



BMT/14/17

ORIGINAL: English

DATE: November 7, 2014

INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

Geneva

**WORKING GROUP ON BIOCHEMICAL AND MOLECULAR TECHNIQUES
AND DNA-PROFILING IN PARTICULAR****Fourteenth Session
Seoul, Republic of Korea, November 10 to 13, 2014****IMPROVING EFFICIENCY OF DUS TESTING OF PERENNIAL RYEGRASS BY COMBINING
MORPHOLOGICAL AND MOLECULAR VARIETY DISTANCES***Document prepared by an Expert from the Netherlands**Disclaimer: this document does not represent UPOV policies or guidance***OBJECTIVE**

1. Limiting the number of reference varieties by a combination of molecular distances (based on SNP-markers) and morphological distances (based on field observations) will improve the efficiency of DUS testing of grasses - a pilot-project.

DESCRIPTION OF THE PROJECT

2. Varieties need to be tested for DUS (i.e. Distinctness, Uniformity and Stability) to be registered for Plant Breeder's Rights or for listing. DUS testing of perennial ryegrass (*Lolium perenne*) is inefficient in terms of labour and cost involved.

3. Perennial ryegrass is an obligate outbreeding species. Varieties are synthetic populations generated by poly-crosses of selected individual clones. Varieties of perennial ryegrass are genetically diverse. Nevertheless in practice it is very hard to distinguish varieties by morphological characteristics only. There are very few useful visual characteristics and new candidate varieties have to be compared with a large and increasing number of varieties in common knowledge.

4. Distinctness is determined by measuring numerous characteristics (such as length and width of the flag leaf, plant height, etc.) over a number of years on 60 individual, spaced plants per variety. Varieties are often not significantly distinct as these characteristics are highly variable not only for genetic reasons but for environmental or methodological reasons also.

5. However varieties which cannot be distinguished by morphological traits are often clearly distinct for agronomical performance (yield, persistence, resistances, etc.), illustrating once more the limited power of the methodology used in traditional DUS testing of outbreeding grass species.

6. Efficiency of DUS testing can be enhanced by reducing the number of reference varieties. Reference varieties are now planted in two out of every three years (cyclic planting). Varieties are directly compared twice in three years and data for missing years are compensated for by the use of historic data from earlier years. Condensing the dataset by direct comparison of the most relevant reference varieties will enhance the discriminative power and reliability of the test.

7. Managing the reference collection by use of a combination of morphological distances and molecular distances is generally accepted by UPOV nowadays (UPOV, 2011). Recent publications by Wang et al.

(2013) in Australia and by Byrne et al.(2013) in Denmark show the potential of variety discrimination of grasses (*Lolium* spp. in particular) by SNP profiles. The Australian set of SNP-markers might be used to reduce the number of reference varieties by combining molecular distances and morphological distances comparable to the methodology developed for potatoes (Bonthuis, 2011).

8. Biosciences Research (Bundoora, Victoria, Australia) will analyse 20 varieties of *Lolium perenne*, based on their set of SNP-markers. For practical and financial reasons (for future use in DUS testing) varieties will be genotyped on the basis of seed bulks. Genotyping seed bulks will generate information on allele frequencies within populations which is thought to be sufficiently powerful for varietal discrimination (Byrne et al., 2013). 20 varieties will make $(20 \times 19 / 2 =)$ 190 variety pairs. Pairwise comparison of fingerprint profiles as well as pairwise comparison of morphological data will result in threshold values for distinctness. Molecular profiles and morphological profiles are not necessarily correlated (Roldán-Ruiz, 2001) but they can be supplementary in decisions on distinctness. Phenotypic data are available from DUS testing at Tollebeek (The Netherlands) in previous years. Molecular data will be analysed by Biosciences Research (Pembleton et al., 2013). Both molecular and morphological data will subsequently be analysed by Biometris (Wageningen UR, Netherlands). Pairwise comparison of varieties by combining morphological and molecular distances will result in a threshold for varietal differences beyond which reference varieties can be excluded from field testing.

9. The project is a pilot project to explore the potential use of today's high-throughput technology in the management of the reference collection. If results are sufficiently positive the project can be expanded in cooperation with other UPOV partners (e.g. in Europe, Australia, New Zealand a.o.).

Henk Bonthuis (projectleader)
Manager DUS/VCU testing of Field crops
h.bonhuis@naktuinbouw.nl
Naktuinbouw, Wageningen, The Netherlands

LITERATURE

Bonhuis H (2011) Combining morphological and molecular distances in the management of the reference collection of Potato. 13th UPOV-BMT, Brasilia. pp. 7.

Byrne S, Czaban A, Studer B, Panitz F, Bendixen C and Asp T (2013). Genome Wide Allele Frequency Fingerprints (GWAFFs) of populations via Genotyping by Sequencing. PLOS one 8(3): e57438. Doi:10.1371/journal.pone.0057438

Pembleton LW, Cogan NOI and Forster JW (2013). StAMPP: an R package for calculation of genetic differentiation and structure of mixed-ploidy level populations. Molecular Ecology Resources - Doi:10.1111/1755-0998.12129.

Roldán-Ruiz I, Van Eeuwijk FA, Gilliland TJ, Dubreuil P, Dillmann C, Lallemand J, De Loose M and Baril CP (2001). A comparative study of molecular and morphological methods of describing relationships between perennial ryegrass (*Lolium perenne*) varieties. Theor. Appl. Genet. 1003: 1138-1150.

UPOV (2011). Possible use of molecular markers in the examination of Distinctness, Uniformity and Stability (DUS). UPOV/INF/18/1. pp.26.

Wang J, Pembleton LW, Baillie RC, Drayton MC, Hand ML, Bain M, Sawbridge TI, Spangenberg GC, Forster JW and Cogan NOI (2013). Development and implementation of a multiplexed single nucleotide polymorphism genotyping tool for differentiation of ryegrass species and cultivars. Mol. Breeding - Doi:10.1007/s11032-013-9961-6

[End of document]