UPOV
International Union for the Protection of New Varieties of Plants

E

Working Group on Biochemical and Molecular Techniques and DNA-Profiling in Particular

BMT/16/7

Sixteenth Session
La Rochelle, France, November 7 to 10, 2017

Original: English
Date: October 19, 2017

TEST OF THE POTENTIAL USE OF SNPS MARKERS ON OILSEED RAPE VARIETIES

Document prepared by an expert from France

Disclaimer: this document does not represent UPOV policies or guidance

1. The project aims to examine the potential use of SNP markers as a tool for the management of OSR reference collection. The objectives are to select and validate a suitable set of SNP markers and to asses the use of bulks of plants or seeds instead of individual plants.

2. This project is a collaborative project funded by the CPVO and work is carried out by GEVES and NIAB.

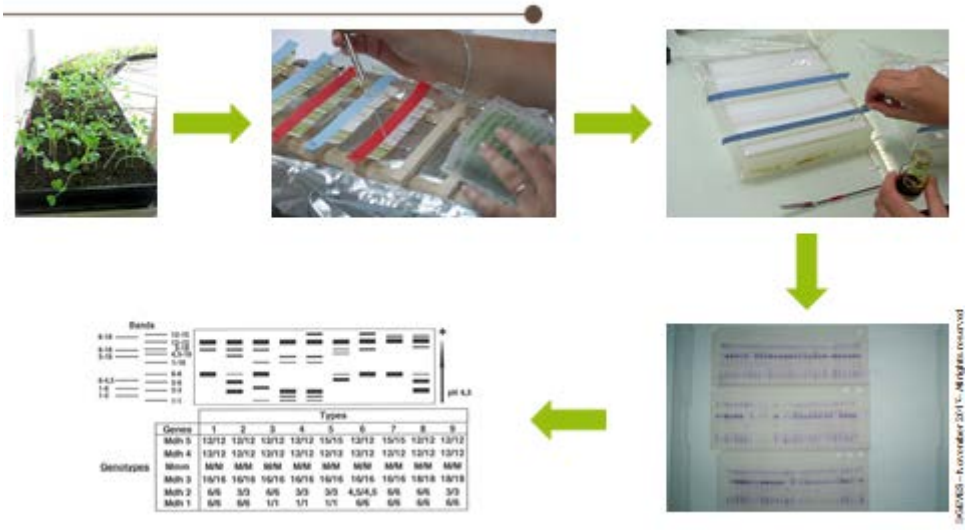
[Annex follows]

TEST OF THE POTENTIAL USE OF SNPS MARKERS ON OILSEED RAPE VARIETIES

→ UPOV_BMT/16_November 2017

Document prepared by French experts (GEVES)

Before DNA: Isozymes



Advantages of Isozymes

- No need of expensive specific lab material
- Relatively cost effective for chemicals
- Normalized methods (technical reference manual)

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

3

Why change from Isozymes to molecular markers?

- Limited number of enzyme loci for which staining protocols are available (6 used in oilseed rape at BioGEVES)
  - Low genomic coverage
  - Low discriminating power (several inbred lines with same profile)
- Time consuming (3 weeks of germination) and no flexibility
- Developmental and seasonally dependent enzyme expression may occur
- Stain constituents are highly toxic
- Increasingly difficult to find laboratories able to run isozymes
- Challenging to read zymograms especially for hybrids
- Ambiguities of reading for some enzyme profils

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

4

Why consider using SNP (Single Nucleotide Polymorphism)

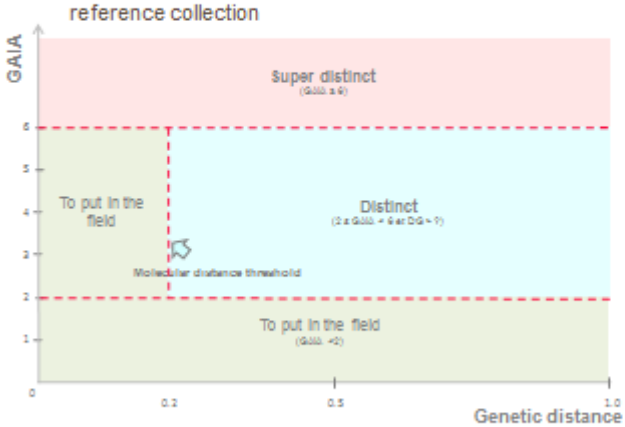
Ind 1	ACGTGTCTGTCTTAA ACGTGTCTGTCTTAA
Ind 2	ACGTGTCCGTCTTAA ACGTGTCCGTCTTAA
Ind 3	ACGTGTCCGTCTTAA ACGTGTCTGTCTTAA

- Bi-allelic
- Co-dominant inheritance
- Randomly and frequently distributed throughout the genome
- Reproducible: easy to compare profiles across different laboratories
- Stable, mutation rate lower than SSR
- Non coding or coding(Synonymous or non-synonymous mutation)
- Problem using SSR markers in an out-crossing tetraploid species and even more when bulk of seedlings (Research project "Management of winter oilseed rape reference collections" CPV5766 (2005-2007))

5

Using molecular marker for DUS test: Maize's example

- Combining molecular and morphological data for field implantation and management of reference collection



- 2 steps: combination morphological distance/genetic distance

1. GAIA

- GAIA ≥ 6: super distinct pairs
- GAIA<2: to put in the field

2. Molecular distance threshold

- If GD <threshold: to put in the field
- If GD > threshold : distinct varieties

6

SNP versus isozymes in Maize

- Comparisons of numbers of varieties to be observed in the field according to the model used for management of the reference collection with electrophoresis or SNP.

