Both genetic and environmental conditions affect wheat grain texture: Consequences for grain fractionation and flour properties

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Both genetic and environmental conditions affect wheat grain texture: consequences for grain fractionation and flour properties

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Abstract

This review summarizes the results of studies on near-isogenic common wheat lines differing in the *Pinb-D1* allele encoding puroindoline B or durum wheat into which both wild-type puroindoline genes were introduced. The material was grown in different environments to evaluate the respective effect of puroindoline genes or of the environmental factors on grain characteristics and milling behavior.

Environmental conditions were found to impact grain porosity (=1/vitreousness) and the presence of both wild-type puroindoline genes was found to reduce the vitreousness threshold under 60%. Hardness measurements with single kernel characterization system were found to differ from near-infrared reflectance spectroscopy analysis and were linearly related to vitreousness but differently depending on the puroindoline allele carried.

Puroindoline genes were found to play a major role in the grain porosity, breaking energy, size of generated particles and in the concentration of phytic acid and damaged starch into flour whereas vitreousness introduced variations in the ability to break and in the level of damaged starch.

Finally, the highest flour yield is obtained from either vitreous common wheat grains carrying the wild-type puroindoline alleles or carrying mutated alleles and displaying low vitreousness. This result was confirmed using common French wheat cultivars whose puroindoline genes were identified.

Keywords: hardness; milling; puroindoline; vitreousness

1. Introduction

The first step of wheat grain processing, i.e. milling, corresponds to successive grinding and sieving operations (Posner, 2009) with the aim of separating the starchy endosperm from the peripheral tissues (the outer layers and the germ). During this process, the mechanical properties of grain tissues play a major role in the grain breaking behavior and in the fate of the different tissues and consequently in the properties of the resulting product (Morris and Rose, 1996; Symes, 1969).

Direct or indirect methods have been used to evaluate the mechanical resistance of the grain (Pasha et al., 2010). One of the indirect methods used is based on the measurement of the percentage of particles passing through a sieve of defined aperture (75 μm) after grinding the grain, resulting in a particle size index (PSI) that is used to classify wheat in two classes (Williams and Sobering 1986) "soft" and "hard" depending on the PSI obtained. The PSI method was progressively replaced by a near-infrared reflectance spectroscopy (NIRS), which measures scattering of the analyzed ground grain sample at specific wavelengths in comparison with wheat grains with contrasted known PSI values and is used to classify wheat samples in the different classes (Norris, 1989; Saurer, 1978). A more direct measurement of grain mechanical resistance uses the Single Kernel Characterization System (SKCS) developed by Martin et al. (1993) to evaluate the crushing force required to break the grains using a hardness index (HI). Further mechanical resistance of specific grain tissues was also developed to specifically characterize their resistance to breaking at a given temperature and water content. In particular, endosperm bricks or cylinders (Haddad et al., 1998; Morris et al., 2011a) were found to be able to correctly evaluate the physical grain resistance to breaking and to be related to the previous hardness classification. Similar measurements of the mechanical properties of tissue samples with well-defined dimensions were also developed for the grain outer layers (Mabille, 2001) and further refined to characterize almost all component tissues (Antoine et al., 2003).

A major locus called *Ha* that controls grain hardness was identified on the short arm of chromosome 5D in common wheat (*Triticum aestivum*) using chromosome substitution lines (Law et al., 1978; Mattern et al., 1973) and its emergence recorded during wheat selection (Chantret et al. 2005; Charles et al. 2009). It was found to encode two specific low molecular weight and tryptophan and cysteine enriched proteins, called puroindolines (PINs) A and B (Bhave and Morris, 2008). These proteins were suspected to play a role in the starch-protein adhesion in the starchy endosperm (Chichti et al., 2015; Pauly et al, 2013; Turnbull and Rahman, 2002). The presence of the wild-type alleles, *Pina-D1a* and *Pinb-D1a*, of the genes

encoding PINA and PINB results in a soft phenotype whereas deletion or mutations of one of the genes results in a hard phenotype. As durum wheat does not contain the D genome, its grain mechanical properties are the hardest.

A number of different sequences of PIN genes have been recorded but their occurrence depends on wheat selection in different countries. The most common mutation of *Pina-D1* in the USA is a null mutation (*Pina-D1b*) that results in the absence of PINA from the endosperm (Morris and King, 2008). In Europe, the most frequent mutation occurs in *Pinb-D1* gene (allele *Pinb-D1b*) and corresponds to a Gly to Ser substitution in position 46 (Huang and Röder, 2005). The two other relatively frequent mutated alleles, *Pinb-D1c* and *Pinb-D1d*, correspond, respectively, to a Leu to Pro change in position 60 (Lillemo and Morris, 2000) or to a Trp to Arg change in position 44 (Huang and Röder, 2005).

