A combined NMR-HRMS approach for discrimination of table grapes

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DICATECh, Politecnico di Bari

La Risonanza Magnetica Nucleare nel Settore Alimentare

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- Table grapes
- Fingerprinting by NMR spectroscopy and HR-MS spectrometry
- Case study 1: discrimination of grape leaves
- Case study 2: discrimination of grape berries
- Advantages in using NMR data for development of expert classification systems

Tables Grapes

ITALY

4°world producer

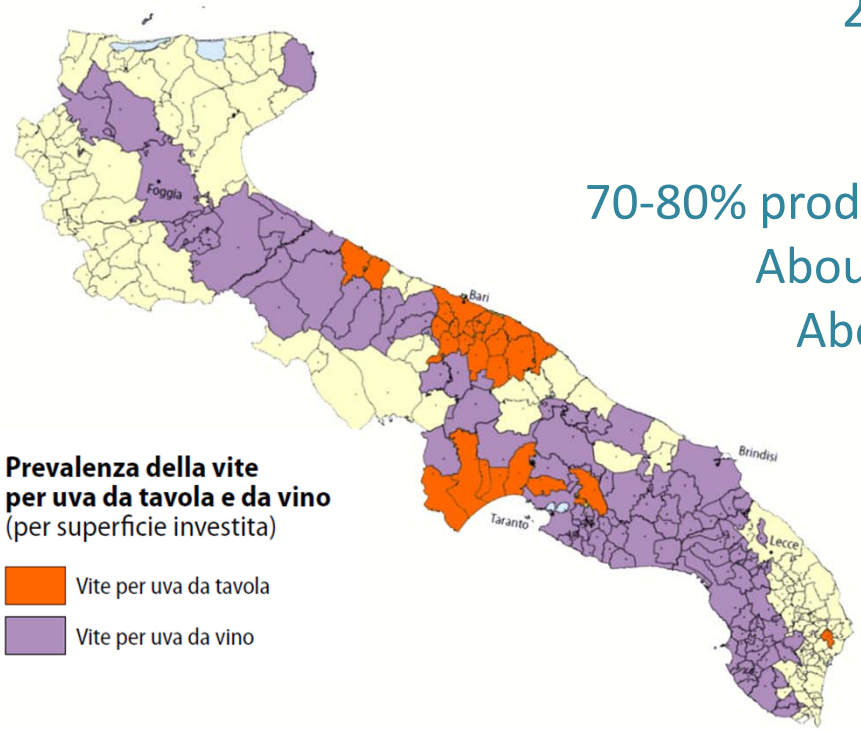
2° world exporter

APULIA

70-80% production of Italian table grapes

About 1 million tons/year

About 1 billion €/year



Tables Grapes

-Bunch weight> 300 g

- Overall diameter>

21 mm (cv. Victoria)
15 mm (cv. Regina)
22 mm (cv. Italia)
22 mm (cv. Palieri)
22 mm (cv. Red Globe)

PGI "Uve di Puglia"

- Soluble solid content>

13 °Brix (cv. Victoria)
14 °Brix (cv. Regina)
14 °Brix (cv. Italia)
13 °Brix (cv. Palieri)
14 °Brix (cv. Red Globe)

- Soluble solid content/total acidity> 22

- Color

Tables Grapes

-Bunch weight> 300 g

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PGI "Uve di Puglia"

- Soluble soli[illegible]

[illegible] Victoria)
[illegible] (cv. Regina)
14 °Brix (cv. Italia)
13 °Brix (cv. Palieri)
14 °Brix (cv. Red Globe)

Are these parameters suitable for classification of the grapes?

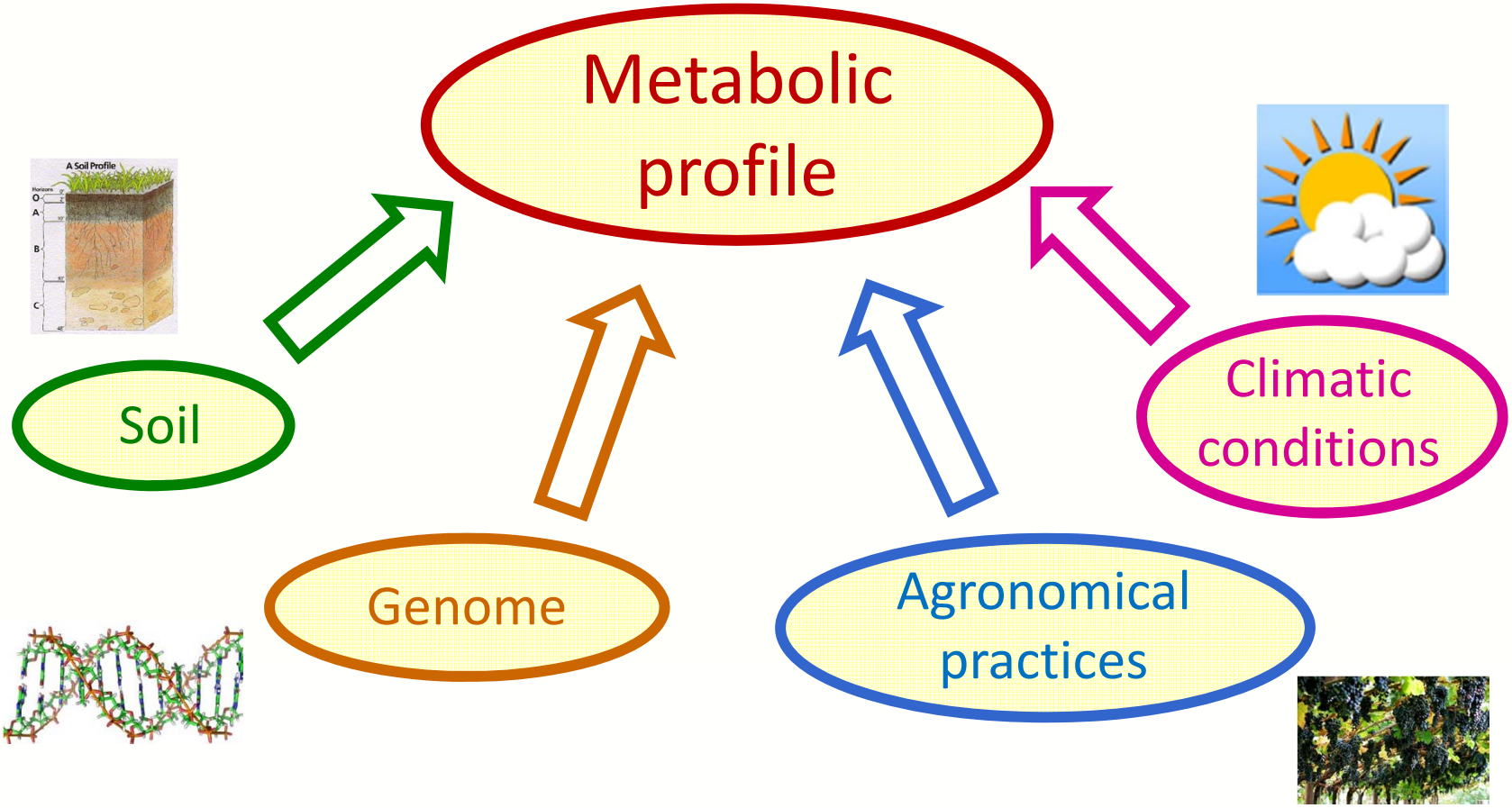
- Soluble solid content/total acidity> 22

- Color

Tables Grapes

May fingerprinting help us in classification of table grapes?

Fingerprinting



Nuclear Magnetic Resonance (NMR)

NMR Performances

> Food samples as solid and/or liquid complex matrixes

> Non-destructive analysis which permits a wide range of experimentals on the same sample

> No calibration curve for the quantitative analysis

> In most cases the preparation of the food sample does not need any manipulations

> Generation of the metabolite profile of the food sample in a single experiment

> Analysis Automation

High Resolution Mass Spectrometry (HRMS)

HRMS Performances

> Food samples as solid and/or liquid complex matrixes

> High sensitivity

> Generation of the metabolite profile of the food sample in a single experiment

>Analysis Automation

NMR and HRMS fingerprinting

Primary metabolites

(sugars and amino acids, biogenic amines, polysaccharides, alcohols, organic acids)

Reproducibility

Sensitivity

Secondary metabolites

(phenolics, simple phenolics and phenylpropanoids, flavonoids, stilbenoids)

