UPOV

International Union for the Protection of New Varieties of Plants

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

BMT/16/3

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INTERNATIONAL GUIDELINES ON MOLECULAR METHODOLOGIES INCLUDING COOPERATION BETWEEN OECD, UPOV, ISTA AND ISO

Document prepared by the Office of the Union

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EXECUTIVE SUMMARY

1. This document reports on developments concerning the Guidelines for DNA-Profiling: Molecular Marker Selection and Database Construction ("BMT Guidelines") and cooperation between OECD, UPOV, ISTA and ISO.

2. The BMT is invited to note that:

(a) a Joint OECD/UPOV/ISTA/AOSA Workshop on Biochemical and Molecular Methods was held in Paris on June 8, 2016, and that the recommendations of the Joint OECD/UPOV/ISTA/AOSA Workshop as reproduced in paragraph 9 of this document, were approved by the Annual Meeting of the OECD Seed Schemes, held in Paris on June 9 and 10, 2016;

(b) the TC, at its fifty-third 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 those organizations;

(c) practical workshops on "DNA Techniques and Variety Identification" were held in Roelofarendsveen, Netherlands, from May 8 to 10, 2017 and from September 20 to 22, 2017;

(d) the TC agreed that UPOV and OECD should consider making progress in the matters reported in this document if ISTA is unable to participate in the near future.

3. The structure of this document is as follows:

4. The following abbreviations are used in this document:

BMT: Working Group on Biochemical and Molecular Techniques, and DNA-Profiling in Particular
TC: Technical Committee
TWPs: Technical Working Parties
AOSA: Association of Official Seed Analysts
OECD: Organization for Economic Co-operation and Development
ISO: International Organization for Standardization
ISTA: International Seed Testing Association

BMT GUIDELINES

5. At its forty-fourth session, held in Geneva, Switzerland, October 21, 2010, the Council adopted document UPOV/INF/17/1 "Guidelines for DNA-Profiling: Molecular Marker Selection and Database Construction" ("BMT Guidelines") (see document C/44/17 "Report", paragraph 34).

6. It is recalled that the purpose of the BMT Guidelines is to provide guidance for developing harmonized methodologies with the aim of generating high quality molecular data for a range of applications as follows.

> "A. INTRODUCTION
>
> The purpose of this document (BMT Guidelines) is to provide guidance for developing harmonized methodologies with the aim of generating high quality molecular data for a range of applications. The BMT Guidelines are also intended to address the construction of databases containing molecular profiles of plant varieties, possibly produced in different laboratories using different technologies. In addition, the aim is to set high demands on the quality of the markers and on the desire for generating reproducible data using these markers in situations where equipment and/or reaction chemicals might change. Specific precautions need to be taken to ensure quality entry into a database."

7. The BMT Guidelines will be reviewed at the sixteenth session of the BMT (see agenda item 11, document BMT/16/4 "Review of document UPOV/INF/17 "Guidelines for DNA-Profiling: Molecular Marker Selection and Database Construction ('BMT Guidelines')").

COOPERATION BETWEEN OECD, UPOV, ISTA AND ISO

8. The background to this matter is provided in document BMT/15/5 "Cooperation between OECD, UPOV, ISO and ISTA".

OECD/UPOV/ISTA Joint Workshop on Molecular Techniques

9. A Joint OECD/UPOV/ISTA/AOSA Workshop on Biochemical and Molecular Methods was held in Paris, France, on June 8, 2016, and the following recommendations of the Joint OECD/UPOV/ISTA/AOSA Workshop were approved by the Annual Meeting of the OECD Seed Schemes, held in Paris, France, on June 9 and 10, 2016:

(a) To 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 and, for mutual understanding, to repeat the joint workshop at relevant meetings of the OECD and ISTA;

(b) 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 National Designated Authorities (NDAs) and continuously collected by the Secretariat;

(c) To develop a list of terms and their definitions as used by OECD, UPOV, AOSA and ISTA and to make an attempt to harmonize these;

(d) To consider organizing another similar workshop in three years' time; and

(e) To consider replacing the term used in the OECD Seed Schemes for the status of DNA based techniques from "internationally validated" to another term such as "internationally harmonized."

