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Certified Reference Materials

AOCS 0222-A

Report of the certification process for

B50027-4

Canola Certified Reference Material

First Batch

OECD Unique ID NS-B5ØØ27-4

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ISO 17034:2016
A2LA Certificate 3438.01

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Contents

Abstract

This report describes the preparation and certification of the canola CRM AOCS 0222-A 1g produced by AOCS Technical Services in 2022. 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. The canola NS-B5ØØ27-4 (hereinafter B50027-4) powder was provided by Nuseed Nutritional US Inc. (hereinafter “Nuseed”), West Sacramento, CA. It was prepared by grinding the bulk seed at Nuseed. The certified value of AOCS 0222-A was based on the purity of the bulk seed material and with 95% confidence, the true value is ≥ 977 g/kg. The powder was aliquoted and bottled in 6-mL glass headspace vials and sealed under a nitrogen gas environment at Illinois Crop Improvement Association. The presence of B50027-4 in AOCS 0222-A was verified using event-specific, qualitative PCR analysis by OMIC USA INC, Portland, OR (an ISO 17025 accredited laboratory). The powder CRM samples should be stored in a dry, sealed container in ambient or cooler conditions in the dark.

Acknowledgements

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Glossary

AOCS	American Oil Chemists' Society
Conventional Crop	Crop variety with no history of transgenic technology and is produced through traditional plant-breeding techniques that rely on selecting and mating parent plants possessing promising traits and repeatedly selecting for superior performance among their offspring
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
ISO	International Organization for Standardization
GMO	Organism that has had genetic sequences modified using molecular-level techniques
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 zero in on a particular target DNA sequence and a special DNA-copying enzyme (DNA polymerase) that makes enough copies of 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 reproducible.
Quantitative PCR	PCR methods that estimate the relative amount of target DNA sequence in a mixture of DNA molecules
Trait: NS-B5ØØ27-4	Synthesis of omega-3 long chain polyunsaturated fatty acids; Herbicide tolerance to glufosinate-ammonium. Also known as B50027-4

Introduction

Plant genetic modification 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 transgenic technology and Certified Reference Material must be available before the EC will consider authorizing acceptance of a new crop derived from transgenic technology. Several nations outside Europe also require grain and ingredients to be labeled above a threshold level before accepting a shipment.

To meet the above regulatory requirements for GMO determination, AOCS 0222-A was manufactured from Canola according to ISO 17034:2016 and in accordance with EC No 1829/2003, EC No 641/2004 and EC No 619/2011. This CRM is available from AOCS.

Material Processing

The B50027-4 canola seeds used to prepare AOCS 0222-A were homozygous resulting from several cycles of self-pollination. Nuseed milled ~1 kg of B50027-4 canola seed. All of the seed powder was passed through a 712 µM mesh sieve. The seed powder was delivered to AOCS who contracted Illinois Crop Improvement Association for packaging the samples. The powder was aliquoted and bottled in 6-mL glass headspace vials and sealed under a nitrogen gas environment.

Trait Verification to Certify Presence of B50027-4

The presence of the B50027-4 trait was assessed on 10 vials of AOCS 0222-A. Sample numbers that were selected were sent to OMIC USA INC, Portland, OR. (an ISO 17025

accredited laboratory) for event-specific, qualitative PCR analysis to verify the presence of B50027-4 in the samples (Table 1).

Table 1. Trait verification testing on AOCS 0222-A B50027-4 Canola performed by OMIC USA INC, Portland, OR (an ISO 17025 accredited laboratory).

AOCS 0222-A Sample	Trait B50027-4 Presence
Sample # 148	Positive
Sample # 277	Positive
Sample # 315	Positive
Sample # 489	Positive
Sample # 551	Positive
Sample # 673	Positive
Sample # 746	Positive
Sample # 862	Positive
Sample # 970	Positive
Sample # 1054	Positive

Certified Value and Measurement Uncertainty

The genetic purity of the seed lot used to produce AOCS 0222-A was assessed by Nuseed. A total of 352 canola seeds were subjected to individual seed testing for the presence of B50027-4 by qualitative event-specific PCR. 352 of the 352 seeds tested positive for the presence of B50027-4.

