

Scientific Notes

Glutenin analysis for the safe screening of self-pollinated wheat seeds when developing doubled-haploid populations

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Abstract – The objective of this work was to apply glutenin analysis, as a simple and reliable method, to identify self-pollinated wheat (*Triticum aestivum*) seeds in F1 crosses, when developing mapping populations using doubled-haploid (DH) technology. F1 seeds were subjected to glutenin analyses using gel electrophoresis to verify and confirm their heterozygotic profiles. Glutenin analyses were carried out using one half of the endosperm of each of the 87 F1 seeds. The other half of the endosperm was used to develop the mapping population through DHs. Plants with band patterns different from the expected ones were discarded. Although the incidence of self-pollinated seeds was relatively low (only 8%), the screening of F1 heterozygotic profiles is extremely important, particularly when creating reliable mapping populations.

Index terms: *Puccinia recondita* f. sp. *tritici*, *Triticum aestivum*, adult plant resistance, leaf rust, microspore culture, Toropi wheat cultivar.

Análise de gluteninas para a identificação segura de sementes autofecundadas de trigo no desenvolvimento de populações duplo-haploides

Resumo – O objetivo deste trabalho foi utilizar a análise de gluteninas, como um método simples e confiável, para identificar sementes F1 de trigo (*Triticum aestivum*) autofecundadas, para o desenvolvimento de populações de mapeamento via duplo-haploides (DH). Sementes F1 foram submetidas a análises de glutenina com eletroforese em gel, para verificação dos perfis heterozigóticos. As análises foram realizadas com a metade do endosperma de cada uma das 87 sementes F1. A outra metade do endosperma foi usada para desenvolver a população de mapeamento via DH. Plantas com padrões de banda diferentes dos esperados foram descartadas. Embora a incidência de sementes autofecundadas tenha sido relativamente baixa (apenas 8%), o rastreamento dos perfis heterozigóticos é extremamente importante, particularmente no desenvolvimento de populações de mapeamento confiáveis.

Termos para indexação: *Puccinia recondita* f. sp. *tritici*, *Triticum aestivum*, resistência de plantas adultas, ferrugem da folha, cultura de micrósoros, cultivar de trigo Toropi.

The doubled-haploid technology, used for the development of completely homozygous plants, is an attractive tool for speeding up selection efficiency for breeding purposes, as well as for basic and applied research. Studying and identifying resistance genes related to major biotic and abiotic problems can effectively contribute to mitigate important constraints that negatively affect wheat (*Triticum aestivum* L.) cultivation. The development of mapping populations through the doubled-haploid technology provides a valuable material for identifying sources of resistance in economically important traits.

Leaf rust (*Puccinia recondita* f. sp. *tritici*) is one of the most important diseases of wheat in South America (Germán et al., 2007). Although fungicides have been used to control it, genetic resistance is the most economical, environmentally friendly, and reliable means of disease control. More than 50 genes were described conferring resistance to leaf rust. However, only some of them confer effective resistance at adult plants growth stages (adult plant resistance, APR). Wheat APR genes are expressed in general, as partial and additive effects, and although the response to infection is essentially susceptibility,

disease development in plants presenting APR genes is slow. These genes may limit the losses caused by the disease, and, in addition, they are considered to confer a more potentially durable resistance (Ellis et al., 2014).

Some of the genes conferring APR have been already comprehensively studied, such as *Lr34* (Tsilo et al., 2014; Fang et al., 2017), while others remain poorly characterized (Dakouri et al., 2013). Toropi is a Brazilian wheat cultivar that shows a durable adult plant resistance (Barcellos et al., 2000; Rosa et al., 2016). The characterization of 'Toropi' genes may help to achieve long-term resistance to wheat leaf rust in Brazil. Using resistance genes originated from 'Toropi' (associated with other biotech tools) can aid breeding programs to cope with and combat the leaf rust disease.

