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Molecular biomarker analysis — SSR analysis of sunflower

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National foreword

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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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ISO copyright office
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
www.iso.org

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Foreword

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

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.

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, MgCl₂, dNTP, primers, etc.)
- Capillary electrophoresis reagents
- Mixer/grinding mill
- Microplate centrifuge
- 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 MM301¹⁾).

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.