10. The Annual Meeting of the OECD Seed Schemes endorsed the proposal of the Netherlands to organize 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.

Developments at the fifty-third session of the TC

11. The TC, at its fifty-third session, noted that the development of a joint document explaining the principal features of the systems of the OECD, UPOV and ISTA could only start after agreement by OECD and ISTA (see document TC/53/31, paragraph 199 to 203).

12. The TC noted that the development of a joint OECD/UPOV/ISTA document containing an inventory of molecular marker techniques used by crop could only start after agreement by OECD and ISTA.

13. The TC 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 those organizations.

14. The TC considered whether to explore circumstances in which the same techniques and information could be used by OECD, ISTA and UPOV, taking into account the different objectives of the organizations, and agreed that the organization by Naktuinbouw of a practical workshop in 2017, in Roelofarendsveen, Netherlands, from May 8 to 10, 2017, could explore such possibilities on the basis of real situations.

15. The TC agreed that UPOV and OECD should consider making progress in the matters above if ISTA was unable to participate in the near future.

16. The TC noted that a Joint OECD/UPOV/ISTA/AOSA Workshop on Biochemical and Molecular Methods had been held in Paris on June 8, 2016, and that the recommendations of the Joint OECD/UPOV/ISTA/AOSA Workshop as reproduced in document TC/53/11, paragraph 25, had been approved by the Annual Meeting of the OECD Seed Schemes, held in Paris on June 9 and 10, 2016 (see document TC/53/31, paragraph 209).

Practical Workshops on "DNA Techniques and Variety Identification"

17. In relation to paragraph 14 of this document, a practical workshop "DNA Techniques and Variety Identification" was held in Roelofarendsveen, Netherlands, from May 8 to 10, 2017 and an "International Workshop on DNA Techniques and Variety Identification" was held in Roelofarendsveen, Netherlands, from September 20 to 22, 2017. The agendas of these workshops are reproduced in Annex I and II to this document, respectively.

18. The BMT is invited to note that:

(a) a Joint OECD/UPOV/ISTA/AOSA Workshop on Biochemical and Molecular Methods was held in Paris on June 8, 2016, and that the recommendations of the Joint OECD/UPOV/ISTA/AOSA Workshop as reproduced in paragraph 9 of this document, were approved by the Annual Meeting of the OECD Seed Schemes, held in Paris on June 9 and 10, 2016;

(b) the TC, at its fifty-third 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 those organizations;

(c) practical workshops on "DNA Techniques and Variety Identification" were held in Roelofarendsveen, Netherlands, from May 8 to 10, 2017 and from September 20 to 22, 2017; and

(d) the TC agreed that UPOV and OECD should consider making progress in the matters reported in this document if ISTA is unable to participate in the near future.

[Annexes follow]

ANNEX I

AGENDA OF PRACTICAL WORKSHOP “DNA TECHNIQUES AND VARIETY IDENTIFICATION”

