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Clonal Variation – Consequences and Methods of Detection

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Genetic Diversity

Diversity

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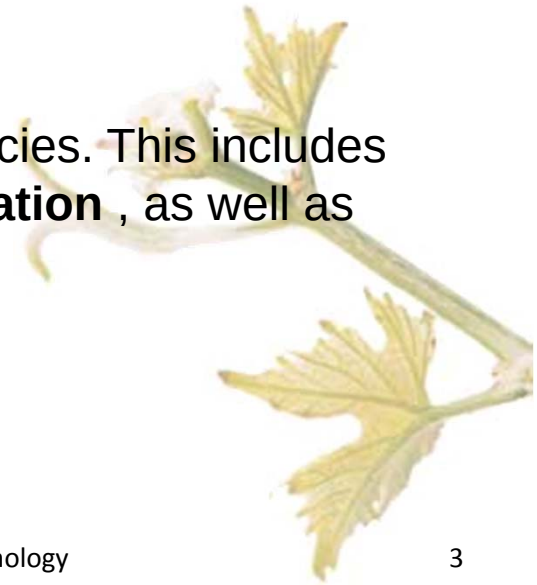
Biodiversity gives the variety of life on earth. This variety can be measured on several different levels:

- **Ecosystem**: Communities of plants and animals, together with the physical characteristics of their environment

- **Species**: Species diversity is the variety of species in a given region or area.

- **Genetic**: Variation between individuals of the same species. This includes genetic variation between individuals in a **single population** , as well as **between different populations of the same species**.



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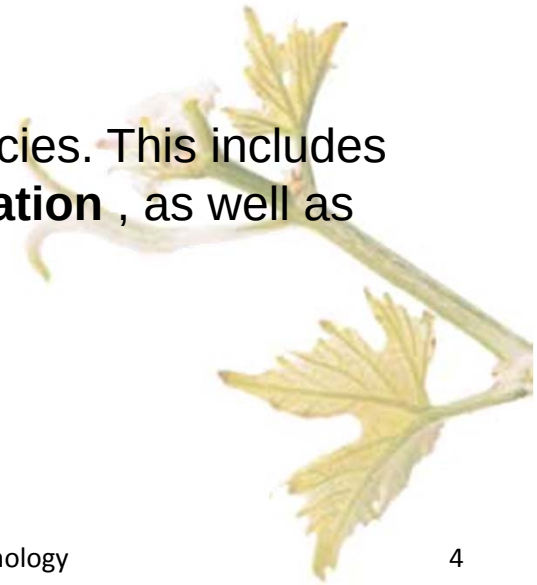

Genetic diversity

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Sources of genetic diversity:

- **Crossing overs** during Meiosis and **Recombination**
- **Mutations** which happen due to environmental influences (stress inducers, like radiation) or failure of DNA repair mechanism

Grape clonal diversity - Vegetative Propagation

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Horticultural clones are asexually derived from **one single individual**, and clonal variation can only occur through mutations.

Molecular markers are an important tool for the differentiation and identification of clones and mutations.

For **breeding purposes** and **clonal selection**, knowledge upon the variability of a given clone is essential.

Mutations

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Clonal diversity occurs only though mutations.

Mutations are **changes of the heritable information** (DNA), like single bases or even whole loci or genes.

Mutations

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Mutations can have different **effects** on clones.
Areas in the genome which are **coding** and **non-coding regions**

If a mutation occur in a non-coding region mutation is ineffective
In a coding region it can affect quality and quantity traits.

Mutations accumulate in the plant over the time

Consequences of clonal diversity

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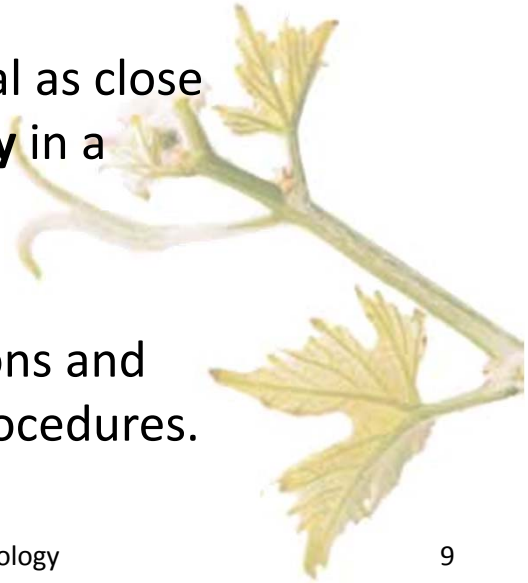
Nature and maintenance breeding oppose with each other if the plant is asexually propagated.