Doubled-haploid (DH) production of wheat has been largely used in many breeding programs to rapidly achieve homozygosity from early generation crosses, increasing the efficiency of breeding selection and fast-tracking the release of new varieties. In Brazil, the production of wheat haploids by cross-pollinating wheat with maize pollen has been done since 1991, with 4–10% frequency of haploid production (Moraes-Fernandes et al., 2002). Despite its advantages, DH system is a labor-intensive and time-consuming process, making it difficult to apply it to the several thousand recombinant inbred lines generated each year in a breeding program. Because of its low efficiency and high costs, the intergeneric method for producing haploids (wheat x maize pollen) has been slowly replaced by more efficient and less laborious techniques, as the isolated microspore culture, using the male gametic cell for producing the haploid plant (Scagliusi, 2014).

Regardless of the chosen methodology for producing DH plants, special attention should be paid to the heterozygotic profile of F1 seeds used as donor plants when developing mapping populations via haploidization. Biparental linkage mapping populations are usually easy to develop, require only a short time for production (via DH), and shows a high power for QTL detection (Xu et al., 2017). However, using unwanted self-fertilized F1 seed as donor plants could hinder, or disrupt the identification of the genomic regions associated with the trait under study.

High-molecular weight glutenin subunits (HMW-GS) are key factors in the bread-making process.

Moreover, the endosperm storage proteins (glutenins and gliadins) have been used for characterizing many bread-wheat cultivars (Payne et al., 1981; Novoselskaya-Dravovich et al., 2011). These proteins are easily evaluated in sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-Page) analyses, which are suitable for rapid screening of a large number of samples. This technique has been employed in Brazil since the 1980s, and its use has been improved and applied for different purposes (Torres et al., 2010; Vancini et al., 2016). The HMW-GS are transmitted to F1 progeny from both parents, and can be easily detected as codominant markers (Burnouf & Bouriquet, 1983). Glutenin analyses from cultivars showing different HMW-GS profiles can be used to check F1 hybrids, and, as a result, self-pollinated seeds can be discarded. To this end, the present study reports, for the first time, the application of glutenin analysis to assist Brazilian wheat breeding programs, using it as a standard procedure for monitoring F1 heterozygotic seed.

Two wheat cultivars were crossed: Toropi (Frontana 1971.37/Quaderna A//Petiblanco 8) and BRS 194 (CEP14/BR23//CEP17). Both are susceptible to wheat leaf rust at seedling stage. However, as adult plants, 'Toropi' shows APR, whereas 'BRS 194' remains susceptible. Glutenin extracts were obtained after a sequential extraction method (Singh et al., 1991) from 87 F1 seed ('Toropi' x 'BRS 194') compared to parental seed. The glutenin subunits were fractionated in SDS-Page, as in Payne et al. (1981), with minor modifications. After the electrophoresis, gels were stained with 0.4% Coomassie Brilliant Blue R 250 and a stain protocol (Blakesley & Boezi, 1977). Gels were scanned with GS-800 densitometer (BioRad). The determination of HMW-GS alleles was carried out using the following wheat cultivars with known electrophoretic mobility (controls): Chinese Spring, Hope, Neepawa, Sappo, Yecora Rojo, and Opata 85.

Bread-wheat is a hexaploid species, with three different but related genomes of seven chromosome pairs named A, B, and D. HMW-GS are encoded by six genes at the loci *Glu-I*, located on the long arms of the wheat chromosomes 1A, 1B, and 1D (Lawrence & Shepherd, 1980). The gene loci are respectively named Glu-A1, Glu-B1, and Glu-D1. In each locus there are two closely linked genes of HMW-GS. SDS-Page showed that each wheat cultivar contains between

three and five HMW glutenin subunits: two of them coded by genes at *Glu-D1*; one, or two, by *Glu-B1*; and one, or none, by *Glu-A1* (Payne, 1987).

