

Technical Committee

TC/58/INF/6

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MOLECULAR TECHNIQUES

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EXECUTIVE SUMMARY

1. The purpose of this document is to report on developments concerning molecular techniques at the Technical Working Parties, at their sessions in 2022.
2. Matters for consideration by the Technical Committee (TC) concerning: (1) cooperation between international organizations; (2) sessions to facilitate cooperation in relation to the use of molecular techniques cooperation; and (3) confidentiality, ownership and access to molecular data are presented in documents TC/58/7 "Molecular techniques" and TC/58/17 "Progress reports on the work of the Technical Working Parties".
3. The structure of this document is as follows:

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4. The following abbreviations are used in this document:

TC:	Technical Committee
TC-EDC:	Enlarged Editorial Committee
TWA:	Technical Working Party for Agricultural Crops
TWC:	Technical Working Party on Automation and Computer Programs
TWF:	Technical Working Party for Fruit Crops
TWM:	Technical Working Party on Testing Methods and Techniques
TWO:	Technical Working Party for Ornamental Plants and Forest Trees
TWPs:	Technical Working Parties
TWV:	Technical Working Party for Vegetables

DEVELOPMENTS AT THE TECHNICAL WORKING PARTIES AT THEIR SESSIONS IN 2022

5. At their sessions in 2022, the TWV¹, TWA², TWO³, TWF⁴ and TWM⁵ considered document TWP/6/7 “Molecular Techniques” (see documents TWV/56/22 “Report”, paragraphs 55 to 62; TWA/51/11 “Report”, paragraphs 68 to 79; TWO/54/6 “Report”, paragraphs 64 to 73; TWF/53/14 “Report”, paragraphs 79 to 87; and TWM/1/26 “Report”, paragraphs 73 to 80).

Presentation on the use of molecular techniques in DUS examination

6. The TWV received a presentation on “International harmonization and validation of a SNP set for the management of tomato reference collection” by an expert from the Netherlands. A copy of the presentation is provided in document TWV/56/21.

7. The TWV discussed the process of authorization from breeders for using varieties in the project and noted the importance of the agreement established to regulate access to genetic information from varieties and confidentiality aspects.

8. The TWA received a presentation on the “Use of molecular techniques in DUS examination: Report from Argentina” by an expert from Argentina. A copy of the presentation is provided in document TWA/51/4.

9. The TWA received a presentation on “Developing a strategy to apply SNP molecular markers in the framework of winter oilseed rape DUS testing” by an expert from France. A copy of the presentation is provided in document TWA/51/4 Add.

10. The TWF received a presentation on “Application of molecular techniques in DUS testing and PBR enforcement of fruit sector in China” by an expert from China. A copy of the presentation is provided in document TWF/53/12.

11. The TWF noted that molecular markers could be used in China as first instance evidence for enforcement of breeders’ rights, followed by a growing trial in case required.

12. Following the presentation from China, the TWF had an open discussion about the use of molecular markers in DUS examination and variety identification. The following aspects were mentioned by participants:

- Possibilities for cooperation on the constitution of common databases, including for authorities receiving relatively few applications for particular crops
- Origin of plant material for DNA extraction (e.g. material provided for DUS testing)
- Selection of markers for each crop, according to intended use (e.g. for PBR and/or variety identification).
- Selecting one or more laboratories capable of providing high-quality molecular profiles (e.g. security back-up);
- High cost for harmonizing methodologies for DNA profiling among different laboratories;
- Difficulties to obtain the same results even for laboratories using harmonized methodologies.

DEVELOPMENTS AT THE FIRST SESSION OF THE TECHNICAL WORKING PARTY ON TESTING METHODS AND TECHNIQUES (TWM)

13. The TWM held its first session organized by electronic means, from September 19 to 23, 2022 (see document TWM/1/26 “Report”, paragraph 1).