Purity estimation was calculated using SeedCalc8 (Remund *et al*., 2008) and corresponded to the lower bound of true % purity. The % purity in the sample was 100%, when 352 seeds were tested. Using a 95% confidence level, the true % purity of the B50027-4 seed lot was at least 97.7%. Consequently, with 95% confidence, the true value is ≥ 977 g/kg.

The Measurement Uncertainty was based on the lower bound of the true % purity and is the expanded uncertainty with a coverage factor of 2 and confidence level of 95%. It is obtained by combining the uncertainties from the purity assessment ($u_{char,rel}$), the homogeneity assessment ($u_{bb,rel}$), the transport stability assessment ($u_{sts,rel}$) and the long-term stability assessment ($u_{lts,rel}$):

$$u_{CRM,rel} = \sqrt{{u_{char,rel}}^2 + {u_{bb,rel}}^2 + {u_{sts,rel}}^2 + {u_{lts,rel}}^2}$$

$$U_{CRM} = 2 \times u_{CRM,rel} \times 1000\ g/kg$$

The expanded measurement uncertainty for AOCS 0222-A is -23 g/kg.

Homogeneity

The homogeneity of AOCS 0222-A is related to the purity of the seeds. 352 out of 352 seeds tested positive for the B50027-4 Canola event by event-specific PCR. Based on the sample purity of 100%, as determined using SeedCalc8, the batch was considered to be homogenous.

In addition, the homogeneity of the B50027-4 trait was confirmed when 10 random vials of AOCS 0222-A were selected and were sent to OMIC USA INC, Portland, OR (an ISO 17025 accredited laboratory) for event-specific, qualitative PCR analysis to verify the presence of B50027-4 in the samples (See Trait Verification section and Table 1).

Stability

Time, temperature and light are regarded as the most relevant influences on the stability of CRM (Linsinger, et al., 2001). The influence of light is mitigated by shipping and storing the vials in boxes, thus minimizing the possibility of degradation due to light. The influence of temperature is mitigated by storing the vials in a temperature-controlled room, and shipping vials at ambient temperature.

The effect of temperature and time are investigated.

A transport (short-term) stability study is conducted to assess the stability of maize CRM during transport. The temperature and time conditions in the study cover the typical conditions and the not so rare situations. The outcome of the study is considered transferable to other CRMs of similar property. Samples were subject to 3 different temperatures (4 °C (fridge), 25 °C (ambient), 60 °C (oven)) for 4 different durations (0, 1, 2, and 4 weeks). The study concluded that samples are stable at 4 °C (fridge) and 25 °C (ambient) for 4 weeks. The estimated uncertainty contribution from transport (short-term) stability is 1.0%.

A long-term stability study is conducted to assess the stability of maize CRM during storage. Samples are stored at 25 °C (ambient) and the stability of the sample is monitored as long as the samples is available. The storage temperate studied is 25 °C and the length of time to be studied is 10 years. The outcome of the study is considered transferable to other CRMs of similar property. In the initial 1-year stability study, samples were subject the storage condition for 4 different durations (0, 1, 3, 6 and 12 months). The study concluded that samples are stable at 25 °C (ambient) for 12 months. The estimated uncertainty contribution from long-term stability is 0.42%.

Stability of these CRMs has been listed as 1 year from the certification date. The materials were processed and are stored at ambient temperature, under nitrogen gas, in 27 mL glass headspace vials. These materials are expected to be stable for longer than the estimated expiration date. The stability of the powder material will be reevaluated at time of expiration. If the samples still test positive for the presence of the intended trait, the certificates will be extended.

References

Illinois Crop Improvement Association, 3105 Research Road, Champaign, IL 61826; Telephone: +1 217 359 4053 Fax: +1 217 359 4075; http://www.ilcrop.com/index.htm

ISO 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

Linsinger T.P.J., Pauwels J., van der Veen A.M.H., Schimmel H., and Lamberty A. 2001. Homogeneity and stability of reference materials. *Accred. Qual. Assur.* 6:20-25.

OMIC USA Inc., 3344 N.W. Industrial Street, Portland, OR 97210, (503) 223-1497, www.omicusa.com

Remund K., Simpson R., Laffont J-L., Wright D., and Gregoire S. Seedcalc8. 2008. https://www.seedtest.org/en/statistical-tools-for-seed-testing-_content---1--3449--1102.html