UPOV
International Union for the Protection of New Varieties of Plants

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Technical Working Party for Agricultural Crops

TWA/55/10

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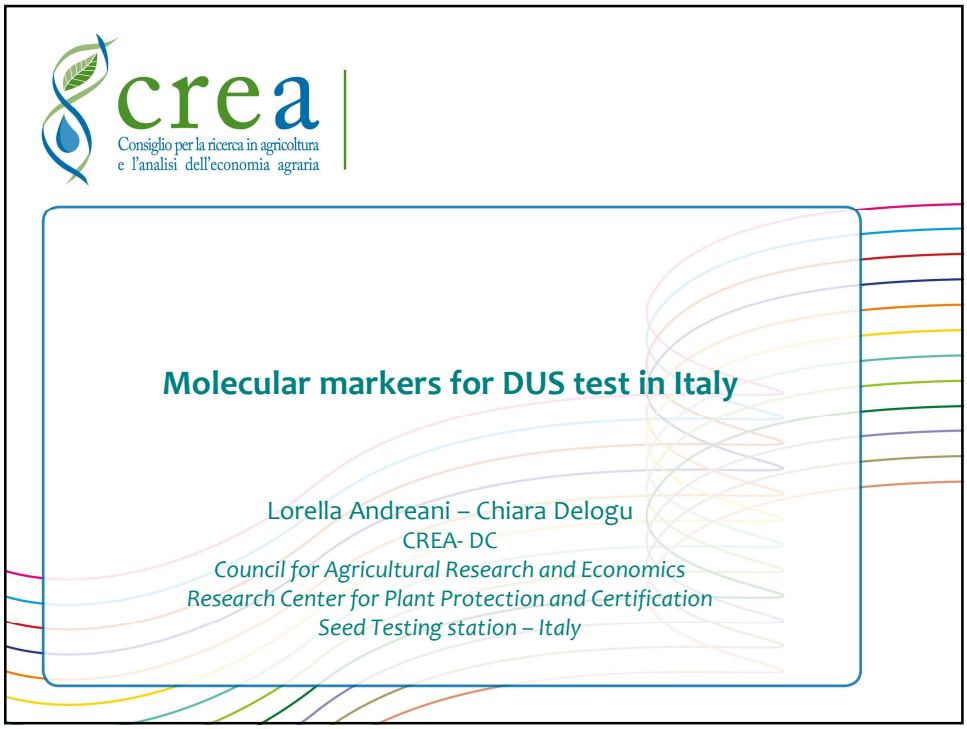
MOLECULAR MARKERS FOR DUS TEST IN ITALY

Document prepared by an expert from Italy

Disclaimer: this document does not represent UPOV policies or guidance

The annex to this document contains a presentation “Molecular markers for DUS test in Italy”, to be made by an expert from Italy, at the fifty-fifth session of the TWA.

[Annex follows]


crea
Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria
Molecular markers for DUS test in Italy
Lorella Andreani – Chiara Delogu
CREA- DC
Council for Agricultural Research and Economics
Research Center for Plant Protection and Certification
Seed Testing station – Italy

