UPOV

International Union for the Protection of New Varieties of Plants

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Technical Working Party on Testing Methods and Techniques **TWM/3/8**

Third Session
Beijing, China, April 28 to May 1, 2025

Original: English
Date: March 26, 2025

USE OF DNA DATABASES AT NAKTUINBOUW TO IMPROVE DUS WORK

Document prepared by an expert from the Netherlands (Kingdom of)

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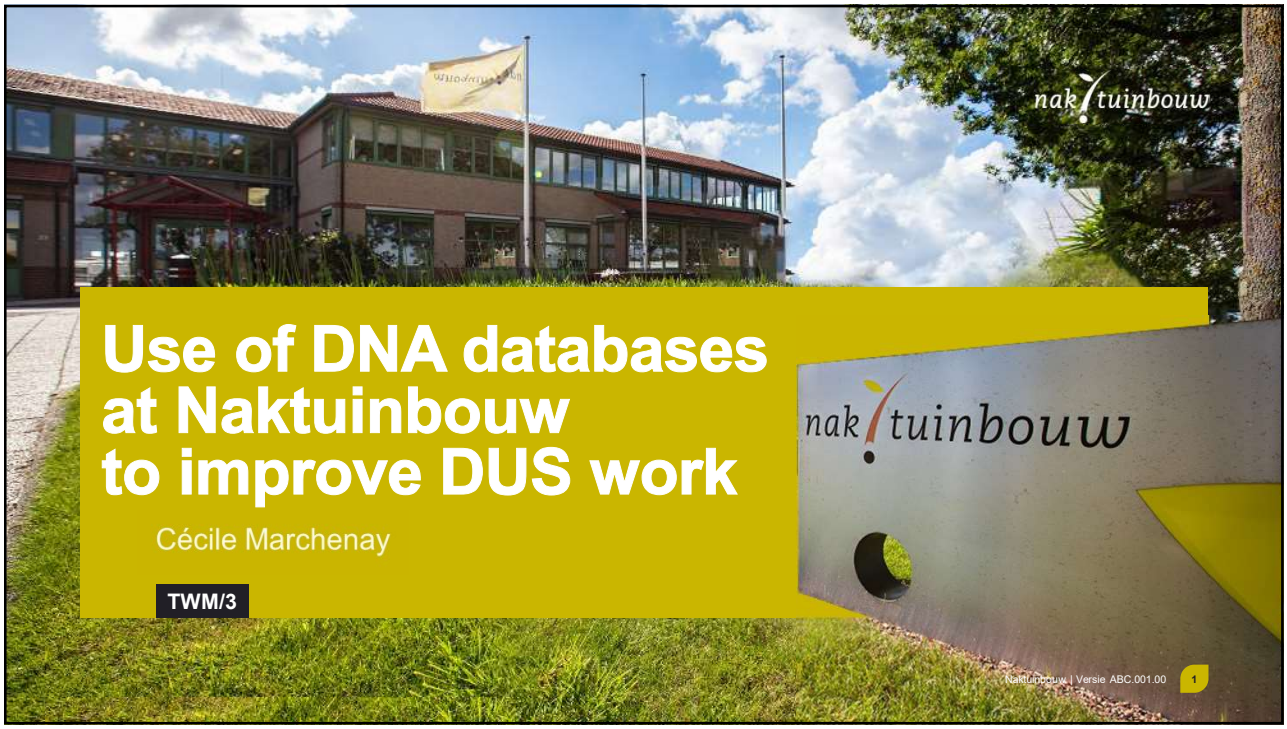
1. Developing and using DNA databases in combination with morphological work for Distinctness, Uniformity, and Stability (DUS) ensures more consistent and reliable results, facilitating the protection of Plant Breeders' Rights. Choice of crops, criteria and process to build a DNA database, with few examples currently used at Naktuinbouw and our vision concerning the future of DNA databases will be illustrated in this presentation. Additionally, international collaboration, data sharing, and open discussion with the audience will play a crucial role in maximizing the benefits of DNA databases for DUS work by incorporating diverse feedback and perspectives.