¹ at its fifty-sixth session, held via electronic means, from April 18 to 22, 2022

² at its fifty-first session, hosted by the United Kingdom and held via electronic means, from May 23 to 27, 2022

³ at its fifty-fourth session, hosted by Germany held via electronic means, from June 13 to 17, 2022

⁴ at its fifty-third session, held via electronic means, from July 11 to 15, 2022

⁵ at its first session, held via electronic means, from September 19 to 23, 2022

Papers presented

14. The papers presented under each of the agenda items of the first session of the TWM were as follows:

Reports on developments in UPOV (document TWM/1/2)

Short presentations on new developments in biochemical and molecular techniques by DUS experts, biochemical and molecular specialists, plant breeders and relevant international organizations document TWM/1/3)

Software and statistical analysis methods for DUS examination

(a) *Statistical tools and methods for DUS examination*

- *Developments on the improved COYU method (splines) (TWM/1/8 and TWM/1/8 Add.)*
- *Combined-over-year uniformity (COYU) criterion: Extrapolation (TWM/1/7 and TWM/1/7 Add.)*

(b) *Exchange and use of software and equipment*

- *Development of Statistical Analysis Software: DUSCEL (TWM/1/10)*
- *PATHOSTAT application (TWM/1/11)*

Phenotyping and image analysis

- *Image Analysis in Plant Variety Testing (TWM/1/4)*
- *Color Imaging Analysis System (TWM/1/5)*
- *DUS characteristics image processor (TWM/1/6)*
- *UAV potential in DUS testing (TWM/1/20)*
- *Machine Learning InnoVar project (TWM/1/25)*

Developments in molecular techniques and bioinformatics

(a) *Latest developments in molecular techniques and bioinformatics*

- *No documents were received for this agenda item.*

(b) *Cooperation between international organizations*

- *ISTA report on the use of molecular techniques (TWM/1/23)*
- *Latest developments in the application of BMT under the OECD Seed Schemes (TWM/1/24)*

(c) *Report of work on molecular techniques in relation to DUS examination*

- *Update on IMODDUS activities (TWM/1/14)*

(d) *Methods for analysis of molecular data, management of databases and exchange of data and material*

- *Application of molecular markers in DUS testing of new varieties of Chinese cabbage (TWM/1/9)*
- *DURDUSTools: Development of a common online molecular database and a genetic distance calculation tool for durum wheat (TWM/1/12)*
- *Development of a SNP marker set in Cannabis to support DUS testing (TWM/1/17)*
- *International harmonisation and validation of a SNP set for the management of tomato reference collection (TWM/1/18)*
- *Cotton genotyping using the TAMU 63KSNPsArray (TWM/1/13)*
- *The US PVPO Soybean molecular marker method (TWM/1/16)*

(e) *Confidentiality, ownership and access to molecular data, including model agreement template (TWM/1/22)*

(f) *The use of molecular techniques in examining essential derivation*

- *No documents were received for this agenda item.*

(g) *The use of molecular techniques in variety identification¹*

- *Variety identification: soybean case in Argentina (TWM/1/15)*
- *Digital PCR for Genotype Quantification: A Case Study in a Pasta Production Chain (TWM/1/21)*
- (h) *The use of molecular techniques for enforcement¹*
- *Variety Tracer: Fraudulent use of parental lines (TWM/1/19)*

Report of work on molecular techniques in relation to DUS examination

15. The TWM received a presentation from Ms. Cécile Collonnier (Community Plant Variety Office (CPVO)) on "Update on IMODDUS activities", a copy of which is reproduced in document TWM/1/14.

Methods for analysis of molecular data, management of databases and exchange of data and material

16. The TWM received a presentation from Mr. Ruixi Han (China) on "Application of molecular markers in DUS testing of new varieties of Chinese cabbage", a copy of which is reproduced in document TWM/1/9.

17. Clarifications were provided on the number of varieties considered for the establishment of the genetic similarity threshold.

18. The TWM received a presentation from Ms. Alexandra Ribarits (Austria) on "DURDUSTools: Development of a common online molecular database and a genetic distance calculation tool for durum wheat", a copy of which is reproduced in document TWM/1/12.

19. Clarifications were provided on aspects related to the use of an external service provider for data extraction, including the perception that updates in the marker array could bring even more information into the database. Data obtained using a reduced number of markers from a prior version of the marker set would still be comparable to that obtained using a later version. The expert explained that molecular information was used during the second year of DUS trial due to difficulty in obtaining data at earlier stage of examination. Molecular data was then used along with morphological data during the second year of examination to ensure comparison with all relevant similar varieties.

20. The TWM received a presentation from Ms. Hedwich Teunissen (the Netherlands) on "Development of a SNP marker set in Cannabis to support DUS testing", a copy of which is reproduced in document TWM/1/17.

