From seed company to supermarket: the benefits of variety traceability

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Overview

- Plant variety;
- Plant variety in the food chain;
- DNA-based method for variety verification;
- A Case Study in a Pasta Production Chain;
- Variety traceability in organic Soymilk production.

Definition: Variety

UPOV Convention 1991

- Variety means a plant grouping within a single botanical taxon of the lowest known rank
- defined by the expression of the **characteristics resulting from a given genotype or combination of genotypes**,
- **distinguished** from any other plant grouping by the expression of at least one of the said characteristics
- and considered as a unit with regard to its suitability for being propagated unchanged

The use of new varieties is responsible for at least 50% of the production increases in many crops

Which Traits define a variety?

- results from a given genotype or combination of genotypes
- is sufficiently consistent and repeatable in a particular environment;
- exhibits sufficient variation between varieties to be able to establish distinctness;
- is capable of precise definition and recognition
- allows uniformity requirements to be fulfilled
- allows stability requirements to be fulfilled, meaning that it produces consistent and repeatable results after repeated propagation

Morpho-physiological traits
detectable in the **field** during the entire life cicle of the plant.
From the sowing to the harvest.

Molecular profile
detectable in the **laboratory** starting from the seeds or any vegetative material (seedlings, leaf, etc) in few days

Variety Testing

DUS test

Plant Breeder Right

Variety listing

- Technical protocol UPOV/CPVO.
- morpho-physiological traits detected in two growing cycles
- Biochemicaland molecular profiles
- Official description of the variety
- Standard sample of the variety retained by the National Designated Authority (Reference sample)
- **Variety description compliance with Distingubility, Uniformity, Stability**