A clear relationship between the presence of both puroindoline wild-type alleles and a soft grain phenotype was further reinforced by complementation of corresponding null (Wanjugi et al., 2007) or mutated alleles (Beecher et al., 2002). Moreover, the introduction of PIN genes in other cereals that lack the corresponding genes was found to reduce grain hardness (Krishnamurthy et al., 2001; Morris et al., 2011b; Zhang et al., 2009). Grain mechanical properties were also found to be affected by endosperm porosity (Dobraszcyk et al., 2002; Haddad et al., 2001), which can be evaluated by visual observation of a white endosperm (mealiness), whereas non-porous grains are glassy in appearance. Different methods for more accurate evaluation of vitreousness have been developed in recent years using either transmitted light (Neethirajan et al., 2006; Venora et al., 2009; Xie et al., 2004), soft X-ray, dual energy X-ray or light reflectance (Neethirajan et al., 2006, 2007; Xie et al., 2004) or near infrared hyper-spectral image analysis (Gorretta et al., 2006; Serranti et al., 2013). All these methods require a well characterized reference sample set for classification of the analyzed sample that consequently needs similar characteristics such as grain geometry, tissue thickness, and color. Recently, a clear relationship between grain porosity and vitreousness was demonstrated through the study of light transmission through wheat grain longitudinal cross sections of different thickness and was shown to strictly follow a Beer-Lambert law (Chichti et al., 2018). This enabled direct quantification of endosperm vitreousness. Environmental conditions during wheat grain growth were suspected to play a role in grain porosity despite the possible influence of the genotype background (Sharp, 1927; Stenvert et al., 1977). In particular, the influence of environmental conditions on protein content and its relationship with vitreousness was pinpointed in durum wheats as it was controversially discussed in the literature (Sieber et al., 2015) and further reinvestigated in durum or common

wheats (Oury et al., 2015; Fu et al., 2018). The relationship between vitreousness and the protein content was shown to be very weak at least in soft common wheat and to depend on the protein content value in hard common wheats (Oury et al., 2015), as well as in durum wheats (Fu et al., 2018).

To clarify the respective roles of puroindoline genes and of environmental conditions in determining the microstructure and mechanical characteristics of wheat grains, as well as their milling behavior, only wheat lines or cultivars whose PIN alleles were known or controlled were grown in different conditions.

2. Environmental conditions affect grain porosity as revealed by measurements of vitreousness

Near isogenic lines (NILs) of common wheat differing only in the puroindoline allele encoding PINB, were grown along with a control cultivar in three consecutive years (2007, 2008, 2009) at seven distinct sites in France (ranging from 45°46'N to 50°28'N and from 1°33'E to 3°12'E) with two different nitrogen supplies applied (one adjusted to obtain a high grain yield of around 9t/ha depending on the measured soil N content, and an identical supply with an additional 50 kg/ha at flowering), and their grain characteristics were measured (Oury et al., 2015). The different NILs carried either the wild-type allele encoding PINB (*Pinb-D1a*) or one of the mutated alleles (*Pinb-D1b* or *Pinb-D1d*). The wheat microstructure was evaluated by visual estimation of vitreousness on grain sections (n=500 for each wheat sample) with a Pohl grain cutter, as described in Lasme et al. (2012).

Analysis of variance including the following factors: year, location and nitrogen supply, revealed a significant effect of all of the factors on vitreousness with a high level of explanation of the model (adjusted r^2=0.67). The wheat grain samples collected (n=304) showed higher vitreousness in 2008 than in the two other years of cultivation (Figure 1A). But marked differences in vitreousness between the different cultivation sites were also observed in each sample in each year, as shown for one of them in Figure 1B. A higher vitreousness level was also observed with the increased supply of nitrogen (Figure 1C).

Figure 1

Concerning the mechanical characteristics measured by NIRS hardness or SKCS HI, only a year effect was found to be highly significant. When measured using NIRS, neither the location nor the nitrogen supply appeared to explain variability of hardness (adjusted r^2=0.06)

and when measured by SKCS HI, location and nitrogen supply were only significant at the 5% level with a quite low level of explanation of the model (adjusted r^2=0.18).

In conclusion, environmental conditions clearly affect the grain microstructure as measured by the level of vitreousness.