21. The TWM received a presentation from Ms. Hedwich Teunissen (the Netherlands) on "International harmonisation and validation of a SNP set for the management of tomato reference collection", a copy of which is reproduced in document TWM/1/18.

22. Clarifications were provided that the SNPs set could be applied to the tomato reference collection in other parts in the world.

23. Clarifications were provided that the selected marker set of 297 SNPs were publicly available and would soon be published. The expert clarified that due to the broad genetic base represented in the project, the selected markers could be used in any geographical region.

24. The TWM received a presentation from Mr. Alberto Ballesteros (Argentina) on "Cotton genotyping using the TAMU 63KSNPsArray", a copy of which is reproduced in document TWM/1/13.

25. The TWM received a presentation from Mr. Jeffery Haynes (United States of America) on "The US PVPO Soybean molecular marker method", a copy of which is reproduced in document TWM/1/16.

26. Clarifications were provided that the method presented had been developed in collaboration with plant breeders and was used as supplementary information in support of distinctness analysis.

The use of molecular techniques in examining essential derivation

27. No documents were received for this agenda item.

The use of molecular techniques in variety identification

28. The TWM received a presentation from Ms. Ana Laura Vicario (Argentina) on “Variety identification: soybean case in Argentina”, a copy of which is reproduced in document TWM/1/15.

29. Clarifications were provided on the different types of samples used in the development of the method, which included seeds from different generations of propagation. The expert explained that a public set of markers had been used. Clarifications were also provided on a previous study that enabled the use of molecular distances to reduce the size of soybean trials in Argentina.

30. The TWM received a presentation from Ms. Chiara Delogu (Italy) on “Digital PCR for Genotype Quantification: A Case Study in a Pasta Production Chain”, a copy of which is reproduced in document TWM/1/21.

31. Clarifications were provided that up to 96 samples could be analyzed in each run of the equipment used by the presenter.

The use of molecular techniques for enforcement

32. The TWM received a presentation by Ms. Hedwich Teunissen (the Netherlands) on “Variety Tracer: Fraudulent use of parental lines”, a copy of which is reproduced in document TWM/1/19.

33. Participants heard that allele frequencies had been requested in some cases and that these could provide additional information to address cases of potential infringement.

[Annex follows]

ANNEX

ELEMENTS FOR DRAFT JOINT DOCUMENT EXPLAINING THE PRINCIPAL FEATURES OF THE SYSTEMS OF THE OECD, UPOV AND ISTA

The Organisation for Economic Co-operation and Development (OECD)*What are the OECD Seed Schemes?*

The OECD Seed Schemes provide an international framework for the varietal certification of agricultural seed moving in international trade. The Schemes were established in 1958 driven by a combination of factors including a fast-growing seed trade, regulatory harmonisation in Europe, the development of off-season production, the seed breeding and production potential of large exporting countries in America (North and South) and Europe, and the support of private industry. Membership of the Schemes is voluntary and participation varies. There are seven agricultural Seed Schemes.

Participating countries

59 countries from Europe, North and South America, Africa, the Middle-East, Asia and Oceania currently participate in the OECD Seed Schemes:

ALBANIA	(2)	LITHUANIA	(2)
ARGENTINA	(2)	LUXEMBOURG	(1)
AUSTRALIA	(1)	MEXICO	(1)
AUSTRIA	(1)	MOLDOVA	(2)
BELGIUM	(1)	MOROCCO	(2)
BOLIVIA	(2)	NETHERLANDS	(1)
BRAZIL	(2)	NEW ZEALAND	(1)
BULGARIA	(2)	NORWAY	(1)
CANADA	(1)	POLAND	(1)
CHILE	(1)	PORTUGAL	(1)
CROATIA	(2)	ROMANIA	(2)
CYPRUS ¹	(2)	RUSSIAN FEDERATION	(2)
CZECH REPUBLIC	(1)	SENEGAL	(2)
DENMARK	(1)	SERBIA	(2)
EGYPT	(2)	SLOVAKIA	(1)
ESTONIA	(1)	SLOVENIA	(1)
FINLAND	(1)	SOUTH AFRICA	(2)
FRANCE	(1)	SPAIN	(1)
GERMANY	(1)	SWEDEN	(1)

¹ Source OECD "Note by Turkey

The information in this document with reference to 'Cyprus' relates to the southern part of the Island. There is no single authority representing both Turkish and Greek Cypriot people on the Island. Turkey recognises the Turkish Republic of Northern Cyprus (TRNC). Until a lasting and equitable solution is found within the context of the United Nations, Turkey shall preserve its position concerning the 'Cyprus issue'.