Example: DUS Trial in 2013

274 candidate lines; 3741 lines in the reference collection
⇒ more than 1 million of pairs to compare side by side in field

Morphological data (GAIA only)
5266 pairwise comparisons to grow side by side

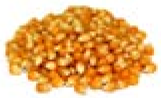
-83%

Morphological data (GAIA) + **Isozymes**
905 pairwise comparisons to grow side by side

-28%

Morphological data (GAIA) + **Genetic distances (SNP)**
647 pairwise comparisons to grow side by side

- This strategy works very well for Maize
  (Diploid and homogeneous)



What about oilseed rape ?
(tetraploid, less homogeneous than Maize …)

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

Pre-Project aims

- Objective:

  TEST OF THE POTENTIAL USE OF SNPS MARKERS ON OILSEED RAPE VARIETIES

  ① Selection and validation of a suitable set of SNP markers

  ② Determination of the analysis matrix
  i.e. Individual seed, seed bulk, individual seedling…

funded by CPVO Community Plant Variety Office

NIAB GEVES Expertise & Performance

molecular & data analysis conducted by NIAB and GEVES

9

Phase 1- Marker selection, primer design/ varieties selection

- Milestone: selection of a set of SNP markers, synthesis of molecular primers and distribution of the primers to the two laboratories
  - Based on publication of Clarke *et al.* 2016[1] and genotypes data (source : INRA)
  - 500 SNP set choice based on:
    - Non multilocus
    - High PIC (Polymorphism Information Content)
    - Homogenous genome coverage
    - Suitable flanking sequence for KASPar design

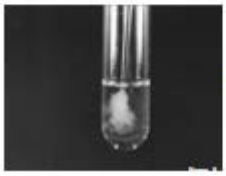
[1] Clarke et al., 2016. A high-density SNP genotyping array for Brassica napus and its ancestral diploid species based on optimised selection of single-locus markers in the allotetraploid genome. Theor. Appl. Genet. 129, 1887-1899

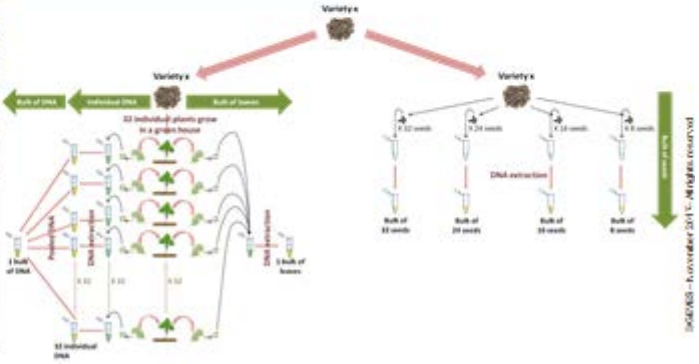
10

Phase 1- Marker selection, primer design/ varieties selection

- Milestone: selection of varieties to be tested and material requirements
  - Selection of 23 varieties (inbred lines and hybrids)

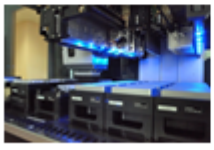
- Milestone: Growing of the materiel and DNA extraction

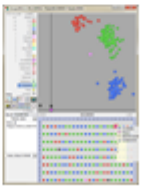




Phase 2- Amplification test/markers screening

- Milestone: amplification test to create a valid set of SNPs to be deployed by the partners
  - Amplification test on the 500 SNP (selected in silico).
  - One sample of each of the 23 varieties to be genotyped.
  - Harmonisation and standardisation of the PCR & reading conditions.





Phase 3 - Working in Bulk and size of the Bulks

- Milestone: scoring bulks on lines & scoring seed and plant bulks with validation from synthetic bulks
- Aims:
  - Test the analysis in bulk
  - Optimize bulk size.
  - Analyze reproducibility through replicated samples.
  - Study impact of bulking different type of tissues (seeds or leaves).

→ genotyping of individual seedlings, seedling bulks, seed bulks
→ genotyping of synthetic bulks of DNA
→ comparison of the results and determination of the best methodology

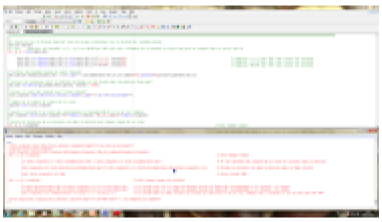
=> Test on 4 varieties and 90 SNPs

13

Phase 4 - Variety screening

- Milestone: data collation by partners for method validation
  - Genotyping with the chosen method in phase 3
    → chosen bulk type and size
    → all the selected SNP
    → all the 23 selected varieties

- Milestone: Data analysis and review of the results



14

Phase 5 – Final Meeting with all entrusted partners

- Milestone: reviewing the results
  - Possibility to work in bulks instead of single plant
  - Recommended size of bulks.
  - An optimized marker set (that could be used for collection description)
  - Proposition of an experimental protocol that could be used for a future project on combining genetic and morphological distances for the management of reference collections.
  - Discussion about a follow-up project.
  - Final meeting 7th December 2017

15

Thanks for your attention

[End of Annex and of document]