UPOV

International Union for the Protection of New Varieties of Plants

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Technical Working Party for Vegetables

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TWV/51/2

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MOLECULAR TECHNIQUES

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The Annexes to this document contain a copy of a presentations to be made at the fifty-first session of the Technical Working Party for Vegetables:
- "Management of variety collections - How we use molecular techniques in France" by an expert from France;
- "Onion- Managing the variety collection with the use of DNA information" by an expert from the Netherlands.

[Annexes follow]

Management of variety collections

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How we use molecular techniques in France

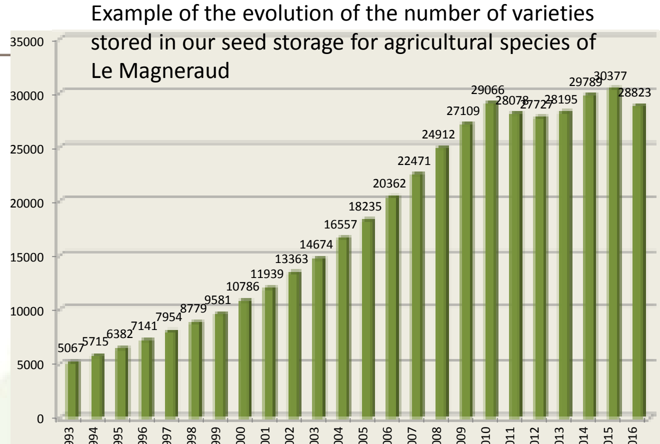
based on UPOV TC/53, Geneva, April 3-5, 2017
TWV, Leiden, July 2-7, 2017

GEVES
Expertise & Performance

Need for a more efficient management of variety collections

- **Increasing size of the variety collections :**
  => Need to improve the management of the collections
  => Need to develop news tools and procedures

Example of the evolution of the number of varieties stored in our seed storage for agricultural species of Le Magneraud



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One possible option is the use of molecular markers

- Following UPOV guidance TGP/15/1

- The objective is to develop an efficient tool, based on **a combination of phenotypic and molecular distances**, to identify within the variety collection, those varieties which need to be compared with candidate varieties in order to improve the selection of "distinct plus" varieties and so to limit the workload without decreasing the quality of the test. The challenge is to develop a secure system that:
  - (a) **only selects varieties which are similar** to the candidate varieties; and
  - (b) **limits the risk of not selecting a variety** in the variety collection which needs to be compared in the field,
    - especially when there is a large or expensive variety collection.

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Combination of morphological and genetic distances for the management of reference collection

- **Used in routine** in GEVES for maize and spring barley

- **On-going projects** in GEVES to develop the use on sorghum and wheat

- **Future possible collaborative** projects on oilseed rape, durum wheat

-> Mainly used for agricultural species,
- with large variety collections,
- with a 2 years DUS test,
- with 2 testing locations per year in France

(Not yet on vegetable species…)

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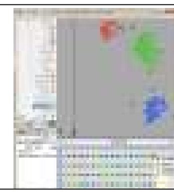
How we use molecular techniques for the management of variety collections

First DUS cycle

Description in the field

Description in our lab BIOGEVES

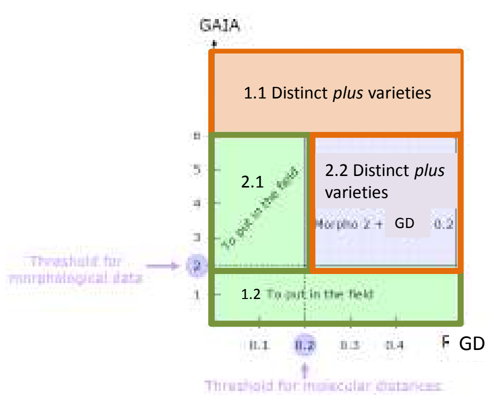


Comparison of the description with our database :
- calculation of **morphological distance** for each pair of varieties
- calculation of **genetic distance** for each pair of varieties

Combination of morphological distance and genetic distance in order to decide which pairs of varieties should be **compared side by side in the field** during **Second DUS cycle**

Combination of morphological and genetic distances for the management of reference collection

- **2 steps:**



1. **Comparison of Morphological Distances** (notes from 1 to 9)

1.1 If MD **≥ 6** — Distinct *plus* varieties

1.2 If MD **< 2** — **To put in the field**

2. **For all pairs with MD between 2 and 6, we look at the Genetic Distance**

2.1 If GD < threshold — **To put in the field**

2.2 If GD >= threshold — Distinct *plus* varieties

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Exemple of efficiency: Maize inbred lines DUS trial in 2013

274 candidate inbred lines ; 3741 inbred lines in the reference collection of GEVES
= **more than 1 million** pairs of lines to compare side by side in the field !