3. Pinb-D1 allele appears to affect both grain hardness and porosity

Comparisons were made between the common wheat grain characteristics depending on the Pinb-D1 allele carried (Oury et al., 2015) and are summarized in Table 1 with their average mean values. Significant differences were found in the NIRS and SKCS mean values between grains from NILs (NIL1) carrying the wild-type allele, *Pinb-D1a*, or the mutated allele, *Pinb-D1b*; the latter presenting 3-fold higher average NIRS or SKCS values. This result was expected as NIRS and SKCS measurements were carried out with the aim of dividing wheat grain samples into soft or hard classes. But surprisingly, the mean vitreousness level was also found to differ between NILs carrying either the wild-type or the mutated allele of *Pinb-D1*. Near isogenic lines carrying the wild-type allele *Pinb-D1a*, and thus displaying a soft phenotype were found to present 1.5-fold lower mean vitreousness values than the lines carrying the mutated allele, *Pinb-D1b,* classified as a hard phenotype. Therefore, the presence of *Pinb-D1a* increases wheat grain porosity regardless of the environmental conditions. A similar decrease in both PSI or SKCS grain hardness and vitreousness due the presence of wild-type alleles of the puroindolines was also reported by Heinze et al. (2016) in a study of durum wheat in which these genes were translocated (Morris et al., 2011b) in comparison with the non-translocated line. In both cases, the differences in vitreousness cannot be linked with differences in protein content as frequently reported. Indeed, the mean protein content in common wheat grains carrying either *Pinb-D1a or Pinb-D1b* was (11.8-11.9%, Oury et al., 2015) whereas it was around 16% (15.8-16.2 %) for durum wheat grains carrying or not the puroindoline genes.

Table 1

Comparison of near-isogenic lines carrying either the mutated allele, *Pinb-D1b* or *Pinb-D1d* (NIL2) which both lead to a hard phenotype, revealed a highly significant difference (*p* value <0.001) in average NIRS values, wheat grains carrying *Pinb-D1b* displaying higher grain hardness measured by NIRS than grains carrying the other mutation *Pinb-D1d* (Table 1). Similarly, grains carrying *Pinb-D1b* also displayed higher SKCS values but with lower

significance while average vitreousness values did not differ significantly. These results reflect the fact that NIRS and SKCS measurements do not evaluate the same hardness characteristics. As no significant differences in vitreousness were observed between wheat samples carrying Pinb-D1b and Pinb-D1d, the impact on the required breaking energy, as measured with SKCS was weakly significant. However, differences in the particle size between grounded samples were observed.

Figure 2

Figure 2 summarizes the effect on SKCS hardness of the wild-type or mutated Pinb-D1 allele in common wheat near-isogenic lines carrying the wild-type Pina-D1 allele compared with SKCS hardness measured in durum wheat with or without the genes encoding wild PINA and PINB introduced by Morris et al. (2011b). Each different sample (n=243) was grown in several locations to explore variations due to the environment compared with variations due to the puroindoline alleles and genetic background. The results clearly point to an impact of puroindoline alleles on SKCS hardness. SKCS hardness values below 25 were found to only correspond to wheat grains carrying the wild-type alleles encoding PINA and PINB, whereas values above 75 were found to only correspond to durum wheat grains. Figure 2 also shows that the presence of the wild-type puroindoline alleles limits grain mechanical resistance measured by SKCS to values below 45. Moreover, SKCS values between 45 and 75 were only obtained for durum wheat or common wheat carrying the mutated puroindoline alleles. Therefore, when SKCS hardness values are between 25 and 45, it is impossible to classify wheat grains according to the puroindoline alleles (these values being obtained either with grains carrying wild-type or mutated alleles of puroindoline b).

4. Relationships between NIRS hardness, SKCS HI and vitreousness

Relationships between indirect hardness measurements (NIRS hardness) based on the size of the particles after grinding, or direct evaluation of the grain mechanical resistance (SKCS hardness) and vitreousness were studied as a function of the allele, wild-type or mutated, of puroindoline B (Fig. 3).

Figure 3

Figure 3 shows that, regardless of the level of vitreousness, NIRS hardness efficiently distinguish between common wheat near-isogenic lines carrying either the wild-type alleles or the mutated alleles encoding PINB. Only very weak relationships (r^2<0.3 for common wheat carrying the wild-type or mutated alleles encoding PINB) were found between NIRS hardness and vitreousness measurements (Fig. 3A). By contrast, a strong relationship was found between SKCS HI values and vitreousness (r^2= 0.73 for soft near-isogenic lines and r^2= 0.75 for hard genotypes), as illustrated in Fig. 3B. Thus, vitreousness increases the energy required to break the grains. Fig. 3B also shows that the presence of both wild-type alleles encoding PINA and PINB reduces grain mechanical resistance. It also clearly demonstrates that SKCS values above 25 and below 45 can be obtained either with vitreous soft wheat grains or hard wheat grains with low vitreousness. Interestingly, vitreousness values higher than 60% were only found in wheat grains carrying the mutated alleles of *Pinb-D1*.

