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INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

Geneva

TECHNICAL WORKING PARTY FOR VEGETABLES

Forty-Seventh Session
Nagasaki, Japan, May 20 to 24, 2013

ADDENDUM TO MOLECULAR TECHNIQUES

Document prepared by the Office of the Union

1. The Annexes to this document contain the following presentations:

ANNEX I Naktuinbouw Project Report on Male sterility detection (in cabbage) using Molecular Techniques

ANNEX II Molecular Techniques in DUS Testing in the Group for Study and Control of Varieties and Seeds (GEVES)

[Annexes follow]

ANNEX I

NAKTUINBOUW PROJECT REPORT ON MALE STERILITY DETECTION (IN CABBAGE) USING MOLECULAR TECHNIQUES

PROJECT REPORT

Subject: Male sterility detection in Cabbage (Cabbage (*Brassica oleracea* L. convar. *capitata* (L.) Alef. var. *sabauda* L., *Brassica oleracea* L. convar. *capitata* (L.) Alef. var. *rubra* (L.) Thell., *Brassica oleracea* L. convar. *capitata* (L.) Alef. var. *alba* DC., *Brassica oleracea* L. var. *gemmifera* Zenker. *Brassica oleracea* L. var. *gongylodes* L.)) using molecular techniques.
Project leader: G. van Hameren

1. INTRODUCTION

The aim of this project is to investigate if a molecular marker can replace an expensive greenhouse test in Red cabbage, White cabbage, Savoy cabbage, Kohlrabi and Brussels sprouts. This molecular marker can also be used in the vegetables Cauliflower and Broccoli (Calabrese), but in those two crops there is no costs reduce, because in Cauliflower also the flower colour needs to be observed and in Broccoli the flowers (head) already develops in an early plant stage (first year).

2. METHOD/MATERIAL

From 47 varieties of 6 different cabbage species samples were taken. The varieties were sown within the framework of the DUS field trials of 2012. 5 pools of 5 individuals were sampled for fertile varieties and 24 individuals were sampled for sterile varieties (total of 668 samples representing 1128 individuals). The collected samples were freeze dried and DNA isolation was carried out following the standard Naktuinbouw CTAB DNA isolation protocol. DNA was not diluted before PCR protocol was carried out. The confidential PCR protocol for detection of the marker for CMS resistance was provided by Syngenta Seeds B.V. Before Naktuinbouw started to pool the fertile samples, tests were carried out (table 1) for investigating if a sterile plant can be found in a fertile pool.
Table 1: Investigation if a sterile plant can be found in a fertile pool.

Sample ID	CQ marker (CMS)	CQ internal control	conclusion
1 plt sterile; 4 fertile	26,19	18,69	Sterile
1 plt sterile; 4 fertile	24,97	19,09	Sterile
1 plt sterile; 9 fertile	30,52	18,08	Unclear

3. RESULTS

Results will be made publicly available in 2014

4. CONCLUSION

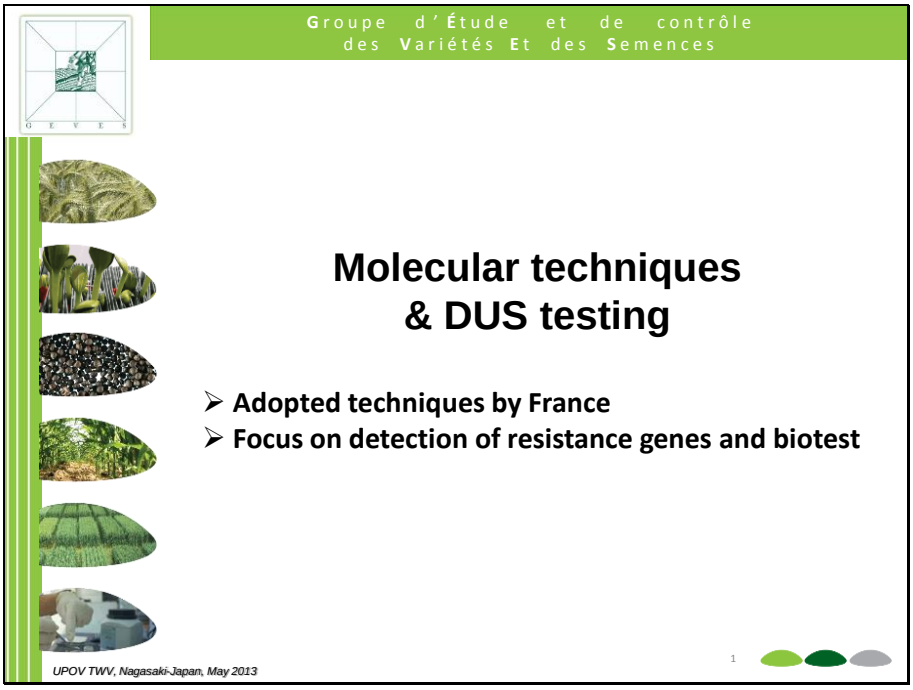
100% correlation between marker presence and Male Sterility is shown in this data set.
A sterile individual can be detected in a pool of 5 (4 fertile) individuals. A sterile plant in a pool of 10 individuals (9 fertile) is too difficult to detect. The presence of the marker is a reliable predictor for the characteristic male sterility (CMS) in cabbage (*Brassica oleracea* L. convar. *capitata* (L.) Alef. var. *sabauda* L., *Brassica oleracea* L. convar. *capitata* (L.) Alef. var. *rubra* (L.) Thell., *Brassica oleracea* L. convar. *capitata* (L.) Alef. var. *alba* DC., *Brassica oleracea* L. var. *gemmifera* Zenker. *Brassica oleracea* L. var. *gongylodes* L.).

