Certified Reference Materials
AOCS 1206-A and AOCS 1206-B

Report of the certification process for

Conventional and Roundup Ready® (H7-1)

Sugarbeet Seed Certified Reference Materials

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Abstract

This report describes the preparation and certification of the Sugarbeet seed CRMs AOCS 1206-A and AOCS 1206-B produced by AOCS Technical Services in 2006. These CRMs have been prepared according to ISO Guides 30-35 and are intended to serve as control material for third party testing of Sugarbeet seed or root for transformation events. The purity of the conventional and Roundup Ready® Sugarbeet was verified using DNA- and protein- based detection methods. AOCS 1206-A and AOCS 1206-B are available in 27 -mL glass headspace vials. The conventional Sugarbeet (RM-HYBCONV-B) and the Roundup Ready® (Line: RM-HYBH7-E) Sugarbeet were clean seed quality provided by Monsanto Company, St. Louis, MO, USA and KWS SAAT AG, Einbeck, Germany. The sugarbeet seed was prepared by grinding the bulk sources through a 0.5 mm screen and was then packaged under a Nitrogen environment. The ground sample shall be stored dry in a sealed container at +4° C in the dark.

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Glossary

AOCS	American Oil Chemists' Society
Conventional Variety	Crop variety with no history of genetic engineering and are produce through plant-breeding techniques that rely on selecting and mating parent plants possessing promising traits and repeatedly selecting for superior performance among their offspring
CP4 EPSPS	Glyphosate tolerance derived by inserting a 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) encoding gene from *Agrobacterium tumefaciens* strain CP4.
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 exist in a sample and be reliably tested by PCR methods. It is typically expressed as a percentage: the ratio of the number of transgenically derived genomes to the number of crop genomes times 100 percent
EC	European Commission
GMO	Organism that has had genetic sequences modified using molecular-level techniques
Genome	The full set of genes and associated DNA characteristic of an organism
IRMM	Institute for Reference Materials and Measurement
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 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.

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 biotech event and Certified Reference Material must be available before the EC will consider authorizing acceptance of a new genetically modified crop. 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 analytical requirements for GM determination, AOCS 1206-A and AOCS 1206-B were manufactured from sugarbeet seed according to ISO Guides 30-35 and in accordance with EC No 1829/2003. The CRMs are available from AOCS.

Materials and Methods

Monsanto Company (St. Louis, MO) and KWS SAAT AG (Einbeck, Germany) delivered 5 kg conventional Sugarbeet seed (Line: RM-HYBCONV-B) and 5 kg of H7-1 Sugarbeet (Line: RM-HYBH7-E) to AOCS. The materials were clean seed quality. The International Seed Testing Association's (ISTA) Seed Science and Technology Rules state a minimum of 5 primary samples (small portion taken from one point in the lot) be taken from batches up to 500 kg. Ten working samples of 100g each were prepared from the composite and sent to Eurofins GeneScan, New Orleans, LA (ISO 17025 Accredited laboratory) for event-specific qualitative PCR analysis, followed with quantitative PCR if qualitative results indicated that H7-1 was present in the sample. This testing was for purity as well as homogeneity purposes.

Four hundred seeds were randomly selected from the composite sample and analyzed with EnviroLogix QuickStix™ Kit for Roundup Ready® Sugarbeet Leaf and Seed to verify seed-lot purity.

The conventional seed (Line: RM-HYBCONV-B) was ground through a 0.5 mm screen and packaged in 27 -mL headspace vials and sealed under a Nitrogen environment. AOCS used the Random Number Generator function of Microsoft Excel 2003 to select samples for verification of purity, homogeneity, and to rule out contamination during packaging. Sample numbers AOCS 1206-A: 21, 26, 32, 34, 52, 74, 75, 80, 87, and 93 were sent to Eurofins GeneScan (New Orleans, LA) for event-specific qualitative PCR analysis to screen for H7-1 presence in the samples.

After the non-modified seed was completely ground and packaged, the H7-1 Sugarbeet was ground through a 0.5 mm screen and packaged in 27 -mL headspace vials and sealed under a Nitrogen environment. AOCS used the Random Number Generator function of Microsoft Excel 2003 to select samples for verification of purity, homogeneity, and to rule out contamination during packaging. Sample numbers AOCS 1206-B: 10, 18, 22, 24, 42, 52, 63, 85, 87, and 96 were sent to Eurofins GeneScan USA (New Orleans, LA) for event-specific qualitative PCR analysis to screen for H7-1 presence in the samples.