Varietal certification

Seed lot varietal identity

Seed lot varietal purity

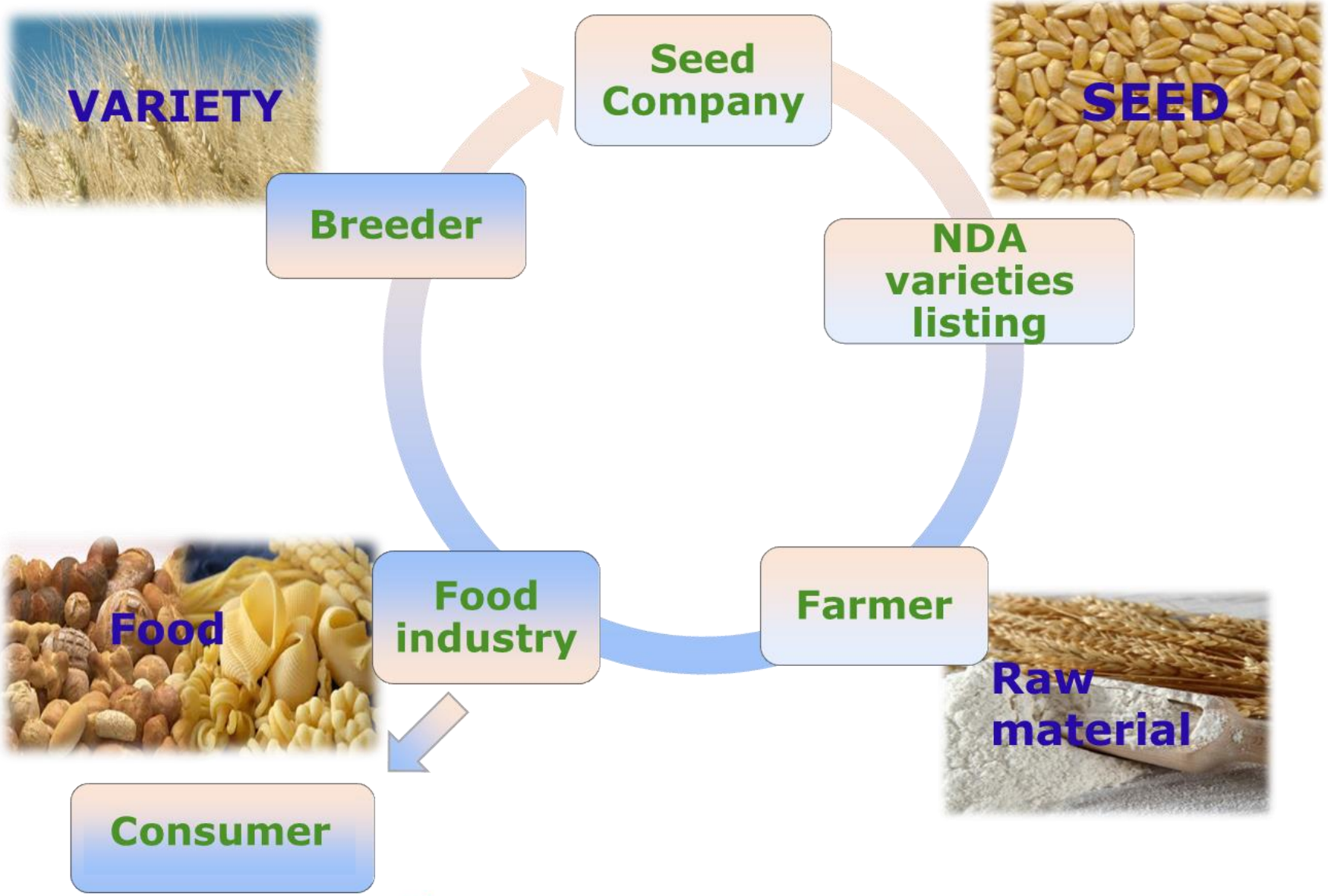
- Field inspection, Post control plots

  UPOV/CPVO/OECD Technical protocol
- Biochemical and molecular markers, ISTA Rules
- **Verification of the Seed lot identity and purity by the comparison of their morpho-physiological characteristics and/or biochemical profiles with a reference sample of the variety**

- The **identification** of the varieties based on the **description (DUS test)** of their morpho-physiological characteristics and the **verification** of the **genetic quality** of seed lots during the certification process (identity and purity) are strictly connected and represent a key issue for the **value chain from seeds to fork.**
- The objective of breeders is to select superior genotypes in the main crops, which has resulted in the release of several varieties.
- Some varieties have technological and/or nutritional characteristics that make them particularly suitable to produce certain foods.
- In addition to the production process, the quality of food is contingent upon the intrinsic characteristics of raw materials.

Plant varieties in the food chain

- Taste,
- Nutritional characteristics,
- Protein content (quality and quantity),
- Fat content (quality and quantity),
- Nutraceuticals (vitamins, polyphenols, prebiotics, fibre, etc.),
- Colour,
- Technological characteristics (processability, shelf life, etc.)


VARIETY
Seed Company
SEED
Breeder
NDA varieties listing
Food
Food industry
Farmer
Raw material
Consumer

Molecular/genetic marker

Genetic marker: is a fragment of DNA that is associated with a certain location within the genome used to identify a particular sequence of DNA in a pool of unknown DNA.

Characteristics:

- Independent of the environment.
- The DNA sequences are stable regardless of the stage of development of the plant and the tissue.
- The number of loci that can be studied is theoretically infinite.

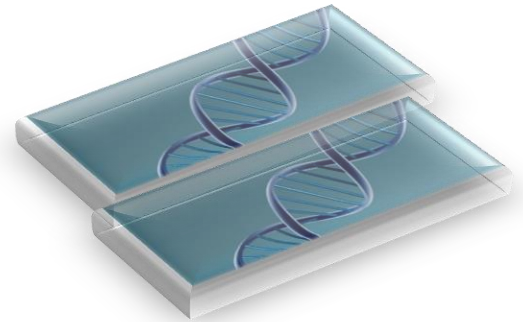
Molecular marker and variety testing

Genetic characteristics

- Good level of polymorphism;
- Well distributed in the genome;
- Codominant (possibility of identifying heterozygous and homozygous individuals);

