Biotechnology Division Newsletter

October 2014, Issue - 1

Contents

Editorial

The office bearers of the Biotechnology Division are pleased to bring this newsletter to the members. At a time when biotechnology is variously being portrayed, it remains a powerful tool for value-added and technology-driven complement to the grain industry. It is essential to pause, reflect and re-assess the immense contribution biotechnology has brought to agriculture. Moving forward it is important that we continue to explore opportunities to further strengthen biotechnological applications to cater to improvement in grain quality and yield, grain testing and certification. The intent of this newsletter is to encourage members to share anecdotes of their research highlighting use of biotechnological tools encompassing among others genomics, molecular genetics and molecular markers and marker-assisted selection in grain crop improvement for enhanced quality, yield, grain testing and analysis, purity and phytosanitary certification.

Pooba S. Ganeshan
Editor
Biotechnology Division

Application of molecular markers beyond breeding

Plant breeding has had a significant impact on agricultural plant improvement, contributing to the sustained food requirements of the world population. Breeding programs for cereal crop improvement have made tremendous strides in enhancing productivity. However, to expedite breeding efforts, newer technologies have become integral complements of breeding programs. The tools provided by biotechnology include areas like *in vitro* culture technology, transgene technology, DNA-based mapping and DNA-based marker-assisted selection (MAS), and gene cloning and sequencing technologies. Tools for the analysis of the transcriptome, proteome and metabolome have also been developed with unprecedented levels of sensitivity, accuracy and throughput. With the availability of all these tools, incorporation of specific genomic blocks can be achieved more effectively and rapidly than conventional breeding practices alone.

Sequencing projects have effectively dissected genomes and provided a wealth of information, with single nucleotide polymorphism (SNP) markers considered as the most valuable for marker-assisted selection in breeding programs. Expression profiling studies are further adding to the repertoire with functional markers. While breeding efforts based on MAS are proving successful, DNA-based markers are also useful in genetic diversity assessment in germplasm collections and in association mapping studies.

The use of molecular markers is not limited to breeding and extends equally to

diagnostic and testing possibilities in crops. For example, the need to monitor and verify the presence and amount of undesirable constituents in agricultural crops and in their derived products has created a need for the development of analytical methods for detecting, identifying and quantifying such constituents in given samples. SNP markers are valuable for addressing these needs, provided sensitive, high-throughput and cost-effective platforms are available. Among the various methods available, quantitative polymerase chain reaction (QPCR) has been proven to be one of the most successful, accurate, and powerful technique to provide accurate quantification. It is the method of choice for GMO quantification and pathogen identification.

At the Saskatchewan Research Council (SRC), Saskatoon, Canada, the Biotechnology group has developed methods using SNP markers for wheat purity testing specifically related to the quantification of wheat midge refuge blends. The wheat midge refuge strategy was initiated by the Midge Tolerant Wheat Stewardship (http://www.midgetolerantwheat.ca) to slow the development of resistance in the midge insects. Midge damage causes yield losses and downgrading of grain quality (**Fig. 1**). Resistance is predominantly governed by a single gene, *Sm1*. Growing only tolerant wheat varieties would likely lead to breakdown of the resistance within 10 years and the *Sm1* gene would become ineffective due to change in the midge

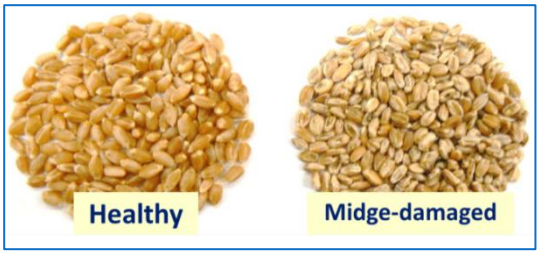

Fig. 1. Healthy and midge-damaged wheat grains

population. Adopting a refuge strategy, wherein 90% of the wheat is tolerant and 10% is susceptible, is expected to prolong the tolerance to over 90 years. This blend composition needs to be adhered to in order to sustain the refuge strategy against midge resistance buildup. To be able to use their farm saved seeds, before each planting season, farmers have to provide a certificate to the Canadian Seed Growers' Association (CSGA) showing that their seeds still contain the acceptable ratio of 90% tolerant to 10% susceptible.

