulization (kg hl^{-1}); HW_n = hectoliter weight following nebulization (kg hl^{-1}); FN = falling number (seconds); GERM = sprouting seed (%). ^{ns} not significant by the F test; ** Significant ($p < 0.01$) by the F test; * Significant ($p < 0.05$) by the F test.

A significant effect between growing seasons ($p < 0.01$) for all traits, was detected, indicating that the growing conditions may influence genotypes response. All trials were conducted in the same location, but in different agricultural years. Environmental conditions varied from year to year, this fact can be seen in Figure 1. A significant effect ($p < 0.01$) for the double interactions Genotype × Year, Control × Year and Group × Year was also detected, suggesting differentiated genotype behavior in the different growing seasons (Table 1). Only the interaction Control × Year for the trait GERM was not significant, since all controls utilized in this study present pre-harvest sprouting tolerance and presented behavior stability on the three years assessed.

Environmental variation coefficients estimates (CV) varied on 0.3% (hectoliter weight prior nebulization) to 24.8% (pre-harvest sprouting percentage), which represents good experimental precision for this study according to Gomes (2000).

Among the triticale genotypes, a hectoliter weight variability (HW) was observed. Seed HW prior to nebulization varied from 72 to 81 kg hl^{-1} (Table 2). HW express indirectly towards grain quality attributes.

Therefore, lines and cultivars identification with high values is desirable. Genotypes BRS Netuno, BRS Saturno, IPR Aimoré, PFT 0609, TCL 15014, TCL 15052, TCL 15102 and TCL 0717 presented HW superior to 78 kg hl^{-1}, in the three assessed years.

The occurrence of harvest raining affects HW negatively, since all genotypes presented HW reduction when submitted to nebulization (Table 2). Due the simulated raining, an average reduction of 9.4% in the HW (Table 2) was verified. A HW magnitude of reduction due to simulated raining, was distinct among triticale and wheat genotypes (Table 2). Triticale cultivars BRS Saturno, BRS 203 and lines TCL 15052 and TCL 15022 presented the lowest HW reductions, following nebulization. On the other side, genotypes TCL 15102, TCL 11014, ITW 11014 and IPR Aimoré were the most effected, since they presented the greatest HW reductions.

Wheat genotypes are set at the greatest tolerance class towards sprouting in all the assessed years (Table 3). Wheat cultivar Frontana, known as a pre-harvest sprouting tolerant source, is much utilized as a parent on breeding programs in Brazil. On the study done by Franco et al. (2009), they reported that dormancy seems to be the most evident mechanism, since the greatest number of dormant seeds detected trough tetrazolium test were associated to the lowest sprouting rates.

Pre-harvest sprouting tolerant cultivars breeding is one of the main challenges to the triticale breeding in the country. The comparative study among the triticales highlighted six promising genotypes: BRS Netuno and BRS Saturno cultivars, TCL 15116 and X 092181 lines and the accesses Tiguera 1 and Tiguera 8. These genotypes had been inserted at the same pre-harvest sprouting control tolerant groups (Frontana, ND 674 e Quartzo) and have presented behavior stability, showing low sprouted grains value on the three years assessed (Table 3). Tolerant genotypes identification permit its use as parents in breeding programs aiming to get future cultivars with great pre-harvest sprouting tolerance levels.

The presence of the Genotype × Environment (G × E) interaction makes it difficult to classify genotypes with intermediate HWS resistance. The triticale lines TCL 15022, TCL 15020 and TCLD 1003 were classified as moderately resistant in one season and as moderately susceptible in another season (Table 3).

ITW 11014 and TCLD 0903 were pre-harvest sprouting susceptible, due to the high sprouting values found in the three years of assessment (Table 3). Pre-harvest sprouting is a serious grain quality concern for millers and farmers alike. Sprouting happends as the seed breaks dormancy, and activates enzymes that begin breaking down starch.

Falling numbers is a test that indicates how well the grain's starch has retained its strength. The FN of the triticale genotypes were lower than the wheat genotypes (Table 3). The FN in the triticale genotypes ranged from 62 seconds to FN > 250 seconds. Climatic conditions influenced this characteristic in different growing season. In 2017, the FN average of triticales was 188 seconds. The average FN in 2016 and 2018 was 99 and 86 seconds, respectively. The higher average FN in 2017 is probably due to absence of rainfall at the end of the grain filling and maturation period (Figure 1).

