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Pericarp thickness of sorghum whole grain is accurately predicted by NIRS and can affect the prediction of other grain quality parameters

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The thickness of grain pericarp, the outer layer of the kernel, is an important breeding criterion for sorghum. This cereal is mainly used through traditional processing in family-based food systems in many regions of the world. We investigated in this study how pericarp thickness could be predicted by Near Infrared Reflectance Spectroscopy (NIRS), a fast and non-destructive measurement method that is commonly used to measure physico-chemical parameters of sorghum grains, and how this trait also influences the prediction of those parameters. We showed that, using a classification approach, it was possible to discriminate thick from thin pericarp whole grain samples with a good accuracy and that the proportion of thin and thick grains in mixed samples could also be predicted. In addition, pericarp thickness had a significant effect on the calibration performance for other grain parameters indicating that the pericarp can distort spectral information of whole grain samples. As a practical consequence, we suggest to develop separate whole grain calibration models for thin and thick pericarp samples, combined with a two-steps prediction approach to improve the accuracy of whole grain NIRS calibrations for grain quality parameters in sorghum.

1. Introduction

Sorghum (*Sorghum bicolor* (L.) Moench) is the fifth most important cereal worldwide, after maize, rice, wheat and barley (FAO, 2014). It is particularly adapted to a wide range of environmental conditions including drylands because of its moderate water requirements and its large genetic diversity (Chantereau et al., 2013; Dendy, 1995; Smith and Frederiksen, 2000). Sorghum grain and stover are used in various ways as food or feed in different geographic areas and countries, at both traditional and industrial levels. In Africa and some regions of Asia and India, traditional uses of sorghum grain are mainly for human consumption. It is traditionally processed into thick (tô, bogobe, ugali) or thin (ogi, motogo) porridges, granulated products (couscous, dégué), flat breads (tortilla, kisra, roti) or beverages (beer, gowé, mahewu) (Anglani, 1998; Kleih et al., 2000; Smith and Frederiksen, 2000).

Most of the food products made from sorghum grain require removing the pericarp, either manually or mechanically, a first processing step known as dehulling. Traditional dehulling is performed by pounding washed grains with a wooden mortar and pestle. During pounding, water is added to soften the pericarp and facilitate its removal (Scheuring et al., 1983). Mechanical dehulling is most often a dry process and abrasive-type dehullers are the most used in Africa for sorghum and millet grain (Reichert, 1982). A good dehulling is defined by a complete removal of the pericarp, of the testa layer if present, and of much of the germ, providing high endosperm recovery with minimum breakage of the endosperm (Anglani, 1998; Bello et al., 1990; Fliedel, 1995; Reichert, 1982).

The thickness of the pericarp is an important factor in both traditional and industrial dehulling. When associated with hard endosperm, a thick pericarp is much easier to remove by pounding which is a common practice in Africa; this is one of the reasons why

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in some case farmers prefer to grow this type of grain in Africa (Murty and Kumar, 1995). Conversely, thin pericarp varieties require two times longer to be traditionally dehulled but they are more suitable for dry mechanical dehulling whatever the nature of the endosperm (Earp et al., 2004; Gomez et al., 1997; Scheuring et al., 1983). However, sorghum varieties with a thick pericarp have some disadvantages such as sensitivity to mold, grain weathering in the field during maturation, and rapid deterioration of grains during storage. Varieties with thin pericarp are usually considered to be more tolerant to these constraints (Glueck and Rooney, 1980). The thickness of the pericarp should thus be an important breeding criterion, and a simple and fast measurement method is needed for analyzing the large number of samples usually handled by breeding programs.

The pericarp is the main envelop covering the kernel. It is subdivided into three layers: the epicarp, which is the outermost layer of the pericarp, the mesocarp, and the endocarp, which is the innermost layer, constituted of cross and tube cell layers transporting moisture throughout the kernel. The thickness of the pericarp depends on the thickness of the mesocarp which refers to the number of layers and the presence of starch granules in the mesocarp (Earp et al., 2004; Hoseney et al., 1974; Rooney et al., 1981; Rooney and Murty, 1982). Mesocarp thickness is genetically controlled by a major gene named Z. Sorghum varieties with thin pericarp carry the dominant allele (Z), while sorghum varieties with thick pericarp are homozygous for the recessive allele (z) (Ayyangar et al., 1934).