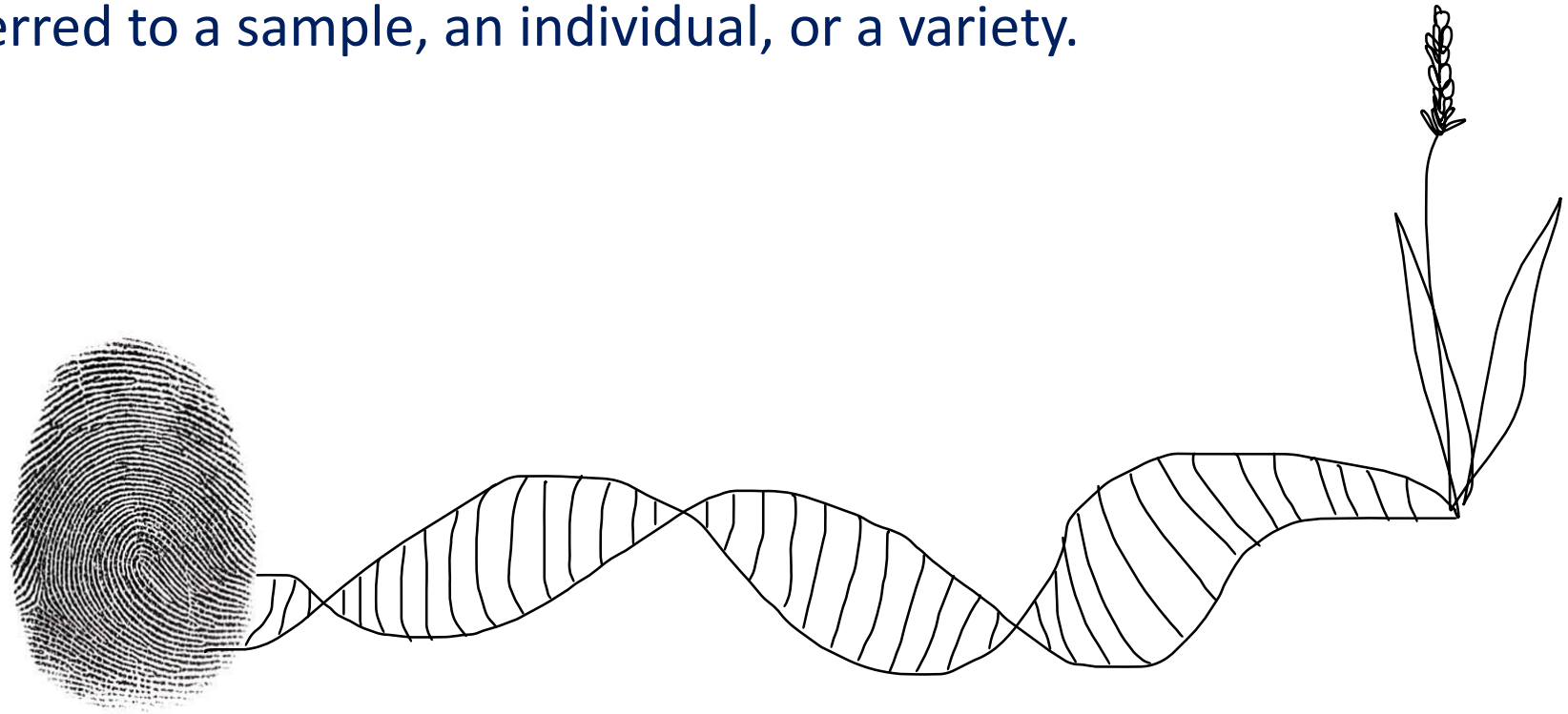
Technical features

- Repeatable, Stable
- Quick execution, Possibility of multiplexing
- Amenable to automation
- Possibility to create genetic profiles' exchangeable database



Molecular marker and variety testing

DNA fingerprinting: a combination of alleles resulting from a specific set of markers referred to a sample, an individual, or a variety.



- DNA is a stable molecule found in all living organisms, and the DNA sequence of each organism is unique, making it possible to identify the species and varieties used to produce a particular food.
- DNA can be recovered in sufficient quality and quantity even in highly processed food matrices.

- Molecular marker-based methods have become powerful and widely used for authenticating agri-food products and tracking raw materials throughout the industrial process.
- The main reasons are

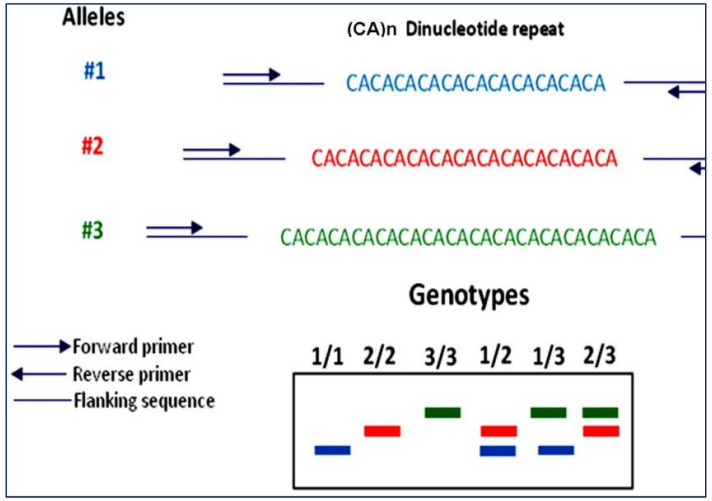
  the ability to analyse several target regions simultaneously

  The ability to provide both qualitative and quantitative information.

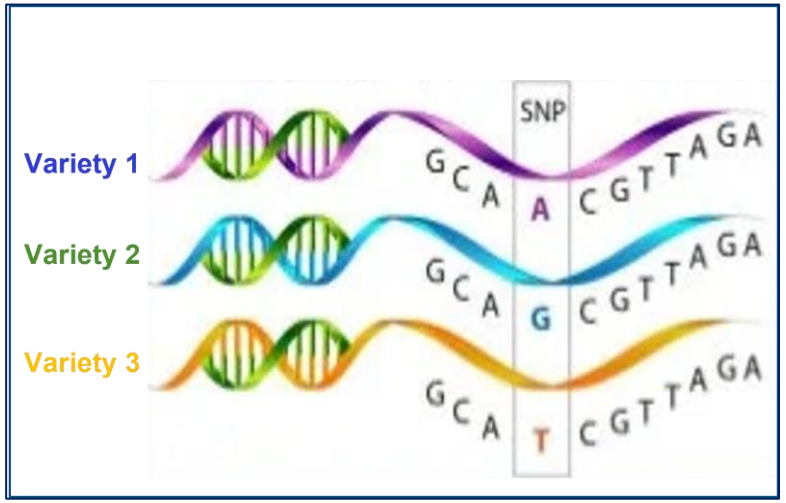
The types of molecular markers most used for traceability purposes are microsatellites or simple sequence repeats (SSRs) and single nucleotide polymorphisms (SNPs).