5. Both puroindoline allele encoding PINB and vitreousness affect milling behavior

The milling behavior of common wheat near-isogenic lines carrying the wild-type allele of puroindoline b gene or the mutated alleles and displaying different vitreousness was studied (Oury et al., 2017). Results were analyzed in each type of allele but also depending on vitreousness while taking its effect on grain mechanical resistance into account. For this analysis, the grain sample population within each near-isogenic line was divided into two groups according to half the maximum vitreousness value. Significant differences in grain or semolina breaking were found between groups and are reported in Figure 4. Vitreousness was shown to significantly reduce the breaking flour yield at milling whatever the genetic background (Fig. 4A and 4B). Reduction of semolina at the reducing step was also studied through the percentage of sizing flour produced from this fraction. Sizing flour production was shown to be reduced only in the case of wheat grains from near-isogenic lines carrying the mutated alleles and displaying a high level of vitreousness (Fig. 4C and 4D). Thus, if vitreousness appeared to mainly reduce the ability to produce flour in the case of hard wheat grains (Fig. 4B and 4D), in the case of soft wheat grains that carry the wild-type alleles of both puroindoline genes, the negative effect of high vitreousness on break flour production (Fig. 4A) appeared to be balanced by a positive effect on the production of sizing flour (Fig. 4C). As a consequence, total flour appeared to both depend on the puroindoline allele of puroindoline b gene carried and on the level of vitreousness.

Figure 4

Analysis of total flour production from French common wheat cultivars (n=197) carrying different alleles encoding PINB (*Pinb-D1a*, *Pinb-D1b*, *Pinb-D1c* or *Pinb-D1d*), together with those from the near-isogenic lines (n=72), carrying either *Pinb-D1a*, *Pinb-D1b*, or *Pinb-D1d,* revealed that the highest yield was obtained using grains displaying SKCS hardness values ranging between 30 and 60, which corresponds to either soft vitreous grains or hard grains with a low level of vitreousness (Oury et al., 2017 and Fig. 5).

Figure 5

6. Starch damage and separation between the starchy endosperm and the envelopes

The involvement of both puroindoline genes and vitreousness in the energy required for breaking grains was shown to impact the yield of total flour through the size of the resulting particles, but was also shown to potentially impact flour composition.

Due to the role of puroindolines in reducing the cohesion between the protein network and the starch particles, particle size distribution of flours obtained from near-isogenic lines (Greffeuille et al., 2006a) or common wheat cultivars carrying both of the wild-type alleles encoding PINA and PINB was found to present a bi-modal curve with a first population of particles around the starch granule size (25 µm) and a second population around the flour particle size (150 µm). Conversely, common wheat cultivars or near-isogenic lines carrying the mutated alleles encoding PINB led to a mono-modal distribution around the flour particle size (150 µm). Consequently, less damage to starch was observed in flours made from grains carrying both wild-types alleles encoding PINA and PINB (Mayer-Laigle et al., 2018).

As vitreousness also impacts mechanical resistance of the starchy endosperm, differences in the level of starch damage between vitreous or non-vitreous grains in common wheat near-isogenic lines carrying the same type of puroindoline b allele have also been reported (Mayer-Laigle et al., 2018). Mealy and vitreous grains carrying both wild-type alleles encoding PINA and PINB displayed a mean starch damage level of 1.9% and 2.2%, respectively, whereas mealy and vitreous grains carrying the mutated allele encoding PINB displayed a mean starch damage level of 3.4% and 5.2%, respectively.

Similarly, the particle size distribution of flours obtained after grain milling of durum wheat (c.v. *Svevo*) was found to display a mono-modal form whereas flours from the same cultivar into which both wild-type puroindoline genes were introduced (*Svevo-Pin*) presented a bi-

267 modal curve (Heinze et al., 2016). Consequently, differences in the starch damage level in
268 milling flours from durum wheat (c.v. *Svevo*) and *Svevo-Pin* were 8.3% and 1.9%,
269 respectively. A similar decrease in flour particle size and reduced starch damage due to the
270 introduction of puroindoline genes into durum wheat (c.v. *Svevo*) has also been observed by
271 other authors (Murray et al., 2016; Quayson et al., 2016).
272 These differences in the level of starch damage may therefore alter the water absorption
273 properties of flours (Morrison and Tester, 1994) when used to make food products.
274 The involvement of both vitreousness (=1/porosity) and of the presence of PINA and PINB,
275 which have been suggested to play a role in reducing adhesion between starch granules and
276 the protein matrix, in the level of starch damage was clearly shown to match results from
277 numerical models (Chichti et al., 2016). In these models, voids reflect porosity and different
278 level of adhesion forces were applied between the two main elements of the starchy
279 endosperm (starch and proteins) to mimic the role of puroindolines.
280 Greffeuille et al. (2005) first pinpointed a different distribution of the aleurone cellular
281 content between cultivars of soft and hard common wheat carrying either wild-type or
282 mutated puroindoline alleles. The higher amount of the aleurone layer cellular content,
283 monitored through phytic acid concentration known to be present in the aleurone globoids
284 (Morrison et al., 1975), was found in milling flours made from the cultivars carrying the
285 mutated alleles of puroindoline b. This content negatively correlated with the maximum
286 tensile strain of the outer layers (Greffeuille et al., 2006b). This difference in behavior of the
287 mechanical properties of the outer layers was further confirmed using common wheat near-
288 isogenic lines differing only in the wild-type or mutated allele encoding PINB (Greffeuille et
289 al., 2007).
290 Recently, Heinze et al. (2016) clearly showed that differences in the aleurone cellular layer
291 behavior at milling were linked with the presence or absence of both wild-type puroindoline
292 genes. Indeed, with durum wheat (c.v. *Svevo*), in which both puroindoline genes are absent,
293 the concentration (or proportion of total content) of phytic acid in the flours after milling was
294 significantly 2-3 fold higher than in flours obtained from durum wheat grains into which both
295 wild-type puroindoline genes (*Svevo-Pin)* were introduced. These differences in the cellular
296 content of the aleurone layer, more precisely the total phytic acid content, in the flours surely
297 impact their technological, nutritional and safety properties. Indeed, the cellular content of the
298 aleurone layer is rich in beneficial micronutrients but may also contain detrimental hydrolytic
299 enzymes (Antoine et al., 2002) or mycotoxins (Rios et al., 2009). Furthermore, phytic acid is
300 known to be a complexing agent for minerals and is thus recognized as an anti-nutrient even if