2. The annex to this document contains a copy of a presentation “Use of DNA databases at Naktuinbouw to improve DUS work”, to be made by an expert from the Netherlands (Kingdom of), at the third session of the TWM.

[Annex follows]



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Outline

- Why to develop and use DNA databases to support DUS examination?
- Choice of crops
- Criteria and process to build a DNA database
- Few examples
- What's next

2

Why to develop and use DNA databases to support DUS examination

- Increase reliability: expand the collection of reference varieties by the use of DNA databases when living reference collection are limited
- Safely exclude varieties of common knowledge from the trial to reduce workload and costs without decreasing quality of the DUS test.
- Efficient choice of similar varieties before the growing trial (from 2 to 1 DUS trial?)
- Check on duplicates

➢Based on an international accepted method: UPOV model 2:

Combining phenotypic and molecular distances in the management of variety collections

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Choice of crops to develop a DNA database

- Vegetatively propagated crops (large investment on maintenance of collection, phytosanitary issues) (e.g. potato, cannabis)
- Crops where current set of morphological characteristics does not distinguish varieties (e.g. grasses)
- More efficiency, more reliability in the process of selecting comparing varieties for the DUS growing trial (e.g. bean)
- Large number of DUS applications. DUS fee has to cover maintenance of database (e.g. tomato)
- Identity confirmation for certified vegetatively propagated material (fruit trees)

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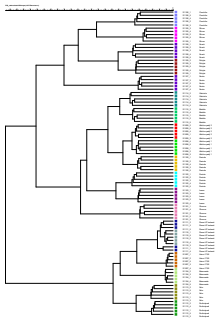
Criteria and process to build a DNA database

- Choice of varieties
  - Representative for maximum diversity in assortiment
  - Known related varieties
- Choice of technology (crop-dependent)
  - Wishes:
    - Fast, reliable, robust, cost effective
    - Harmonization between labs
    - Genotypes of protected varieties is confidential information
- Choice of maker system (depends on genome size, complexity…)
  - SSR (Simple sequence repeats): labor intensive, expensive, limitations in discriminative power
  - SNP (Single Nucleotide Polymorphism): Bi-allelic markers that are scored co-dominantly, occur frequently throughout the genome



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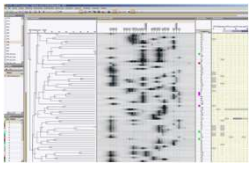
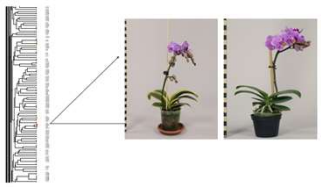
Orchid

DNA database created (SSR-markers based):

- To minimize the risk to grant PBR on existing varieties
- To help in the management of the variety collection
- To check stability when renewing reference material
- To avoid sending plant material over the world
- As fast reference for suspected infringment





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Rose (greenhouse cut flowers)

Challenges:

- No living reference collection, no full overview of common knowledge
- Photodatabase of limited value, lack of variation other than colour
- Distance between varieties become smaller
- Phytosanitary restrictions of importing living reference material

DNA database created (after SNP set validation)

➢ To make the quality of the distinctness decision even more reliable, reduces the risk of unjustified granting of PBR

➢ Wish to enlarge the database in the future with outdoor garden roses

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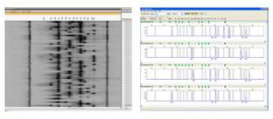
Potato

DNA database (SSR-markers based)

- Use to support DUS examination, +/- 4000 varieties already included
- Threshold values (similarity = >85%) have been determined, only a limited set of varieties needed in the field for comparison

➢ In most cases the duration of DUS trial can be reduced (cost reduction) and compensated by added value molecular profile

➢ Expanding a DNA database for potato is easier than continuously exchanging/storing potatoes, which means a larger “up to date” reference collection is available



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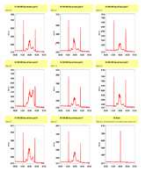
French bean (follow-up project ongoing)

- Selection of 96 bean varieties representing the genetic diversity for GBS analysis.
- Optimization and validation of a SNP set with enough distinguishing power.
- Creation of a SNP profile for all the varieties of the collection at Naktuinbouw (+/- 700)
- Genotyping new applications

➢ Goal: less time for selection of comparing varieties, better quality of side-by-side comparison, from 2 to 1 DUS trial ?



