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ADDENDUM

**USE OF A MICROSATELLITE-BASED SYSTEM FOR THE CONSTRUCTION OF A
REFERENCE COLLECTION IN GRAPEVINE**

Document prepared by experts from Spain

Use of a microsatellite-based system for the construction of a reference collection in grapevine

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BMT 11. Madrid, September 16 to 18, 2008



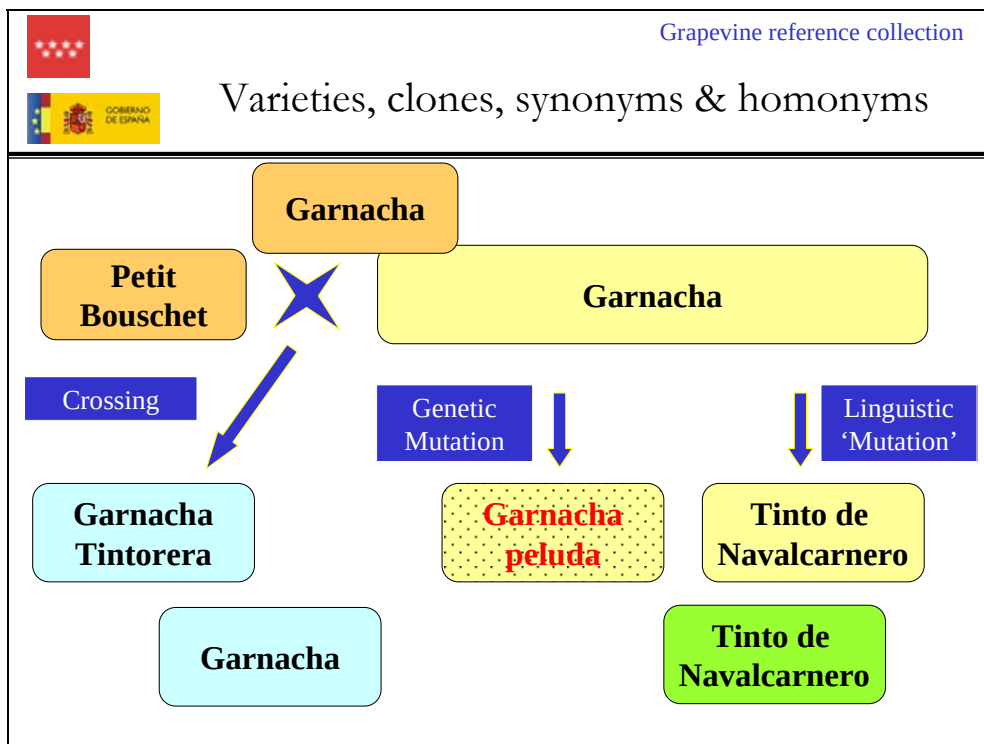
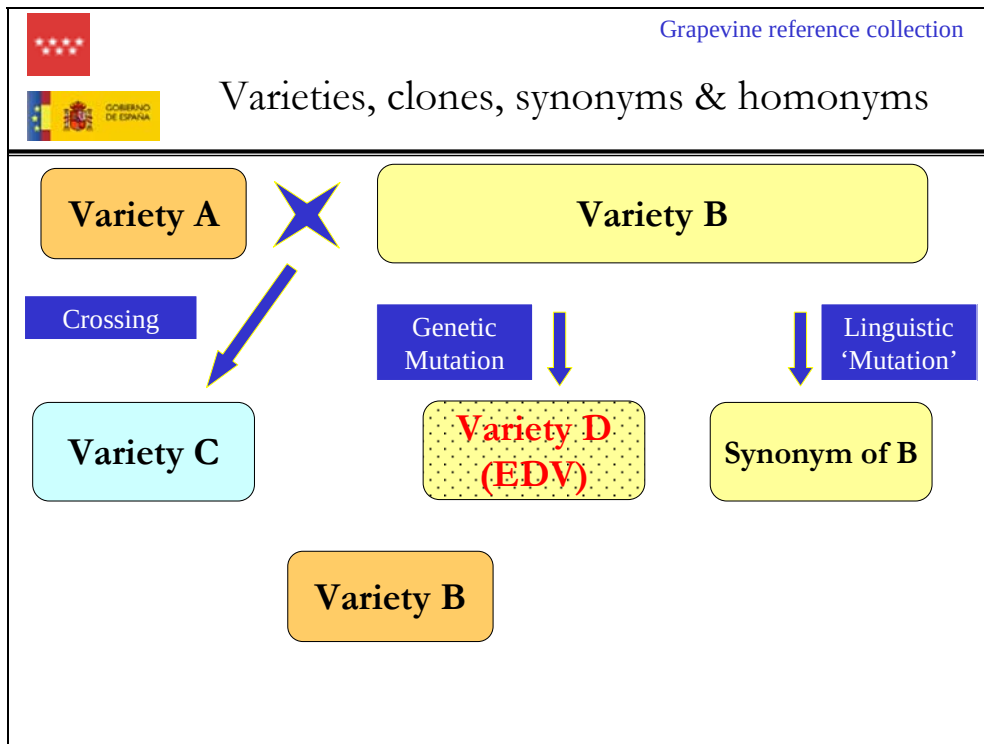
Grapevine reference collection



Vitis vinifera L.