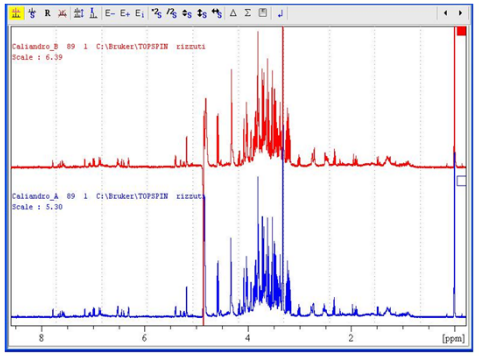
NMR and HRMS fingerprinting

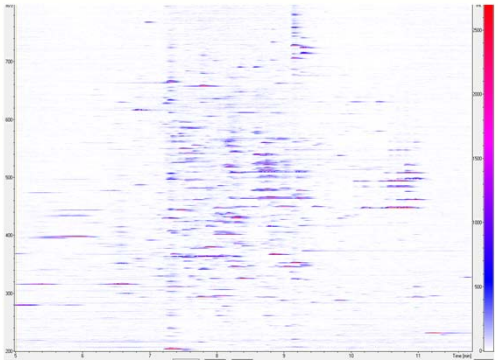
Sample preparation and analysis

NMR

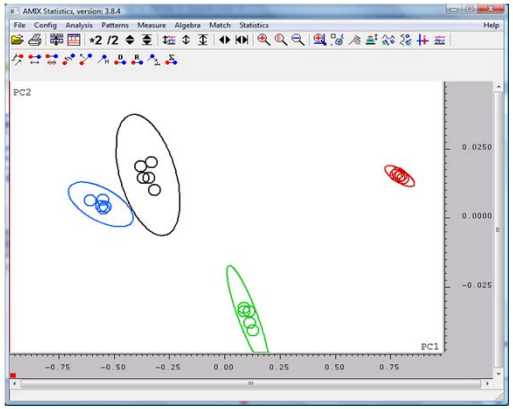
HRMS

Spectra acquisition and processing





Statistics
Multivariate Analysis
Classification algorithms



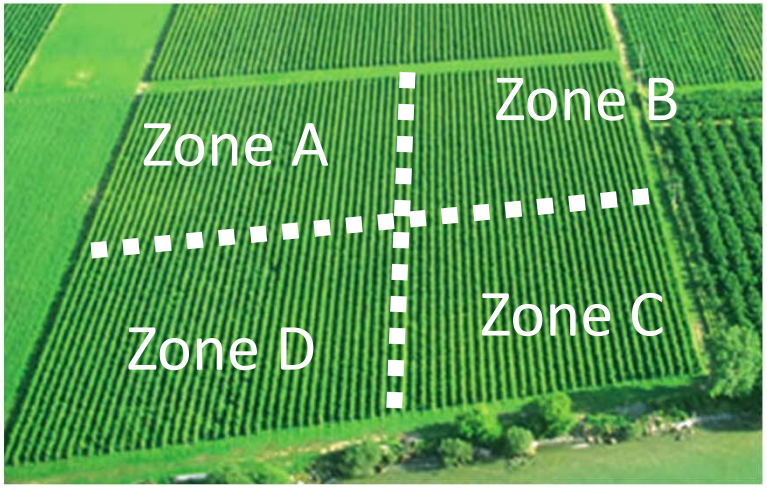
Experimental part

Classic approach

- Subdivision of the "experimental" vineyards in zones

- Cultivation of table grapes according to well defined protocols for each zone

- Comparison of the zones response



In most cases, research outputs fail when applied to "commercial" vineyards

Experimental part

New approach

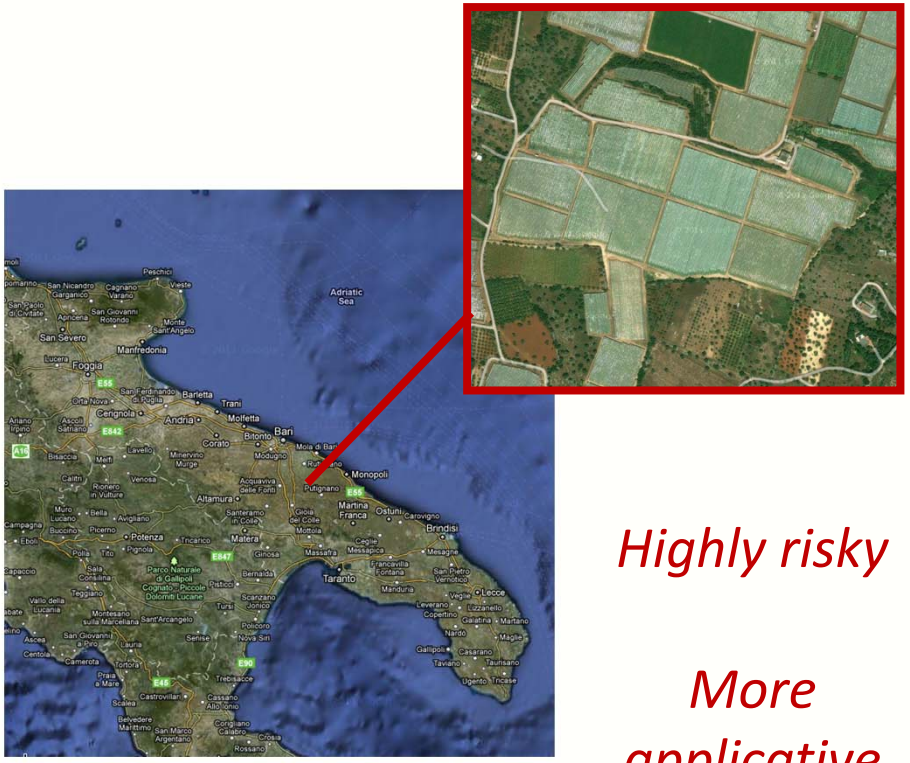
- No “experimental” vineyards

- Study of “commercial” vineyards

- No preliminary knowledge of the agronomical practices

- *Ex post* evaluation of differences between vineyards

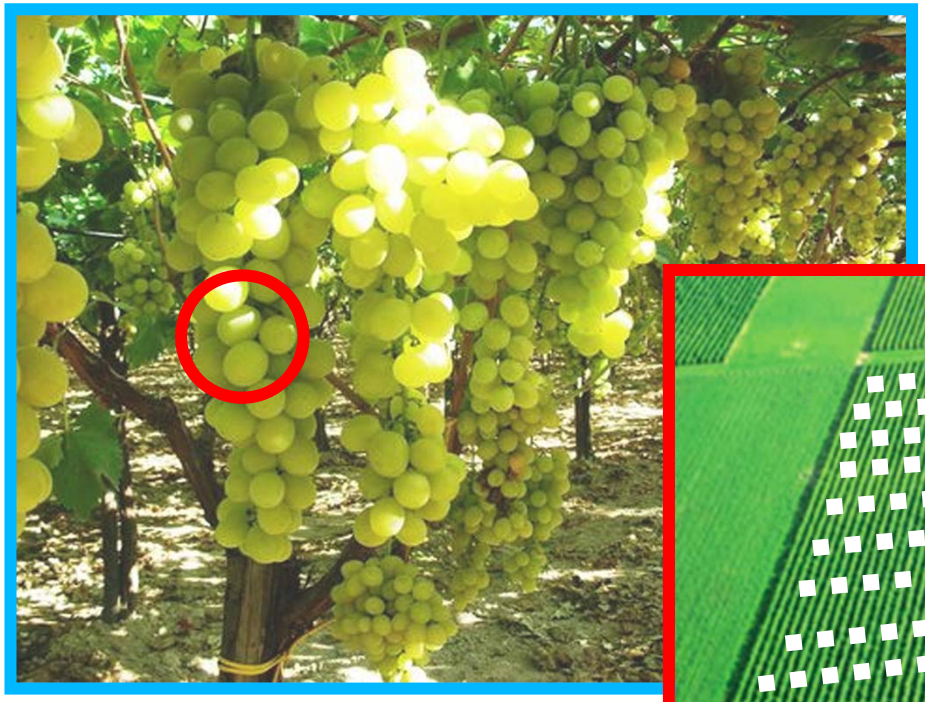
- Individuation of the factors causing the differences