Stability of these CRMs has been listed as 1 year from the introduction date. The materials have been ground and are stored frozen under Nitrogen gas in a sealed, glass vial. These materials are expected to be stable for longer than the estimated expiration date. The stability of the ground material will be reevaluated at time of expiration. If the samples are still representative of the certified value, the certificates will be extended.

Results and Discussion

Sample Homogeneity

The following tables are the purity data for the homogeneity samples. The non-modified Sugarbeet (Line: RM-HYBCONV-B) is presented in Table 1. Results for the Roundup Ready® (H7-1) Sugarbeet are presented in Tables 2 and 3.

Table 2 includes the data generated from the EnviroLogix QuickStix Kit for Roundup Ready® Sugarbeet Leaf and Seed. Four hundred seeds were test and 91.87 % (373 out of 396) were positive for the Monsanto trait CP4 EPSPS. The 10 (100 g) samples sent to GeneScan USA for H7-1 screening by qualitative PCR analysis are presented in Table 3. These results, coupled with the results from seeds tested by the EnviroLogix's test strips, conclude that the only quantifiable biotech event present in Roundup Ready® Sugarbeet is H7-1.

Table 1. Results from Eurofins GeneScan for the homogeneity of conventional; Sugarbeet seed (Line: RM-HYBCONV-B).

Sample	H7-1 Presence
MC-SB-1	Negative
MC-SB-2	Negative
MC-SB-3	Negative
MC-SB-4	Negative
MC-SB-5	Negative
MC-SB-6	Negative
MC-SB-7	Negative
MC-SB-8	Negative
MC-SB-9	Negative
MC-SB-10	Negative

Table 2. Results from administering EnviroLogix's QuickStix™ Kit for Roundup Ready® Sugarbeet Leaf and Seed for the presence of CP4 EPSPS in 396 Roundup Ready® Sugarbeet seeds.

Seeds Tested	Results
Conventional	23
H7-1 Sugarbeet	373
Unreacted Strips	0
91.87% of the seeds in this line exhibit the H7-1 trait (373/396) seeds with 95% confidence	

Table 3. Results from Eurofins GeneScan for the homogeneity of H7-1 Sugarbeet (Line: RMHYBH7-E) seeds.

Sample	H7-1 Presence
MC-SB-11	Positive
MC-SB-12	Positive
MC-SB-13	Positive
MC-SB-14	Positive
MC-SB-15	Positive
MC-SB-16	Positive
MC-SB-17	Positive
MC-SB-18	Positive
MC-SB-19	Positive
MC-SB-20	Positive

Prepared Sample Verification

Once the seeds were packaged, 10 samples of each variety were identified by the Microsoft Excel Random Number Generator and sent to Eurofins GeneScan (New Orleans, LA) for event-specific qualitative PCR analysis. Table 4 verifies that no contamination was introduced during the packaging phase of AOCS 1206-A. The 10 Roundup Ready® Sugarbeet samples are presented in Table 5. These data show no contamination occurred during the packaging of AOCS 1206-B. These results are in agreement with the homogeneity data presented in Tables 1, 2, and 3.

Table 4. Results for the verification of AOCS 1206-A [conventional sugarbeet seed (Line: RM-HYBCONV-B)] as tested by Eurofins GeneScan.

Sample AOCS 1206-A	H7-1 Presence
21 of 100	Negative
26 of 100	Negative
32 of 100	Negative
34 of 100	Negative
52 of 100	Negative
74 of 100	Negative
75 of 100	Negative
80 of 100	Negative
87 of 100	Negative
93 of 100	Negative

Table 5. Results for the verification of AOCS 1206-B [H7-1 Sugarbeet (Line: RM-HYBH7-E) seeds as tested by Eurofins GeneScan.

Sample AOCS 1206-B	H7-1 Presence
10 of 100	Positive
18 of 100	Positive
22 of 100	Positive
24 of 100	Positive
42 of 100	Positive
52 of 100	Positive
63 of 100	Positive
85 of 100	Positive
87 of 100	Positive
96 of 100	Positive

References

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