Fitness and clonal variation is important to react to and survive environmental changes and are important for breeder to achieve superior traits.

Maintenance breeders want to keep their plant material as close as they can to the original selection to insure **uniformity** in a vineyard and to keep characteristic **flavor** of the grape.



It is therefore important to analyze very careful mutations and their importance for the breeding and maintenance procedures.

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Project Description

Endangered Grapes

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Preservation of endangered Grapes in Croatia and Montenegro

Project between:

University of Zagreb – SSR cultivar identification, ampelographic evaluation of Croatian Clones and Cultivars

Research Institute Geisenheim – Virus testing

University of Montenegro – ampleographic evaluation of Montengrian Clones and Cultivars

BOKU – genetic variability between clones of cultivars

Genetic analysis

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Main structure of a genetic analysis:

- Study design
- Phenotyping
- Sample collection and processing
- Genotyping
- Statistical analysis

How to Analyse Genetic Diversity in the Laboratory

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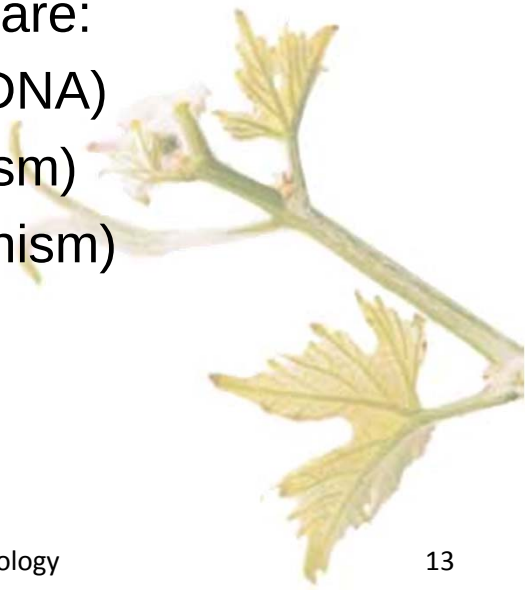
Marker analysis via Polymerase Chain Reaction (PCR) approach:

Various markers but common for Genetic Diversity are:

- RAPDs (Random Amplification of Polymorphic DNA)
- AFLPs (Amplified Fragment-Length Polymorphism)
- SSAPs (Sequence Specific Ampilfied Polymorphism)
- SNPs (Single Nucleotide Polymorphism)



Analysis of Data (1)

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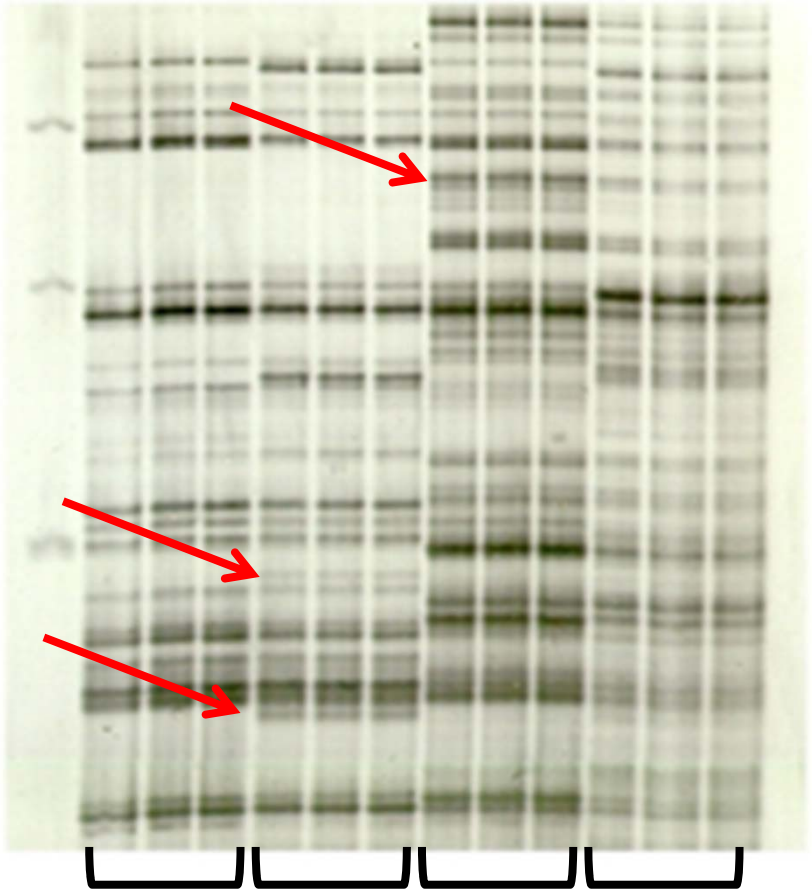
Since the fragment size give the marker pattern, occurence or non-occurence of a band gives the diversity.