Highly risky

More applicative results

Experimental part

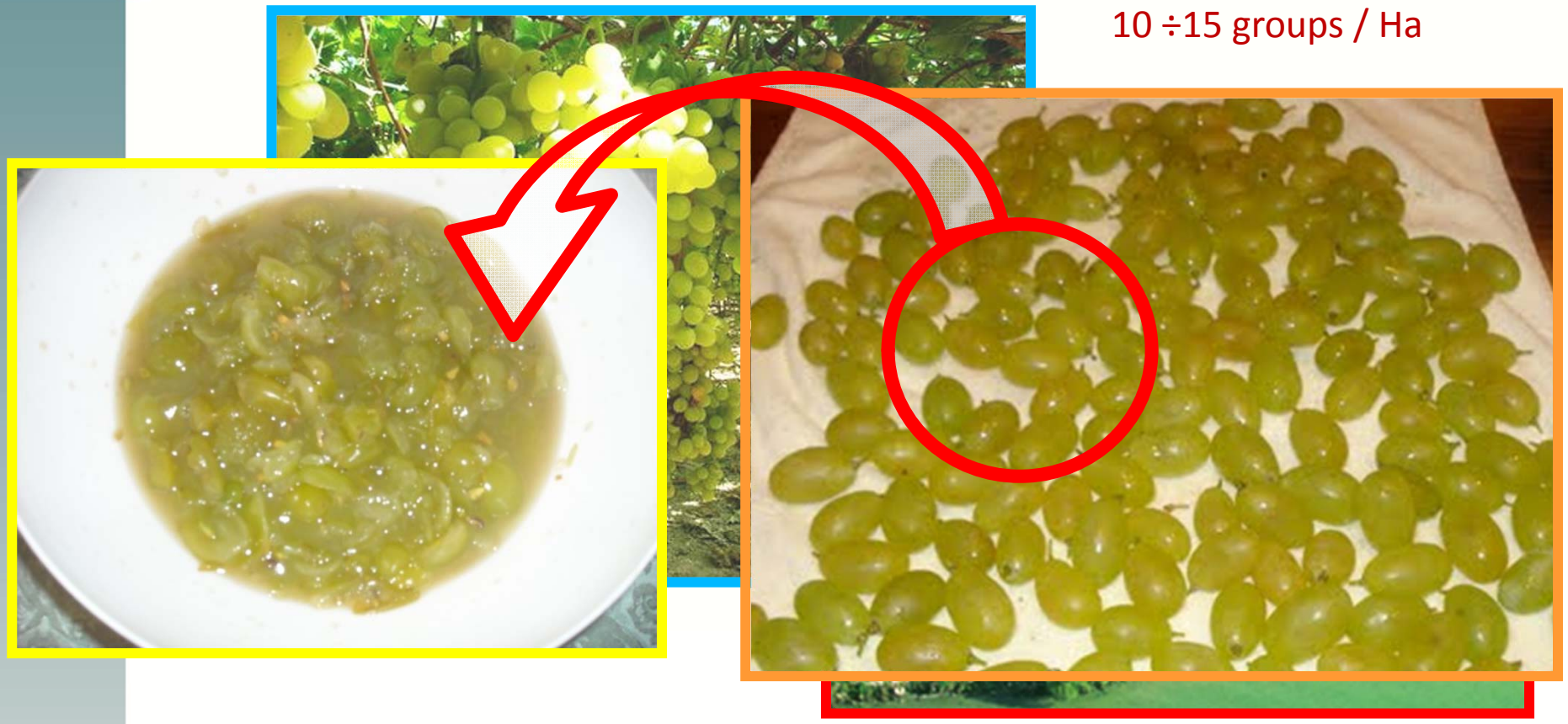


Sampling

> 2 Kg / Ha

Experimental part

10 ÷15 groups / Ha



Experimental part

500µL of centifuged juice + 300 µL TSP in D_2O + 200 µL oxalate buffer (pH = 4.2) in H_2O + NaN_3

NMR

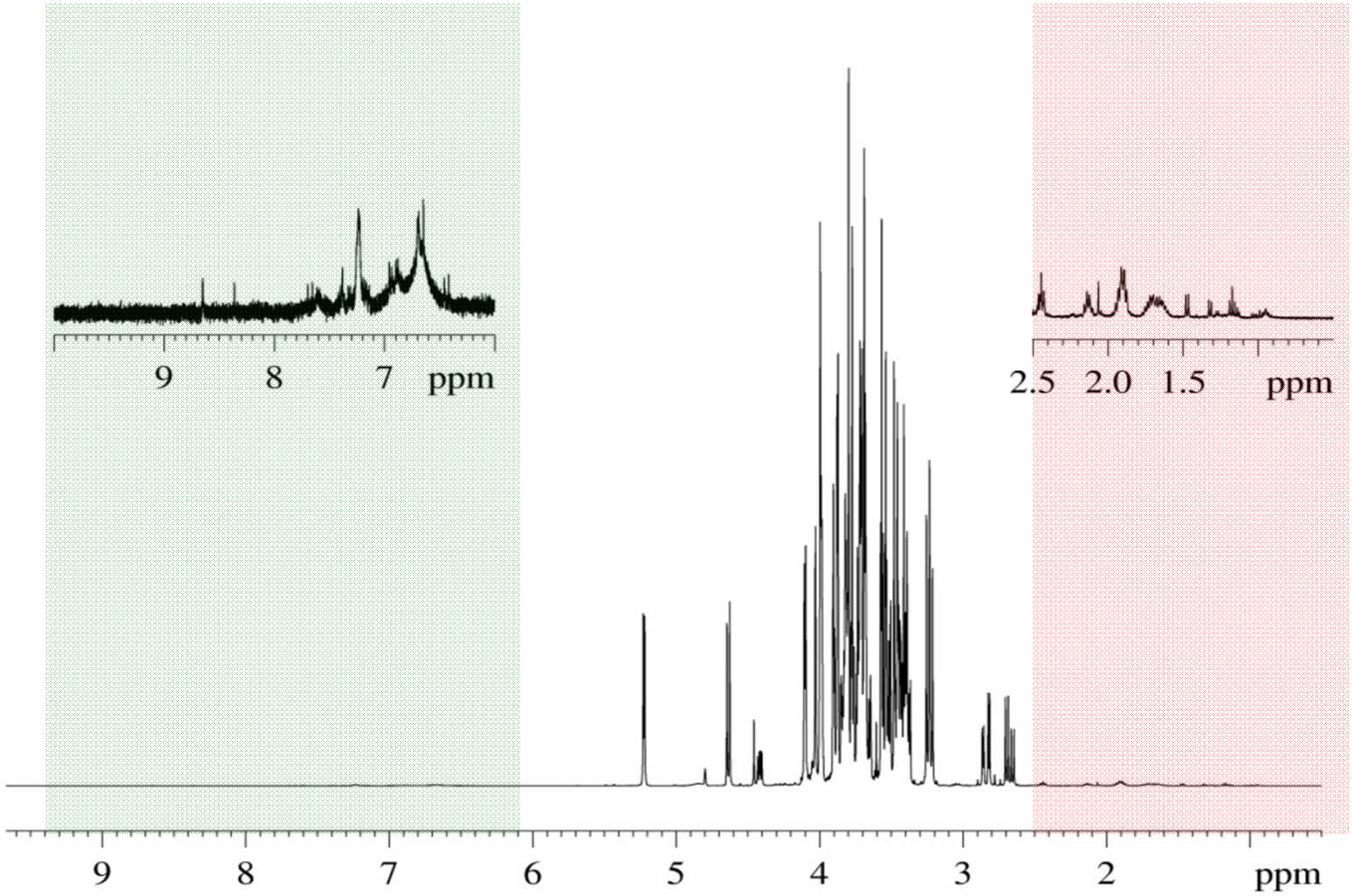
No pre-treatment

HRMS

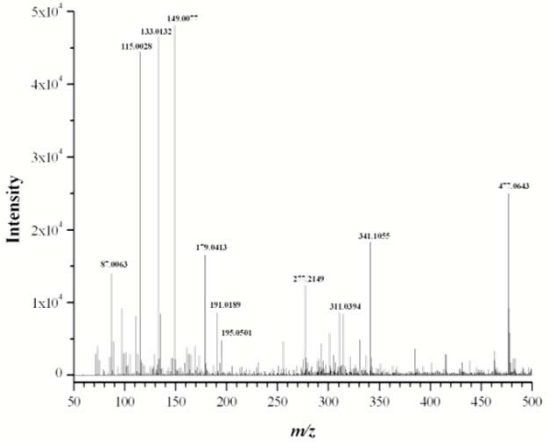
100µL of centifuged juice + 1 ml of CH_3OH:H_2O:HCOOH=70:30:1 + NaN_3