8 – 10 May, 2017, Naktuinbouw, Roelofarendsveen, Netherlands

AGENDA				***DNA techniques and variety identification***		***parallel Program***	
				Group 1		***Group 2***	
Monday, May 8, 2017				*Subject*	*By*	*Subject*	*By*
focus: introduction and DNA basics			*8:30*	*Transportation to Naktuinbouw*	*Bus*		
	9:00	-	*9:15*	*Registration*	*All*		
	9:15		*9:30*	*Introduction of participants*	*All*		
	9:30	-	*10:00*	*Lecture: Introduction to Naktuinbouw*	*Bert Scholte*		
	10:00		*10:20*	*Film about Naktuinbouw*	*Bert Scholte*		
Coffee	*10:20*	-	*10:50*	*coffee break*			
	10:50		*11:00*	*Agenda of the workshop*	*Bert Scholte*		
	11:00	-	*11:40*	*Lecture: DNA; the basics*	*Hedwich Teunissen*		
	11:40	-	*11:45*	*Video: sampling of potato*	*video*		
	11:45	-	*12:05*	*sample own potato*	*Menno Hoekstra / Alex Reid*	*make description of own potato and photographs of plants*	*Amanda van Dijk / Jan Kees Schipper*
	12:05	-	*12:25*	*make description of own potato and photographs of plants*	*Amanda van Dijk / Jan Kees Schipper*	*sample own potato and watch film of Variety reseach at Naktuinbouw*	*Menno Hoekstra / Alex Reid*
Lunch	*12:30*	-	*13:30*	*buffet*			
	13:30	-	*14:00*	*Lecture: Introduction to ISTA and developments on the use of DNA for variety identification within ISTA system*	*Chiara Delogu*	*Hands-on DNA extraction*	*Menno Hoekstra / Daniel Deinum / Alex Reid / Hedwich Teunissen*
	14:00	-	*14:30*	*Lecture: introduction to OECD and developments on the use of DNA for certification within OECD system*	*Gerry Hall*	*Hands-on DNA extraction*	*Menno Hoekstra / Daniel Deinum / Alex Reid / Hedwich Teunissen*
	14:30	-	*15:00*	*Lecture: introduction to UPOV and developments on the use of DNA for PVR within UPOV system*	*Leontino Traveira*	*Hands-on DNA extraction*	*Menno Hoekstra / Daniel Deinum / Alex Reid / Hedwich Teunissen*
Coffee	*15:00*	-	*15:30*	*coffee break*			
	15:30	-	*16:00*	*Hands-on DNA extraction*	*Menno Hoekstra / Daniel Deinum / Alex Reid / Hedwich Teunissen*	*Lecture: Introduction to ISTA and developments on the use of DNA for variety identification within ISTA system*	*Chiara Delogu*
	16:00	-	*16:30*	*Hands-on DNA extraction*	*Menno Hoekstra / Daniel Deinum / Alex Reid / Hedwich Teunissen*	*Lecture: introduction to OECD and developments on the use of DNA for certification within OECD system*	*Gerry Hall*
	16:30	-	*17:00*	*Hands-on DNA extraction*	*Menno Hoekstra / Daniel Deinum / Alex Reid / Hedwich Teunissen*	*Lecture: introduction to UPOV and developments on the use of DNA for PVR within UPOV system*	*Leontino Traveira*
	17:00	-	*21:00*	*Dinner in Kaag en Braassem*	*all*		
				Transportation to hotel	*Bus*		
Tuesday, May 9, 2017				***Group 1***		***Group 2***	
focus: genotyping technologies			*8:30*	*Transportation to Naktuinbouw*	*Bus*		
	9:00	-	*9:15*	*video on DNA quanitification and discusion on results DNA extraction previous day, compare different DNA extraction methods*	*video*		
