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AN OVERVIEW OF DNA-BASED VARIETY IDENTIFICATION AT THE CANADIAN GRAIN COMMISSION

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BACKGROUND

1. The Canadian Grain Commission (CGC) is a federal government agency that regulates all aspects of grain handling in Canada and certifies the quality, safety and weight of Canadian grain destined to domestic and export markets. Operating within the CGC, the Grain Research Laboratory (GRL) carries out scientific research to understand grain quality and grain safety and develops methods and tests for measuring and evaluating grain quality and grain safety. The GRL also monitors grain in Canada's grain handling system for quality and safety.

2. Canadian wheat is marketed by class; varieties within a class share specific functional characteristics such that class is a reliable indicator of the processing and end-use quality. The CGC maintains variety designation lists that specify which varieties are eligible for the top grades of each class. Tolerances for the presence of wheats of other classes, including non-registered varieties, are specified for each class and grade in Canada's Official Grain Grading Guide.

3. Malting barley is marketed on a variety-specific basis. Each variety has unique quality characteristics that may affect how it responds to the malting process. To achieve uniform production of high quality malt customers often stipulate varietal purity, typically 95% or greater, as a contract specification.

4. The GRL develops molecular methods for variety identification and uses these methods to assess variety composition of wheat and barley shipments to support Canada's grain quality assurance system. Monitoring of variety composition of hexaploid wheat (bread wheat) shipments is primarily accomplished using protein electrophoresis, with some supplementary DNA-based testing. For variety monitoring of durum wheat and certification of varietal purity of malting barley, DNA-based methods are used exclusively.

MICROSTATELLITE METHODS

5. Microsatellite markers have been selected from a variety of published sources. The general approach has been to develop one or two multiplexed sets of markers for each application by selecting informative markers that are compatible with respect to product sizes and co-amplification. Each forward primer is tailed with an M13 sequence such that all amplification products may be labelled by a single dye-labelled primer. Fragment analysis is performed on a Li-Cor DNA Analyzer.

6. Analyses are conducted on single kernels. DNA is extracted using an SDS-based procedure, generally in a 96-well plate format (two plates, or 192 kernels concurrently). Multiple individual kernels are examined per sample for assessment of varietal composition

or purity. Reference databases are also constructed based on multiple kernels per variety in order to allow representation of intravarietal polymorphism.

Barley

7. DNA profiling has been the exclusive basis for malting barley cargo varietal purity certification since 2006. The marker system established for two-row barley includes eight microstatellite markers that are grouped in two four-marker multiplexes. The corresponding two-row reference database currently includes more than 80 varieties. When first developed in 2003, all but one pair of non-malting two-row varieties were distinguishable using these markers. But some recently registered varieties have presented additional challenges; therefore, supplementary markers are being investigated to enhance this set.

8. Although originally developed specifically for analysis of two-row barley, this marker system is also effective for six-row malting barley cargo certification purposes; all currently recommended six-row malting varieties and most of the more than 100 other varieties represented in the corresponding six-row barley database are uniquely identified.

Durum wheat

9. All variety monitoring of durum wheat has been carried out using microsatellite-based methods since early 2009. The marker system consists of seven microsatellites multiplexed into a single PCR (Perry 2004). The corresponding database has grown to include each of the 24 currently or formerly registered Canadian varieties. All are uniquely identified with the exception of one closely related pair ('AC Pathfinder' and 'Commander') that is only partially distinguished and may require application of supplementary markers for a more specific determination. 'Pathfinder' and 'Commander' are rarely encountered; 'Pathfinder' has been deregistered and 'Commander' represented less than one half of one percent of acres seeded to durum wheat in the last crop year, a decrease relative to the previous year (Canadian Wheat Board, 2009).

Bread wheat

10. The majority of routine variety monitoring of wheat other than durum wheat continues to be conducted using protein electrophoresis. However, in some cases protein electrophoresis can only narrow down the identity of a kernel to a group of varieties and a more specific identification is required. DNA is then extracted from these same kernels for supplementary microsatellite-based testing. Two sets of multiplexed markers are used for this purpose; the seven-marker set originally established for durum wheat and an additional set of three multiplexed markers that improve the resolution among hexaploid wheat varieties. The corresponding databases include over 350 varieties, within which almost 90 percent are uniquely identified.

SNP RESEARCH

11. In recent years, research at the GRL has shifted focus to single-nucleotide polymorphisms (SNPs), which are seen to offer advantages over microsatellites for variety identification. In single-kernel applications, SNP data analysis requires less user intervention than microsatellites because computer algorithms can characterize the states of bi-allelic markers more reliably than multiple, size differentiated products; reduced user intervention facilitates higher throughput. The bi-allelic nature of SNPs may also contribute to greater portability of methods, both to alternative detection platforms and to other laboratories. Perhaps most notably, SNPs are amenable to quantitative analyses, allowing allele frequencies

to be estimated directly from pooled samples rather than from the qualitative analyses of multiple individual kernels.

12. A prototype quantitative system that operates on a ground sample of grain has been developed for two-row barley. This system consists of a set of real-time PCR assays that target 16 polymorphisms, a reference database of allele frequency profiles of pure varieties, and an algorithm to estimate the combination and proportions of varieties in a test sample based on an observed allele frequency profile. The prototype system has performed well in trials with grain mixtures of known composition and blind samples. Modifications to the marker set may be required to accommodate some of the more recently registered varieties.

13. The variety composition of commodity wheat samples is potentially much more complex than that expected for variety-specific shipments such as malting barley because a number of different wheat varieties may be delivered into the same class and individual deliveries are combined in a bulk handling system. The resulting complexity may often exceed the capabilities of a quantitative PCR-based approach. Until more suitable quantitative technologies are available, assessment of the variety composition of wheat samples will continue to rely upon single-kernel analyses and the development of single-kernel methods that offer higher throughput and reduced cost will remain a priority. Current efforts in the GRL are directed toward development of an SNP-based method for wheat using the TaqMan OpenArray genotyping system (Applied Biosystems). This represents the first application of this relatively new, high-throughput SNP genotyping technology to analysis of individual kernels of wheat.

CONCLUDING REMARKS

14. Strict control of varieties is an important component of Canada's grain quality assurance system. The CGC has supported this system through the development and implementation of DNA-based variety identification and will continue to explore new technologies in its pursuit of improved, more efficient approaches to determining the variety composition of grain.

REFERENCES

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