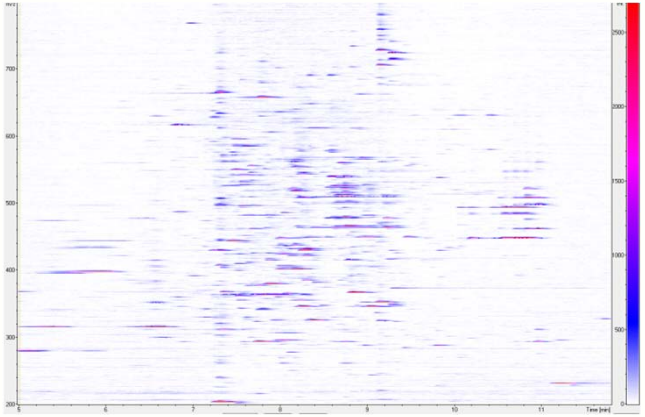
NMR spectra

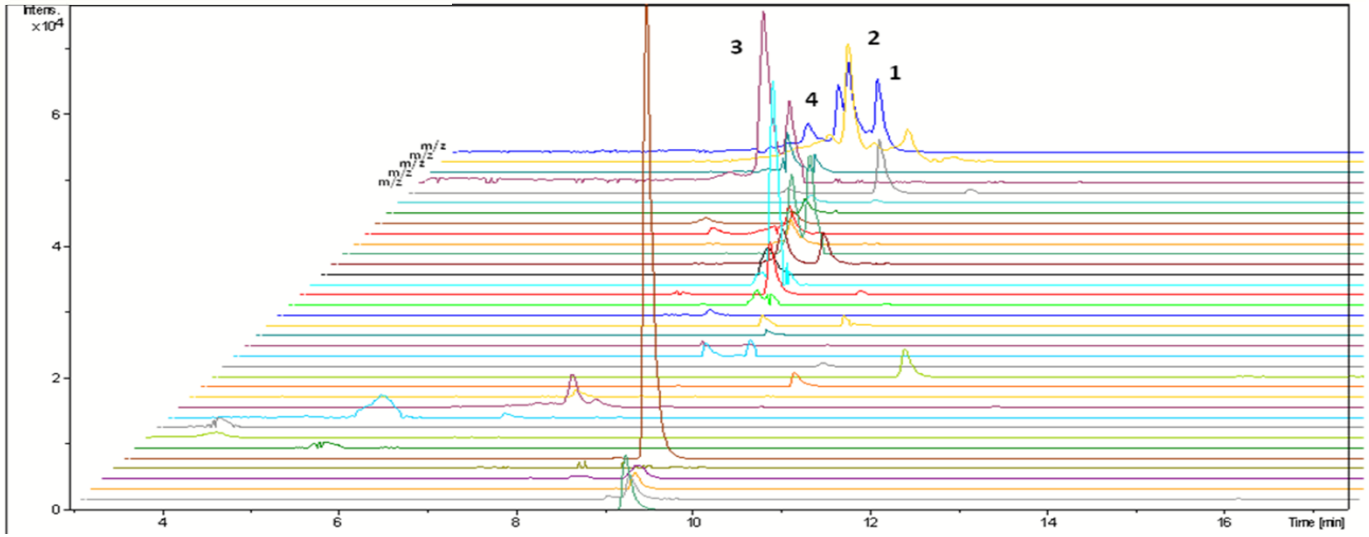
^{1}H NOESY 1D with presaturation of the solvent



HPLC-HRMS spectra







Case study 1: discrimination of grape leaves

Food Chemistry 141 (2013) 1908–1915

Contents lists available at SciVerse ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

A combined approach for characterisation of fresh and brined vine leaves by X-ray powder diffraction, NMR spectroscopy and direct infusion high resolution mass spectrometry

Antonino Rizzuti [a], Rocco Caliandro [b], Vito Gallo [a,c,*], Piero Mastrorilli [a,c], Giuseppe Chita [b], Mario Latronico [a,c]

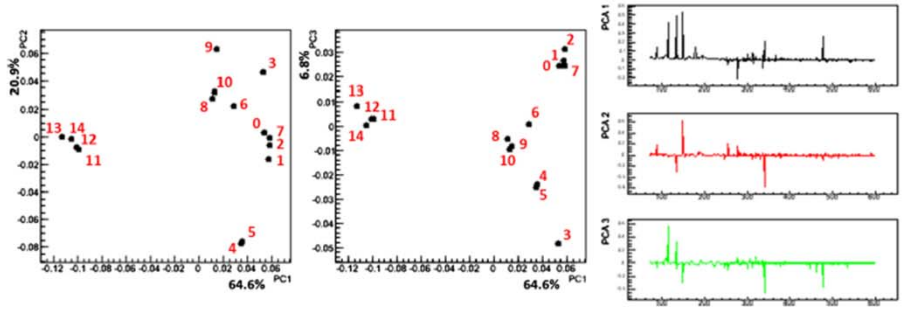
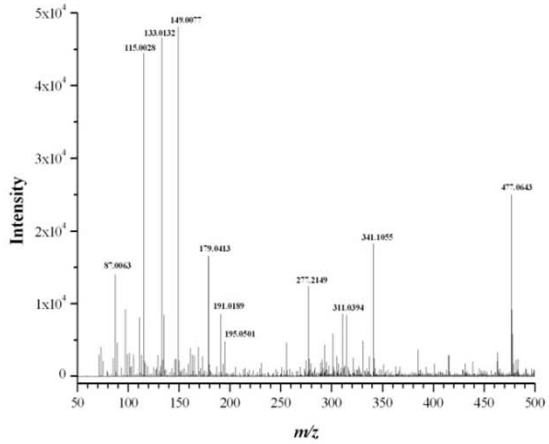
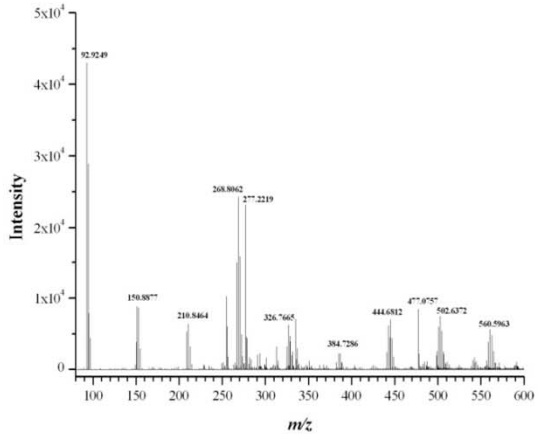


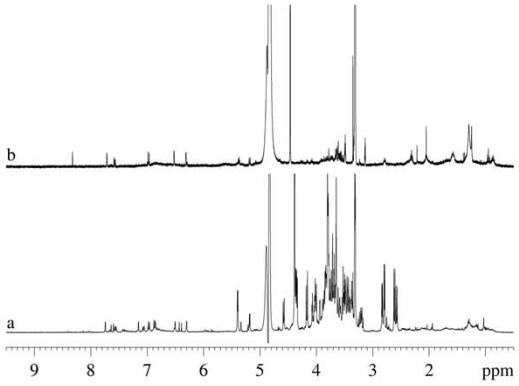
Fig. 2. Scores plots and loading vectors of the first three principal components of the MS spectra, after removal of NaCl contribution. The percentage of variance explained by the principal components is reported on the axes.





Case study 1: discrimination of grape leaves

Figure S8. Typical ^{1}H NMR spectra of vine leaves (a: fresh; b: brined)



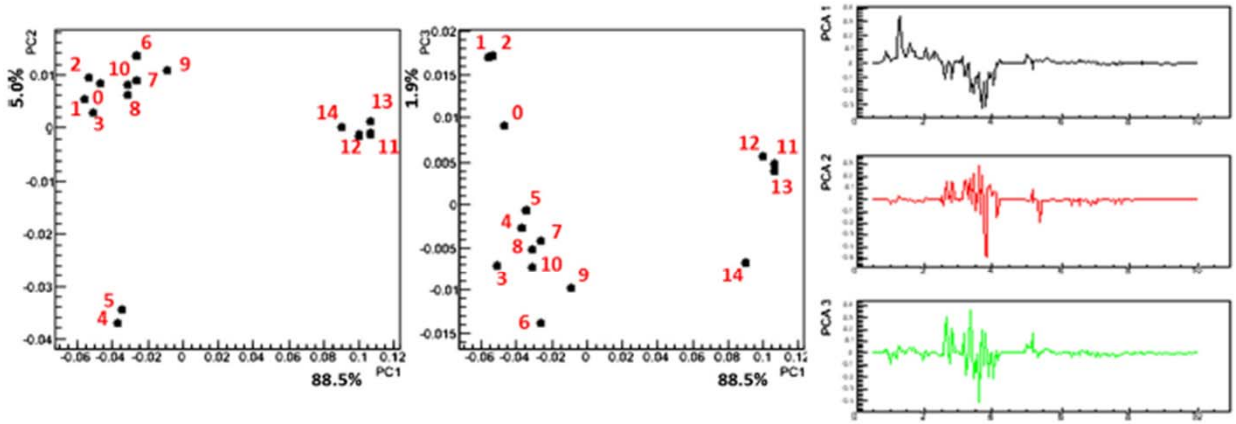
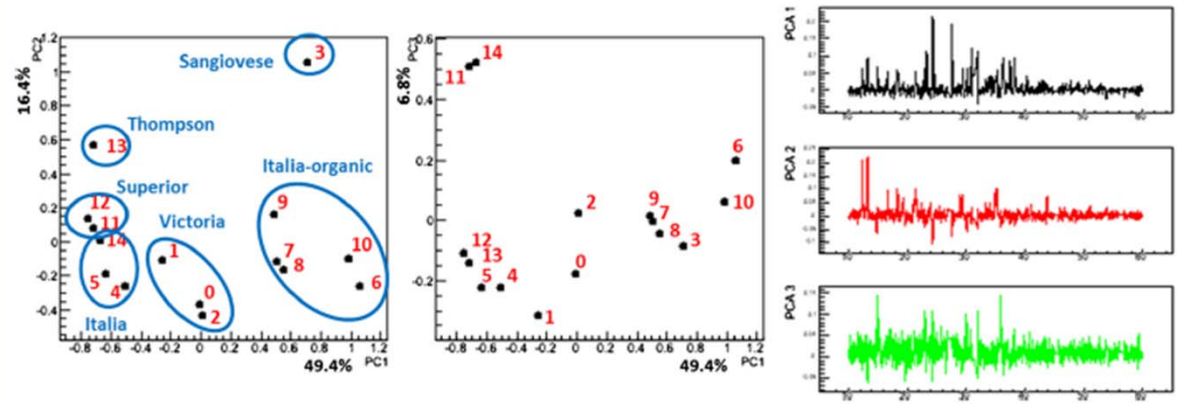


Fig. 3. Scores plots and loadings vectors of the first three principal components of the NMR spectra. The percentage of variance explained by the principal components is reported on the axes.