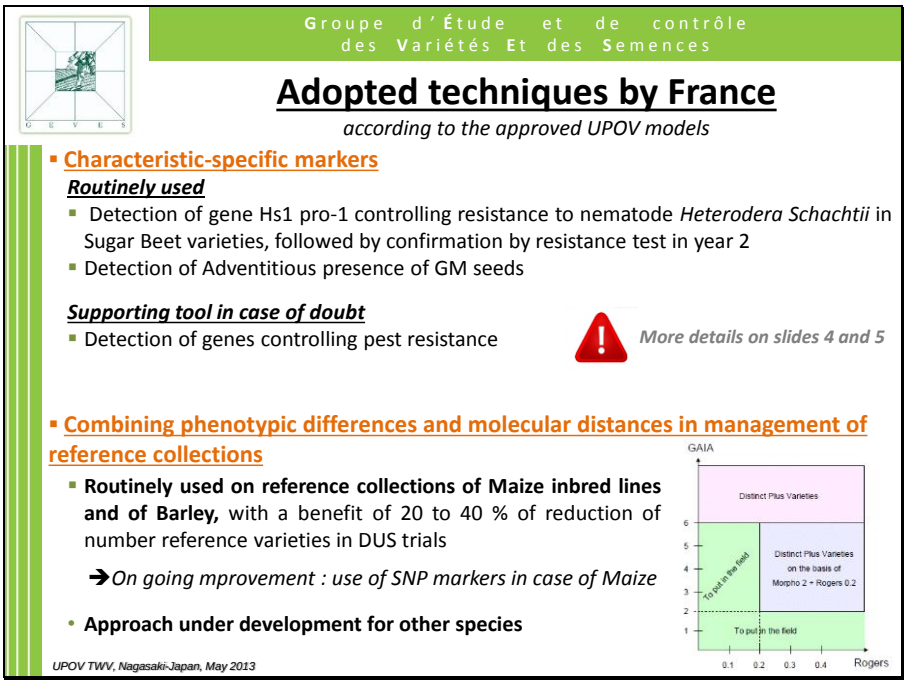
Daniël Deinum and Hedwich Teunissen
Roelofarendsveen

[Annex II follows]

ANNEX II

MOLECULAR TECHNIQUES IN DUS TESTING IN THE GROUP FOR STUDY AND CONTROL OF VARIETIES AND SEEDS (GEVES)





Groupe d'Étude et de contrôle des Variétés Et des Semences

Adopted techniques by France

according to the approved UPOV models

- **Identity control**

Routinely used

- Renewal of reference samples *(Maize, Sunflower, Sorghum, Strawberry,…)*
- Check of VCUS samples *(Pea,…)*
- Seed certification *(varietal identity, varietal purity)*
- Arbitration

On request

- Comparison of samples

➔ *Technique not applicable on mutants*
➔ *In case of differences between reference and new samples, control on phenotypic characteristics*

- **Check of hybrid conformity**
  for Sunflower, Sorghum, Wheat, Barley
- **Description of reference collections based on neutral markers**
  of Peach, Apricot, Lettuce, Wheat, Pea, Sorghum, …

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Focus on detection of resistance gene and biotest

- **Characteristic-specific markers**
  - Detection of genes Tm1, Tm2 and TM2² controlling resistance to mosaic virus in Tomato varieties

➔**100% correlated to phenotype**

  - Detection of genes controlling resistance to *Verticillium* and *Fusarium oxysporum* in Tomato and resistance to mosaïc virus in Lettuce

➔ **correlated to phenotype except for few varieties**

  - Detection of genes controlling resistance to *Meloïdogyne incognita* in Tomato

➔ **not correlated to phenotype**

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Focus on detection of resistance gene and biotest

- **Markers targeted on genes**
  - Genes known by breeders, but not described in technical files
  - Could favour some genes and decrease genetic diversity
  - A gene/marker could be present but not expressed
  - Effect of genetic background on phenotype (Mi/tomato)

  ➔ ***Only used in case of doubt to test for heterogeneity and help decision on phenotype***

- **Higher cost than biotest for 20-30 plants**

- **Resistance genes can not be used in case of description of reference collections**
  as markers are not 100% correlated to phenotype : risk of mistakes in variety description

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Thank you for your attention

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[End of Annex and of document]