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A wheat grain quantitative evaluation of vitreousness by light transmission analysis

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ABSTRACT

Light transmission through wheat (*T. aestivum* L.) grain longitudinal cross sections of different thickness was used to study the endosperm microstructure and was shown to strictly follow a Beer-Lambert law allowing a non ambiguous quantification of the endosperm vitreousness. Therefore similar samples obtained from near-isogenic lines differing by hardness and grown in two distinct environments affecting their vitreousness were analyzed and confirmed the relationship between light transmission and the endosperm microstructure. In each sample, moreover analysis of light transmission within the different grain parts highlighted the greater compactness of the central endosperm cheeks in comparison with the distal and the proximal regions. These results helps a better understanding of the endosperm microstructure.

1. Introduction

The protein-starch adhesion and the microstructure of the common wheat (*Triticum aestivum* L.) starchy endosperm were found to differ depending on genetic and environmental factors (Turnbull and Rahman, 2002). These factors are both found to affect the grain mechanical resistance i.e. endosperm hardness (Haddad et al., 2001) and thus play a key role in grain milling behavior. Recently, an effort was made to experimentally clarify the role of these factors using genetically well-defined grains grown under different cultural conditions (Greffeuille et al., 2006; Oury et al., 2017). The main genetic locus controlling the starchy endosperm texture, called *Ha*, was located on the short arm of chromosome 5D (Chantret et al., 2005) where two important genes encoding specific proteins called puroindoline A (PINA) and puroindoline B (PINB), were found (Bhave and Morris, 2008; Morris, 2002). Presence of the wild-type alleles of both puroindoline genes (*Pina-D1a/Pinb-D1a*) leads to both functional PINA and PINB and results in a soft mechanical behavior, whereas mutation or deletion of one or both of the puroindoline genes was found to lead to a hard texture (Morris, 2002). Moreover, translocation of the wild type puroindoline genes in a durum background (Morris et al., 2011), which lacks D genome, leads to mechanical resistance and similar characteristics to soft common wheat *i.e.* a higher production of the finest particles with low starch damage after milling (Morris et al., 2015; Murray et al., 2016; Heinze et al., 2016). Conversely, removal of the chromosome 5D distal part (which carries the puroindolines genes) in a soft hexaploid wheat led to hard vitreous grains (Morris and Beecher, 2012).

Differences at the interface between protein and starch granules are suggested to explain the mechanical differences between soft and hard cultivars (Barlow et al., 1973) and puroindolines are believed to affect the starch-protein adhesion. Indeed recent studies showed mechanical changes at the interface using near-isogenic lines differing only by the wild-type or mutated allele of the gene encoding PIN B (Chichti et al., 2015).

Besides genetic factors, environmental conditions were found to affect the starchy endosperm appearance the so-called vitreousness (Oury et al., 2015) which is an optical property attributed to differences in endosperm porosity (Dobraszczyk et al., 2002). Grains are classified as mealy when the starchy endosperm is porous and appears white and floury, or as vitreous when it is transluscent and glassy. Vitreousness is generally estimated through examination of a number of grain cross-sections made with a Pohl grain cutter. However, this method is operator-dependent and time consuming. Therefore, other methods were recently developed for rapid classification of wheat grains depending on their vitreousness level. Transmitted light images were found

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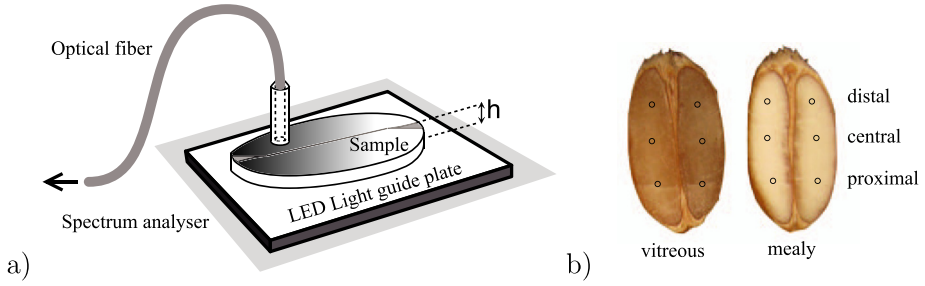

Fig. 1. (a) Schematic representation of the experimental assembly used for the spectroscopic analysis of the grain microstructure. h is for the sample thickness (b) Example of the analyzed grains differing by vitreousness. Circles represent the six areas where the transmission spectra were collected into the starchy endosperm.

allowing correct classification according to vitreousness even if percentages of accuracy relative to the visual inspection differ between authors (Neethirajan et al., 2006; Venora et al., 2009). Soft X-ray, dual energy X-ray or light reflectance coupled with image analysis were also found to be potentially efficient in the differentiation between vitreous and non-vitreous grains (Neethirajan et al., 2006, 2007; Xie et al., 2004). Near infrared hyper-spectral imaging was also used to satisfactory classified vitreous from non-vitreous grains (Gorretta et al., 2006; Serranti et al., 2013). These classification methods are rapid and non-destructive. However, they are based on reference wheat samples which need to be well characterized and are only valid for grains which share the same characteristics as the references (grain geometric characteristics, tissue thickness, colors, hardness, etc.). These recent methods are hardly used to quantify the level of vitreousness as a function of the microstructure.