SRC's Biotechnology group has identified SNP markers capable of distinguishing the two constituent varieties (tolerant and susceptible) of a given wheat midge blend and developed tests to quantify them. Initial assays were based on the Invader assay technology. This assay, while easy to perform and reliable, was based on counts of seeds having the corresponding SNP marker of the respective varieties of the blend. The method was effectively adapted to 96-well plates, with crude DNA extraction performed from individual seeds of the blend, followed by PCR and cleavase reaction. To further streamline the process, a Taqman-based QPCR approach was investigated

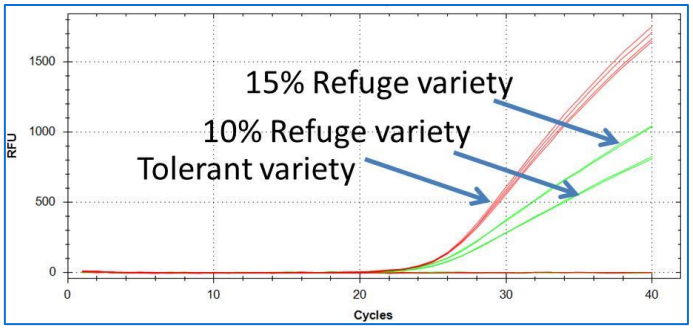

Fig. 2. QPCR amplification curves for tolerant and refuge blends

on DNA extracted from a sample of seeds of the blend. Quantification of the amount of DNA from each variety in the blend was based on a standard curve. Expected blend percentages were obtained and attested to the validity of the QPCR assay (**Fig. 2, Table 1**).

Table 1. Actual and QPCR and ddPCR quantification of blend

Blend	Actual Refuge (Susceptible) %	QPCR determination of Refuge %	ddPCR determination of Refuge %
Varietal blend A	10	10.5 (±0.24)	10.15 (±0.35)
Varietal blend B	15	15.1 (±0.31)	14.65 (±0.25)

To capture the latest technology for improving on the QPCR capabilities, we have explored the droplet digital PCR (ddPCR) platform (QX200 ddPCR System, Biorad, Inc.). The conventional QPCR, while sensitive and accurate, is still limited by the use of standard curves. The latter are time-consuming to generate and often prone to limiting the accuracy of assays due to pipetting errors and inadequate dilution series preparation. The advent of the ddPCR effectively eliminates the need for standard curves. In ddPCR, target DNA molecules in a 20 µL PCR are distributed across 20,000 nL droplets, such that some droplets have no template while other droplets have one or more copies of template DNA. At the end of 40 cycles of amplification, droplets with template exhibit high fluorescence (green HEX signal and blue FAM signal) and negative droplets show no fluorescence (grey droplets) (**Fig. 3**).

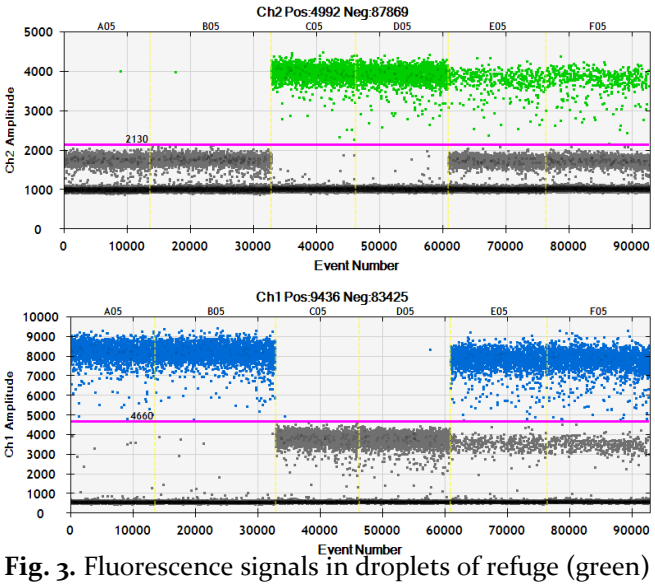

Fig. 3. Fluorescence signals in droplets of refuge (green) and tolerant (blue) midge varieties. Grey droplets are negatives.

Using the QPCR/ddPCR platforms, the SNP marker approach can be extended to the detection/diagnostics of plant pathogens and detection/quantification of allergens. For the detection of allergens, ELISA based methods are generally very valuable. However, occurrence of cross-reactivity and sometimes lack of sensitivity may preclude identification of allergens. While possibly debatable, a complementary approach of indirectly detecting DNA from a specific allergen may prove to be equally informative. Such allergen species-specific DNA detection can be applied to gluten, mustard, soybean, sesame, nuts, etc. The drawback to a DNA-based test is that in highly processed foods the ability to detect DNA may be compromised. Nonetheless, a DNA-based approach may be valuable for increased sensitivity of detection to complement current ELISA-based allergen detection methods.