Note by all the European Union Member States of the OECD and the European Union

The Republic of Cyprus is recognised by all members of the United Nations with the exception of Turkey. The information in this document relates to the area under the effective control of the Government of the Republic of Cyprus."

GREECE	(1)	SWITZERLAND	(1)
HUNGARY	(1)	TUNISIA	(2)
ICELAND	(1)	TURKEY	(1)
INDIA	(2)	UGANDA	(2)
IRAN	(2)	UKRAINE	(2)
IRELAND	(1)	UNITED KINGDOM	(1)
ISRAEL	(1)	UNITED STATES	(1)
ITALY	(1)	URUGUAY	(2)
JAPAN	(1)	ZIMBABWE	(2)
KENYA	(2)		
KYRGYZSTAN	(2)		
LATVIA	(2)		

(1) OECD Member Country
(2) Non OECD Member Country

Figure 1 Map of Participating Countries in the OECD Seed Schemes (2016)



Objectives

The objectives of the Schemes are to encourage the production and use of “quality-guaranteed” seed in participating countries. The Schemes authorise the use of labels and certificates for seed produced and processed for international trade according to agreed principles ensuring varietal identity and purity.

The Schemes facilitate the import and export of seed, by the removal of technical barriers to trade by assuring identification and origin through internationally recognised labels (“passports”) for trade. They also lay down guidelines for seed multiplication abroad, as well as for the delegation of some control activities to the private sector (“authorisation”). The quantity of seed certified through the OECD Schemes has grown rapidly in recent years and now exceeds 1 million tonnes.

How do the Seed Schemes operate

The success of international certification depends upon close co-operation between maintainers, seed producers, traders and the designated authority (appointed by the government) in each participating country. Frequent meetings allow for a multi-stakeholder dialogue to exchange information, discuss case studies, revise rules and update the Schemes. A wide range of international and non-governmental organisations as well as seed industry networks participate actively in the Schemes.

Benefits of the Schemes

- To facilitate international trade by using harmonised certification procedures, crop inspection techniques and use of control plots. The varietal purity standards for the appropriate species are also agreed and standardised by all member states.
- To provide a framework to develop seed production with other countries or companies.
- To participate in the elaboration of international rules for seed certification.
- To develop collaboration between the public and private sectors.
- To benefit from regular exchanges of information with other national certification agencies and Observer organisations.

Annual List of Varieties

The Annual List of Varieties eligible for OECD certification includes varieties which are officially recognized as distinct, uniform and stable, and possess an acceptable value in one or more participating country. The List contains the seed varieties internationally traded using the OECD seed Schemes. The number of varieties included has grown steadily over the last thirty years. Currently, the number of listed varieties amounts to over 62 000, corresponding to 200 species. The List is available online and updated frequently.

Outlook

As seed “consumers” become more demanding, there are greater needs for uniform seed standards, while at the same time public financial resources for regulation and quality control are limited.

Co-operation among countries and stakeholders in the framework of the Schemes is a response to the concern for a market-responsive regulatory approach. Every country is confronted with a different legal framework, institutional barriers and trade relations whilst the different approaches must remain consistent between countries entering international markets as importers or exporters of seed.

Maintainers and seed companies are responsible for ensuring their varieties remain pure and true to the description and the definitive sample (which is the ‘living description’ of the variety) not only domestically, but also across borders. However, there is a need for minimum criteria to be commonly defined, endorsed and enforced when multiplying seed in large quantities for the trade. The OECD Seed Schemes provide this legal framework at international level.

Status of Biochemical and Molecular Techniques (BMT) in the OECD Seed Schemes

The OECD Seed Schemes do not specifically endorse any laboratory method for determining varietal identity or for determining varietal purity. The traditional OECD methods of using field inspection techniques together with pre- and post- control plots are to be regarded as the required methods of determining varietal identity and varietal purity.