Sample replicates

Example of Graphical Interpretation of Diversity Data (1)

Aradhya *et al*. (2003)

- Neighbour joining cluster analysis

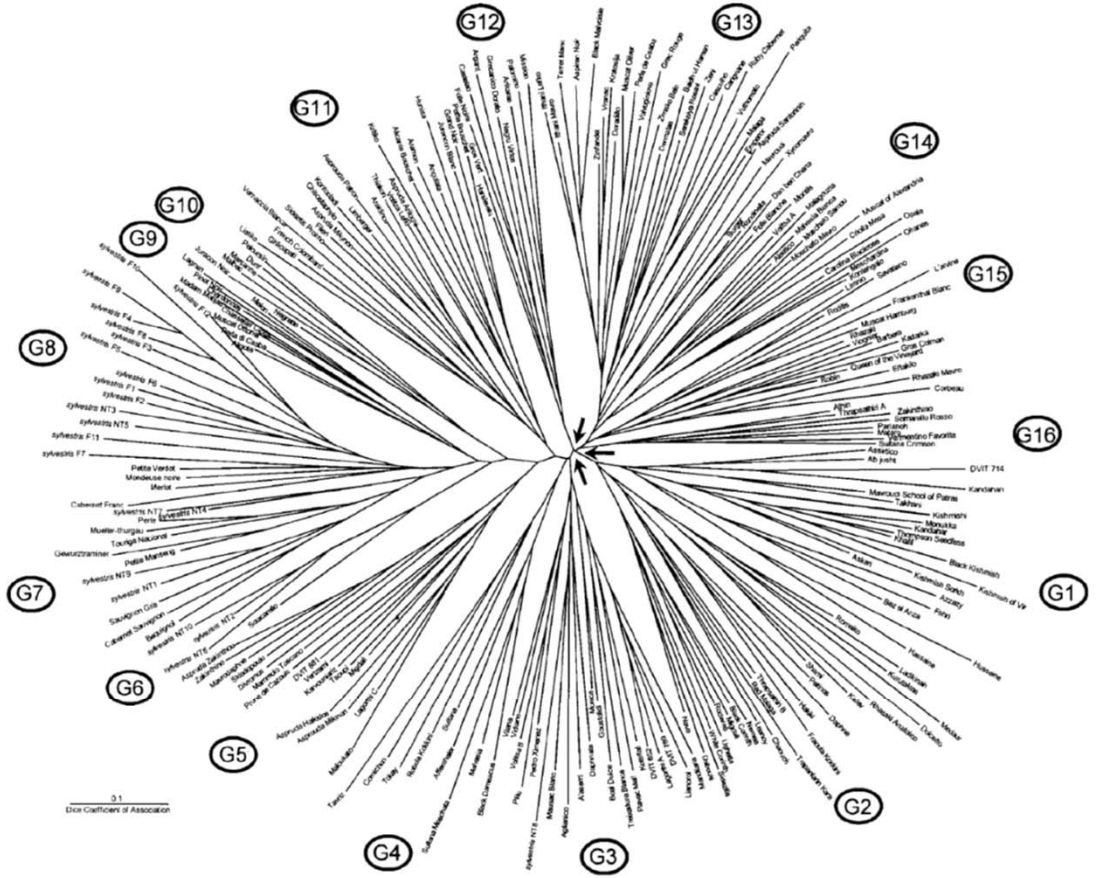


Fig. 1. Neighbour-joining cluster analysis based on the pairwise Dice coefficient of association showing the genetic relationships among grape cultivars. G1 G16 represent the genetic groups recognized for further analyses of genetic structure and differentiation within the cultivated grapes. Arrows at the centre of the phenogram demarcate the three clusters (G1 and G2, Mediterranean table grape cluster; G3 G12, Western European grape cluster; G13 G16, Central European grape cluster).

