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INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

Geneva

TECHNICAL WORKING PARTY FOR VEGETABLES

Fiftieth Session
Brno, Czech Republic, June 27 to July 1, 2016

REVISED ADDENDUM TO

MOLECULAR TECHNIQUES

Document prepared by the Office of the Union

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This document contains presentations that were made at the fiftieth session of the Technical Working Party for Vegetables (TWV), as follows:

- Annex I: "Reports on developments within UPOV", by the Office of the Union;
- Annex II: "Efficient DUS Test in French Bean *(Phaseolus vulgaris L.)* by using Molecular Data", by an expert from the Netherlands

[Annexes follow]

ANNEX I

REPORTS ON DEVELOPMENTS WITHIN UPOV
BY THE OFFICE OF THE UNION

OECD/UPOV/ISTA JOINT WORKSHOP ON MOLECULAR TECHNIQUES

WORKSHOP 1

(UPOV BMT/14 - Seoul, Republic of Korea, November 12, 2014)

Agreed:

1. would be useful to develop a joint document explaining the principal features (e.g. DUS, variety identification, variety purity, etc.) of the systems of OECD, UPOV and ISTA.

WORKSHOP 2

(OECD Seed Schemes- Paris, June 8, 2016)

OECD Annual Meeting approved:

1. develop a joint document explaining the principal features (e.g. DUS, variety identification, variety purity, etc.) of the systems of OECD, UPOV, **AOSA** and ISTA

WORKSHOP 1

Agreed:

2. to propose an inventory by UPOV, OECD and ISTA of the use of molecular marker techniques, by crop, with a view to developing a document containing that information, in a similar format to UPOV document UPOV/INF/16 “Exchangeable Software”.

WORKSHOP 2

OECD Annual Meeting approved:

2. to carry out a joint inventory by UPOV, OECD, AOSA and ISTA of the use of molecular marker techniques, by crop, with a view to developing a document containing that information. The OECD will contribute to the document by sharing the ongoing list of molecular techniques used by NDAs and continuously collected by the Secretariat.

WORKSHOP 1

Agreed:

3. to propose to invite UPOV, OECD and ISTA to develop lists of possible joint initiatives in relation to molecular techniques. It was noted that, in the case of UPOV, the list could be drafted by the BMT at its fifteenth session, subject to approval by the Technical Committee.
   - The UPOV TC, at its fifty-second session, agreed that the BMT should include the development of a list of terminology (definitions) used by OECD, UPOV and ISTA in the list of joint initiatives
   - The UPOV BMT, at its fifteenth session, agreed that possible future collaboration between UPOV, OECD and ISTA might include the harmonization of terms and methodologies used for different crops and the possible development of standards, after agreement by these organizations.

WORKSHOP 2

OECD Annual Meeting approved:

3. To develop a list of terms and their definitions as used by OECD, UPOV, AOSA and ISTA and to make an attempt to harmonise these.

WORKSHOP 1

Agreed:

4. would be useful for mutual understanding, to repeat the joint workshop at relevant meetings of the OECD and ISTA

WORKSHOP 2

OECD Annual Meeting approved:

4. to consider organising another similar workshop [in OECD] in three years time

UPOV BMT/15

- OECD, ISTA and UPOV had different objectives and cooperation between the organizations in the use of molecular techniques would need to reflect that. However, the BMT agreed that it would be important to explore circumstances in which the same techniques and information could be used. In the first instance, it agreed that it would be more effective to explore such possibilities on the basis of real situations rather than at a theoretical and institutional level.
- welcomed the proposal by the Netherlands to organize a practical workshop in 2017, with support from UPOV, OECD and ISTA, to explore how molecular techniques might be applied in an efficient way for UPOV, OECD and ISTA purposes.