Two main methods of pericarp measurement have been reported. The first method uses electronic microscopy. Grains are scanned and the amount of starch granules in the mesocarp is investigated to determine pericarp thickness. Three classes (thin, thick or very thick) are generally distinguished by this method which is precise but complex and extremely time consuming (Earp et al., 2004; Scheuring et al., 1983) and thus not adapted to breeding. The second method is a visual appraisal commonly used by breeders. Grains are scraped with a scalpel and pericarp thickness is observed using a magnifying glass (Gomez et al., 1997). This method identify two classes of thickness (thin and thick) and is simple and fast but more error-prone.

Near Infrared Reflectance Spectroscopy (NIRS) is an indirect method that can be applied to a large number of samples and allows measuring many grain parameters through a single assay. As a preliminary step, spectral data of a reference set of samples characterized for their biochemical or physical properties measured through standard methods are used to develop a predictive model. This calibration model can then be routinely applied to the spectra of new samples. Whole grain calibrations are more appropriate for breeding applications because they do not require any grinding step and are thus faster and nondestructive.

The use of NIRS on sorghum to assess grain quality has been reported, in particular to measure starch content, amylose content, protein content, lipid content, total phenols content, condensed tannin content, grain hardness and endosperm texture (de Alencar Figueiredo et al., 2006; Davrieux et al., 2007; Dykes et al., 2014; Hicks et al., 2002; Hooks et al., 2006; Rami, 1999). These studies were conducted on ground grains, on whole grain, or on both. Calibrations based on ground grains generally performed slightly better than calibrations on whole grain (de Alencar Figueiredo et al., 2006). In all conditions, the most accurate predictions were obtained for protein content, followed by grain hardness or lipid content, while amylose content showed consistently poor calibration performances (de Alencar Figueiredo et al., 2006). However, to our knowledge, no study reported so far the use of NIRS to predict pericarp thickness of sorghum grain. Considering the biochemical nature and the simple genetic determinism of pericarp thickness, it is likely that this trait might be predictable using NIRS. Furthermore, as the Z gene can segregate in breeding populations, bulk of grains harvested in breeding programs may contain a mixture of grains with thin and thick pericarp in varying proportions. It is thus of interest to identify samples showing mixture of thick and thin pericarp grains and even quantify the proportion of each type of grain. Finally, in the case of NIRS calibrations established on whole grains, the pericarp, as the peripheral part of the grain, can possibly alter spectral acquisition of endosperm. It should be useful to know if the endosperm components of a thick pericarp variety could be still accurately represented by the NIRS spectrum of whole grains and consequently, if pericarp thickness could affect the performance of whole grain calibrations for endosperm parameters.

The objective of this study was to assess how pericarp thickness could be accurately predicted by NIRS, both qualitatively and quantitatively and to investigate the effect of pericarp thickness on non-destructive prediction of several biochemical and physical grain parameters by NIRS.

2. Materials and methods

2.1. Plant material

Two sets of material were used for this study: a core collection and a breeding population. The core collection was developed by CIRAD (Deu et al., 2006) and consisted of 278 accessions belonging to the five basic races of cultivated sorghum and five intermediate races. The accessions originated from 39 countries representing sorghum production areas. The methods used to produce seed samples were described by de Alencar Figueiredo et al. (2006).

The breeding population was derived from a cross between two sorghum inbred lines named Lata3 and Tiandougou. Lata3 is a guinea type with a thin pericarp, vitreous endosperm, small size, and translucent grains, while Tiandougou is a caudatum type with a thick pericarp, softer endosperm, large size, and chalky grains. The breeding population included 404 progenies in F_3 generation. The whole population was grown in the field in 2010 in Mali at Sotuba IER research station. Each plot included two rows of 10 F_5 plants obtained through bulk multiplication of F_3 progenies ($F_{3:5}$ families). A total of 403 progenies and 60 replications of the two parents grain samples were harvested and used in this study.

2.2. Sample preparation

Grain samples were cleaned by hand by removing stones, straw, moldy, broken, or insect-damaged kernels, and dust. Flour samples were produced for all the accessions of the core collection and for a random subset of 139 individuals of the breeding population. About 20 g of cleaned grains were ground using a Perten Mill 3100 with 0.8 mm sieve (Laboratory Mill 3100, CEMOTEC 1090, Tecator).

2.3. NIRS instrumentation and measurement

A monochromator Foss NIRS instrument (NIRS 6500) was used to scan whole grain and ground grain samples. A quartz ring cup of 47 mm outside diameter and 36 mm inside diameter was filled with 5 g of whole grains or with 3 g of ground grain. The values of reflectance from 1100 to 2500 nm at 2 nm intervals were collected as log (1/R). A second spectrum was acquired for each sample after refilling the cup. The mean of the two spectra was used for further calculation.

2.4. Pericarp thickness measurement

Pericarp thickness was evaluated on all samples of the breeding