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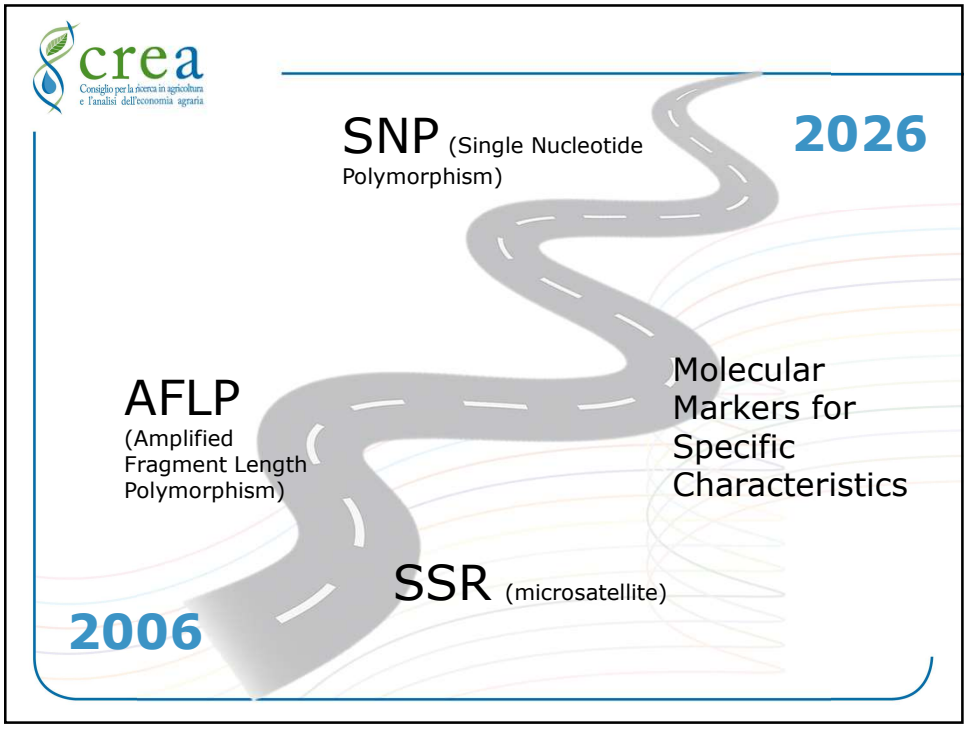
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SNP (Single Nucleotide Polymorphism)
2026
AFLP
(Amplified Fragment Length Polymorphism)
Molecular Markers for Specific Characteristics
SSR (microsatellite)
2006

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When we introduced the use of molecular markers, what were the new challenges?

- Growing number of varieties
- New breeding tecniques
- Essentially Derived Varieties (EDVs)
- Increasingly large variety collections
- A high number of field plots
- A significant increase in costs
- Complex phenotypic characteristics and strong environmental influence

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MOLECULAR MARKERS, A COMPLEMENTARY TOOL

How molecular markers can help?

- To support objective and reproducible variety identification
- To complement phenotypic DUS assessment, especially for complex or environmentally influenced characteristics
- To manage an increasing number of varieties and variety collections more efficiently
- To respond to new breeding techniques and increasingly subtle genetic differences

The use of molecular markers aims **to support, not replace**, phenotypic assessment, ensuring efficiency while preserving the principles of the UPOV system. UPOV, CPVO, ISTA and OECD are actively exploring molecular data integration.

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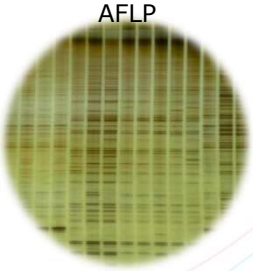
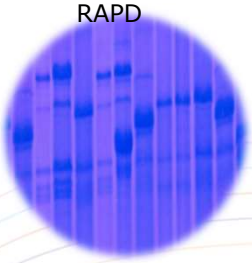
MOLECULAR MARKERS, A COMPLEMENTARY TOOL

We assessed the performance of different molecular markers in multiple species.

AFLP



RAPD



SSR

Many marker systems were progressively discontinued over time; **SSR markers** are those that have been retained and continue to be used to date.

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SSR: ISTA and UPOV

SSR markers satisfy the requirements defined by UPOV

ISTA introduced SSR markers for Maize (2018), Wheats (2017), Oat and pea (2023)

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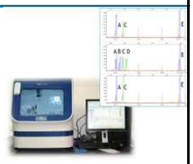
Molecular Data and Their Analysis

What type of data do we obtain?

SSR molecular markers produce, for each analyzed individual, a numerical series expressing the length of DNA fragments obtained for each SSR locus analyzed.



	Bo 1897		Bo 1652		Bo 1740		Bo 0836		Bo 0808		Bo 1909		Bo 2499		Bo 1795		Bo 3139	
PATRESI	216	216	202	222	220	220	139	147	236	236	215	215	209	227	205	205	186	192
PATRESI	216	216	202	202	220	220	139	155	236	236	215	215	209	227	205	205	186	192

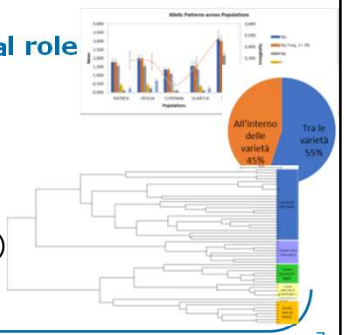
Bioinformatic analysis plays a fundamental role

Discrimination power of the markers, allele frequency, sample heterozygosity, etc.;

Assessment of inter- and intra-varietal genetic variability (e.g. AMOVA, etc.);

Assessment of genetic similarity through multivariate analyses (e.g. UPGMA, PCoA, etc.)