Falling number trait does not demonstrate correlation with pre-harvest sprouting grains percentage (GERM) on triticale genotypes (Table 4). Such an issue might be explained, by the fact that, triticale genotypes scored the minimum FN the equipment "Falling Number" can register. Triticales' FN cannot be directly compared to wheat, because factors besides α-amylase decrease triticale' solution viscosity a lot. What would be considered a low wheat FN number, not necessarily indicates high α-amylase activity in triticale. A possible cause is the endogen enzymes action, which does not have α-amylase, particularly those which act on non-starch polysaccharides (Randhawa et al., 2015).

A high correlation was detected, although negative, between pre-harvest sprouting grains percentage (GERM) and nebulized grains hectoliter weight (HW_n) on the three assessed years (Table 4). Therefore, grain HW quantifying following simulated rain may be used to the aid screening pre-harvest sprouting tolerant triticale genotypes.

Table 2. Hectoliter weight average (kg hl^{-1}) on triticale and wheat seeds assessed prior (HW_s) and following nebulization (HW_n). 2016, 2017 and 2018 growing seasons

Genotype	HW_s (kg hl^{-1})			HW_n (kg hl^{-1})		
	2016	2017	2018	2016	2017	2018
BRS 203	79 f	76 h	76 j	72 c	69 c	74 e
BRS Harmonia	76 i	76 h	79 f	66 g	70 c	72 f
BRS Netuno	78 f	78 f	79 f	72 c	69 d	75 d
BRS Saturno	79 f	79 d	79 g	74 b	74 a	74 e
IPR 111	73 l	77 g	75 k	66 g	67 e	68 h
IPR Aimoré	79 e	78 e	79 g	71 d	65 f	73 f
ITW 11014	74 k	76 h	79 e	65 g	65 f	72 f
PFT 0609	79 e	80 a	78 g	72 c	72 b	75 d
TCL 13003	81 c	80 a	77 i	71 d	68 d	69 h
TCL 15008	76 i	77 f	78 g	71 d	67 e	75 d
TCL 15014	78 f	78 f	78 g	70 d	69 c	71 g
TCL 15019	78 f	77 g	77 i	69 e	67 e	73 e
TCL 15020	77 g	78 e	77 i	71 d	69 d	74 e
TCL 15022	75 k	75 j	78 h	69 e	67 e	75 d
TCL 15052	79 d	78 e	80 d	73 b	71 c	77 c
TCL 15063	82 b	77 f	76 i	70 d	70 c	75 d
TCL 15102	79 f	78 e	80 e	68 f	66 f	72 f
TCL 15104	78 g	78 e	77 i	68 f	64 f	73 e
TCL 15116	80 d	78 e	77 i	68 f	72 b	74 e
TCLD 0717	80 d	79 c	78 g	69 e	68 d	74 e
TCLD 0903	76 j	79 c	77 i	71 d	67 e	72 f
TCLD 1003	80 d	74 k	74 l	68 f	68 d	72 f
Tiguera 1	76 j	75 j	76 j	67 f	67 e	75 d
Tiguera 5	79 e	76 h	78 h	72 c	66 e	75 d
Tiguera 6	76 j	73 l	78 h	68 f	67 e	76 d
Tiguera 8	80 d	75 j	79 e	71 d	68 d	73 e
TLD 1204	77 h	79 d	79 g	69 e	67 e	73 f
TPOLO 0611	72 m	75 j	76 j	64 h	63 g	73 e
TPOLO 0628	73 l	77 g	78 h	68 f	69 d	71 g
TPOLO 61	76 j	78 f	78 h	65 h	66 e	71 g
TPOLO 66	81 b	77 g	78 h	73 c	67 e	75 d
X 092181	72 m	75 i	80 d	66 g	67 e	76 d
Frontana (WTC*)	81 c	80 b	82 c	76 a	72 b	77 c
ND 674 (WTC*)	83 a	76 h	84 a	76 a	67 e	81 a
Quartzo (WTC*)	84 a	79 c	83 b	75 b	75 a	79 b

Note. Averages followed by the same letter in column are not different among itself according to Scott Knott test at 5%. * WTC = Wheat Tolerance Control.