it has also been found to display beneficial antioxidant properties due to its iron binding properties (Kumar et al., 2010; Urbano et al., 2000). It has also been suspected to interact with proteins, thereby reducing their digestibility (Bye et al., 2013; Kumar et al., 2010).

Similarly, the phytic acid content present in the aleurone layer cells has been shown to be higher in durum (c.v. *Svevo*) wheat flour samples in comparison with corresponding wheat samples (*Svevo-Pin)* into which both wild-type puroindoline genes were introduced (Heinze et al., 2016). It was also shown that conversely starch content in bran from durum wheat obtained after milling, as well as proportion of the total starch content, was significantly lower in comparison with the starch content in the bran fraction originating from corresponding *Svevo-Pin* sample (Heinze et al., 2016). This suggests a greater loss of starchy endosperm, whose starch is a molecular marker, in the bran fractions milling from grains carrying both wild-type puroindolines. Therefore, separation between the starchy endosperm in grains carrying both wild-type puroindoline genes appeared to preferentially occur in the sub-aleuronic area, whereas in durum wheat, which does not contain the puroindoline genes and in common wheat grains carrying a mutated puroindoline allele, this separation appeared to occur closer to the aleurone layer.

7. Conclusions

Our review clearly demonstrates that the milling behavior (breaking energy, generated particle size, tissue distribution) of wheat grain depends on both the type of puroindoline genes (wild-type or mutated) whereas grain vitreousness was determined by environmental growth conditions. Grain vitreousness values were found to depend on the type of *Pinb-D1* gene present (from lowest to highest vitreousness grains carrying both wild type puroindoline genes, or carrying the mutated allele *Pinb-D1d,* or *PinbD1b* in common wheat, or no puroindoline genes as in durum wheat). SKCS HI values was found to display a linear relationship with vitreousness and was found to differ depending on the presence of wild-type or mutated alleles encoding PINB. The vitreousness level of the former was found to reach a threshold of around 60% corresponding to SKCS values between 35 and 40, therefore presence of PINA and PINB increases grain porosity and lower the breaking energy. The highest total flour yield was obtained at SKCS values ranging between 30 and 60 which correspond to either vitreous grains carrying the wild-type alleles of both puroindolines or grains carrying a mutated allele encoding PINB and displaying low vitreousness. Indeed a negative effect of high vitreousness on break flour production for soft wheats appears to be

balanced by a positive effect on the sizing flour production. As both vitreousness and puroindoline alleles have an impact on grain breaking resistance, they consequently also affect the particle size of the milling products and the level of starch damage. Differences in flour and bran composition depending on the presence of wild-type or mutated alleles encoding PINB were also reported and was suggested to result from differences in the grain rupture location. These differences in composition will affect the milling fraction properties.

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Figure captions

Figure 1. Boxplots to compare data obtained from grain vitreousness evaluation of the different samples: in different years of cultivation (A), in 7 different growth sites in 2007 with two different nitrogen input for one of the near-isogenic line containing *Pina-D1a-Pinb-D1b* (B) and with two different level of nitrogen used for wheat growth (C). For (A) and (C) the number (n) of samples is 304 and for (B), n=14. The graph summarizes the distribution of

data as the following: boxes enclose 50% of the data with the median value for variables displayed as a line, boxes span from the first quartile to the third quartile and vertical lines display 1.5 times the interquartile distance from the box. Outliers are positioned as a dot outside the box and the vertical lines. Mean values are marked with a cross.

Figure 2. Boxplots to compare data from SKCS HI measurement for different samples. Number of samples n are 34, 36, 43, 75 and 55 for, respectively, durum wheat (*c.v. Svevo)*, same durum wheat in which both wild-type alleles encoding PINA and PINB were introduced by Morris et al. (2011b), common wheat near- isogenic line carrying the wild-type allele of gene encoding PINB, near-isogenic line carrying the mutated allele *Pinb-D1b* leading to the following substitution in the amino acid sequence: G46S, near isogenic line carrying the mutated allele *Pinb-D1d* leading to the following substitution in the amino acid sequence: W44R. See Figure 1 for boxplot description.