Example of Graphical Interpretation of Diversity Data (2)

- Aus Aradhya *et al*. (2003) UPGMA Cluster Analyse

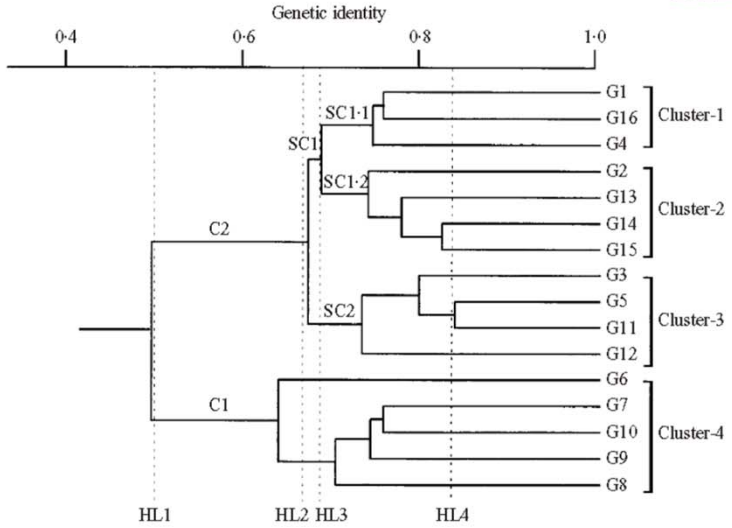


Fig. 2. UPGMA cluster analysis depicting the intergroup relationships at different levels of genetic identity (cophenetic correlation = 0·750). HL1 HL4 refer to differentiation at different hierarchical levels. Cluster 1, predominantly table type belonging to *orientalis* and some to *pontica*; clusters 2 and 3, mostly wine type with some table type belonging to *pontica*; cluster 4, the French wine types representing the group *occidentalis*.

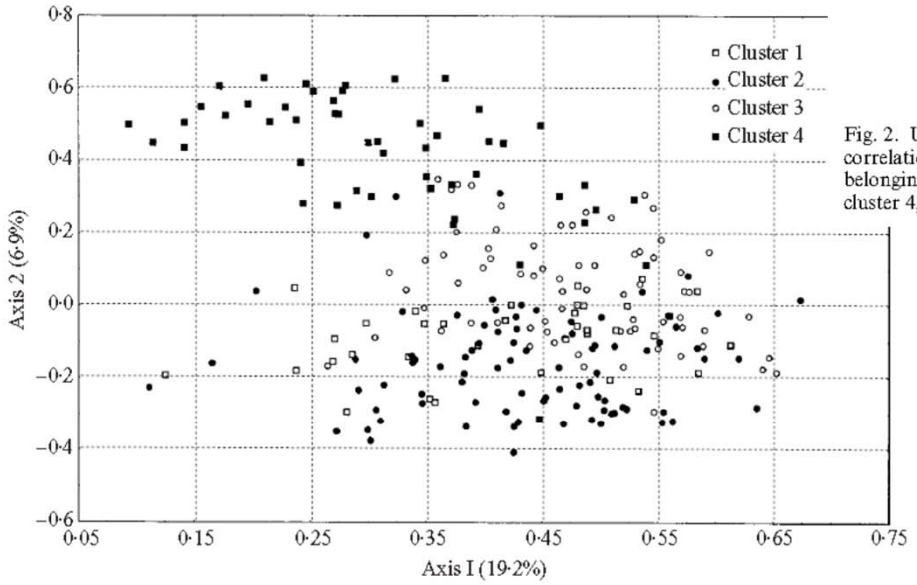
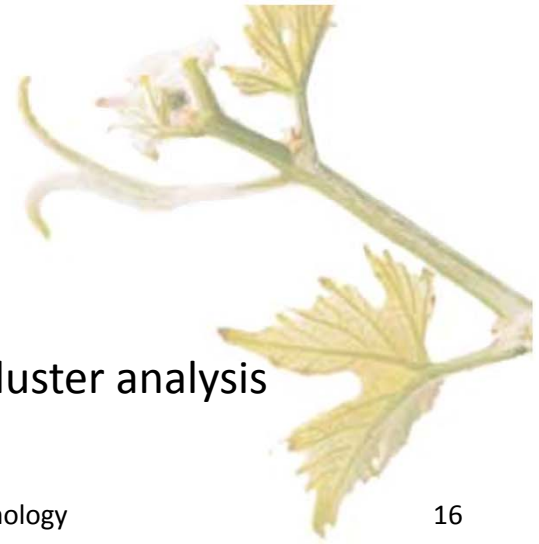