	9:15	-	*10:00*	*Lecture: DNA amplification by PCR, real-time PCR and primer/probe design, optimization and validation*	*Alex Reid*		
Coffee	*10:00*	-	*10:30*	*coffee break*			
	10:30	-	*11:30*	*Lecture: overview PCR based genotyping technologies*	*Hedwich Teunissen / Alex Reid*	*Hands-on: performing SSR - PCR*	*Laboratory*
	11:30		*12:00*	*Lecture: The potato system*	*Alex Reid*	*Hands-on: performing SSR - PCR*	*Laboratory*
	12:00	-	*12:45*	*excursion: Naktuinbouw ISTA accreditated laboratory*	*Marco Hofman*	*excursion: Naktuinbouw CPVO accreditated Examination office- variety centre*	*Amanda van Dijk*
Lunch	*12:45*	-	*13:30*				
	13:30		*14:30*	*Hands-on: performing SSR - PCR*	*Laboratory*	*Lecture: overview PCR based genotyping technologies*	*Hedwich Teunissen / Alex Reid*
	14:30	-	*15:00*	*Hands-on: performing SSR - PCR*	*Laboratory*	*Lecture: The potato system*	*Alex Reid*
Coffee	*15:00*	-	*15:30*				
	15:30	-	*16:00*	*Lecture: KASP and conversion SSR markers to KASP markers*	*Joris Parmentier (LGC)*		
	16:00	-	*16:45*	*excursion: Naktuinbouw CPVO accreditated Examination office- variety centre*	*Amanda van Dijk*	*excursion: Naktuinbouw ISTA accreditated laboratory*	*Marco Hofman*
	16:45	-	*17.15*	*Lecture: DNA sequencing*	*Hedwich Teunissen*		
	17:15	-	*17:30*	*Video: Loading SSR reactions on LICOR gel and running*	*video/webcam*		
			17:30	*Transportation to hotel*	*Bus*		
Wednesday, May 10, 2017				***Group 1***		***Group 2***	
focus: data analysis and applications of genotyping			*8:30*	*Transportation to Naktuinbouw*	*Bus*		
	9:00	-	*9:30*	*Lecture: data analysis*	*Hedwich Teunissen*		
	9:30	-	*10:30*	*Demonstration: Bionumerics*	*iemand van Applied Maths, bijvoorbeeld Johan Goris of Bruno Pot, via live stream*		
Coffee	*10:30*	-	*11:00*	*Coffee break*			
	11:00	-	*12:00*	*Hands-on: exercise 1: translation of own potato fingerprint in similarity values with whole group and visualisation in dendrogram*	*Menno Hoekstra / Daniel Deinum*	*Hands-on: exercise 1: translation of own potato fingerprint in similarity values with whole group and visualisation in dendrogram*	*Menno Hoekstra / Daniel Deinum*
	12:00	-	*12:30*	*Discussion: results on potato analysis both groups*	*Hedwich Teunissen / Amanda van Dijk*		
Lunch	*12:30*	-	*13:30*	*buffet*			
	13:30	-	*14:15*	*Lecture: genotyping for QC by breeding industry*	*Mike Heimerikx (ENZA Zaden)*		
	14:15	-	*15:00*	*Lecture: management of reference collection: example French Bean*	*Amanda van Dijk*		
Coffee	*15:00*	-	*15:30*	*Coffee break*			
	15:30	-	*16:00*	*Lecture: Variety Tracer and Green Forensics*	*Hedwich Teunissen*		
	16:00	-	*16:45*	*Discussion: the future of genotyping for variety identification in certification, seeds testing and DUS testing*	*All*		
	16:45	-	*17:00*	*concluding remarks*	*Bert Scholte*		
				Transportation to Schiphol Airport/Trainstation	*Bus*		