Figure 3. Relationships between NIRS hardness (A) or SKCS hardness (B) and vitreousness data for common wheat near-isogenic lines carrying the wild-type allele *Pinb-D1a* (soft) or the mutated allele, *Pinb-D1b* or *Pinb-D1d* (hard).

Figure 4. Percentages of total breaking flour yield, obtained with a LabMill (Chopin technologies, Villeneuve-la-Garenne, Fr., Dubat et al., 2015), depending on the class of vitreousness within near-isogenic lines carrying the wild-type allele *Pinb-D1a* (A) or the mutated alleles *Pinb-D1b* or *Pinb-D1d* (B) encoding PINB. Percentage of sizing flour produced from semolina reflecting the ability of wheat particles to be reduced into flour depending on vitreousness and on the wild-type (C) or the mutated allele (D) of the gene encoding PINB. Grains within each near-isogenic line was divided into two groups (high and low) according to half of the maximal vitreousness value (V.) obtained for soft and hard near-isogenic lines, which was equal to 30 % and 40 %, respectively. See Figure 1 for boxplot description.

Figure 5. Boxplot to compare total flour yield depending on grain SKCS values (0-90) from French common wheat cultivars (n=197) carrying different alleles encoding PINB (*Pinb-D1a*, *Pinb-D1b*, *Pinb-D1c* or *Pinb-D1d*) and near-isogenic lines (n=72), carrying either *Pinb-D1a*, *Pinb-D1b*, or *Pinb-D1d.* Each defined class of SKCS values represents between 17 and 20 % of the total wheat samples. See Figure 1 for boxplot description.

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Fig. 1

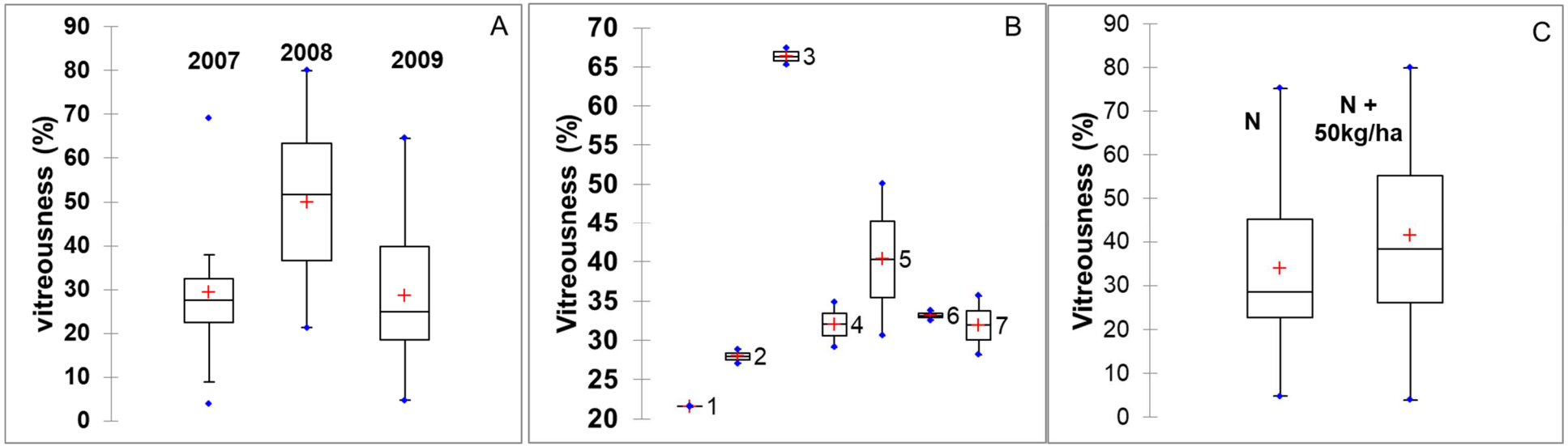

A
vitreousness (%)
90
80
70
60
50
40
30
20
10
0
2007
2008
2009
B
Vitreousness (%)
70
65
60
55
50
45
40
35
30
25
20
1
2
3
4
5
6
7
C
Vitreousness (%)
90
80
70
60
50
40
30
20
10
0
N
N +
50kg/ha