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Some presentations of interest at AACCI

Monday Morning (8:30 – 10:10 a.m.)
Cereal Foods: Opportunities in the Oriental World •
Symposium • 551 AB, CC
Scientific Initiatives: Engineering & Processing; Health & Nutrition;
Ingredients & Innovations
9:50 a.m. • 12-S
Genetic variation and molecular improvement of the quality of
Japonica rice.Q. LIU (1). (1) Yangzhou University, Yangzhou,
China

Monday Afternoon (2:00 – 4:00 p.m.)
Starch Bioengineering, Structure, and Function • Science Café •
555 AB, CC
Scientific Initiatives: Biotechnology & Sustainability
27-S
Understanding starch biosynthesis in order to control its structure,
composition and properties. S. ZEEMAN (1). (1) ETH
Zurich, Switzerland
28-S
Understanding roles of starch synthases in the determination
of starch structure and function in barley. Z. LI (1), A. Regina
(1), M.K. Morell (2), S.A. Jobling (3). (1) CSIRO Plant Industry,
Canberra, ACT, AB, Australia; (2) International Rice Research
Institute, Manila, AB, Philippines; (3) CSIRO Plant Industry, Canberra,
ACT, AB, Australia
30-S
Influence of starch synthase III on the cluster structure of
maize starch.F. ZHU (1), E. Bertoft (2), K. Seetharaman (2). (1)
University of Auckland, Auckland, New Zealand; (2) University
of Minnesota, St. Paul, MN, U.S.A.

Tuesday Morning (10:40 a.m. – 12:20 p.m.)
Emerging Genetic Methods in Cereal Grain Quality
Improvement • Symposium • 552 AB, CC
Scientific Initiatives: Biotechnology & Sustainability
Organizer: Andreas Blennow, University of Copenhagen,
Frederiksberg C, Denmark
Moderators: Andreas Blennow, University of Copenhagen,
Frederiksberg C, Denmark; Christian Ruzanski, Carlsberg
Laboratory, Copenhagen, Denmark
10:40 a.m. • 70-S
Increasing resistant starch content in wheat grain using TILLING.
B. HAZARD (1), X. Zhang (1), M. Naemeh (1), J. Dubcovsky (1).
(1) University of California, Davis, Davis, CA, U.S.A.
11:00 a.m. • 71-S
Targeted mutagenesis using TALENs as means to improve cereal
grain quality. I.B. HOLME (1). (1) Aarhus University, Slagelse,
DC, Denmark
11:20 a.m. • 72-S
TALENs and CRISPR/Cas9: Genome editing tools for wheat. J.
GIL-HUMANES (1), N.J. Baltes (1), D.F. Voytas (1). (1) Univ.of
Minnesota, St. Paul, MN, U.S.A.
12:00 p.m • 73-S
Epitope tagging to study gluten deposition, interactions and
impact on grain functionality. P. TOSI (1), P.R. Shewry (2),
C. Sanchis Gritsch (2), J. Freeman (2), W. Funatsuki (3). (1)
University of Reading, Reading, United Kingdom; (2)
Rothamsted Research, Harpenden, United Kingdom; (3) NARO
Western Region Agricultural Research Center, Hiroshima, Japan

Wednesday Morning (8:30 – 10:10 a.m.)
The Use of New Technologies in the Determination and
Enhancement of Rice Grain Quality • Symposium • 551 AB, CC
Scientific Initiatives: Engineering & Processing; Quality & Analytical
Methods

9:10 a.m. • 86-S
Genetics of eating and cooking qualities revealed by genome-
wide association mapping. J. BAO (1). (1) Zhejiang University, Hangzhou, AB, China

Posters

13-P The Molecular BioMarkers for Grain Technical
Committee.
R.D. SHILLITO (1). (1) Bayer CropScience LP, Morrisville, NC, U.S.A.

114-P Molecular properties of wheat lines containing combinations
of three HMW-GS transgenes.
T. Aussenac (1), L. RHAZI (2), F. BARRO (3). (1) Inst Polytechnique LaSalle
Beauvais, Beauvais France; (2) LaSalle Beauvais, Beauvais France; (3) Instituto
de Agricultura Sostenible, Cordoba Spain

165-P Genetic differences in starch synthases control starch fine
structure and gelatinization properties in rice.
K. WANG (1), J. Hasjim (2), E. Li (1), R.J. Henry (1), R.G. Gilbert (3).
(1) Univ.of Queensland, Brisbane Australia; (2) Centre for Nutrition and
Food Sciences, Queensland Alliance for Agriculture and Food Innovation,
University of Queensland, Brisbane Australia; (3) Univ.of Queensland/
Huazhong Uni of Science & Technology, Brisbane Australia

Please join us for the: Biotechnology Division Business Meeting; Tuesday October 7, 2014 4:30 – 5:30 PM; Washington, Omni

Agenda will be provided at the meeting

Do not miss this opportunity to enjoy some beverages and network with fellow members at the: Biotechnology and Protein Division Social; October 7, 2014, 2014 5:30 – 6:30 PM; Bristol, Omni