They are highly informative due to their large number and even distribution throughout the genome and can highlight both inter and intra-species diversity.

Simple Sequence Repeats markers (SSR)



Single nucleotide polymorphism (SNP)



Microsatellites or SSR (Simple Sequence Repeats) markers

Simple sequence repeats are tandem repeated motifs of 2–6 bp flanked by highly conserved sequences.

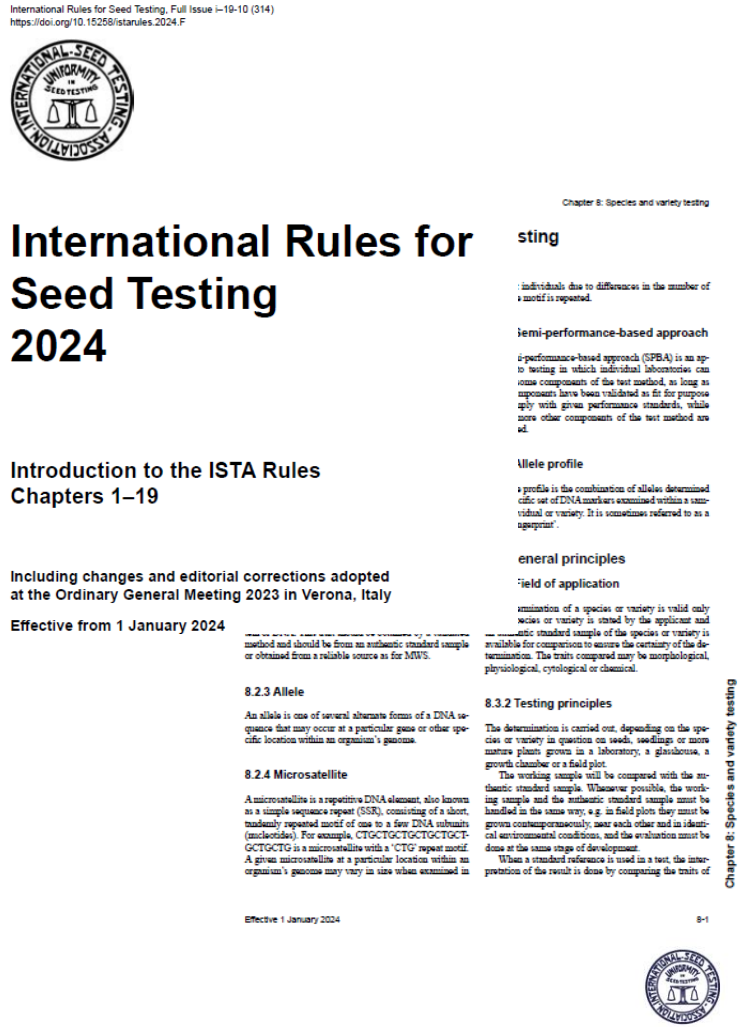
The polymorphism is due to the different number of repeats in the microsatellite region and can be easily detected by PCR.

Their high reproducibility and polymorphism degree make them a marker of choice for many applications including varietal identification and adulteration detection.

ISTA Rules
DNA-based methods: SSR markers for variety testing

ISTA introduced SSR markers for Maize (2018), Wheats (2017), Oat and pea (2023)
ISTA adopted a semi-performance approach:

- One common marker set will be PRESCRIBED by ISTA, in addition:
  - laboratories will be free to add as many markers of the same type as they need,
  - ISTA can suggest a second set of molecular markers aiming to improve the discrimination power of the method.
- Laboratory will use its “in-house validated” methodology including DNA extraction, PCR protocols, **additional markers** and data collection


International Rules for Seed Testing, Full Issue i–19-10 (314)
https://doi.org/10.15258/istarules.2024.F

International Rules for Seed Testing 2024

Introduction to the ISTA Rules Chapters 1–19

Including changes and editorial corrections adopted at the Ordinary General Meeting 2023 in Verona, Italy

Effective from 1 January 2024

Chapter 8: Species and variety testing

8.2.3 Allele

8.2.4 Microsatellite

8.3.2 Testing principles

Effective 1 January 2024

8-1

ISTA SSR protocol in wheat

- 8 SSR **prescribed**;
- 6 SSR **recommended**;
- Addition of at least 4 SSRs to the analysis protocol (**CREA-DC protocol**).

The selected SSR markers are distributed across all wheat genomes, allowing for the identification of soft wheat (AABBDD) and durum wheat (AABB).