With GAIA only morphological data
5266 pairs grown side by side

With GAIA morphological + isoenzymes data
905 pairs grown side by side

Reduction in the number of plots and varieties grown side by side in field

With GAIA morphological + genetic distances
647 pairs grown side by side

An additional reduction of 28 % of plots grown side by side by switching from isoenzymes to genetic distances calculated from ***SNP markers.***

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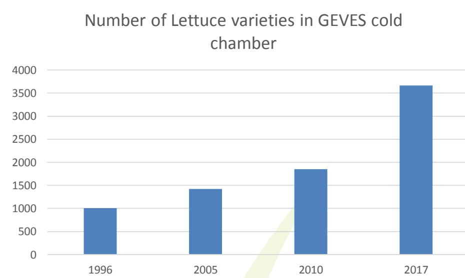
An other example : the Lettuce reference collection

- **Increasing size of the variety collections :**
  => Need to improve the management of the collections
  => Need to develop news tools and procedures



➔ **On the basis of the Maize approach**

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Raw data produced

- 2010 – 2011 Study on :
  - **500 UE varieties**, belonging to **all registrated cultigroups**, between 1950 and 2010.
    - 13 **phénotypical characterics** (QN and QL)

440 test plots (1 400 m²) ➔ 9 280 plants

- And **6 disease resistance characteristics**

69 tests *Bl* 16	462 tests *Bl* 26
253 tests *Bl* 24	310 tests *Bl* 25
35 tests LMV	

➔ **33 520 plants**

- **30 selected SSR primers**
  (10 US, 12 NL, 8 breeding companies)

500 molecular profils
➔ Bulk of 30 seeds / variety

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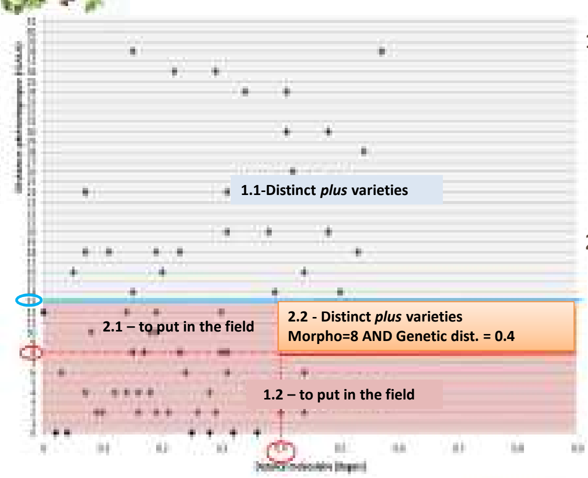
2011- Lettuce combined approach



1. **Comparison of Morphological Distances**

1.1 If MD ≥ 13 — Distinct *plus* varieties

1.2 If MD < 8 — To put in the field

2. **For all pairs with MD between 8 and 13, we look at the Genetic Distance**

2.1 If GD < 0.4 — To put in the field

2.2 If GD ≥ 0.4 — Distinct *plus* varieties

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Lettuce Conclusion and Prospects

Close genetic distances between varieties are identified. The threshold of 0.4 does **not allow** the development of an **effective tool** to **structure the reference collection**.

The global treatment of all culti groups is not more effective in structuring than the structuration thanks to morphological and diseases resistance characteristics.

This result is not surprising because Lettuce is a diploid, autogamous, highly worked species, whose gene pool is not very extensive.

Nevertheless, a new approach focus on a large cultigroup, such as Butterhead Lettuce or Crisphead Lettuce, could perhaps allow additional structuring elements. *To follow…*

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Conclusion… Which use of the molecular markers ?

- **Efficient tool to co-manage (*combined approach*) variety collections ?**

Depending on factors : species, diversity range in the cultigroup, primer types…

According the retained Genetic Threshold (GenTh), the strength of the phenotypical characteristics, the **combined approach** can be MORE or LESS effective:

- in **maize** (GenTh= 0.2, which allows 75% saving of implantation)
- in **barley** (GenTh= 0.3, which allows 50% saving of implantation)
- in **lettuce** (GenTh= 0.4, which not really allows saving of implantation)

- **Interest of molecular markers for OTHER purposes** such as
  - Maintenance control,
  - Sample identity control,
  - Hybrid conformity,
  - Essential derivation
  - Infringement proceeding
  - ...