Genetic distance.



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Use of SSR Markers in DUS Testing in Italy

Species where SSR molecular markers are currently applied in routine DUS testing:

Rice

Oryza sativa

Hybrid Formula Validation

SSR markers used to verify the genetic purity of hybrid seed lots and validate the hybrid formula declared by breeders.

Soybean

Glycine max

Variety Identification

A panel of SSR loci applied for genetic characterisation and variety discrimination within the national variety collection.

Khorasan Wheat

Triticum turgidum subsp. turanicum

Species Verification

SSR markers used for variety description and species verification in accordance with ISTA Rules and MIPAAF decree.

Molecular markers aim to support, not replace, phenotypic DUS assessment — in line with UPOV principles.

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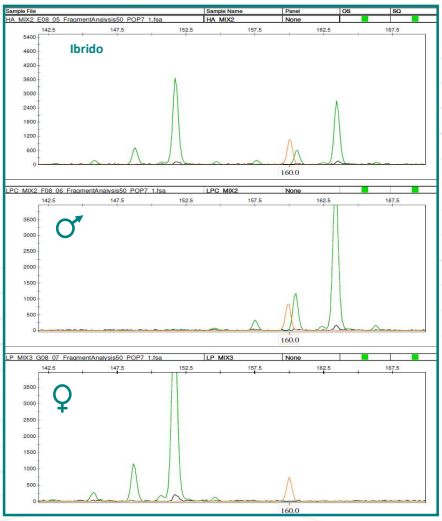
Rice -12 SSR loci

Hybrid formula validation

Technical criteria and procedures for inclusion in the National Register of rice – Decree of the Italian Ministry of Agricultural and Forestry Policies of 25 February 2014, published in the Official Gazette No. 91 of 18 April 2014



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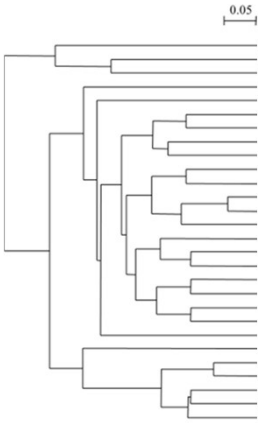
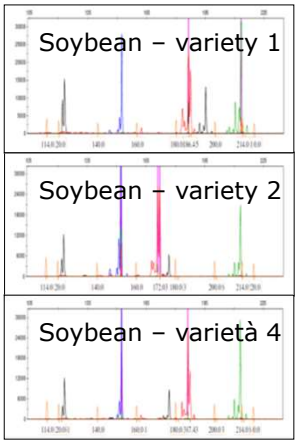
Soybean - Varietal description (20 SSR markers)





Technical criteria and procedures for inclusion in the National Register of soybean varieties – Decree of the Italian Ministry of Agricultural and Forestry Policies of 13 January 2017, published in the Official Gazette No. 50 of 1 March 2017

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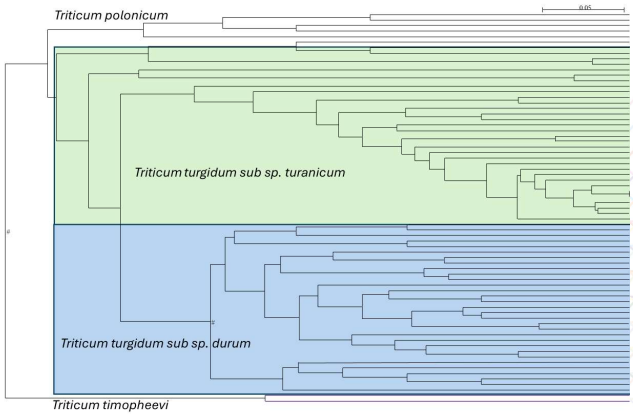
Triticum turgidum sub.sp. *turanicum*

Variety description and species verification



Ista Rules method 8.10.2
18 SSR + 4 additional SSR selected by the laboratory

Voluntary Register and Evaluation Criteria for Korasan Wheat (*Triticum turgidum subsp. turanicum*) MiPAAF Decree of October 24, 2019, published in the Official Gazette No. 265 of November 12, 2019