Fig. 3. Two-dimensional projection of grape cultivars along the first two principal axes accounting for ~26% of the total variation. (Clusters correspond to the UPGMA analysis in Fig. 2.)



PCA: Cluster analysis

Method of the current SEE-ERAnet project

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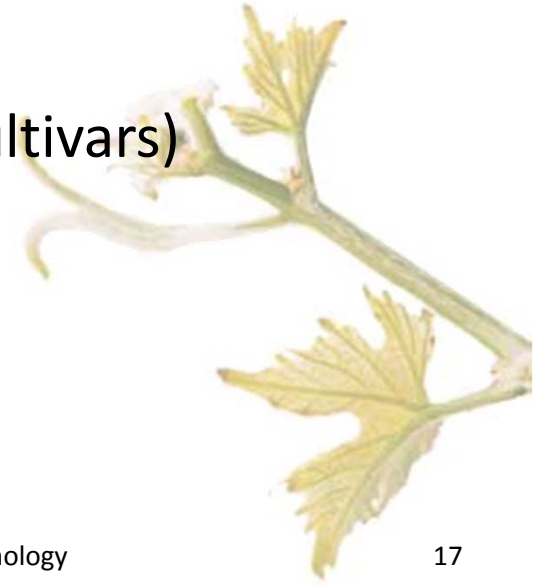
Method:

modified S-SAP (sequence-specific amplified polymorphism) method by Wegscheider et al. 2009 with universal primers for retrotransposons

214 clones from Croatia and Montenegro (11 cultivars)

