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Characterization of wheat cultivars for pre-harvest sprouting

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SUMMARY

Sprouting or germination in wheat takes place as a result of the rupture of the dormancy in grain, expressed in physiological terms as the increase of α-amylases and proteases activity, which has been associated with low values of falling number. Grain sprouting has been related with high moisture and temperature at harvest and it is a serious problem in the north of Argentina. The objectives of this study were to characterize introduced and local wheat cultivars for sprouting response through falling number and, tentatively, to identify adapted materials with good behavior for this trait. A set of thirty introduced and local late and early heading wheat varieties was tested through falling number in two experiments conducted at Marcos Juárez, Argentina in 2005 and 2006. Significant differences in falling number between tested cultivars were detected with a strong interaction with environment. Late heading cultivars introductions showed significantly higher falling number values than local cultivars, and early heading cultivars introductions and local cultivars showed similar values. In the early heading cultivars group, the highest falling number value was observed in Klein Proteo, a local cultivar.

Key words: *Triticum aestivum* L., pre-harvest sprouting, falling number

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RESUMEN

En trigo el término brotado se refiere a la ruptura de la dormición en el grano, lo que expresado en términos fisiológicos es el incremento de las actividades proteásica y α-amilásica, que se asocia con valores bajos del test falling number. El brotado de grano se ha relacionado con elevada temperatura y humedad a cosecha. En este estudio se propuso caracterizar la respuesta a brotado de culti-

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vares de trigo, locales e introducidos, a través de falling number y tentativamente, identificar materiales adaptados con buen comportamiento a brotado de grano en precosecha. Treinta variedades de trigo locales e introducidas con espigazón precoz y tardía fueron evaluadas a través del falling number en dos experimentos conducidos en Marcos Juárez, Argentina, durante 2005 y 2006. Se detectaron diferencias significativas en falling number entre los cultivares evaluados con una fuerte interacción con el ambiente. Los cultivares introducidos con espigazón tardía mostraron valores de falling number significativamente mayores que los cultivares locales, y en el grupo de espigazón precoz no se observaron diferencias significativas en falling number de cultivares introducidos y locales. Dentro del grupo de espigazón precoz el valor más alto de falling number fue observado en el cultivar local Klein Proteo.

Palabras clave: *Triticum aestivum* L., brotado en precosecha, falling number

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Pre-harvest sprouting (PHS) can be defined as the germination of grains in the ear before harvest (Groos *et al.*, 2002). This event reduces the quality of wheat and the economic value of the grain. The sprouting or germination occurs as a result of the breakdown of the grain dormancy, which is physiologically expressed as an increase in protease and alpha-amylase enzyme activity. PHS expression in susceptible cultivars is observed when rainfall and high temperature happen together with ripeness and harvest. The flour of a sprouted grain has lower falling number because the active alpha-amylases degrade starch, resulting in poor baking quality (Humphreys and Noll, 2002). PHS tolerance and grain dormancy are complex traits affected by different environmental factors (Trethowan *et al.*, 1996; Johansson, 2002) and controlled by several genes and QTLs (Bailey *et al.*, 1999; Mares *et al.*, 2005; Yang *et al.*, 2007).

In the north of Argentina (30° S latitude or lower), wheat harvest is frequently performed in warm, humid and rainy conditions, and PHS is a serious problem, but in the last years there has been a significant increase of frequency in pre-harvest rainfalls in the Central Pampa (economically, the most important wheat production area in Argentina, between 32° and 38° S latitude) and PHS may acquire more relevance in this region. PHS tolerance has become an important trait in wheat breeding programs in Argentina, but unfortunately, preliminary data suggest that most of the local germplasm is susceptible to PHS mainly explained by the low dormancy detected in tested cultivars (Reartes *et al.*, 2004). A simple strategy to identify germplasm with PHS tolerance can be selecting material based on falling number values evaluated on seed kept in environmental conditions favouring sprouting (high humidity, high temperature). In these conditions, materials with higher falling number values will probably show higher PHS tolerance than materials with low falling number values.

Based on that hypothesis, the present study reports the characterization of a set of introduced and local wheat cultivars using the falling number test information and the interaction of falling number in tested cultivars with the environment.

Two different sets of wheat cultivars were used in this study. (1) Late heading cultivars, including ACA 302, BIOINTA 2003, BIOINTA 3000 and Buck Mataco from Argentina; AC Barrier, AC Domain, Cadet, Columbus and Rescue from Canada. (2) Early heading cultivars, including BIOINTA 1000, BIOINTA 1001, BIOINTA 1002, BIOINTA 1003, Klein Proteo, ProINTA Elite from Argentina; AC Splendor, AC Vista, Bluesky, Cutler, Foremost, Garnet, Glenlea, Laser, Prelude and Oslo from Canada; Pavon 76 and Potam S70 from CIMMyT and Sunlin from Australia. Late and early heading cultivars were phenotyped for falling number during the 2005 and 2006 at Marcos Juárez experimental field (32° 42' S, 62° 07' W), Córdoba, Argentina. Cultivars were planted at optimal sowing date in a completely randomized block design with split plots with 3 replicates, considering cultivars as the main plot and harvesting date as subplot. Each subplot consisted of 7 rows 3 m long spaced 0.2 m apart. The trials were kept free of pests, weeds and diseases. Harvest was carried out at two different times: the first time, when each cultivar reached the dough-yellow ripening stage (Maturity), and the second time, in field 30 days after first date. Falling number was determined using the standard AACC method 56-81B with 7 g of flour at the Cereals and Oils Quality Laboratory of the EEA Marcos Juárez. Falling number analysis of variance were conducted to late and early heading cultivars by GLM procedures using Statistical Analysis System version 9.3 (SAS Institute Inc., 2004), considering cultivars, harvesting dates, year and their interactions as variation sources.

