Internationally recognized methods currently commonly used by the National Designated Authorities

154. The OECD Seed Schemes does not develop or carry out any independent testing to determine the effectiveness of laboratory techniques for the determination of varietal identity, and therefore cannot specifically endorse any specific laboratory techniques for determining varietal identity.

155. The traditional OECD field inspection techniques together with pre- and post-control plots as described in Parts I and II of the Guidelines for Control Plot Tests and Field Inspection of Seed Crops are to be regarded as the standard techniques for determining varietal identity and varietal purity.

156. However, the OECD Seed Schemes do recognise that there are occasions where these traditional techniques limit the certainty of the varietal determination, and in certain cases varieties of some species cannot be identified with certainty using these traditional techniques. In these specific circumstances, it might be beneficial to use non-field based tests, which must be seen as supplementing and not replacing the more traditional techniques.

157. The Annual Meeting of the Seed Schemes has agreed that any supplementary techniques that are used must be included in internationally recognised and documented methods. This is to ensure that any techniques used by an NDA have been shown to be accurate and repeatable.

158. The Annual meeting has therefore approved the methods listed in the annex below for inclusion in the Guidelines for Control Plot Tests and Field Inspection of Seed Crops and for use by Member Countries to support the traditional field-based techniques, only in those cases where these traditional techniques have been implemented fully but still leave some doubt as to the identity of a variety.

159. It should be noted that all characteristics that are included on the Official Description of a variety, whether they are field characters or laboratory characters, can be used by an OECD NDA for the purposes of determining varietal identity and the issue of an OECD Seed varietal Certificate.

160. These methods can be used at the discretion of the National Designated Authority, and may be applied to any category of seed.

161. Where an NDA, other than the certifying NDA, is planning to reject a seed lot based on results obtained through the use of these methods, this should be undertaken in agreement with the certifying National Designated Authority.

162. Where a technique is used that is not documented in the variety description, an authenticated standard sample of the variety must be available for comparison and must be treated and examined in the same way as the sample under test.

163. Annex A lists those internationally recognised methods that have been found reliable in identifying varieties in some member countries National Certification, in situations where the traditional field inspection and control plot techniques have been inconclusive.

Annex .A. INTERNATIONALLY RECOGNISED METHODS CURRENTLY COMMONLY USED BY THE NATIONAL DESIGNATED AUTHORITIES

The following methods were approved by the 2023 Annual meeting, and NDA's are able to use these methods for varietal identity evaluation in accordance with the Guidelines for Control Plot Tests and Field Inspection of Seed Crops in Part III. All of these methods have been used by at least 1 OECD NDA as part of their National certification programme and providing accurate results, and were in the Molecular and Biochemical survey and included in document [TAD/CA/S/RD(2016)6/REV4] " Final List of BMT Methods in use by Participating Countries", noted by the 2023 Annual Meeting.