Table 8B. Prescribed microsatellite markers and PCR primers for verification of wheat varieties

Marker	Forward primer	Reverse primer	Source
DuPw167	CGGAGCAAGGACGATAGG	CACCACACCAATCAGGAACC	[a]
DuPw217	CGAATTACACTTCCTTCTTCCG	CGAGCGTGTCTAACAAGTGC	[a]
DuPw004	GGTCTGGTCGGAGAAGAAGC	TGGGAGCGTACGTTGTATCC	[a]
DuPw115	TGTTTCTTCCTCGCGTAACC	CCTCGAATCTCCCAGTTATCG	[a]
DuPw205	ATCCAGATCACACCAAACGG	CTTCCGCTTCATCTTCTTGC	[a]
Xgwm155	CAATCATTTCCCCCTCCC	AATCATTGGAAATCCATATGCC	[b]
Xgwm413	TGCTTGTCTAGATTGCTTGGG	GATCGTCTCGTCCTTGGCA	[b]
Xgwm003	GCAGCGGCACTGGTACATTT	AATATCGCATCACTATCCCA	[b]

[a] Eujayl *et al.* (2002). Isolation of EST-derived microsatellite markers for genotyping the A and B genomes of wheat. *Theoretical and Applied Genetics* **104**, 399–407.
[b] Röder *et al.* (1998). A microsatellite map of wheat. *Genetics* **149**, 2007–2023.

Table 8C. Recommended supplementary microsatellite markers and PCR primers for verification of wheat varieties

Marker	Forward primer	Reverse primer	Source
Xgwm372	AATAGAGCCCTGGGACTGGG	GAAGGACGACATTCCACCTG	[b]
Xbarc347	GCGCACCTCTCCTCACCTTCT	GCGAACATGGAAATGAAAACTATCT	[a]
Xbarc184	TTCGGTGATATCTTTTCCCCTTGA	CCGAGTTGACTGTGTGGGCTTGCTG	[a]
Xbarc074	GCGCTTGCCCCTTCAGGCGAG	CGCGGGAGAACCACCAGTGACAGAGC	[a]
Xgwm052	CTATGAGGCGGAGGTTGAAG	TGCGGTGCTCTTCCATTT	[b]
Xgwm095	GATCAAACACACACCCCTCC	AATGCAAAGTGAAAAACCCG	[b]

[a] Song *et al.* (2005). Development and mapping of microsatellite (SSR) markers in wheat. *Theoretical and Applied Genetics* **110**, 550–560.
[b] Röder *et al.* (1998). A microsatellite map of wheat. *Genetics* **149**, 2007–2023.

Case study1
PASTA – An Italian tradition

- One of the pillars of the Italian durum wheat pasta production chain is grain identity, which is mandatory in Italy at species level.

- Not only the species, but also the variety can influence the quality of the final product.

- Commercial interest in bread and pasta made from a single variety or using one or a few specific varieties is growing.

Raw material authenticity

The question is: the processed flour or the Pasta lot derived by high-value monovarietal grains?

The protocol:

- ISTA Rules 8.10.2, 18 SSR
- Samples:

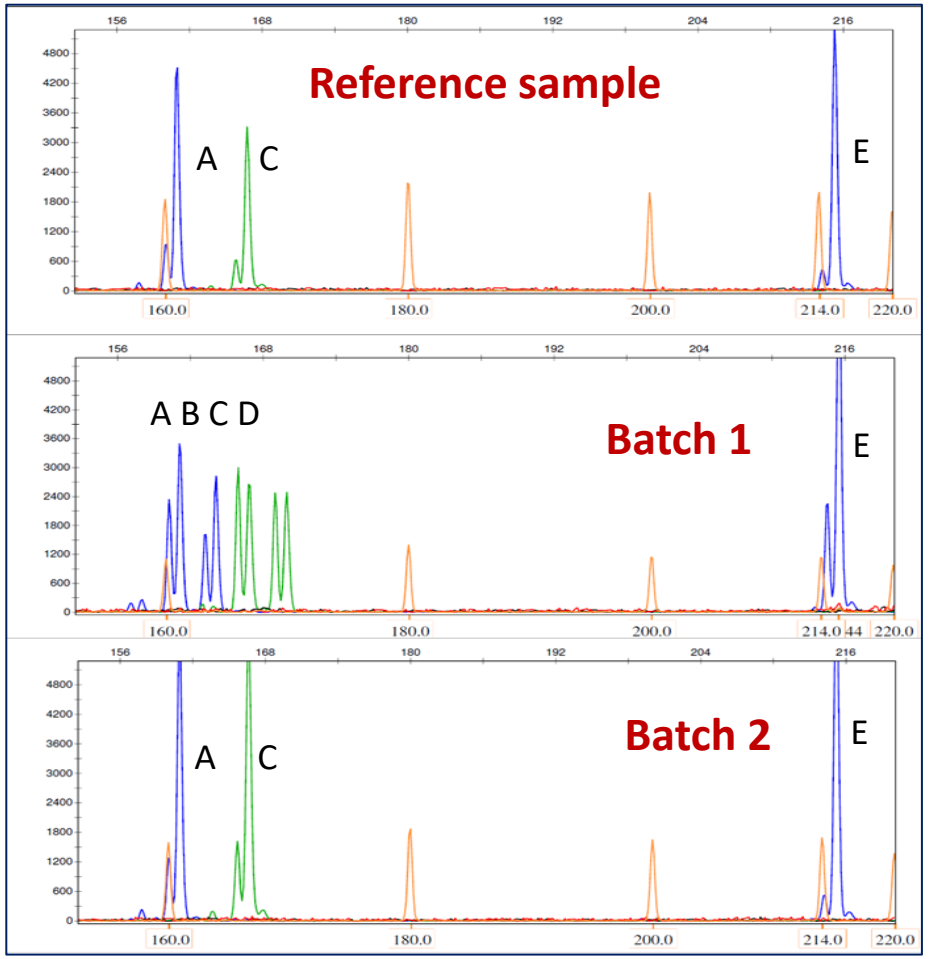
100 mg Flour (2 replicates)