The allelic protein subunits can be easily distinguished by SDS-Page. Band 1 was allocated to the HMW subunit with the slowest electrophoretic mobility during SDS-Page, and the following ones were numbered consecutively with increasing mobility using a number system (Payne et al., 1981). Any subsequent work may present new bands of

intermediate mobilities to those already described. Newly discovered subunits thereafter were described with special characters as 2*, which has a slightly slower mobility than subunit 2, or the band 2.2, which is related to bands 2, 3, and 4.

'Toropi' and 'BRS 194' differed for their HMW-GS profiles (Figure 1 A). 'Toropi' showed *Glu-A1b* (band 2*), *Glu-B1b* (bands 7+8), and *Glu-D1a* (bands 2+12) alleles, whereas 'BRS 194' had *Glu-A1b* (band 2*), *Glu-B1i* (bands 17+18), and *Glu-D1d* (bands

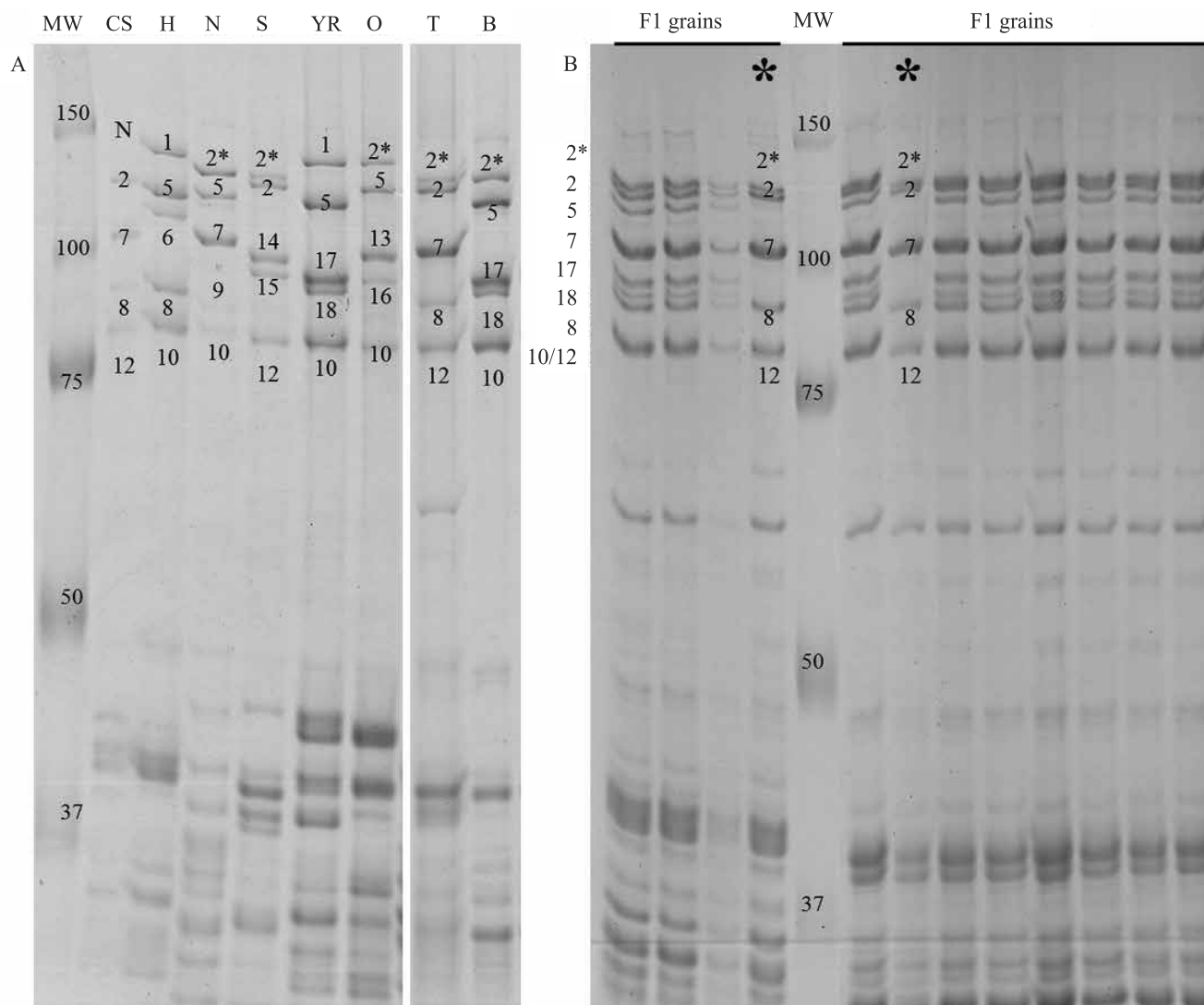


Figure 1. Glutenin profiles of parental lines (A) and F1 grains (B) in SDS-Page analyses. Numbers in the gel represent glutenin subunits, designated according to the nomenclature described by Payne et al. (1981). Different wheat cultivars used as references for electrophoretic mobility: CS ('Chinese Spring'), H ('Hope'), N ('Neepawa'), S ('Sappo'), YR ('Yecora Rojo'), and O ('Opata 85'); followed by 'Toropi' (T) and 'BRS 194' (B) showing different HMW-GS profiles. Lanes of F1 grains with black stars indicate the occurrence of self-pollination. MW, Precision Plus Protein Standards, BioRad.

5+10) alleles. Eighty-seven F1 seeds were analyzed, and 92% of them showed a heterozygosity profile regarding HWM-GS (Figure 1 B). F1 seed showing the same profile as the female parent ('Toropi') were discarded because they resulted from self-pollination. Kammholz et al. (2001) used glutenin analysis and some phenotypic traits to discard plants that contained foreign alleles, or that were contaminated at some point during the development of seven doubled-haploid wheat populations. In their study, the number of eliminated plants ranged from 2.3 to 11.6%, considering the final size of the mapping populations (between 172 and 180 lines).