WORKSHOP 2

OECD Annual Meeting approved:

- endorsed the proposal of the Netherlands to organise a practical workshop in 2017, with support of the OECD, UPOV and ISTA, to explore how molecular techniques might be applied in an efficient way for UPOV, OECD and ISTA purposes.

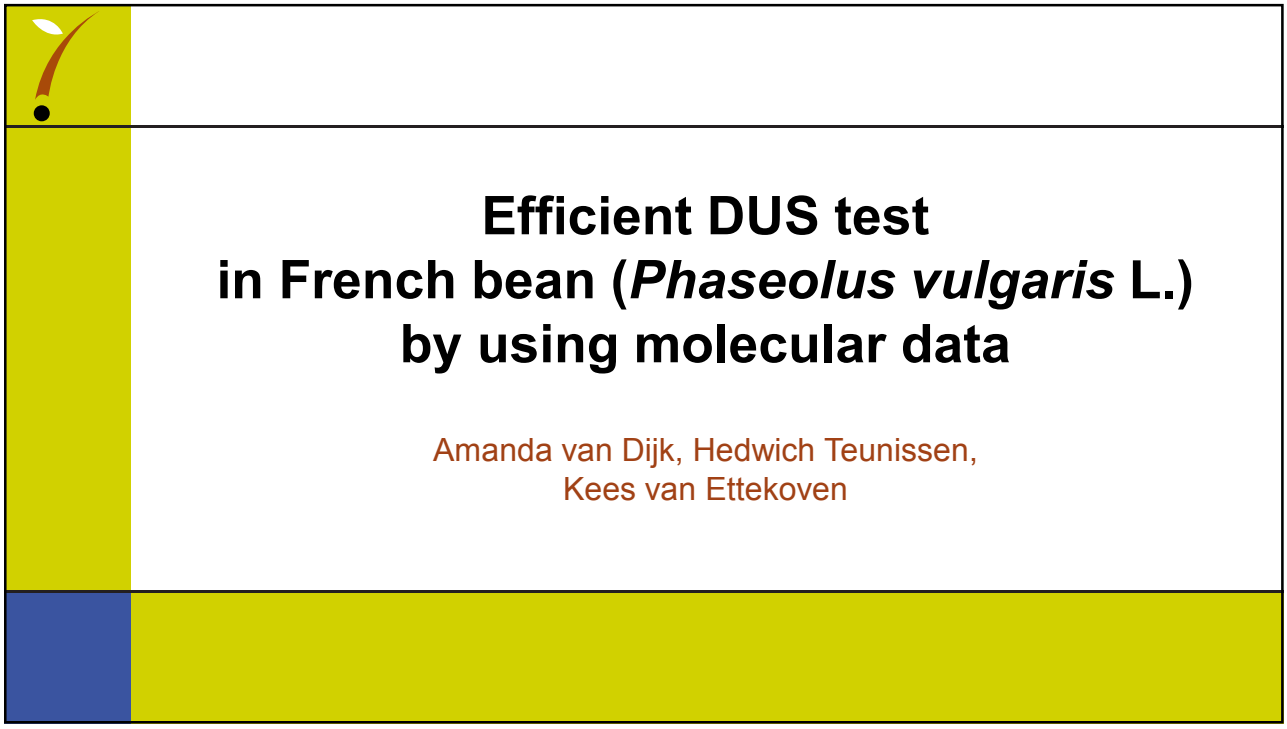
for consideration

- Organize OECD/UPOV/ISTA JOINT WORKSHOP ON MOLECULAR TECHNIQUES (proposal 4)
- Proposals 1, 2 and 3
- Support for Netherlands to organise a practical workshop in 2017, with support of the OECD, UPOV and ISTA, to explore how molecular techniques might be applied in an efficient way for UPOV, OECD and ISTA purposes

[Annex II follows]

ANNEX II

EFFICIENT DUS TEST IN FRENCH BEAN (Phaseolus vulgaris L.) BY USING MOLECULAR DATA
BY AN EXPERT FROM THE NETHERLANDS



Efficient DUS test in French bean (1)

- Many varieties in same group (TG/12/9 Rev. 2): dwarf, white flower, round, green pod without string, white seed, resistant to BCMNV. And many of them also resistant to *Colletotrichum* and to *Pseudomonas*.