SPECIES	NAME OF TEST	DOCUMENTED BY	REFERENCE
Avena sativa **(Oats)**	A-PAGE (Acid-Polyacrylamide Gel Electrophoresis)	ISTA	ISTA Rule 8.8.6
	SSR based method	ISTA	2023 ISTA Rules 8.10.5 *Avena sativa* (oats) Microsatellite Markers Inclusion of DNA-based test for testing Avena sativa. Proposal supported by validation study. Proposal developed and approved by Variety TCOM.
Brassica juncea (Mustard)	Fat acid composition and erucic acid quantification	ISO	Standards NF EN ISO 17059: "Oilseeds - Extraction of oil and preparation of methyl esters of triglyceride fatty acids for analysis by gas chromatography" and NF EN ISO 12966-4: "Gas chromatography of fatty acid methyl esters — Part 4: Determination by capillary gas chromatography"
Brassica napus **(Rapeseed)**	Isozymes in starch gels	CPVO	Annex II.2 " Description of the SGE Method (Starch Gel Electrophoreses) for the Analysis of Isoenzymes from Brassica napus " of CPVO-TP/036/2 Final " protocol for Distinctness, Uniformity and Stability tests"
	GMO detection and identification	ISTA	Internal methods validated with reference to EURL validation studies and to the standards ISO 21569 and ISO 21571
	Determination of glucosinolates content	ISO	Standard NF EN ISO 9167-1 Rapeseed and rapeseed meals — Determination of glucosinolates content — Method using high-performance liquid chromatography
	SSR Molecular Markers	ISO	ISO 13495:2013 Foodstuffs — Principles of selection and criteria of validation for varietal identification methods using specific nucleic acid
	Fat acid composition	ISO	Standards NF EN ISO 17059: "Oilseeds - Extraction of oil and preparation of methyl esters of triglyceride fatty acids for analysis by gas chromatography" and NF EN ISO 12966-4: "Gas chromatography of fatty acid methyl esters — Part 4: Determination by capillary gas chromatography"
Brassica nigra (Black mustard)	Fat acid composition and erucic acid quantification	ISO	Standards NF EN ISO 17059: "Oilseeds - Extraction of oil and preparation of methyl esters of triglyceride fatty acids for analysis by gas chromatography" and NF EN ISO 12966-4: "Gas chromatography of fatty acid methyl esters — Part 4: Determination by capillary gas chromatography"
Glycine max	Seed: coloration due to peroxidase activity in seed coat	UPOV	Ad. 16: "Seed: coloration due to peroxidase activity in seed coat" to the UPOV TG/80/6 guidelines for the conduct of tests for Distinctness, Uniformity and Stability

(soyabean)	GMO Detection, Identification and Quantification	ISTA/ EURL	Internal methods validated with reference to EURL validation studies and to the standards ISO 21569, ISO 21570 and ISO 21571
***Helianthus annuus* (Sunflower)**	SGE Method for the Analysis of Isoenzymes (Starch Gel Electrophoreses)	UPOV	Part III " SGE Method (Starch Gel Electrophoreses) for the Analysis of Isoenzymes " of the UPOVTG/81/6guidelines for the conduct of tests for Distinctness, Uniformity and Stability
	SSR based method	ISO	ISO/TR 17622:015(E)
***Hordeum vulgare* (Barley)**	A-PAGE (Acid-Polyacrylamide Gel Electrophoresis)	UPOV / ISTA	"Acid PAGE Method for Analysis of B- and C-Hordeins from *Hordeum vulgare*" in Part III "Description of the method to be used" of the UPOV tg/19/10 guidelines for the conduct of tests for distinctness, uniformity and stability / ISTA Rule 8.8.3
	SDS-PAGE (Sodium Dodecyl Sulfate - Polyacrylamide Gel Electrophoresis)	UPOV	"Hordein composition: allele expression at loci Hor-3(30), Hor- 1(31) and Hor-2(32) - SDS PAGE Method for Analysis of Hordeins from *Hordeum vulgare*" in Part III "Description of the method to be used" of the UPOV tg/19/10 guidelines for the conduct of tests for distinctness, uniformity and stability