The year 2005 was characterized by lower humidity and cooler temperatures during crop cycle than 2006. In late heading cultivars mean relative humidity and temperature in 2005 were 59.9% and 19.8 °C, and in 2006 were 61.2% and 23.2 °C. In early heading cultivars mean relative humidity and temperature in 2005 were 59.3% and 19.1 °C, and in 2006 61.7% and 20.2 °C. Heading time in late heading cultivars was similar in 2005 and 2006 (289 ±4.5 Julian days in 2005 and 290 ±4.8 Julian days in 2006), but slightly shorter in 2006 vs. 2005 in early heading cultivars (277 ±4.5 Julian days in 2006 and 282 ±5.7 Julian days in 2005).

The analysis of variance considering late heading cultivars showed that falling number was significantly affected by cultivar (P < 0.0001), harvest time (P < 0.0001), year (P < 0.0001) and replicate (P < 0.0076) and their interactions (Table 1).

The low variation observed in heading time between cultivars and years (289 ±4.5 Julian days in 2005 and 290 ±4.8 Julian days in 2006) would suggest that most of the variation assigned to cultivar in the ANOVA could be associated to genetic differences between cultivars instead of differences in phenology. All tested cultivars from Canada except Rescue, showed significantly higher falling number (mean of 551 considering 2005-2006 average second harvest) than selected local cultivars (mean of 269 similar conditions) confirming the PHS susceptibility in local germplasm (Table 3). These data agree with Kato *et al.* (2001) defining AC Domain as PHS tolerant, and also posicionate the germplasm from Canada as a promising source of PHS tolerance for local breeding programs.

Table 1. Analysis of variance of falling number in late heading wheat cultivars

Source	DF	F Value	P > F
Replicate	2	5.24	0.0076***
Cultivar	8	89.27	<.0001***
Year	1	43.69	<.0001***
Harvest time	1	45.19	<.0001***
Cultivar*harvest time	8	2.96	0.0066***
Cultivar*year	8	25.49	<.0001***
Year*harvest time	1	58.24	<.0001***
Cultivar*year*harvest time	8	1.79	0.0930

Probability numbers for falling number. Symbols *, **, *** indicate P<0.05, P<0.01, P<0.001, respectively.

Table 2. Analysis of variance of falling number in early heading wheat cultivars

Source	DF	F Value	Pr > F
Replicate	2	3.16	<.0001***
Cultivar	20	52.50	<.0001***
Year	1	528.18	<.0001***
Harvest time	1	1314.10	<.0001***
Cultivar*harvest time	20	14.13	<.0001***
Cultivar*year	20	6.79	<.0001***
Year*harvest time	1	664.47	<.0001***
Cultivar*year*harvest time	20	7.06	<.0001***

Probability numbers for falling number. Symbols *, **, *** indicate P<0.05, P<0.01, P<0.001, respectively.

The second source of variation affecting falling number in the ANOVA of late heading cultivars was harvest time. Falling number variation (expressed as mean ± standard device) observed in the second harvest was higher than variation in the first harvest (394 ± 155 vs. 547 ± 114). This data would posicionate falling number measured in second harvest (thirty days in field after maturity) as a better tool to detect differences between cultivars for this trait.

The third source of variation in the ANOVA affecting significantly falling number was year. As previously expressed, environmental conditions in 2005 and 2006 were quite different, the year 2005 was characterized by lower humidity and cooler temperatures during crop cycle than 2006. Higher humidity and temperature would posicionate 2006 as a more favorable year for the occurrence of PHS in grain which could be observed as lower falling number values in cultivars in comparison to 2005. In line with this hypothesis, average falling numbers detected in first and second 2005 harvests were higher than average falling numbers in 2006 (473 and 482 vs.

441 and 305). Moreover, 7 tested cultivars showed significant differences in falling number between first and second harvest in 2006 and no cultivar showed significant differences in 2005 (Table 3).

The fourth source of variation affecting falling number significantly was replicate; however, with almost an order of magnitude lower than the other source variation.

The interactions affecting significantly falling number in late heading experiment "cultivar * year" and "year* harvest time", and in lower magnitude, "cultivar * harvest time" would suggest a complex genetic control of falling number influenced by many environmental factors.