The following have been agreed as useful grouping characteristics:

(a) Plant: growth type (characteristic 3)
(b) Flower: color of standard (characteristic 16)
(c) Pod: shape in cross section (through seed) (characteristic 22)
(d) Pod: ground color (characteristic 24)
(e) Pod: stringiness of ventral suture (characteristic 29)
(f) Seed: number of colors (characteristic 43)
(g) Seed: main color (largest area) (characteristic 44)
(h) Seed: secondary color (characteristic 45)
(i) Resistance to *Bean common mosaic necrosis virus* (BCMNV) (characteristic 50)

- In total 209 varieties known in this group, yearly 6 to 12 new applications at Naktuinbouw.

Efficient DUS test in French bean (2)

- Information on other characteristics, as stated in the (national) TQ, is being used for a careful selection of reference varieties for the field trial
  - Leaf: green color
  - Flower: size of bracts
  - Pod: length
  - Pod: width
  - Pod: intensity of ground color
  - Seed: weight

G	2.	Plant: growth type	1 dwarf/2 climbing	[ ]
	8.	Leaf: intensity of green colour	1 very light/3 light/5 medium/7 dark/9 very dark	[ ]
	14.	Flower: size of bract	1 very small/3 small/5 medium/7 large/9 very large	[ ]
G	15.	Flower: colour of standard	1 white/2 pinkish white/3 pink/4 violet	[ ]
	17.	Pod: length (excluding beak)	1 very short/3 short/5 medium/7 long/9 very long (..... cm)	[ ]
	18.	Pod: width at maximum point	1 very narrow/3 narrow/5 medium/7 broad/9 very broad (..... mm)	[ ]
G	21.	Pod: shape of cross section (through seed)	1 narrow elliptic/2 elliptic to ovate/3 cordate/4 circular/5 eight shaped	[ ]

part of NL TQ

- Information in TQ not always complete and/or accurate: e.g.
  - very dark green leaves (9) and pods 14,5 cm in DUS test,
  - dark green leaves (7) and pods 12-13 cm in TQ

Trial results

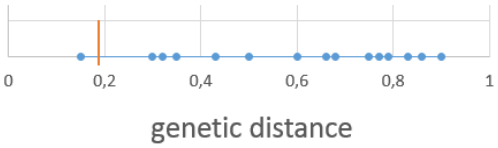
Image analysis

Efficient DUS test in French bean (3)

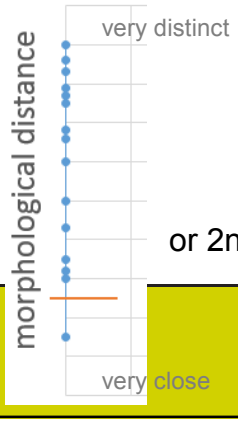
- Based on grouping characteristics and careful use of other information in TQ **15 to 20** reference varieties selected per application.
  - Expensive (**2 – 3 hours** per application for an expensive DUS expert)
  - Too many to have a good side by side comparison
  - Risk of mistakes in selection due to inaccurate information on TQ.
  - In case of mistakes (2015: 3 cases on 12 new applications) again check on reference varieties, but now based on own, complete description. Risk on 3 years of testing.