Case study 1: discrimination of grape leaves



MS-XRPD			
Metabolite	**m/z [M-H]⁻**	**XRPD signal (2θ)**	**Correlation**
Tartaric acid	149.01	13.3, 24.3, 36.4	Positive
Linolenic acid	277.21	12.4, 21.4, 43.8 13.3, 24.3, 36.4	Positive Negative
Sucrose	341.11	13.3, 24.3, 36.4	Negative

MS-NMR			
Metabolite	**NMR signal (Chemical shift, δ)**	**XRPD signal (2θ)**	**Correlation**
α-Glucose	5.2 3.7	13.3, 24.3, 36.4	Positive
α-Linolenic acid	1.3	12.4, 21.4, 43.8 13.3, 24.3, 36.4	Positive Negative
Sucrose	5.4	13.3, 24.3, 36.4	Negative

Case study 2: discrimination of grape berries

Evaluation of use of plant-growth regulator Ethephon (ETH) on cv. Crimson seedless table grapes

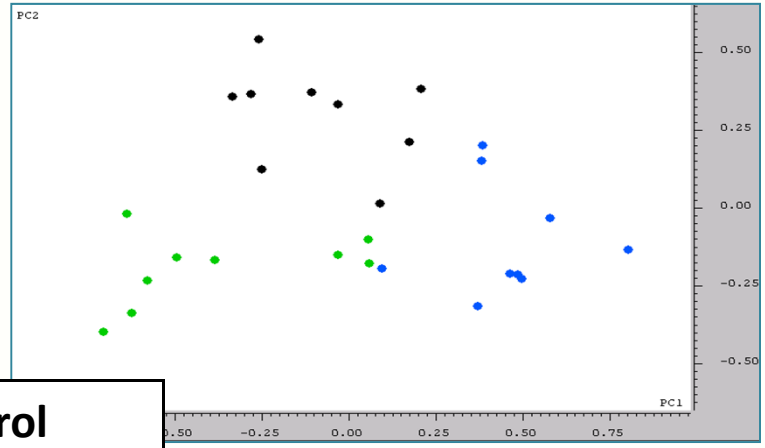
Sample	Plant-growth regulator content (ppm)	ETH application date	Harvesting date
Control	-	-	05 oct 2010
ETH10	10	19 Sept 2010	05 oct 2010
ETH20	20	19 Sept 2010	05 oct 2010

- Grapes can be marketed as "ready to use" food product.
- The berry abcission can be facilitated by the use of plant-growth regulators

Quality control of treated grapes

Case study 2: discrimination of grape berries

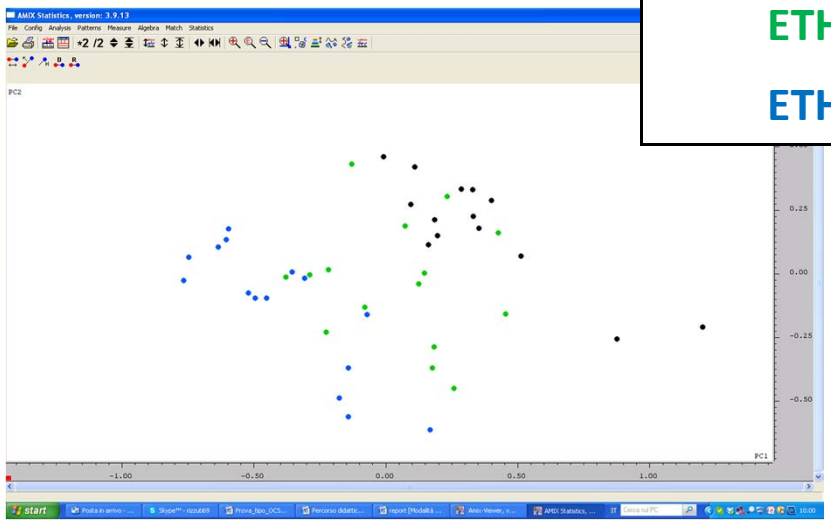
PCA applied to HRMS spectra



Control

ETH10

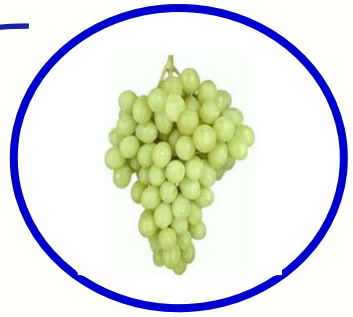
ETH20



PCA applied to NMR spectra

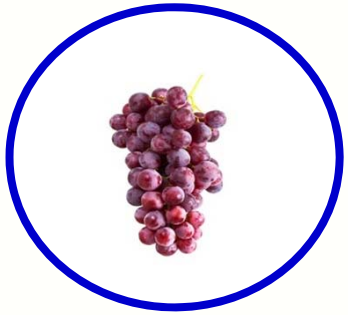
Advantages in using NMR data for development of expert classification systems

Cultivars



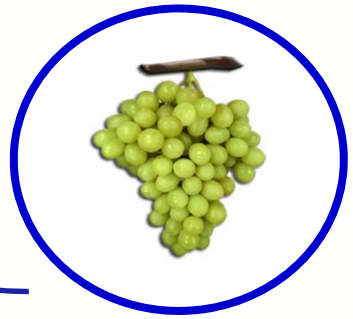
Superior Seedless

Samples from two vineyards and harvested in 2009



Red globe

Samples from six vineyards located in the provinces of Bari and Taranto and collected in 2009



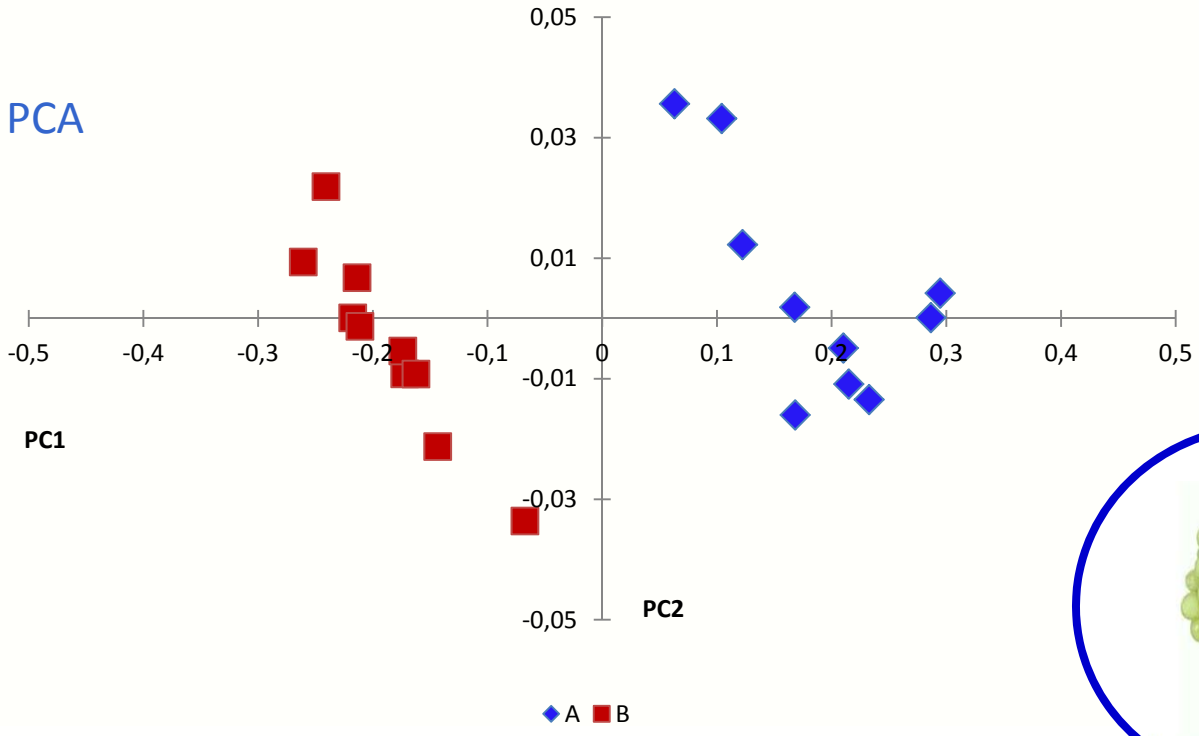
Italia

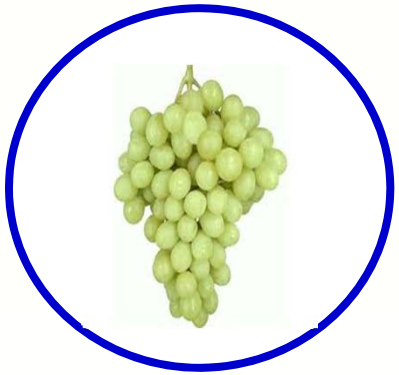
Samples from 18 vineyards located in the provinces of Bari and Taranto and collected in 2009 and 2010

V.Gallo, P. Mastrorilli, I. Cafagna, G. I. Nitti, M. Latronico, V. A. Romito, A. P. Minoja, C. Napoli, F. Longobardi, H. Schäfer, B. Schütz, M. Spraul , Journal of Food Composition and Analysis (2012) submitted