11 primer combinations were used so far



Description of methods S-SAP

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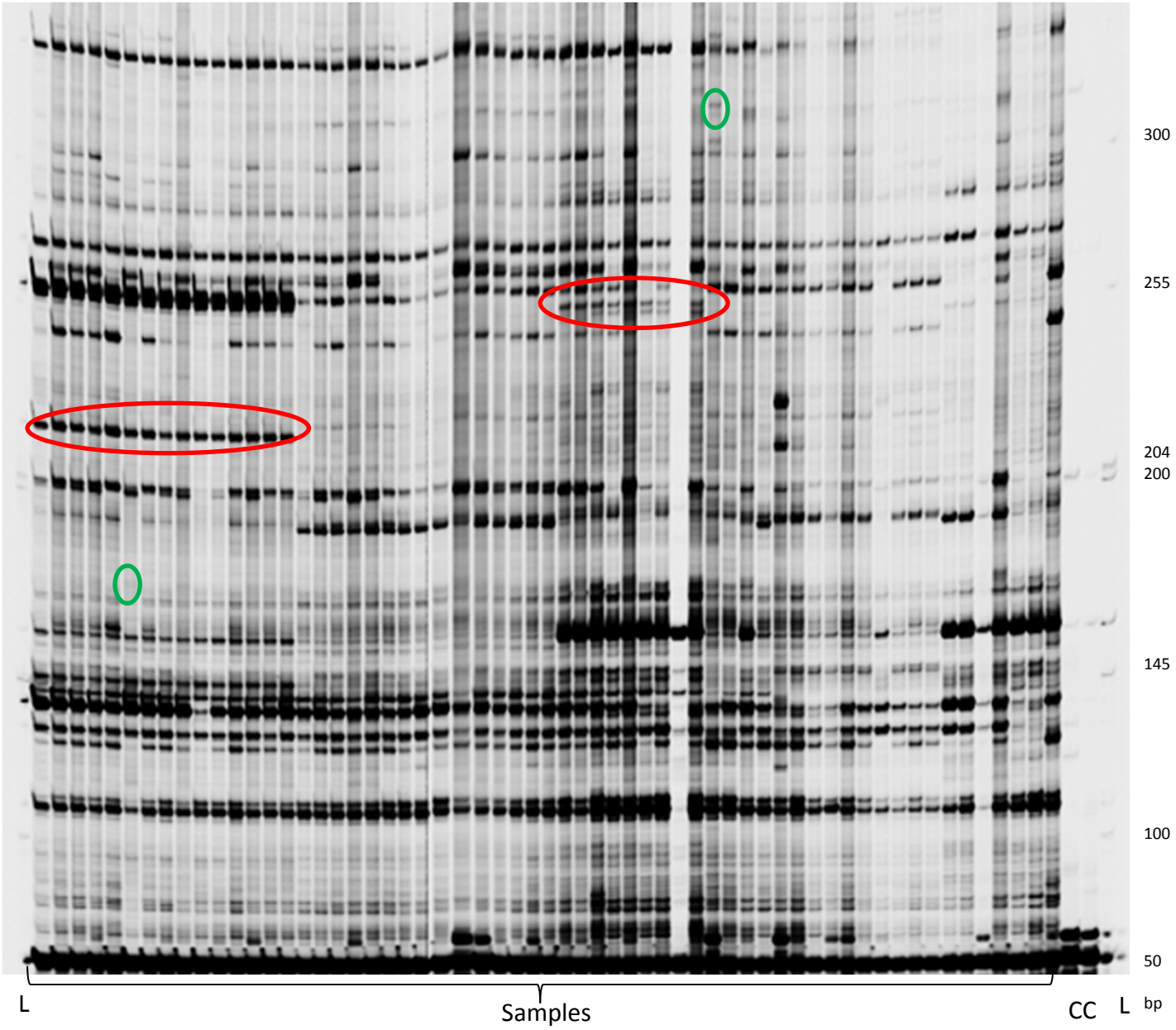
Transposable elements are jumping genes which **cause mutations** in the genome.

Transposable elements which are mainly stress-induced have been demonstrated to be the **most important source of mutations** because of their high mobility.

S-SAP method based on universal retrotransposon specific sequences.