100 mg grounded pasta (2 replicates)

Results:

- Target variety profile: **ACE**
- Batch 1 genetic profile:
  ABCDE-not conform
- Batch 2 genetic profile:
  ACE- conform

Batch 1 was refused by the retailer



SSR Not only qualitative evaluation but also quantitative evaluation

SSRs are very robust and flexible markers, in some conditions they be can use not only for a qualitative evaluation of the genetic profile (alleles composition at a specific Locus) but also for quantification.

Four commercial grain samples containing different percentages of a Target Variety (TV) were evaluated by:

- ✓ dPCR assay based on the private SNP of the Target Variety
- ✓ SSR genetic profile – Target Variety (TV) showed two different polymorphic alleles at two SSR loci, considered "specific marker alleles".

Actual TV% in Pasta	Mean TV% in Flour (dPCR)	Std Dev	Absolute Error	Relative Error
90%	88.7	1.34	1.25	0.01
70%	63.4	2.69	6.6	0.09
50%	48.4	2.05	1.55	0.03
20%	26.1	0.92	6.15	0.31

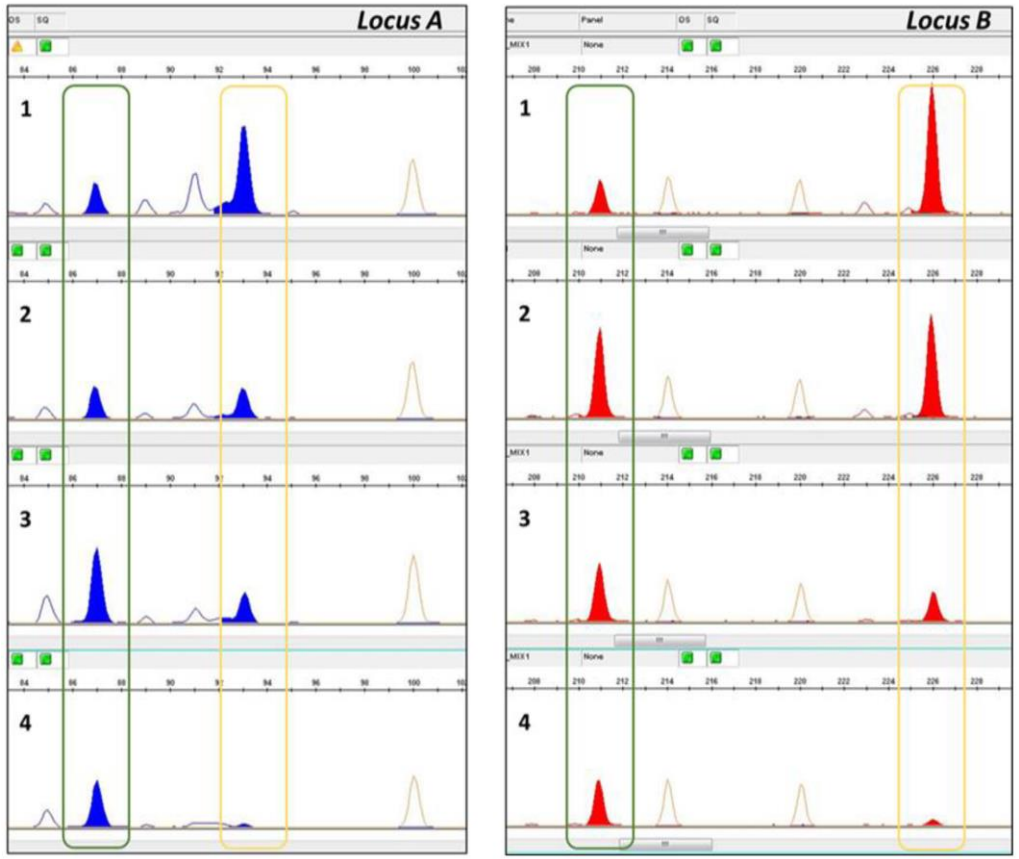
dPCR assay

Actual TV% in Pasta	Mean TV% in Flour (SSR)	Std Dev	Absolute Error	Relative Error
90%	89	0.02	1	0.01
70%	66	0.01	4	0.06
50%	49	0.03	1	0.02
20%	20.5	0.01	0.5	0.025