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

For more information on the OECD Seed Schemes see: www.oecd.org/tad/seed

International Union for the Protection of New Varieties of Plants (UPOV)

Type of Organization: Intergovernmental

Membership

[List of UPOV members](#) / [Situation in UPOV](#)

What is UPOV?

The International Union for the Protection of New Varieties of Plants (UPOV) is an intergovernmental organization based in Geneva, Switzerland. UPOV was established in 1961 by the International Convention for the Protection of New Varieties of Plants (the "UPOV Convention").

The mission of UPOV is to provide and promote an effective system of plant variety protection, with the aim of encouraging the development of new varieties of plants, for the benefit of society.

The UPOV Convention provides the basis for members to encourage plant breeding by granting breeders of new plant varieties an intellectual property right: the breeder's right.

What does UPOV do?

UPOV's mission is to provide and promote an effective system of plant variety protection, with the aim of encouraging the development of new varieties of plants, for the benefit of society. The main objectives of UPOV are, in accordance with the UPOV Convention, to:

- provide and develop the legal, administrative and technical basis for international cooperation in plant variety protection;
- assist States and organizations in the development of legislation and the implementation of an effective plant variety protection system; and
- enhance public awareness and understanding of the UPOV system of plant variety protection.

What are the benefits of plant variety protection and UPOV membership?

The UPOV Report on the Impact of Plant Variety Protection demonstrated that in order to enjoy the full benefits which plant variety protection is able to generate, both implementation of the UPOV Convention and membership of UPOV are important. The introduction of the UPOV system of plant variety protection and UPOV membership were found to be associated with:

- (a) increased breeding activities,
- (b) greater availability of improved varieties,
- (c) increased number of new varieties,
- (d) diversification of types of breeders (e.g. private breeders, researchers),
- (e) increased number of foreign new varieties,
- (f) encouraging the development of a new industry competitiveness on foreign markets, and
- (g) improved access to foreign plant varieties and enhanced domestic breeding programs.

In order to become a UPOV member the advice of the UPOV Council in respect of the conformity of the law of a future member with the provisions of the UPOV Convention is required. This procedure leads, in itself, to a high degree of harmony in those laws, thus facilitating cooperation between members in the implementation of the system.

Does UPOV allow molecular techniques (DNA profiles) in the examination of Distinctness, Uniformity and Stability ("DUS")?

It is important to note that, in some cases, varieties may have a different DNA profile but be phenotypically identical, whilst, in other cases, varieties which have a large phenotypic difference may have the same DNA profile for a particular set of molecular markers (e.g. some mutations).

In relation to the use of molecular markers that are not related to phenotypic differences, the concern is that it might be possible to use a limitless number of markers to find differences between varieties at the genetic level that are not reflected in phenotypic characteristics.

On the above basis, UPOV has agreed the following uses of molecular markers in relation to DUS examination:

- (a) Molecular markers can be used as a method of examining DUS characteristics that satisfy the criteria for characteristics set out in the General Introduction if there is a reliable link between the marker and the characteristic.
- (b) A combination of phenotypic differences and molecular distances can be used to improve the selection of varieties to be compared in the growing trial if the molecular distances are sufficiently related to phenotypic differences and the method does not create an increased risk of not selecting a variety in the variety collection which should be compared to candidate varieties in the DUS growing trial.

The situation in UPOV is explained in documents TGP/15 “Guidance on the Use of Biochemical and Molecular Markers in the Examination of Distinctness, Uniformity and Stability (DUS)” and UPOV/INF/18 “Possible use of Molecular Markers in the Examination of Distinctness, Uniformity and Stability (DUS)”.

<https://www.upov.int/about/en/faq.html#QB80>

International Seed Testing Association (ISTA)

ISTA’S VISION: UNIFORMITY IN SEED TESTING

Founded in 1924, with the aim to develop and publish standard procedures in the field of seed testing, ISTA is inextricably linked with the history of seed testing. With member laboratories in over 80 countries/distinct economies worldwide, ISTA membership is truly a global network.

Our association produces internationally agreed rules for seed sampling and testing, accredits laboratories, promotes research, provides international seed analysis certificates and training, and disseminates knowledge in seed science and technology on behalf of our membership and governed by its member countries/distinct economies. This facilitates seed trading nationally and internationally, and therefore contributes to food security.