In the present work, we chose grains from two near-isogenic lines with a known profile of puroindoline alleles, encoding either native or mutated PINB in order to fix hardness due to the genetic background, respectively soft or hard, and selected two different environments from a previous study (Oury et al., 2015) allowing obtaining contrasted levels of vitreousness for the starchy endosperm. Then, locally distinct regions within the endosperm were explored thanks to an original experimental assembly allowing measurement of transmitted light through grain cuts. It was thus possible to objectively compare the endosperm microstructure between the four wheat grain samples depending on genetic or environmental conditions, as well as within each type of sample depending on intra-grain location.

2. Material and methods

2.1. Wheat grains

Near-isogenic lines (NIL) of *Triticum aestivum* L. were produced by Institut National de la Recherche Agronomique (INRA) and displayed either the wild-type *Pinb-D1a* or the mutated *Pinb-D1b* allele (leading to single amino acid change in PINB, *Gly46Ser*), which respectively conferred to grains the soft or the hard phenotype. They were derived from a cross after selection of the two allelic forms at the F6:F7 selfing generation (F7 siblings issued from the same F6 parent plant, construction and genetic similarity testing detailed in (Greffeuille et al., 2006)). Grains from the two lines were cultivated in different locations in France, collected and cleaned to remove broken kernels or impurities and stored at $4°C$ before analysis. Percentage of vitreousness in each collected location was determined on grain cross sections (n = 500) obtained with a Pohl grain cutter (Versuchs and Lehranstalt, Brauerei, Berlin, Germany) depending on the proportion of glassy (translucent) area observed on the surface after visual analysis as described in (Lasme et al., 2012). Briefly, grains were classified into five groups according to the percentage of vitreous surface in the analyzed grains (i.e., grains that displayed less than 25% vitreousness were placed in the first group; those with around 25% vitreousness constituted the second group; and those with around 50, 75 or 100% vitreousness were placed in the third, fourth, and fifth groups, respectively). The number of grains in each class (N_1 represents number of grains in class 1, for example) was multiplied by the corresponding factor of vitreousness for that class, and the percentage of vitreousness in the grain sample was calculated according to the following equation: $\frac{N_1 \times 0.00 + N_2 \times 0.25 + N_3 \times 0.50 + N_4 \times 0.75 + N_5 \times 1}{N_1 + N_2 + N_3 + N_4 + N_5}$, where the sum of the analyzed grains in each class was around 500. From a Student test the maximum error on this measurement was estimated, to be lower than 2 units. Grains harvested from two different locations (Cappelle ($50°29N/3°10E$) and Maule ($48°54N/1°51E$)) were retained for this study as they were found to display contrasting endosperm vitreousness (Oury et al., 2015).

2.1.1. Light transmission through endosperm samples

Wheat kernels were abraded longitudinally from the back and the ventral sides with a 240 grit extra fine sandpaper (grit size 58.5 μm). Abraded grains were placed above a plate light (flat dome Light, LFX-100, CCS, Japan) that diffuses uniform white LED lighting (Fig. 1a). In order to avoid straight light the plate light was covered with a black paper except at the kernel position. Then light transmission through grain cut was collected in the visible range using a 400 μm diameter optical fiber and measured using a spectrometer (Ocean Optics USB 2000 + XR extended range, FL, USA). The number of photons (Intensity in counts) per unit of time (100 ms) was obtained as a function of the wavelength (nm) between 400 and 800 nm and analyzed with the SpectraSuite software (Ocean Optics, Dunedin, Florida, USA). For each analyzed grain samples, thirty kernels were randomly selected and 3 grain locations were probed into the endosperm cheeks (Fig. 1b): distal part (close to the brush), central, and proximal part (close to the germ). Therefore 180 measurement values were obtained for each wheat sample.

2.2. Statistical analysis

The statistical analysis, such as statistical tests and box-plots, were performed with R software (R Core Team (2016), R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/).

3. Results

Wheat samples were selected from a previous study (Oury et al., 2015). This study drove us to select grains with a defined puroindoline genome leading to either soft (native puroindoline a and b alleles) or hard (mutated puroindoline b) phenotype and grown in two contrasted environments resulting in different levels of vitreousness for the starchy endosperm. Wheat grains were characterized with a Pohl grain cutter as already described (Lasme et al., 2012). Considering the previous study (Oury et al., 2015) which described the vitreousness range within different wheat grain samples grown in two years and in seven different sites, samples displaying vitreousness below 40%, were classified as mealy. Conversely, grains having a vitreousness score above 40% were