Fig. 2

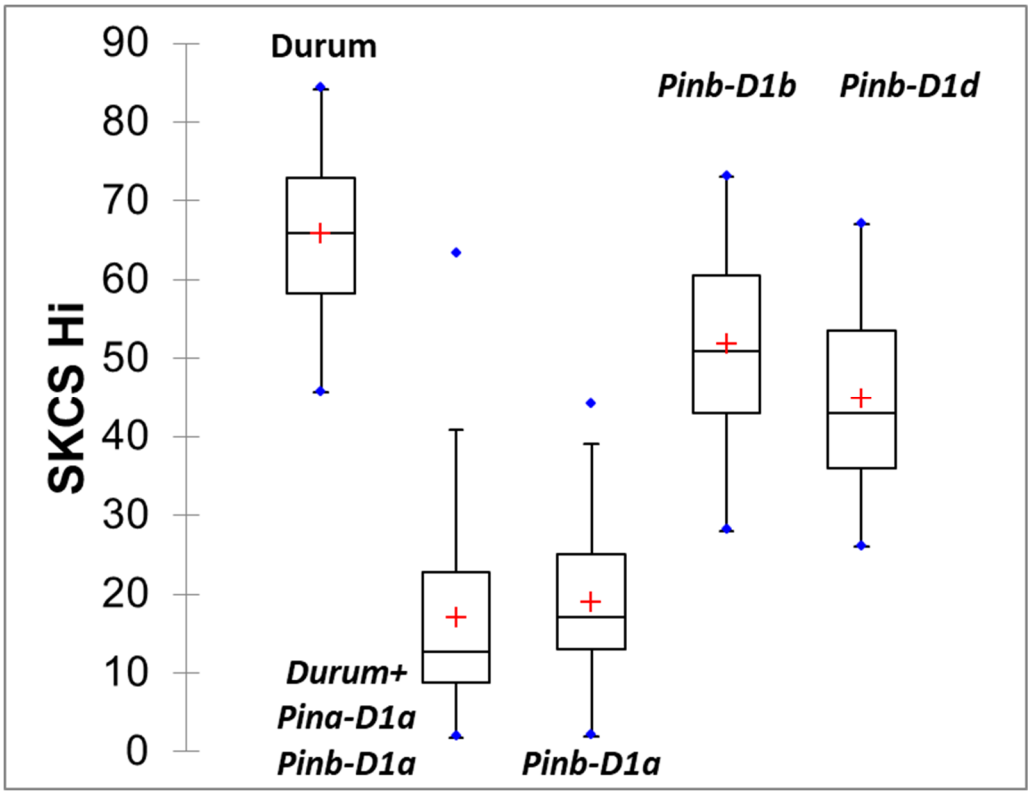

SKCS Hi
90
80
70
60
50
40
30
20
10
0
Durum
Durum+
Pina-D1a
Pinb-D1a
Pinb-D1a
Pinb-D1b
Pinb-D1d