[Annex II follows]

ANNEX II

AGENDA OF INTERNATIONAL WORKSHOP ON DNA TECHNIQUES AND VARIETY IDENTIFICATION

20 – 22 September, 2017, Naktuinbouw, Roelofarendsveen, Netherlands

AGENDA				***DNA techniques and variety identification***			***parallel Program***		
				Group 1			***Group 2***		
Wednesday, September 20, 2017				*Subject*	*By*	*Location*	*Subject*	*By*	*Location*
focus: introduction and DNA basics			*8:30*	*Transportation to Naktuinbouw*	*Bus*	*R'veen*			
	9:00	-	*9:10*	*Registration*	*All*	*Eik*			
	9:10		*9:15*	*Agenda of the workshop*	*Bert Scholte*	*Eik*			
	9:15		*9:30*	*Introduction of participants*	*All*	*Eik*			
	9:30	-	*9:40*	*film to introduce Naktuinbouw*	*Bert Scholte*	*Eik*			
	9:40		*10:00*	*Lecture: Introduction to Naktuinbouw and Variety testing*	*Bert Scholte*	*Eik*			
Coffee	*10:00*	-	*10:30*	*coffee break*					
	10:30	-	*11:15*	*Lecture: DNA; the basics*	*Hedwich Teunissen*	*Eik*			
	11:15	-	*11:30*	*Lecture: sampling and DNA extraction*	*Hedwich Teunissen*	*Eik*			
	11:30	-	*11:35*	*Video: sampling of potato and freeze-drying*	*Hedwich Teunissen*	*Eik*			
	11:35	-	*11:45*	*sample own potato*	*Sebastiaan Flanderhijn / Daniel Deinum*	*Eik*	*sample own potato*	*Hedwich Teunissen*	*kantine achterin*
	11:45	-	*12:15*	*make description of own potato*	*Amanda van Dijk*	*Eik*	*make description of own potato*	*Jan Kees Schipper*	*kantine achterin*
	12:15		*12:45*	*Lecture: molecular markers: the basics*	*Hedwich Teunissen*	*Eik*			
Lunch	*12:45*	-	*13:30*	*buffet*					
	13:30	-	*14:00*	*Lecture: Introduction to ISTA and developments on the use of DNA for variety identification within ISTA system*	*Chiara Delogu*	*Eik*	*Hands-on DNA extraction*	*Daniel Deinum / Sebastiaan Flanderhijn*	*S25 lab*
	14:00	-	*14:30*	*Lecture: introduction to OECD and developments on the use of DNA for certification within OECD system*	*Gerry Hall*	*Eik*	*Hands-on DNA extraction*	*Daniel Deinum / Sebastiaan Flanderhijn*	*S25 lab*
	14:30	-	*15:00*	*Lecture: introduction to UPOV and developments on the use of DNA for PVR within UPOV system*	*Leontino Traveira*	*Eik*	*Hands-on DNA extraction*	*Daniel Deinum / Sebastiaan Flanderhijn*	*S25 lab*
	15:00		*15:10*	*Photo of the whole group*	*All*	*Outside?*			
Coffee	*15:10*	-	*15:30*	*coffee break*					
	15:30	-	*16:00*	*Hands-on DNA extraction*	*Daniel Deinum / Sebastiaan Flanderhijn*	*S25 lab*	*Lecture: Introduction to ISTA and developments on the use of DNA for variety identification within ISTA system*	*Chiara Delogu*	*Eik*
	16:00	-	*16:30*	*Hands-on DNA extraction*	*Daniel Deinum / Sebastiaan Flanderhijn*	*S25 lab*	*Lecture: introduction to OECD and developments on the use of DNA for certification within OECD system*	*Gerry Hall*	*Eik*
	16:30	-	*17:00*	*Hands-on DNA extraction*	*Daniel Deinum / Sebastiaan Flanderhijn*	*S25 lab*	*Lecture: introduction to UPOV and developments on the use of DNA for PVR within UPOV system*	*Leontino Traveira*	*Eik*
	17:00	-	*21:00*	*Dinner in Kaag en Braasum*	*all*	*restaurant*			
				Transportation to hotel	*Bus*				
Thursday, September 21, 2017				***Group 1***			***Group 2***		
focus: genotyping technologies			*8:30*	*Transportation to Naktuinbouw*	*Bus*	*R'veen*			
	9:00	-	*9:10*	*video on DNA quantification and discusion on results DNA extraction previous day*	*Hedwich Teunissen*	*Eik*			
	9:10	-	*10:00*	*Lecture: DNA amplification by PCR, real-time PCR and primer/probe design, optimization and validation*	*Hedwich Teunissen*	*Eik*			