To be considered independently …

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[Annex II follow]

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Onion varieties

Managing the variety collection with the use of DNA information

Background and goal of this project

Background:
- We use, to manage our Onion Variety Collection, types of onion that refer to their geographical or regional origin
- We need a confirmation that those types can be used for grouping the varieties in the collection
- In onion we usually have to select a large number of similar varieties

Goal:
- The goal of this project was to find out whether there are markers that correlate with these different types, and so: can we identify groups on the basis of genetics

In practice: Grouping of onion varieties and selecting similar varieties

- Use of TQ information
  - Grouping characteristics:
    - Seed propagated varieties only: Bulb: tendency to split into bulblets
    - Bulb: shape (in longitudinal section)
    - Bulb: basic color of dry skin
    - Bulb: number of growing points per kg
    - Male sterility
  - Other TQ characteristics
  - Similar varieties
  - Extra information in paragraph 7:
    - Type: 1 onion set production/2 silver skinned/3 normal sowing onion/4 overwintering/5 other
    - Day length conditions which favour full bulb development:
    - Suitability for storage
  - Usually no information in 4.1 given on the origin of the variety

Grouping of onion varieties: Geographical types

- From experience in the trials and extra info from applicants during trial visit we often have an idea or know about the geographical origin of the application. We group our varieties and applications according to geographical origin of the genetics, like Rijnsburger, Spanish, American, Australian/New Zealand, Japanese or crosses between.
- Within those types we finetune the order of the varieties using TQ information for the applications and our description of varieties
- A complication is that most of the characteristics are QN, some are PQ

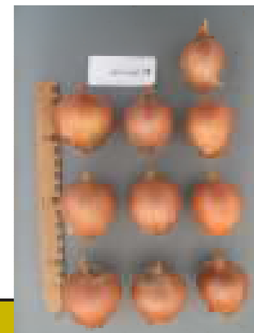
Grouping of onion varieties: A solid basis for Geographical types

However we need a solid basis for our typing of onion.

- We had the opportunity to test 105 varieties of onion using SNP markers:
  - 93 markers out of 2271 were selected, at random positioned on 8 chromosomes, and considering their differentiating ability.
  - Per variety 12 individuals were tested.
  - SNP’s and samples with too many missing data were deleted from the analysis.

Choice of onion varieties

- We chose varieties of which we quite sure they are more or less purely belonging to our ‘geographical genetic types’, and varieties we consider to be of mixed origin.



Choice of Onion varieties

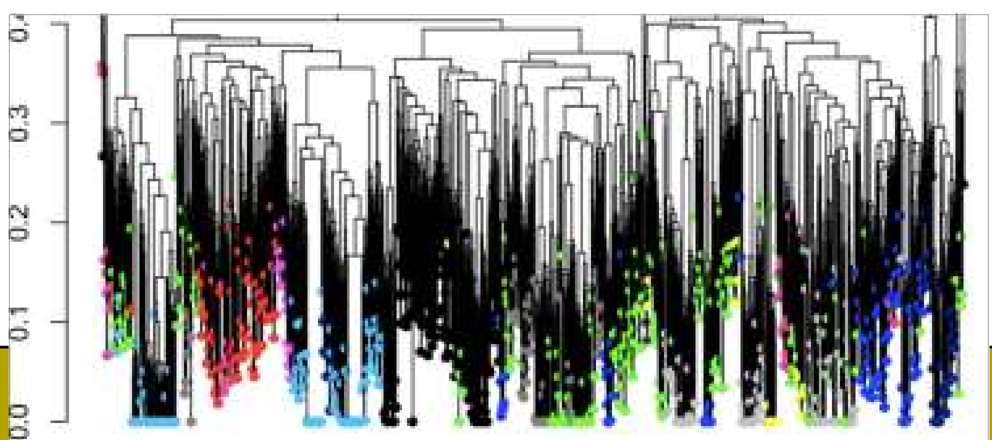
- Varieties of many different types
- Per type a few varieties, preferably of different maintainers

Choice of Onion varieties

Type	Number of varieties
Tropical red	5
Grano	15
Short day white	3
Japanese	16
No class (mixed)	31
Ailsa Craig	1
Spanish	14
American	8
Pukekohe Long Keeper	2
Rijnsburger	10
Long day white	4

Dendrogram (condensed!)

Varieties cluster together, 'pure' types cluster, mixed types (light green) can be found throughout the dendrogram



Conclusions

- We can identify Geographical groups on the basis of their genetics.
- Varieties which need the same day length conditions group together.
- Skin color was not ‘detected’ by the markers used: In Rijnsburger type yellow as well as white and red varieties could be found.

Follow up

- In this year’s trial we put the varieties that belong according to their genetics to another type, in this type
- Analysis of the data without the ‘no class’ mixed type varieties
- Analysis of the ‘no class’ mixed type varieties
- Possibly in future:
  - More study about reduction of number of similar varieties
  - Study about use and reliability of genetical characteristics for more efficiency in DUS testing

Credits

to our colleagues:

Hedwich Teunissen
Miriam van der Wee
Menno Hoekstra
Daniël Deinum

Thank you

Quality in Horticulture

[End of Annex II and of document]