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Specific molecular markers for trait characterization in Routine Analysis

UPOV Model

Characteristic-specific markers ➡ fully predictive ➡ support DUS assessment

SPECIES	TRAIT	Bibliographic Reference
MAIZE	Male sterility type in maize	Zhiyong Liu et al. Crop Sci. 2002, 42:566-569
SOYBEAN	Peroxidase activity in soybean	Gijzen, M., 1997. A deletion mutation at the ep locus causes low seed coat peroxidase activity in soybean. Plant J. 12, 991–998.
RICE	Resistance to blast (Pyricularia oryzae)	Zampieri, E.; et al Marker-Assisted Pyramiding of Blast-Resistance Genes in a japonica Elite Rice Cultivar through Forward and Backgrund Selection. Plants 2023, 12, 757. https://doi.org/10.3390/plants12040757

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Perspectives and future developments

CREA laboratory is transitioning to SNP markers for variety characterisation — in line with the broader shift towards SNP-based molecular characterization.

SNP Technology Maturing
High-density SNP arrays and sequencing platforms now offer robust, reproducible data suitable for variety identification and relatedness testing.

CREA Internal Studies
Experimental SNP marker sets tested in selected species.

CPVO IMODDUS projects on tomato:
- International validation of a SNP set for genetic distinctness testing of tomato varieties of common knowledge.
- Development and implementation of an internationally accepted SNP database to assist tomato DUS testing.

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SNP markers: main limitations

Low individual informativeness — Marker type
Bi-allelic markers → many loci required to match SSR discriminatory power

High cost for small projects — Budget
Few samples or loci → high cost per genotype (SNP arrays or WGS): hundreds to thousands of loci required

Bioinformatic complexity — Technical
Requires specialised expertise, data storage and significant computational capacity

Very high initial set-up cost — Infrastructure
Requires SNP array scanner or NGS platform → high capital investment, often outsourced

Often outsourced — Governance
Limitations in control, flexibility, data governance and long-term harmonisation

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Challenge 1 — Producing SNP Data In-House

Instrumentation & Technology Choice

- Which platform? SNP arrays vs. reduced-representation sequencing vs. whole-genome, kASP markers, etc.
- Compatibility with variety datasets already under development
- Scalability: can the chosen system handle seasonal variety submission peaks?

Cost Sustainability

- Can we reduce field trial plot numbers — and does the genomic cost offset the saving?
- Is in-house production economically justified vs. outsourcing?

Key question: How do we produce the data we need at an accessible and sustainable cost?

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Challenge 2 — Outsourcing to a Service Provider

When delegating SNP data generation to an external service, new risks emerge:

Stability of Marker Panels

- SNP arrays used by providers may be updated or retired. Changes to the marker set compromise comparability of variety fingerprints.
- Continuity of data generation must be guaranteed in contractual terms
- Selection of a custom SNP panel to be outsourced

Data Privacy & Confidentiality

- Applicants' proprietary data must be protected under robust legal and technical safeguards.

Quality Control & Accreditation

How do we verify that external laboratory results meet the quality standards required by official examination procedures? Who audits the provider?

Outsourcing can be cost-effective, but only with strong governance frameworks in place.

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Challenge 3 — Harmonisation & Database Management

Protocol Harmonisation

- Standardised lab protocols across Designated Authorities are essential for cross-border data comparison

Enormous Variety Databases

- SNP datasets will grow exponentially as submissions increase year on year
- Storage, query speed and long-term archival infrastructure are non-trivial investments
- Who hosts, maintains and pays for the central repository?

Shared Databases — A Solution?

- Joint databases could dramatically reduce costs
- Governance: access rights, data ownership, confidentiality between member states
- Data quality control across contributing laboratories

Database Costs vs. Field Savings

- Reducing field plot costs is an incentive — but database infrastructure has its own price
- Net cost benefit depends on crop, scale, and data-sharing model
- Transparent economic modelling is urgently needed

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SNPs and Molecular Markers: A Powerful Tool

Continuous innovation in varietal analysis relies on the development and validation of robust protocols and guidelines, including DNA markers.

Progress in this field can be driven by strengthened collaboration among Designated Authorities and international organizations.

Shared expertise, harmonised protocols, and open data platforms are essential to unlock the full potential of DNA-based variety characterisation across borders.

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[End of Annex and of document]