- Woody, asexually multiplied
- Very old culture
- Widely spread culture
- Very high number of varieties
- Very old varieties
- Synonyms and homonyms







Grapevine reference collection

Set of Microsatellite Markers

ZAG67	VVMD5	VVMD27
ZAG29	ZAG62	ZAG112
VVS2	ZAG83	VVMD28

Multiplex PCR of 9 microsatellites



Grapevine reference collection

Characterization of grapevine varieties with
9 microsatellites

- Set of 9 microsatellites used in:
 - Study of a grapevine collection: 991 accessions (2 plants / accession)
 - Study of the Uniformity and Stability: More than 3,200 plants of 19 varieties



Grapevine reference collection

Study of a grapevine collection

Locus	Nº Alleles	He	Ho	PIC
ZAG67	22	0.8352	0.7832	0.8163
VVMD27	22	0.8430	0.8241	0.8233
VVMD5	19	0.8737	0.8732	0.8604
ZAG29	15	0.3752	0.3313	0.3572
ZAG62	18	0.8165	0.8139	0.7963
ZAG112	16	0.8146	0.8016	0.7915
VVS2	21	0.8591	0.8773	0.8449
ZAG83	15	0.7644	0.7771	0.7259
VVMD28	23	0.8724	0.8732	0.8600



Grapevine reference collection

Study of the Uniformity and Stability

- 9 microsatellites and 19 varieties (wine and table)
- About 3,200 plants (185 plants per variety)
- Microsatellite x variety x plant:
 - Nº analyzed: 29.356
 - Nº correct (as expected): 29.305 (99.8%)

	Grapevine reference collection
	Characterization of grapevine varieties with 9 microsatellites
• Conclusions: – 9 microsatellites highly polymorphic – 9 microsatellites uniform and stable – All different varieties (except somatic mutants) were distinguished by 2 or more alleles	

	Grapevine reference collection
	OEVV Grapevine reference collection
• OEVV: Spanish Plant Variety Office • In charge of: – Register of Commercial Varieties (RCV) – Granting plant breeders' rights • DUS examination • Centre of Trials for Variety Assessment (INIA)	



Grapevine reference collection

OEVV Grapevine reference collection

- Hold by INIA (Murcia)
- 331 grapevine varieties grafted on Richter 110 as rootstock
- 39 rootstock varieties, including the 24 listed in the RCV
- 4 varieties under evaluation for plant breeders' rights
- 20 for the Register of Commercial Varieties



Grapevine reference collection

DUS testing in grapevine

Morphological descriptions:

- Long time (1-3 years) before the grafted scions can be described
- Complete description takes at least 2 years to be completed
- Description not easy



	Grapevine reference collection
	Collaboration between OEVV and IMIDRA
• Use of 9 microsatellites: – Management of the reference collection – Technical examination of DUS	

	Grapevine reference collection
	Collaboration between OEVV and IMIDRA
• Use of 9 microsatellites: – Management of the reference collection • To detect mistakes through the identification of the different plants of a given variety • To confirm the true-to-type status of certain varieties that have been fully characterized in El Encín collection • To confirm or reject synonymies and homonymies	

 	Grapevine reference collection Collaboration between OEVV and IMIDRA
• Use of 9 microsatellites: – Technical examination of DUS: • to detect mistakes through the identification of the different plants of a given variety • to assist to choose which will be the most suitable varieties to compare with for distinctness Distinctness is always based on morphological characteristics	

 	Grapevine reference collection Collaboration between OEVV and IMIDRA
• Use of 9 microsatellites: – 645 samples completed – 375 could be identified using as a reference the El Encín collection database – 159 different genotypes – other 270 not identified: • Not available • Not completed	



Grapevine reference collection

Collaboration between OEVV and
IMIDRA

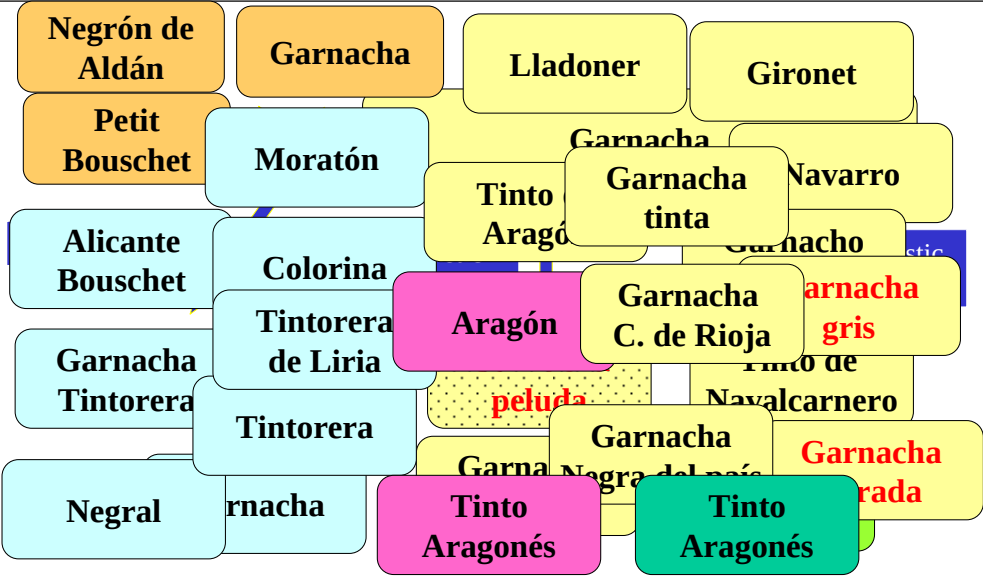
Conservative approach

1. to re-analyze the samples when finding only between one and three different alleles
2. to analyze more microsatellites if such a difference persists



Grapevine reference collection

Varieties, clones, synonyms & homonyms



Use of a microsatellite-based system for
the construction of a reference
collection in grapevine

Thank you for your attention

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