Table 3. Falling Number (FN) and pre-harvest sprouting (GERM) assessed in 32 and 3, triticale and wheat genotypes, respectively. Londrina, PR, Brazil. 2016, 2017 and 2018 growing seasons

Genotype	FN (s)			GERM (%)		
	2016	2017	2018	2016	2017	2018
BRS 203	253 c	284 f	70 j	8 c	6 f	15.5 c
BRS Harmonia	62 l	89 q	66 j	46 a	44 c	31 a
BRS Netuno	62 l	239 h	69 j	3 d	7 f	6 d
BRS Saturno	79 k	301 d	210 e	2 d	14 f	4.5 d
IPR 111	63 l	114 o	62 k	2 d	45 c	30.5 a
IPR Aimoré	64 l	118 o	62 k	12 c	86 a	37.5 a
ITW 11014	124 h	163 m	72 j	36 a	88 a	32.5 a
PFT 0609	100 j	124 o	62 k	16 c	21 e	14.5 c
TCL 13003	192 d	125 i	62 k	13 c	78 a	36.5 a
TCL 15008	156 f	211 j	68 j	3 d	35 d	11 c
TCL 15014	62 l	157 m	63 k	1 d	2 f	12 c
TCL 15019	62 l	100 p	62 k	6 d	29 d	5.5 d
TCL 15020	96 j	181 l	65 k	8 c	24 e	20.5 b
TCL 15022	62 l	103 p	62 k	10 c	21 e	9.5 c
TCL 15052	64 l	245 h	62 k	6 d	26 d	24 b
TCL 15063	134 g	267 g	108 h	1 d	42 c	9 d
TCL 15102	62 l	141 n	62 k	7 d	60 b	6.5 d
TCL 15104	68 l	139 n	69 j	12 c	58 b	6.5 d
TCL 15116	111 i	137 n	62 k	4 d	4 f	3.5 d
TCLD 0717	72 k	184 l	62 k	4 d	16 e	6.5 d
TCLD 0903	62 l	230 i	62 k	38 a	86 a	40.5 a
TCLD 1003	62 l	159 m	62 k	18 c	19 e	15 c
Tiguera 1	183 e	295 e	248 c	2 d	2 f	6.5 d
Tiguera 5	73 k	292 e	71 j	12 c	5 f	1.5 d
Tiguera 6	132 g	192 k	63 k	2 d	20 e	6 d
Tiguera 8	115 i	85 q	70 j	2 d	5 f	8 d
TLD 1204	62 l	290 e	107 h	26 b	42 c	37.5 a
TPOLO 0611	89 j	281 f	94 i	23 b	60 b	8.5 d
TPOLO 0628	63 l	77 r	62 k	38 a	27 d	17.5 c
TPOLO 61	67 l	238 h	148 g	18 c	32 d	31 a
TPOLO 66	67 l	137 n	62 k	4 d	22 e	11.5 c
X 092181	256 c	317 c	237 d	1 d	6 f	1.5 d
Frontana (WTC*)	365 b	354 a	176 f	1 d	2 f	5 d
ND 674 (WTC*)	438 a	307 d	287 b	1 d	6 f	0 d
Quartzo (WTC*)	443 a	344 b	313 a	3 d	7 f	2.5 d

Note. Averages followed by the same letter in column are not different among itself according to Scott Knott test at 5%. * WTC = Wheat Tolerance Control.

Table 4. Pearson correlation among traits of pre-harvest sprouting in wheat and triticale genotypes in the years 2016, 2017 and 2018

Trait	HW_s	HW_n	FN	GERM
Year 2016				
HW_s	1	0.74**	0.40*	-0.43*
HW_n		1	0.47**	-0.41*
FN			1	ns
GERM				1
Year 2017				
HW_s	1	0.44**	ns	ns
HW_n		1	ns	-0.50**
FN			1	ns
GERM				1
Year 2018				
HW_s	1	0.65**	0.53**	ns
HW_n		1	0.58**	-0.54**
FN			1	ns
GERM				1

Note. **, * Significant at 1 e 5%, respectively. ns: not significant. T test with n-2 freedom degree.

HW_s: hectoliter weight without nebulization; HW_n: hectoliter weight with nebulization; FN: falling number; GERM: germination percentage.

4. Conclusion

There is a variability for pre-harvest sprouting tolerance among triticale genotypes assessed in this study. Triticale genotypes BRS Netuno, BRS Saturno, TCL 15116, X 092181, Tiguera 1 and Tiguera 8, standout by showing comparable tolerance, to the one observed in pre-harvest sprouting tolerant wheat acknowledged cultivars. The most tolerant genotypes could be used as parents in the future breeding programs, towards developing varieties with pre-harvest sprouting tolerance.

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