ISTA’S MEMBERSHIP 2019

With member laboratories in 82 countries/distinct economies worldwide, ISTA membership is a truly global network. Currently, ISTA membership consists of:

- 235 Member Laboratories, out of which 136 are ISTA accredited
- 63 Associate Members
- 39 Personal Members

ISTA’S TECHNICAL WORK

The principle objective of ISTA Technical Committees is to develop, standardise and validate methods for sampling and testing of seed quality, using the best scientific knowledge available. They enhance the **ISTA ‘International Rules for Seed Testing’** and develop ISTA Handbooks on seed methods including sampling and testing. Further they are responsible for the organisation of Symposia, Seminars and Workshops. ISTA Technical Committees regularly hold workshops which provide a platform for training as well as the exchange of information, experience and ideas.

There are 20 Technical Committees in ISTA:

	Technical Committees
1.	Advanced Technologies Committee
2.	Bulking and Sampling Committee
3.	Editorial Board of Seed Science and Technology
4.	Flower Seed Testing Committee
5.	Forest Tree and Shrub Seed Committee
6.	Germination Committee
7.	GMO Committee
8.	Moisture Committee
9.	Nomenclature Committee
10.	Proficiency Test Committee
11.	Purity Committee

12.	Rules Committee
13.	Seed Health Committee
14.	Seed Science Advisory Group
15.	Statistics Committee
16.	Seed Storage Committee
17.	Tetrazolium Committee
18.	Variety Committee
19.	Vigour Committee
20.	Wild Species Working Group

ISTA ACCREDITATION PROGRAMME:

ISTA Accreditation verifies whether a laboratory is technically competent to carry out seed sampling and testing procedures in accordance with the ISTA International Rules for Seed Testing. Accredited laboratories must run a quality assurance system, fulfilling the requirements of the ISTA Accreditation Standard. Accreditation can be granted for:

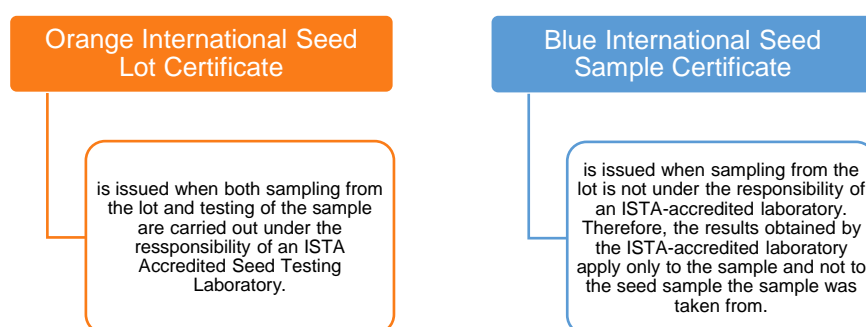
- entities performing sampling only
- laboratories performing testing only
- laboratories performing sampling and testing.

ISTA CERTIFICATES: PASSPORT FOR INTERNATIONAL SEED TRADING

Only ISTA-accredited laboratories are authorised to issue ISTA certificates for seed analysis.

By reporting seed test results on ISTA Certificates, the issuing laboratory assures that the sampling and testing has been carried out in accordance with the ISTA Rules. ISTA Certificates are accepted by most authorities and are mentioned in the seed Acts of several countries.

The ISTA certificates are assuring that the results are reproducible, true and represent the quality of the seed.



More than 200,000 ISTA Orange and Blue Certificates are issued every year, facilitating trading of seed internationally.

THE STATUS OF BIOCHEMICAL AND MOLECULAR TECHNIQUE (BMT) IN ISTA.

The ISTA International Rules for Seed Testing have included BMTs for many years. For example, BMTs are acceptable for GMO testing under a "performance-based approach"; methods that are frequently used include qualitative and quantitative protein detection analyses and various DNA-based methods. BMTs are used as diagnostic and quantitative assessment tools in seed health testing methods. Testing for species and varieties verification also makes use of BMTs by analysing storage protein profiles for sunflower, maize, oat, barley, wheat, rye grass and pea or by DNA fingerprint using molecular markers for maize and wheat. As the versatility

of these methods increases and the cost of utilizing them decreases, they may in the future play an even larger role in seed testing.

To learn more about ISTA, visit our website: www.seedtest.org

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