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What's next

- Molecular technologies used in synergy with morphological examination, make use of the best of both methods
- For vegetable and agricultural crops, wish to increase the use of DNA databases
  - ➢ Reduction in time and cost of DUS examination:
    Combining genetic distances + one DUS trial
- Importance of international cooperation on this matter

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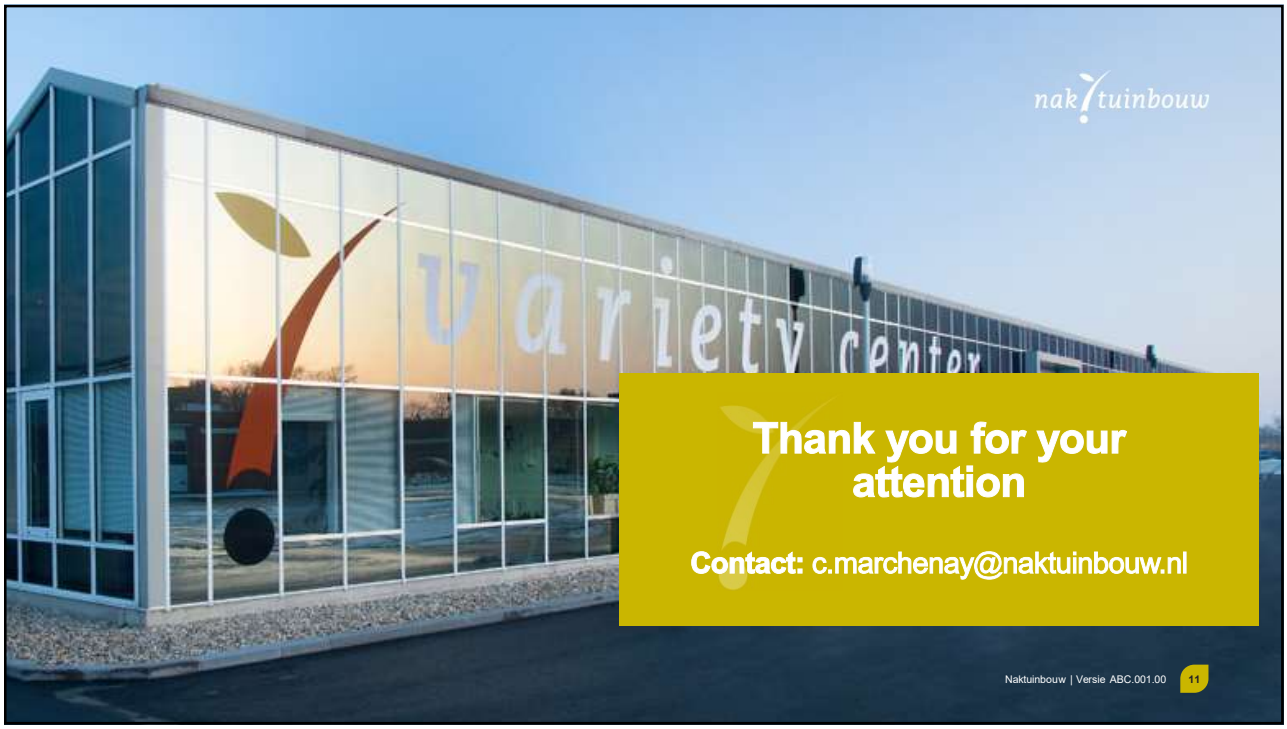

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