Lolium **(Ryegrass)**	SDS-PAGE (Sodium Dodecyl Sulfate - Polyacrylamide Gel Electrophoresis)	ISTA	ISTA Rule 8.8.4 for Principle & Apparatus, and for Specific Procedure: 8.8.4.3.2
Lolium multiflorum	SDS-PAGE (Sodium Dodecyl Sulfate - Polyacrylamide Gel Electrophoresis)	ISTA	ISTA Rule 8.9.4
Pisum sativum **(Pea)**	SDS-PAGE (Sodium Dodecyl Sulfate - Polyacrylamide Gel Electrophoresis)	ISTA	ISTA Rule 8.8.4 for Principle & Apparatus, and for Specific Procedure: 8.8.4.3.1
	SSR based method	ISTA	2023 ISTA Rules 8.10.4 *Pisum* (peas) Microsatellite Markers Inclusion of DNA-based test for testing *Pisum* varieties. Proposal developed by working group within Variety TCOM and supported by validation study. Proposal approved by Variety TCOM.
Solanum lycopersicum **(Tomato)**	Tomato Spotted Wild Virus resistance Sw-5 gene	UPOV/CPVO	Ad. 55: Resistance to Tomato spotted wilt virus (TSWV) of 2016 protocol (CPVO-TP/044/4 Rev2 Guidelines for the conduct of tests for DUS
Triticosecale	SDS-PAGE (Sodium Dodecyl Sulfate - Polyacrylamide Gel Electrophoresis)	ISTA	ISTA Rule 8.8.9
	Method for Determination of Phenol Reaction	UPOV	Ad. 20 " Method for Determination of Phenol Reaction " UPOV (Ad. 25 "Method for Determination of Phenol Reaction" of UPOV TG/121/3 Guidelines for the conduct of tests for DUS)
	Seed: coloration with phenol " of the UPOV TG/121/4 Guidelines for the conduct of tests for Distinctness, Uniformity and Stability	UPOV	UPOV TG/121/4
Triticum aestivum **(Wheat)**	SDS-PAGE (Sodium Dodecyl Sulfate - Polyacrylamide Gel Electrophoresis)	UPOV	Part III - Description of the Method to be Used - Glutenin composition: allele expression at loci Glu-A1 (27), Glu-B1 (28) and Glu-D1 (29) - SDS PAGE Method for Analysis of HMW Glutenins from *T. aestivum* of the UPOV TG3/11 Guidelines for the conduct of tests for distinctness, homogeneity and stability

	A-PAGE (Acid-Polyacrylamide Gel Electrophoresis)	ISTA/UPOV	ISTA Rule 8.8.8 UPOV tg/19/10 guidelines for the conduct of tests for distinctness, uniformity and stability /

	Method for Determination of Phenol Reaction	UPOV	Ad 24 " Grain: coloration with phenol - Method for Determination of Phenol Reaction" UPOV TG : Ad.2 Seed coloration with phenol of UPOV TG/3/12 for Distinctness, Uniformity and Stability tests *Triticum aestivum* L. (wheat)
	Seed: coloration with phenol" of the UPOV TG/3/12 Guidelines for the conduct of tests for Distinctness, Uniformity and Stability	UPOV	UPOV TG/3/12
	GMO detection	ISO/EURL	Internal methods validated with reference to EURL validation studies and to the standards ISO 24276, ISO 21569, ISO 21570 and ISO 21571
	SRR based method	ISTA	ISTA Rule 8.10.2 Triticum (wheat) Microsatellite Markers
Triticum durum **(Durum Wheat)**	A-PAGE (Polyacrylamide Gel Electrophoresis)	ISTA, UPOV	ISTA Rules, Chapter 8 UPOV Guidelines TG/19/10
	Method for Determination of Phenol Reaction	UPOV	Ad. 2: Seed: coloration with phenol of Ad27 TG120/4 Guidelines for the conduct of tests for DUS
Zea mays **(Maize)**	IEF PAGE (Ultra Thin Layer – IsoElectroFocusing - Polyacrylamide Gel Electrophoresis)	ISTA	ISTA Rule 8.8.5
	GMO Detection, Identification, Quantification	ISO/EURL	Internal methods validated with reference to EURL validation studies and to the standards ISO 24276, ISO 21569, ISO 21570 and ISO 21571
	SSR based method	ISO	ISO/TR 17623:2015(E)
	A-PAGE (Polyacrylamide Gel Electrophoresis)	ISTA	ISTA Rules, Chapter 8; Poperelja, F.A. (1989) Elekroforez zeinov kukuruzy v PAGE
	SGE Method for the Analysis of Isozymes (Starch Gel Electrophoreses)	**UPOV**	Part III "Description of the SGE (Starch Gel Electrophoreses) Method for the Analysis of Isozymes from *Zea mays* L." of the UPOV TG/2/7 Guidelines for the conduct of tests for Distinctness, Uniformity and Stability
Sorghum **(Sorghum)**	SSR Molecular Markers	ISO	ISO 13495:2013 Foodstuffs — Principles of selection and criteria of validation for varietal identification methods using specific nucleic acid