Advantages in using NMR data for development of expert classification systems

Cv Superior Seedless Samples from two vineyards and harvested in 2009





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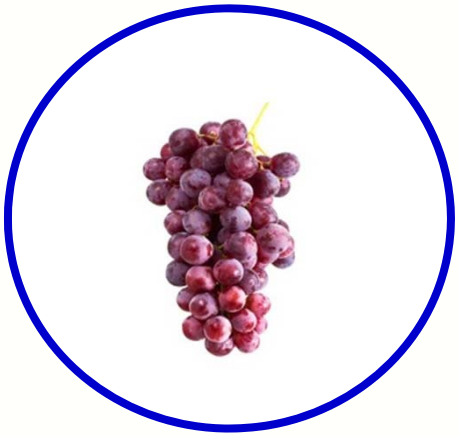

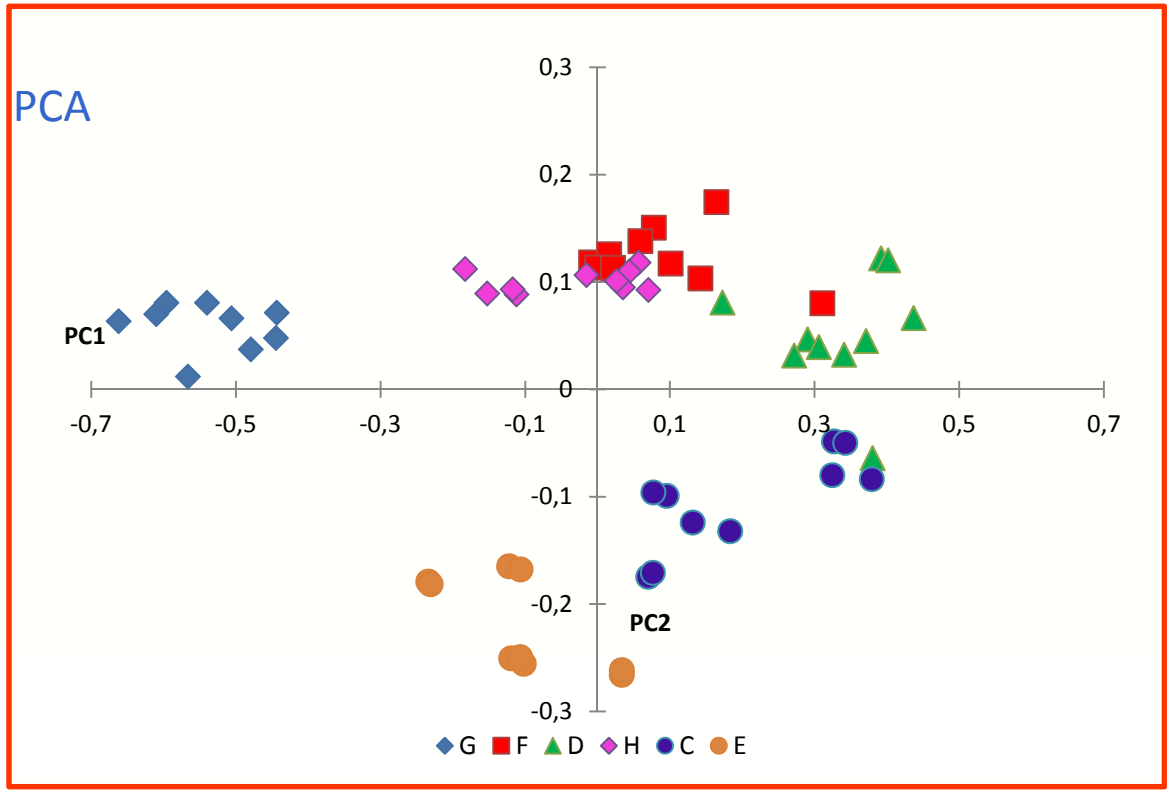

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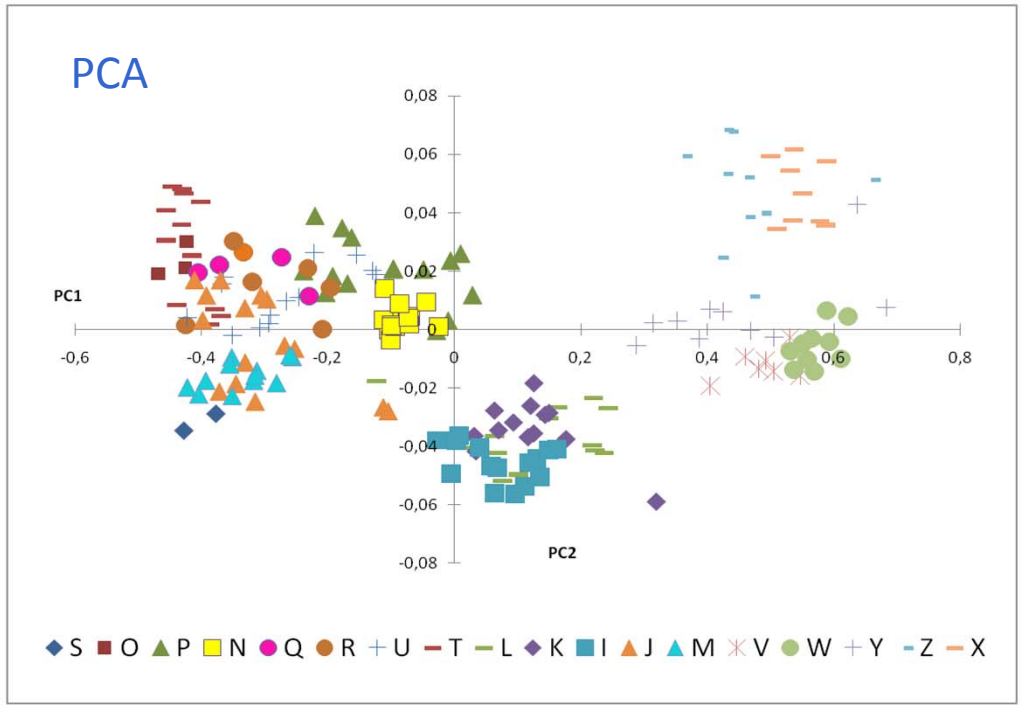

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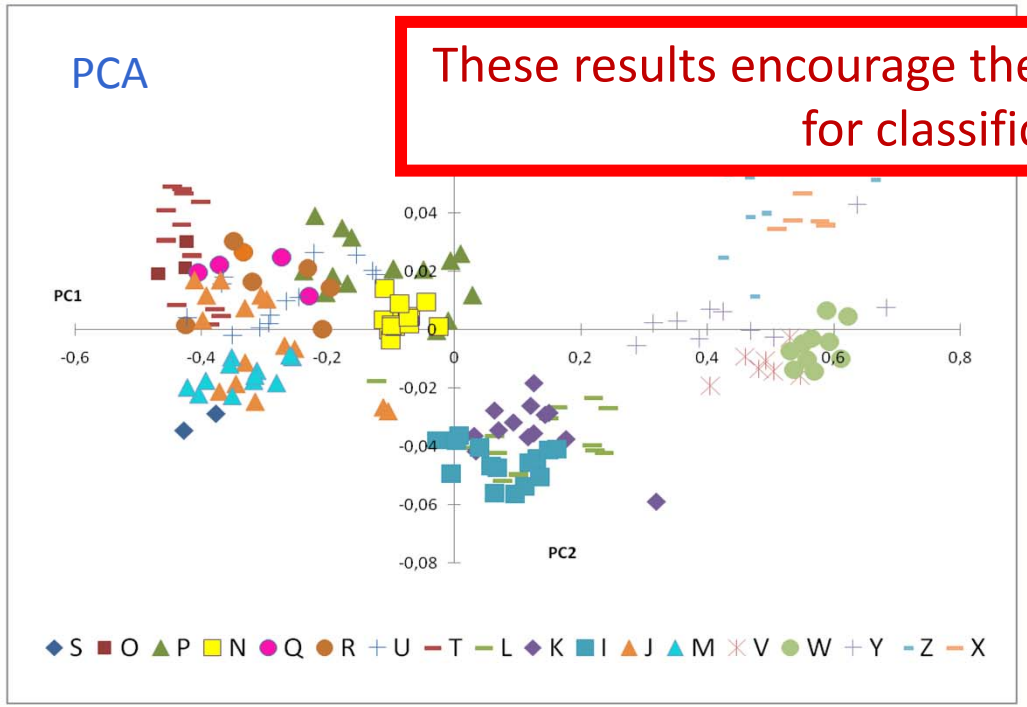

These results encourage the use of expert systems for classification

V.Gallo, P. Mastrorilli, I. Cafagna, G. I. Nitti, M. Latronico, V. A. Romito, A. P. Minoja, C. Napoli, F. Longobardi, H. Schäfer, B. Schütz, M. Spraul , Journal of Food Composition and Analysis (2012) submitted

