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Certified Reference Materials

AOCS 0711-B3

Report of the certification process for

Rf1

Canola Certified Reference Materials

Third Batch

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ISO 17034:2016
A2LA Certificate 3438.01

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Abstract

This report describes the preparation and certification of the canola CRM AOCS 0711-B3 produced by AOCS Technical Services in 2016. The CRMs have been prepared according to ISO 17034:2016 and are intended to serve as control material for third party testing of canola for transformation events and for no other purpose. The purity of the Rf1 canola was verified using event-specific, qualitative PCR analysis by Eurofins-GeneScan, New Orleans, LA (an ISO 17025 Accredited laboratory). AOCS 0711-B3 is available in 0.5 ml skirted screw-cap self-sealing tubes. The canola (Rf1) DNA was extracted from clean leaves provided by BASF Agricultural Solutions Seed US LLC. The leaf DNA extract sample shall be stored in the self-sealing tube at +4 °C in the dark.

Acknowledgements

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Glossary

AOCS	American Oil Chemists' Society
Conventional Crop	A related organism/variety, its components and/or products for which there is experience of establishing safety based on common use as food
DNA	Deoxyribonucleic Acid is the linear, double-helix macromolecule that makes up the genetic material of most organisms
Detection Limit	Lowest level at which target DNA can be detected in a sample.
EC	European Commission
Genome	The full set of genes and associated DNA characteristic of an organism
GMO	Genetically modified/engineered organism: an organism in which the genetic material has been changed through modern biotechnology in a way that does not occur naturally by multiplication and/or natural recombination.
ISO	International Organisation for Standardisation
PCR	Polymerase Chain Reaction: technique used to determine whether a sample of plant tissue contains a particular DNA sequence. PCR relies on primer sets that bind to a particular target DNA sequence and a special DNA-copying enzyme

(DNA polymerase) that exponentially amplifies the target sequence for identification and measurement

Qualitative PCR PCR methods that determine the presence or absence of a specific target DNA sequence at a particular level of detection

Quantitation Limit Lowest level at which the amount of target DNA sequence in a sample can be reliably quantitated

Quantitative PCR PCR methods that estimate the relative amount of target DNA sequence in a mixture of DNA molecules

Trait: Rf1 Confers glufosinate herbicide tolerance, fertility restoration, and antibiotic resistance

Introduction

Plant biotechnology is an extension of traditional plant breeding. It allows plant breeders to develop crops with specific traits including insect, disease, and herbicide resistance; processing advantages; and nutritional enhancement. An important component for identifying these new traits is a Certified Reference Material created from leaf, seed, or grain containing the new trait as well as a CRM created from the conventionally bred matrix. The European Commission has mandated that from 18 April 2004, a method for detecting a new event derived from modern biotechnology and a CRM must be available before the EC will consider authorizing acceptance of a new crop derived from modern biotechnology. Several nations outside Europe also require grain and ingredients to be labeled above a threshold level ranging from 0.90 to 5% of authorized biotech events before accepting a shipment.

To meet the above regulatory requirements for measurement standards, AOCS 0711-B3 was manufactured according to ISO 17034:2016 and in accordance with EC No 1829/2003. The CRMs are available from AOCS.

Materials and Methods

BASF Agricultural Solutions Seed US LLC delivered 2.0 mg of Rf1 canola leaf DNA extract to AOCS. Five (5) working samples of DNA (10 µg) each were prepared from the composite and sent to Eurofins-GeneScan, New Orleans, LA (an ISO 17025 Accredited laboratory) for event-specific, qualitative PCR analysis to screen for the presence of the intended event, Rf1. This testing was for purity as well as homogeneity purposes.

The source leaf material was taken from plants which had been tested individually using a number of quality standards and was grown from seeds harvested from plants that had themselves passed the same criteria. Plants not meeting the quality standards were removed and destroyed. Leaf material was harvested from the plants which met the quality standards and frozen immediately, stored at -70 °C. The genomic DNA was

extracted from leaves of one or more plants according to CTAB-based (Doyle JJ and Doyle JL, 1987) protocol. The integrity and concentration of the genomic DNA was determined by electrophoresis in a 1.0% agarose gel and ethidium bromide-staining and compared to lambda molecular weight standards by digital imaging quantification. The concentration measurement was done in triplicate, repeated in three different gels. No indications for physical degradation were apparent and the DNA migrated at positions higher than 40 Kb.

The leaf used to manufacture the Rf1 materials was shown to contain the Rf1 event as well as the homozygosity of the event; and the absence of P35S, BXN, 2mEPSPS, cp4EPSPS , cp4EPSPS, NPTII, Barstar, and PAT sequences using PCR protocols at BASF Agricultural Solutions Seed US LLC. The Rf1 canola leaf DNA was packaged by SGS-Midwest Seed Services in sterile, 0.5 ml skirted screw-cap self-sealing tubes in aliquots of 10 µg.

AOCS used the Random Number Generator function of Microsoft Excel to select samples for verification of purity, and homogeneity. Sample numbers AOCS 0711-B3: 25, 58, 98, 140, and 207 were sent to Eurofins-GeneScan, New Orleans, LA (an ISO 17025 Accredited laboratory) for event-specific, qualitative PCR analysis to screen for Rf1 presence in the samples.

Stability

Stability of these CRMs has been listed as 1 year from the certification date. The materials were sealed and stored under refrigerated conditions, therefore not exposed to air and are expected to be stable for longer than the estimated expiration date. The stability of the leaf DNA extract material will be reevaluated annually. If the samples are still representative of the certified value, the certificates will be extended.

Results and Discussion

Sample Homogeneity

The purity data for the Rf1 homogeneity samples is presented in Table 1.

Sample	Rf1 Presence
Homogeneity Sample 1	Positive
Homogeneity Sample 2	Positive
Homogeneity Sample 3	Positive
Homogeneity Sample 4	Positive
Homogeneity Sample 5	Positive

Prepared Sample Verification

Once the bulk material was processed and packaged, five (5) samples were identified by the Microsoft Excel 2010 Random Number Generator and sent to Eurofins-GeneScan, New Orleans, LA (an ISO 17025 Accredited laboratory) for event-specific, qualitative PCR analysis. These results are presented in Table 2. These data show no contamination occurred during the packaging of AOCS 0711-B3. These results are in agreement with the homogeneity data presented in Table 1.

Table 2. Results for the verification of AOCS 0711-B3 [Rf1 canola] material as tested by Eurofins-GeneScan with Rf1 event-specific, qualitative PCR analysis.	
Sample	Rf1 Presence
AOCS 0711-B3 25	Positive
AOCS 0711-B3 58	Positive
AOCS 0711-B3 98	Positive
AOCS 0711-B3 140	Positive
AOCS 0711-B3 207	Positive

References

Center for Environmental Risk Assessment GM Database

http://www.cera-gmc.org/?action=gm_crop_database

Eurofins-GeneScan; 2219 Lakeshore Drive, Suite 400, New Orleans, LA 70122;
Telephone: +1 504 297 4330 Toll Free: +1 866 535 2730 Fax: +1 504 297 4335
<http://www.gmotesting.com>

ISO Guide 17034:2016 (E) General requirements for the competence of reference material producers

ISO 17025:2005 and ISO 17025:2017, General Requirements for the Competence of Testing and Calibration Laboratories

International Seed Testing Association, International Rules of Seed Testing: Seed Science and Technology Rules, 2012

Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed; <https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX%3A32003R1829&from=en>

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