Fig. 3

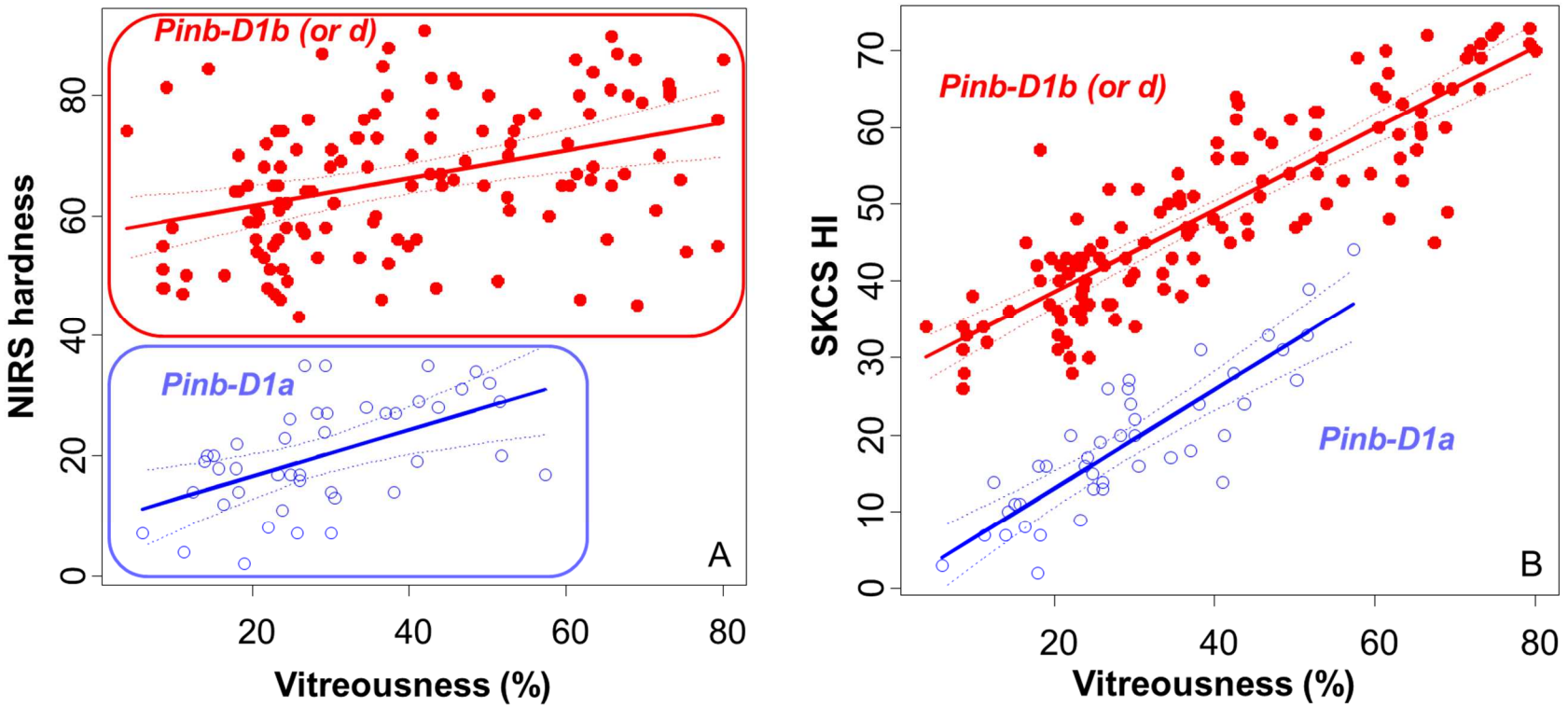
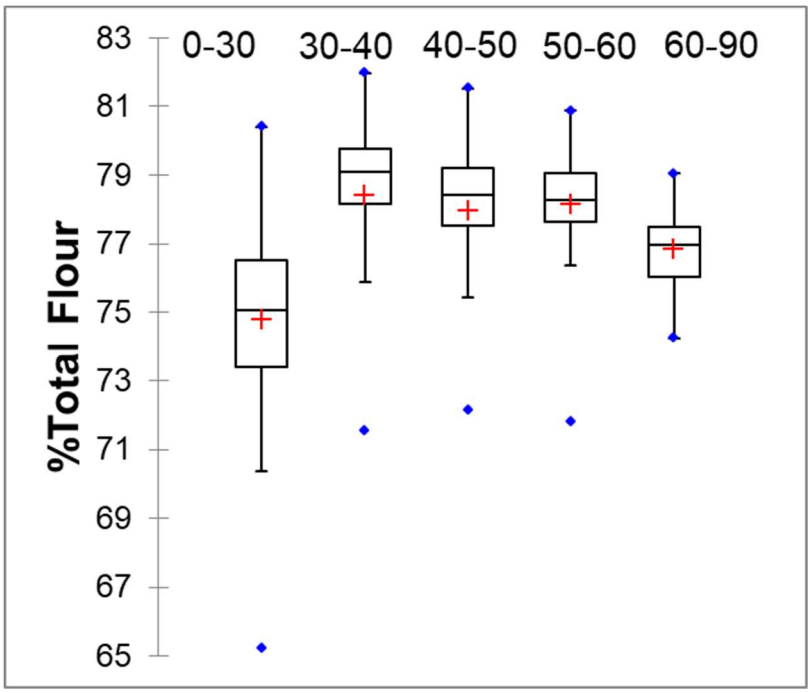

Pinb-D1b (or d)
Pinb-D1a
NIRS hardness
Vitreousness (%)
A
SKCS HI
Vitreousness (%)
B

Fig. 5



	Grains from wheat near-isogenic lines carrying :	NIRS	SKCS	Vitreousness (%)
NIL1	*Pina-D1a-Pinb-D1a*	20.2	18.9	29.2
	Pina-D1a-Pinb-D1b	61.6	52.3	41.5
	Significance	***	***	***
NIL2	*Pina-D1a-Pinb-D1b*	73.3	51.1	44.9
	Pina-D1a-Pinb-D1d	67.1	46.9	42.1
	Significance	***	*(*)*	*ns*

Table 1: NIRS and SKCS hardness and vitreousness average values from two different types of wheat near-isogenic lines differing by the Pinb-D1 allele.

*NIRS was measured using a Percon NIRS apparatus (Inframatic 8620) according to AACC method 39-70A. SKCS values were obtained with a Perten SKCS 4100 according to AACC method 55-31 as described in Oury et al. (2015). Vitreousness was estimated through visual observation using a Pohl grain cutter according to Lasme et al. (2012) on grains from two different types of wheat near-isogenic lines encoding PINB conferring respectively a soft or a hard phenotype or both a hard phenotype due to different Pinb-D1 alleles. Tukey's test was used for comparison of means. Number of observed samples were equal to 164 for NIRS and vitreousness data and to 64 for SKCS measurements for NIL1, and equal to 86 and 64 respectively for NIL2. ***pvalue<0.001, (*) pvalue<0.1.*

Wheat samples with
defined puroindoline genes

Different growth
conditions

NIRS and SKCS hardness +
vitreousness characterisation

Milling
behavior

Flour &
Bran characterisation