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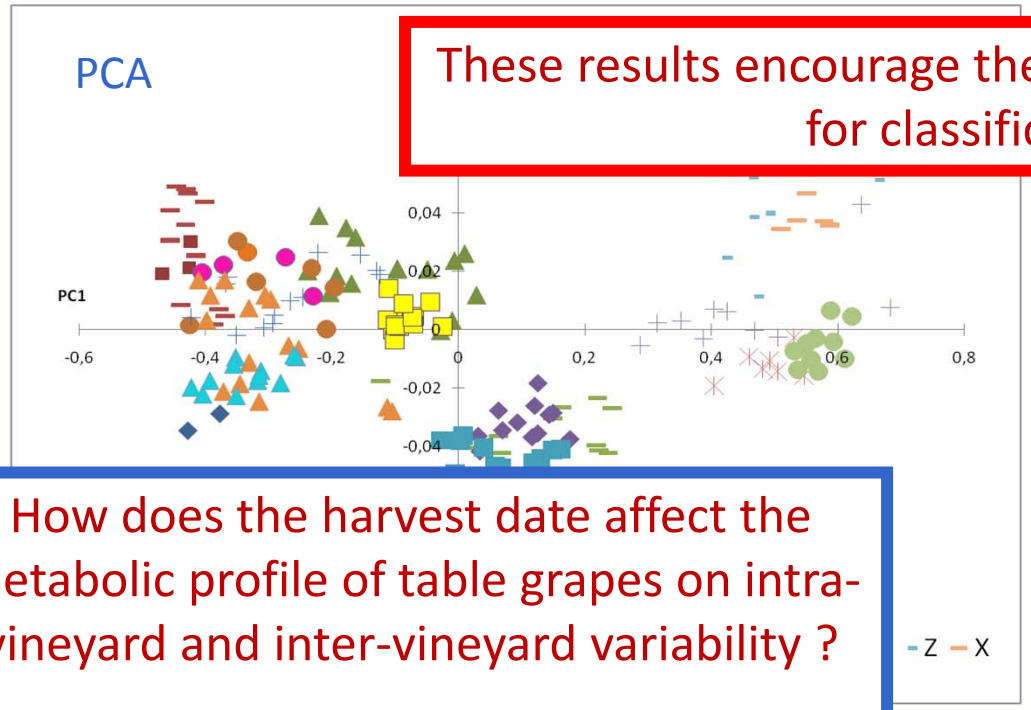

These results encourage the use of expert systems for classification

How does the harvest date affect the metabolic profile of table grapes on intra-vineyard and inter-vineyard variability ?

V.Gallo, P. Mastrorilli, I. Cafagna, G. I. Nitti, M. Latronico, V. A. Romito, A. P. Minoja, C. Napoli, F. Longobardi, H. Schäfer, B. Schütz, M. Spraul , Journal of Food Composition and Analysis (2012) submitted

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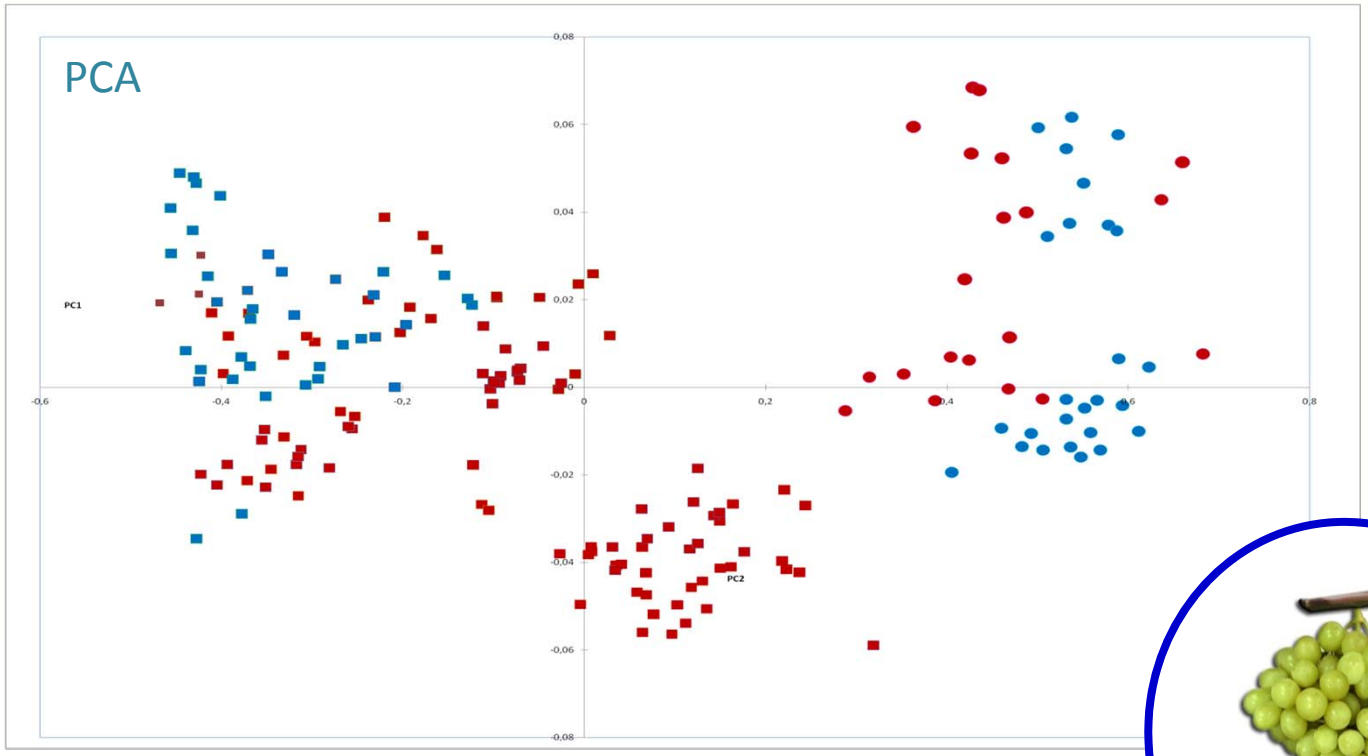
Cv Italia