In the case of early heading cultivars falling number was more affected by harvest time, year, cultivar and replicate, as well as by the interactions "year * harvest time", "cultivar * harvest time", "cultivar * year*harvest time" and "cultivar * year" (Table 2).

Table 3. Falling numbers obtained in late heading cultivars considering two different times of harvesting.

Cultivar	Year 2005		Year 2006		2005-2006 average	
	1st. Harvest	2nd. Harvest	1st. Harvest	2nd. Harvest	1st. Harvest	2nd. Harvest
Columbus	571	614	677	675	624	644.5
AC Domain	523	556	552	528	537.5	542
Cadet	512	534	**594**	**487**	553	510.5
AC Barrier	521	505	**630**	**506**	575.5	505.5
Buck Mataco	394	450	**396**	**177**	395	313.5
ACA 302	454	457	**276**	**83**	365	270
Rescue	428	411	**309**	**115**	368.5	263
BIOINTA 3000	442	423	**320**	**91**	381	257
BIOINTA 2003	416	386	**216**	**88**	316	237
Average	*473*	*482*	*441*	*305*	*457*	*394*
CV %	*6.8*		*7.3*			
LSD (0,05)	*38,7*	*75,3*	*103,8*	*111,7*		

* Data in bold showed significant differences between harvesting date (p=0.05)

Table 4.Falling numbers obtained in early heading cultivars considering two different times of harvesting.

Cultivar	Year 2005*		Year 2006*		2005-2006 average	
	1st. Harvest	2nd. Harvest	1st. Harvest	2nd. Harvest	1st. Harvest	2nd. Harvest
Klein Proteo	599	645	**762**	**363**	680.5	504
AC Splendor	516	478	**596**	**354**	556	416
AC Vista	463	413	**472**	**408**	467.5	410.5
Foremost	421	475	**410**	**264**	415.5	369.5
Glenlea	461	476	**502**	**247**	481.5	361.5
Sunlin	421	421	**457**	**271**	439	346
BIOINTA 1001	434	421	**415**	**247**	424.5	334
BIOINTA 1000	477	452	**484**	**129**	480.5	290.5
BIOINTA 1003	420	399	**362**	**181**	391	290
ProINTA Elite	450	483	**544**	**90**	497	286.5
Potam S70	418	418	**421**	**154**	419.5	286
AC Taber	388	407	**356**	**155**	372	281
BIOINTA 1002	462	420	**433**	**100**	447.5	260
Biggar	429	360	**411**	**152**	420	256
Laser	385	371	**285**	**130**	335	250.5
Oslo	**410**	**264**	**377**	**130**	393.5	197
Garnet	**421**	**297**	**469**	**93**	445	195
Bluesky	**449**	**310**	**357**	**67**	403	188.5
Pavon 76	**428**	**280**	**424**	**69**	426	174.5
Cutler	**406**	**251**	**498**	**84**	452	167.5
Prelude	**430**	**215**	**503**	**65**	466.5	140
Average	*442*	*395*	*454*	*179*	*448*	*286*
CV %	*7.48*		*10.05*			
LSD (0,05)	*55,45*	*58,04*	*70,12*	*44,69*		

* Data in bold showed significant differences between harvesting date (p=0.05)

In contrast to late heading cultivars, harvest time and year explained most of the falling number variation followed by cultivar. In relation with harvest time, falling number variation was higher in second harvest than in first (286 ± 94 vs. 448 ± 71), confirming the second harvest as a better source to find differences in falling number. The year effect was similar to the one observed in late heading cultivars, confirming 2006 as a better year to detect the occurrence of PHS expressed as lower falling number average values than 2005, particularly in the second harvest (Table 2). The smaller effect of cultivar in falling number variation suggests a smaller variation in tested cultivars, at least in comparison to late heading group (Table 4). In contrast to late heading cultivars, the cultivar with higher falling number was the local Klein Proteo, followed by the introductions from Canada AC Splendor, AC Vista, Foremost, Glenlea, the Australian Sunlin and the local BIOINTA 1001 (Table 4). These data suggest that tested early heading local cultivars would not be as susceptible to PHS as tested late heading cultivars, further studies including a higher number of late and early heading time local cultivars will confirm that hypothesis. Furthermore, these data posicionate Klein Proteo as a novel local source with certain degree of PHS tolerance, and confirm PHS tolerance of AC Vista (De Paw *et al.*, 1998).

In recent years there has been several genetic studies focused on fine mapping of genes or QTLs controlling PHS tolerance and/or associated traits like seed dormancy (Bailey *et al.*, 1999; Mori *et al.*, 2005; Yang *et al.*, 2007). In these studies the main gene associated to seed dormancy (*Vp-1*) was detected on group 3 chromosome and main QTL associated to PHS tolerance was detected on group 4 chromosomes. Unfortunately, to date, there is not basic information about the inheritance of PHS tolerance detected in Klein Proteo. The development of mapping populations based on this cultivar will provide tools to determine the genetics of its PHS tolerance as well as the identification of molecular markers associated to this important agronomic trait.

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