Gel image

- Random mutation
- Specific mutation



Cultivars analyzed in 2011 and 2012

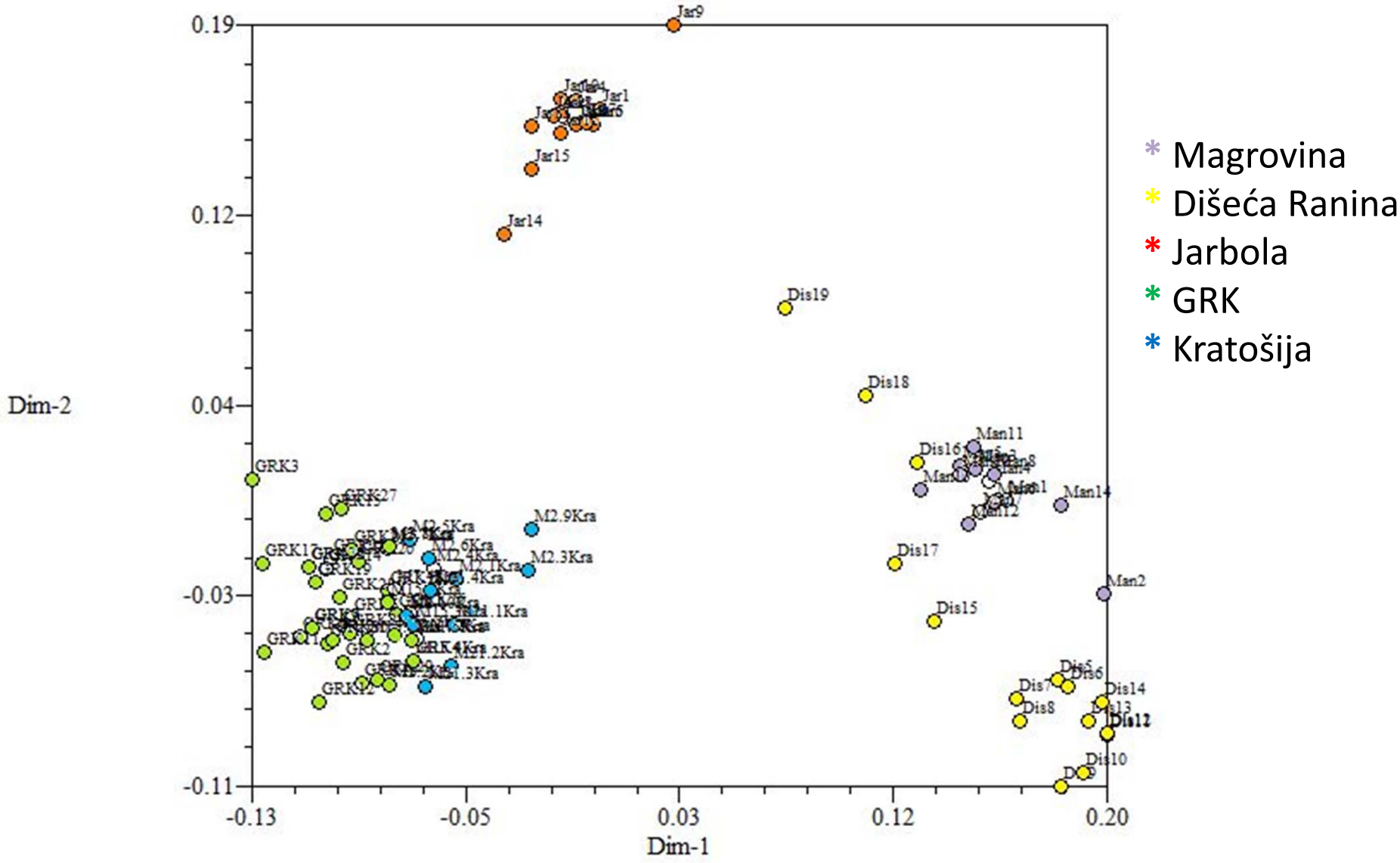
2011	Magrovina	Draganela	Sansigot	Dišeća Ranina	Jarbola	Sokol	Dobričić	Vranac	GRK	Kratošija	Čubrica
No. Individuals	*15*	*15*	*8*	*15*	*15*	*15*	*14*	*19*	*27*	*22*	*9*
Total Polym. Marker	14	7	8	20	1	11	6	7	15	10	6
Specific Mutations*[1]	7	1	5	15	1	4	6	5	9	9	3
Random Mutation*[2]	7	6	3	5	0	7	0	2	6	1	3
Total Marker	**322**										

Table 2 shows polymorphism and mutations of the cultivars separate in project year 2012, choosing the 4 most variable cultivar and one stable cultivar

2012	Magrovina			Dišeća Ranina	Jarbola				GRK	Kratošija	
No. Individuals	*13*			*15*	*15*				*28*	*22*	
Total Polym. Marker	40			40	18				30	24	
Specific Mutations*[1]	32			35	12				17	14	
Random Mutation*[2]	8			5	6				13	10	
Total Marker	**526**										

[1] Mutation in several clones of one cultivar
[2] Single mutation in only one clone

PCA all cultivars of 2012



Summary of Project Results

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- We found genetic variability between cultivars but as well within cultivars
- Mostly specific mutations which could be found in several clones of one cultivar.
- Magrovina and Dišeća Ranina showed the most polymorphic marker.
- Cultivars GRK and Kratošija showed more similarities to each other than to the other cultivars