SSR assay

SSR Not only qualitative evaluation but also quantitative evaluation

Electropherograms showing amplicons at two polymorphic loci between the target variety (TV) and other variety.



The **green line** highlights the **Target Varieties alleles**, while the **yellow line** highlights the **contamination with other variety.**

Plots of Locus A and Locus B from 1 to 4 show the electropherogram obtained from the pasta samples
1: TV 20%– other variety 80%,
2: TV 50%– other variety 50%;
3: TV70%– other variety 30%;
4: TV 90%– other variety 10%

Case study 2 - Variety traceability in organic Soymilk production

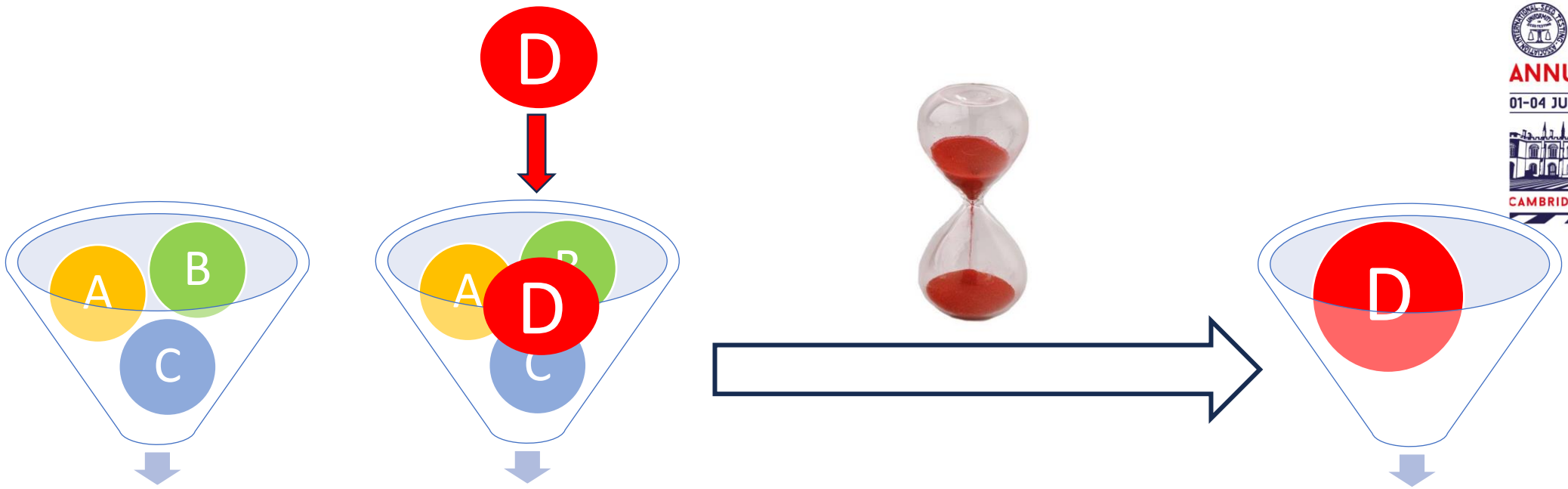
We were approached by a major manufacturer of soya-based food to ask the following question:

In the production of soy milk, how can contamination of organic soy with conventional soy be avoided?

Conventional soya milk produced from varieties A, B and C

Organic soya milk produced from variety D

How long does it take to get a milk with only organic variety D?



Sample1
Conventional soy milk only
Time 1

Sample2
Mix soy milk
Time 2

Sample 3
Mix soy milk
Time 3

Sample 4
Mix soy milk
Time 4

Sample 5
Organic soy milk only
Time 5

Case study 2 - Variety traceability in organic Soymilk production

✓**Analysis samples**:

5 soymilk samples taken at different times after introducing the D variety used to produce organic soymilk.