We observed 8% of self-pollinated seed from 'Toropi' x 'BRS 194' (Figure 1). Their elimination is crucial when developing mapping populations especially designed for genetic studies. Furthermore, monitoring F1 seed can improve selection efficiency in breeding programs, besides avoiding false F1 hybrids in the several thousand recombinant inbred lines developed per year, since only heterozygotic plants will be used for further tests.

A recent study, conducted with the same objective as the present one, used a different approach to screen haploids and parental lines from unwanted individuals (Hofinger et al., 2013). The authors used enzymatic mismatch cleavage in barley plants for the evaluation of heterozygosity in parental plants, as well as in F1 and in putative DH plants. Comparing both methodologies, HMW-GS analyses are simpler and more affordable than the enzymatic cleavage, since there is no need for enzyme extraction, PCR tests, and primer synthesis.

The production of DH plants offers the possibility to reduce the time for developing completely homozygous genotypes from heterozygotes. In order to use this undoubtable advantage for achieving homozygosity (in a single generation), the haploid production system should be efficient, produce many fertile plants from a single spike, and reduce time and costs for production. As a labor-demanding approach, it is important to carefully screen seed parents before generating mapping populations. Therefore, after HMW-GS analyses, only the heterozygotic F1-derived embryos are used for the production of the DH plants.

Glutenin analyses and their grain quality effects have been successful and widely applied for genetic diversity of storage protein and technological quality studies since their first description (Payne et al., 1979). The

present study, however, reports a different application of this method. We developed a cheap, fast, and reliable system for screening unwanted self-pollinated seed in a Brazilian wheat breeding program for the first time. Using this approach, false F1 hybrids can be avoided, either for developing mapping populations aiming at genetic studies, or even to assist breeding programs, improving the selection efficiency.

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