Theory towards more efficiency

- Year 1 test 1



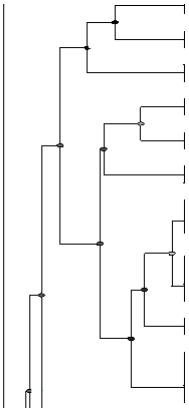
- Year 1 test 2
  - Genetically similar varieties in field
  - Other varieties check on paper



- Conclusion on D after 1 year or 2nd year with 'paper' varieties

Molecular data

- AFLP database for many French bean varieties available at Naktuinbouw
- 4 primer combinations (approximately 500 bands/loci) 78 bands are polymorph in the database. 230 varieties (528 entries in the database).
- No database yet with SSR or SNP, no whole genome sequencing.
- Dendrogram with genetic distances for 230 varieties



Accepted UPOV models

Combining phenotypic and molecular distances in the management of variety collections

Calibrated molecular distances in the management of variety collections

- Not in all crops good correlation
- Large data set needed
- Per crop large preparation phase

Theory as proposed in bean seems (not) to fit (the present examples) in these models.

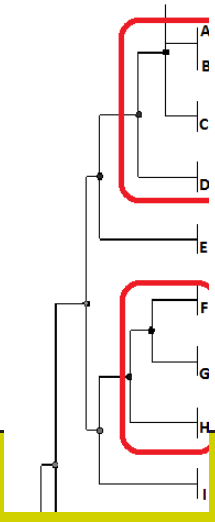
Example French bean (1)

Test 1- year 1:

- seedling check on DNA
- compare DNA pattern with dendrogram
- Set threshold
- decide on 1 to 5 genetically most similar varieties

Example:

- Application A to compare with B, C and D
- Application F to compare with G and H



Example French bean (2)

Test 2 – year 1:

- Field trial of the application with as reference varieties:
  - the 1 to 5 genetically most similar varieties
  - the similar variety, variety indicated by the breeder on the TQ, unless this similar variety is in a different group (example different resistance)
- First year of DUS trial with only 1 to 6 reference varieties, instead of 15 to 20: good side by side comparison possible.

- Conclusion of year 1: compare own complete description with all descriptions in database

Example French bean (3)

As the description of the application is complete and all descriptions are made by the examination office itself, one can be strict in selecting: not coming to 15 to 20 reference varieties, but none or only a few in a short time (less than 30 minutes).

→ Clearly distinct in year 1 and (based on check of morphological data in database) no extra reference varieties needed in year 2: 1 year of testing is sufficient to declare the variety Distinct.

Example French bean (4)

Year 2:

- Field trial with
  - one or no similar reference variety (of the 1 to 6) of year 1
  - other reference varieties selected from the database based on own, reliable description made in year 1
- Conclusion on Distinctness

Results in French bean 2015

	2 step example French bean				Traditional
Applica-tion	Number of genetically similar varieties	Number of references in year 1	Number of references to be added in year 2 (similar on paper)	Total number of references in 2 years trials	Total number of references in 2 years trials
A	3	3	5	8	21
B	3	4	7	11	14
C	1	1	2	3	6
D	2	3	1	4	5
E	1	2	1	3	12
F	1	3	0 D year 1	3	25
G	1	4	2	6	13
H	5	7	1	8	15
I	4	5	0	5	13
			TOTAL	51	124

Implementation

Needed before implementation is possible:

- Availability of own morphological data in database of ‘all’ varieties in common knowledge
- Availability of database with molecular data of ‘all’ varieties in common knowledge, based on a well defined and robust marker system. High resolution and validated.
- Validated threshold for similar varieties to put in the field.
- Availability of seed samples of the applications prox. 3 weeks before preparation of the field trial

Costs and benefits, example French bean

+ less trial plots (51/124 = 41% compared to traditional method)
+ less visual observations to make
+ better quality of side by side comparison
+ less hours for the process of selecting reference varieties for the growing trial (at least 1,5 h less)
+++ when TQ is not very informative (only information on grouping characteristics)

- costs for DNA test (costs will decrease importantly)
- 3 years of test not wanted, but what to do if a ‘paper’ reference appears to be very close in year 2 and genetical similarity is low?
- Submission of seed should be 3 weeks earlier

[End of Annex II and of document]