Reference sample of A, B, C, and D varieties

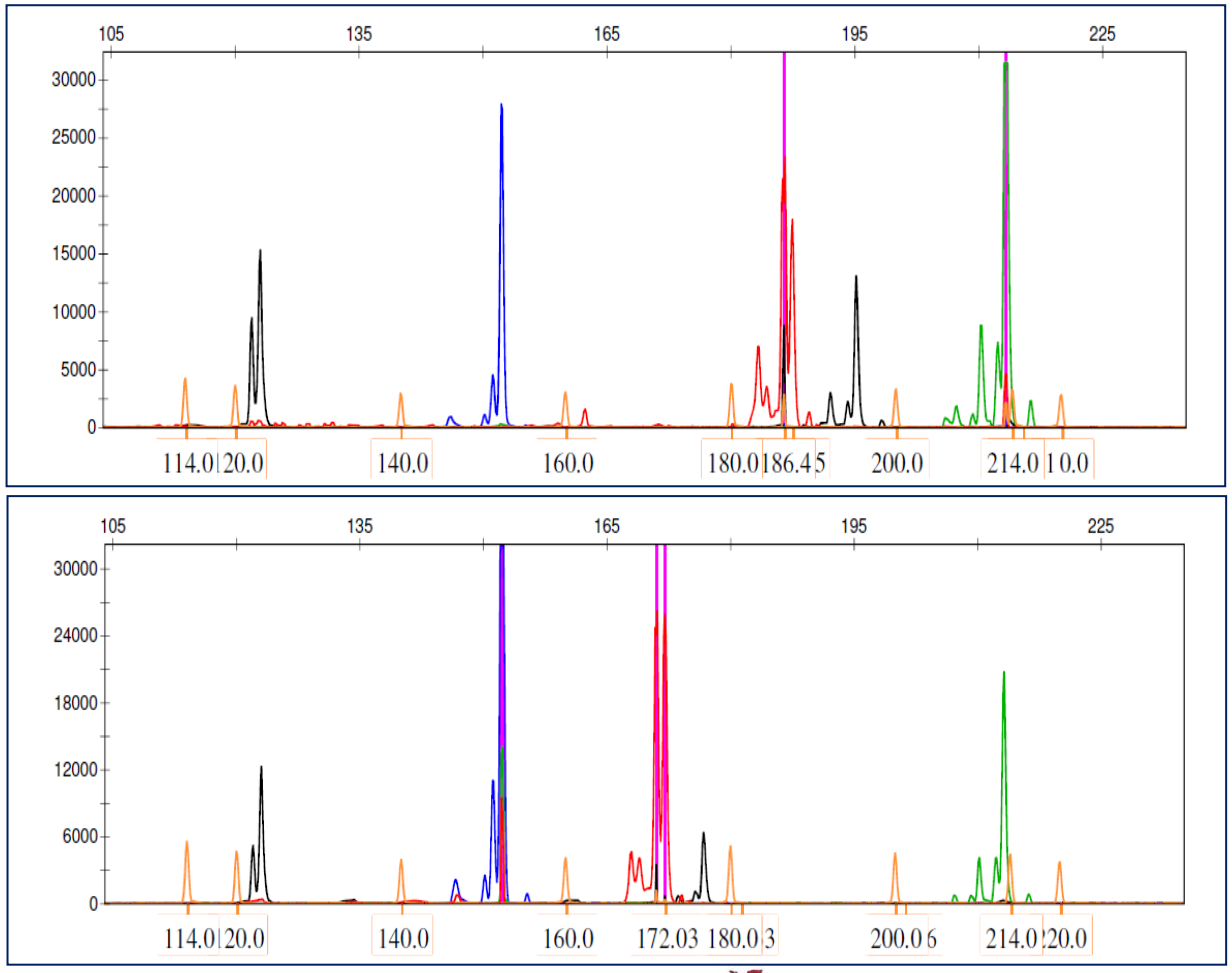
✓**Methods:**

DNA extraction using a CTAB protocol

Amplification of 20 selected SSR markers to describe soybean varieties - Official national protocol developed at CREA-DC for the registration of new soybean varieties

https://www.gazzettaufficiale.it/do/atto/serie_generale/caricaPdf?cdimg=17A01645001000101100001&dgu=2017-03-01&art.dataPubblicazioneGazzetta=2017-03-01&art.codiceRedazionale=17A01645&art.num=1&art.tiposerie=SG

Case study 2 - Variety traceability in organic Soymilk production



Conventional soya milk produced from varieties A, B and C

Organic soya milk produced from variety D

Conclusion

Traceability and authentication of agri-food products requires reliable and accurate methods for the unambiguous identification of plant species and varieties in a wide range of fresh and processed foods.

Molecular approaches offer accurate, sensitive, and reproducible methods for food authentication.

The ability to provide robust and accurate results is strengthened by internationally harmonised methods and accredited laboratories.

ISTA's validation of varietal identity verification methods is a starting point for controlling the entire supply chain, from seed to fork.

Even in a dish of pasta there is DNA!

ISTA ANNUAL MEETING 2024
01-04 JULY
CAMBRIDGE, UNITED KINGDOM

Thank you!
Grazie!

Credits: https://blog.slowfoodeditore.it/2019/10/25/pasta-alla-scoperta-dei-formati-regionali/