TECHNICAL REPORT

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Molecular biomarker analysis — SSR analysis of sunflower

Analyse moléculaire de biomarqueurs — Méthode d'analyse SSR sur le tournesol

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Ch. de Blandonnet 8 • CP 401
CH-1214 Vernier, Geneva, Switzerland
Tel. +41 22 749 01 11
Fax +41 22 749 09 47
copyright@iso.org
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Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT) see the following URL: Foreword - Supplementary information.

The committee responsible for this document is ISO/TC 34, *Food products*, Subcommittee SC 16, *Horizontal methods for molecular biomarker analysis*.

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Introduction

Varietal identification testing requires high-quality markers, which are able to provide reproducible data using a variety of equipment, chemistries, and reagents. Accordingly, this Technical Report only addresses specific amplification methods for sunflower.

The aims of this Technical Report are to provide a list of simple sequence repeat (SSR) markers and methods of analysis for sunflower. The set of SSR markers was established based on expert advice from molecular biologists using lists of publicly-available markers (for ORS markers: Tang et al., 2002: TAG 105:1124-1136 and for SSL markers: GIE Cartisol – Paris – France), and then validated through an intralaboratory study at GEVES (Laboratoire BioGEVES, Domaine du Magneraud, CS40052, 17700 SURGERES). The method is applied in officially testing hybrid conformity as part of the process of registering sunflower varieties in the French national varieties catalogue.

This document is linked to ISO 13495 where the different steps towards method validation are listed, and acceptance criteria are defined.

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Molecular biomarker analysis — SSR analysis of sunflower

1 Scope

The methods and SSR markers included in this Technical Report can be used for testing hybrid conformity and other applications such as molecular fingerprinting of varieties and checking variety identity.

2 Principle

SSR analysis is based on the amplification and visualization of the polymorphism caused by variation in the number of repeats in a sequence motif that is two to five base-pairs in length, also known as a microsatellite. SSR analysis consists of the following steps: sample preparation, DNA extraction, PCR amplification, separation and detection of the PCR products.

3 Consumables and equipment

— 96-well or 384-well microplate

— PCR reagents (DNA polymerase, buffer, $MgCl_2$, dNTP, primers, etc.)

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— Capillary electrophoresis reagents

— Mixer/grinding mill

— Microplate centrifuge

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— Adjustable-volume micropipettes

— Micro-centrifuge for microtubes

— Capillary electrophoresis system with fluorescence detection

— Thermocycler

4 Procedure

4.1 Sample preparation

For each sample, either individual seeds or seed mixes depending on the context are ground using a suitable mill (such as an IKA A10 or a Retsch MM301[1)]).

1) IKA A10 and Retsch MM301 are examples of suitable products available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the product named. Equivalent products may be used if they can be shown to lead to the same results.

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4.2 DNA Extraction and Quantification

a) Obtain an aliquot of each homogenously ground sample; the amount required will depend upon the extraction protocol employed.

b) Extract DNA using in house protocol or equivalent

NOTE Collaborative study has been carried out with QIAGEN DNeasy® 96 Plant Kit[2)].

c) The laboratory will validate that the quantity of DNA extracted is appropriated to ensure a reliable result.

4.3 PCR amplification

Conditions optimized for ABI 9700 thermocycler.

a) Mix preparation (see Table 1).

Table 1 — Mix preparation

	Concentration	**Volume for 1X**
H_2O		3,125 µl
Buffer 10X	1X	1 µl
dNTP (10 mmol/l)	125 µmol/l	0,125 µl
$MgCl_2$ (25 mmol/l)	3 mmol/l	1,2 µl
Taq DNA polymerase (5 U/µl)	0,25 U	0,05 µl
Forward primer (10 µmol/l)	0,25 µmol/l	0,25 µl
Reverse primer (10 µmol/l)	0,25 µmol/l	0,25 µl
Vol 1x mix		***6 µl***
DNA (2,5 ng/µl)		4 µl
Final PCR vol		***10 µl***

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b) Amplification conditions (see Table 2).

A touchdown (TD) program is used: the hybridization temperature is lowered from 64 °C to 55 °C in decrements of 1 °C per cycle.

Table 2 — Amplification conditions

	10 cycles			**30 cycles**				
94 °C	94 °C	TD		94 °C				
10:00	0:30	*	72 °C	0:30		72 °C	72 °C	
		64 °C	0: 30		55 °C	0:30	10:00	10 °C
		0:30			0:30			∞

NOTE Units for times in Table 2 are in “minutes: seconds”.

2) QIAGEN DNeasy® 96 Plant Kit is an example of suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the product named. Equivalent products may be used if they can be shown to lead to the same results.

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5 Established list of SSR markers for sunflower hybrid conformity testing

5.1 Characteristics of the SSRs

Data obtained with a 3130 Genetic Analyser[3)] (Applied Biosystems).

See Table 3.

Table 3 — Characteristics of the SSRs

No.	SSR	Linkage group	Number of alleles recorded	Range of estimated allele sizes (bp)	Nei's diversity index[a]
1	ORS309	4	2	121 – 131	0,48
2	SSL003	14	6	118 – 142	0,70
3	ORS342	2	5	307 – 345	0,42
4	ORS547	5	7	178 – 191	0,68
5	ORS613	10	8	201 – 230	0,62
6	SSL171	—	6	129 – 162	0,62
7	ORS432	3	3	160 – 164	0,52
8	ORS510	9	3	248 – 259	0,37
9	ORS605	1	8	174 – 203	0,66
10	ORS329	8	2	231 – 236	0,41
11	ORS621	11	7	232 – 250	0,63
12	SSL283	—	4	130 – 141	0,76
13	ORS307	14	4	109 – 137	0,53
14	ORS811	17	3	106 – 155	0,62
15	ORS502	12	5	92 – 165	0,38
16	ORS407	16	4	426 – 447	0,43

a Values for the Nei's diversity index were obtained on 124 male and female lines.

NOTE Source is Zhang et al., 2005 [2].

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3) 3130 Genetic Analyzer is an example of suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the product named. Equivalent products may be used if they can be shown to lead to the same results.

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