Coffee	*10:00*	-	*10:30*	*coffee break*					
	10:30	-	*11:30*	*Lecture: overview PCR based genotyping technologies*	*Hedwich Teunissen*	*Eik*	*Hands-on: performing SSR - PCR*	*Laboratory*	*S25-lab*
	11:30		*12:00*	*Lecture: Harmonization between labs: The potato system*	*Hedwich Teunissen*	*Eik*	*Hands-on: performing SSR - PCR*	*Laboratory*	*S25-lab*
	12:00	-	*12:45*	*excursion: Naktuinbouw ISTA accreditated laboratory*	*Erik van Egmond*	*Lab S22*	*excursion: Naktuinbouw CPVO accreditated Examination office- variety centre*	*Amanda van Dijk*	*Variety Centre*
Lunch	*12:45*	-	*13:30*	*buffet*					
	13:30		*14:30*	*Hands-on: performing SSR - PCR*	*Laboratory*	*S25 lab*	*Lecture: overview PCR based genotyping technologies*	*Hedwich Teunissen*	*Eik*
	14:30	-	*15:00*	*Hands-on: performing SSR - PCR*	*Laboratory*	*S25 lab*	*Lecture: Harmonization between labs: The potato system*	*Hedwich Teunissen*	*Eik*
	15:00	-	*15:45*	*excursion: Naktuinbouw CPVO accreditated Examination office- variety centre*	*Amanda van Dijk*	*Variety Centre*	*excursion: Naktuinbouw ISTA accreditated laboratory*	*Erik van Egmond*	*Lab S22*
Coffee	*15:45*	-	*16:15*	*Coffee break*					
	16:15	-	*17:15*	*Lecture: DNA sequencing*	*Hedwich Teunissen*	*Eik*			
			17:15	*Transportation to hotel*	*Bus*				
Friday, September 22, 2017				***Group 1***			***Group 2***		
focus: data analysis and applications of genotyping			*8:30*	*Transportation to Naktuinbouw*	*Bus*	*R'veen*			
	9:00	-	*9:05*	*Video: Loading SSR reactions on LICOR gel and running*	*Hedwich Teunissen*	*Eik*			
	9:05	-	*9:40*	*Lecture: data analysis*	*Hedwich Teunissen*	*Eik*			
	9:40	-	*10:30*	*Hands-on exercise: translation of own potato fingerprint in allele scores, similarity values with whole group and visualisation in dendrogram*	*Sebastiaan Flanderhijn / Daniel Deinum*	*Eik*	*Hands-on exercise: translation of own potato fingerprint in allele scores, similarity values with whole group and visualisation in dendrogram*	*Amanda van Dijk / Hedwich Teunissen*	*apple / paprika*
Coffee	*10:30*	-	*11:00*	*Coffee break*					
	11:00	-	*11:30*	*Hands-on exercise: translation of own potato fingerprint in allele scores, similarity values with whole group and visualisation in dendrogram*	*Sebastiaan Flanderhijn / Daniel Deinum*	*Eik*	*Hands-on exercise: translation of own potato fingerprint in allele scores, similarity values with whole group and visualisation in dendrogram*	*Amanda van Dijk / Hedwich Teunissen*	*apple / paprika*
	11:30	-	*12:15*	*Discussion: results on potato analysis both groups*	*Hedwich Teunissen / Amanda van Dijk / Sebastiaan Flanderhijn / Daniel Deinum*	*Eik*			
Lunch	*12:15*	-	*13:00*	*buffet*					
	13:00		*13:45*	*Lecture: The Maize system*	*Muriel Thomasset (bioGEVES)*	*Eik*			
	13:45	-	*14:30*	*Lecture: databases containing DNA profiles in UPOV*	*Hend Madhour (UPOV)*	*Eik*			
	14:30	-	*15:00*	*Lecture: management of reference collection: example French Bean*	*Amanda van Dijk*	*Eik*			
Coffee	*15:00*	-	*15:30*	*Coffee break*					
	15:30	-	*16:00*	*Lecture: Variety Tracer and Green Forensics*	*Hedwich Teunissen*	*Eik*			
	16:00	-	*16:45*	*Discussion: the future of genotyping for variety identification in certification, seeds testing and DUS testing*	*All*	*Eik*			
	16:45	-	*17:00*	*concluding remarks*	*Bert Scholte*	*Eik*			
				Transportation to Schiphol Airport/Trainstation	*Bus*	*Schiphol*			

[End of Annex II and of document]