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BSI Standards Publication

Molecular biomarker analysis — SSR analysis of maize

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National foreword

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A list of organizations represented on this committee can be obtained on request to its secretary.

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Molecular biomarker analysis — SSR analysis of maize

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

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Contents

Page

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 maize.

The aims of this Technical Report are to provide a list of simple sequence repeat (SSR) markers and methods of analysis for maize. The SSR marker set has been validated through an intralaboratory study at GEVES (Laboratoire BioGEVES, Domaine du Magneraud, BP.52, 17700 SURGERES). Properties and sequences of these SSR markers are publicly available on the website www.maizegdb.org.

This Technical Report is linked to ISO 13495, which lists the different steps toward method validation and defined acceptance criteria.

Molecular biomarker analysis — SSR analysis of maize

1 Scope

The methods and SSR markers included in this Technical Report are suitable for applications such as testing hybrid conformity, molecular fingerprinting of varieties, and checking variety identity.

2 Principle

Simple sequence repeat (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:

a) sample preparation;

b) DNA extraction;

c) PCR amplification;

d) separation;

e) 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.]

— 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 MM301).

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.