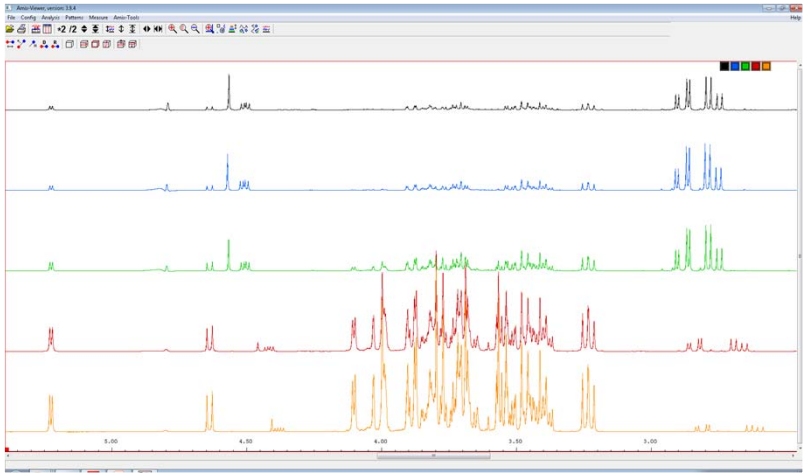


04/09/2009 14/10/2009 06/10/2010 09/12/2010

Advantages in using NMR data for development of expert classification systems

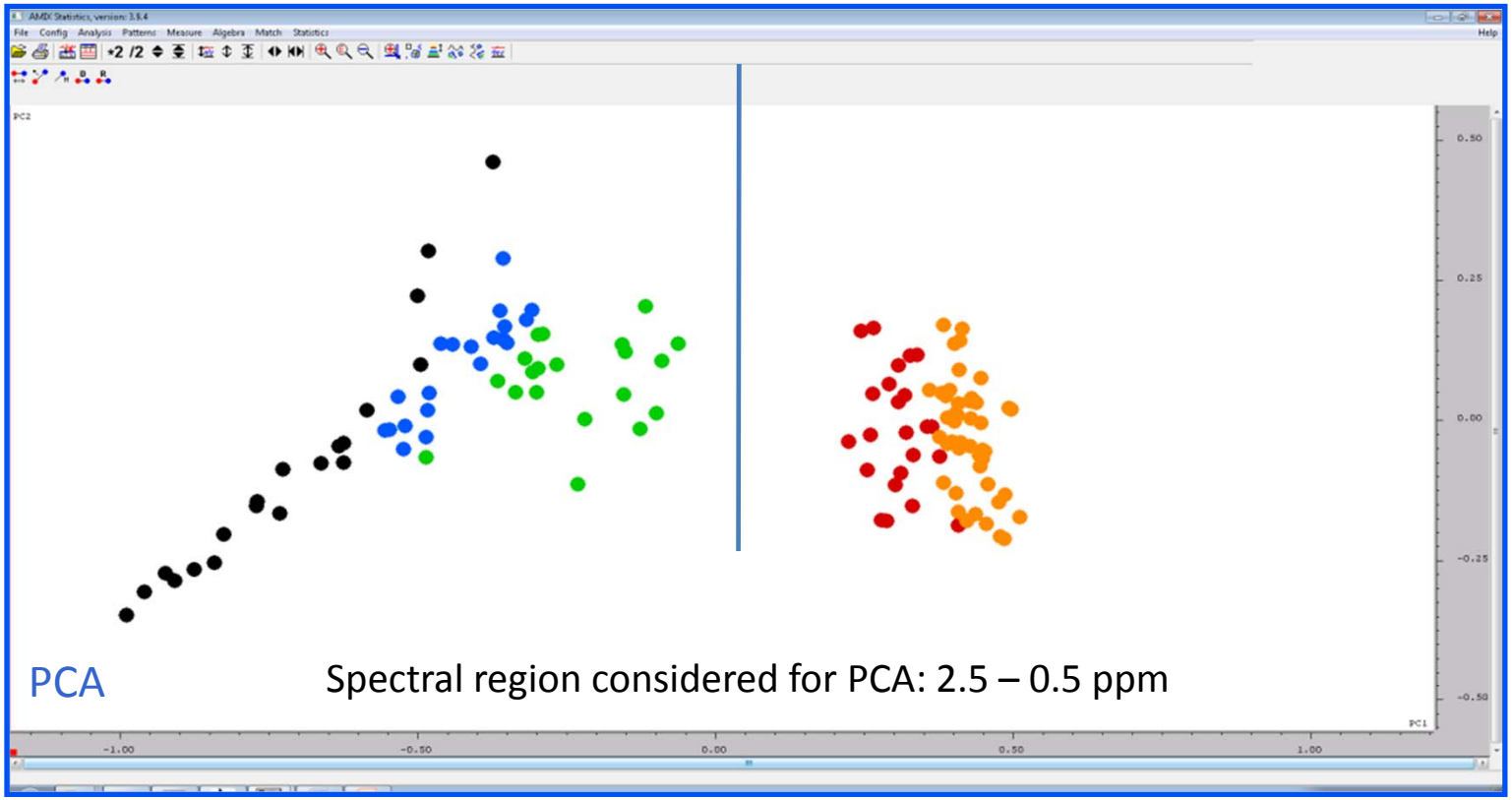
Cv. Italia

- Pre-veraison period
- Veraison period
- Harvest period



Advantages in using NMR data for development of expert classification systems

Cv Italia 2011



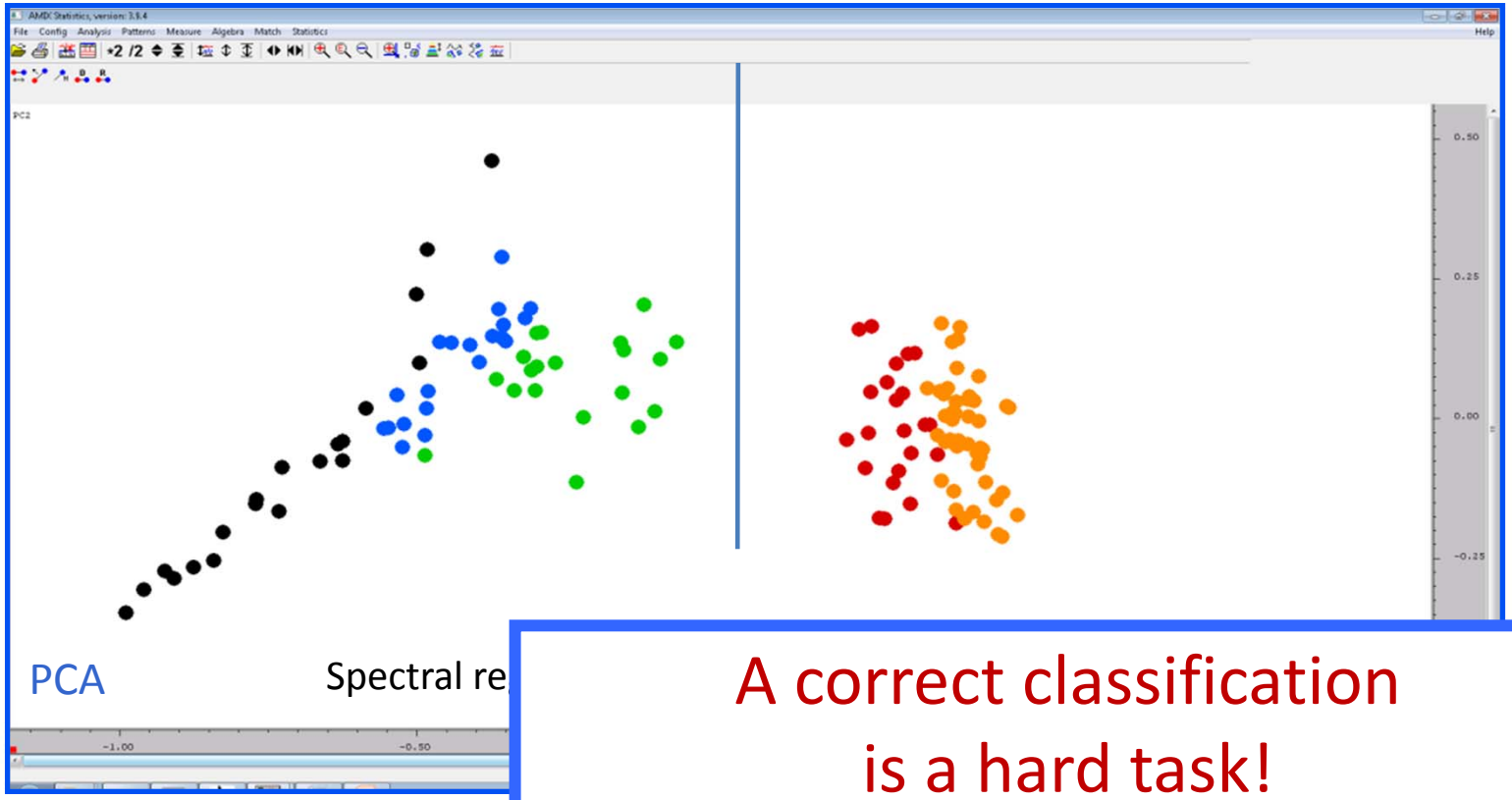
Pre veraison

Veraison

Harvest

Advantages in using NMR data for development of expert classification systems

Cv Italia 2011



Pre veraison

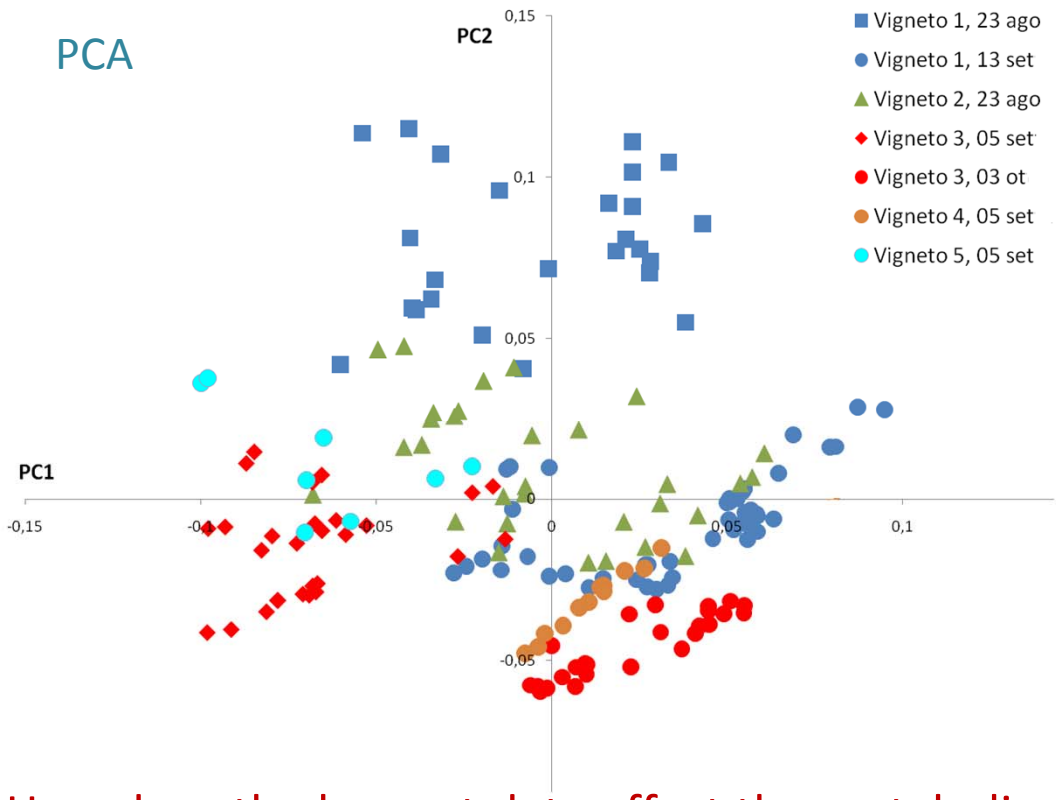
Veraison

A correct classification is a hard task!
Attention must be paid!

Advantages in using NMR data for development of expert classification systems

Cv Italia 2011

PCA



PC1 = 72,11 %
PC2 = 7,49 %

How does the harvest date affect the metabolic profile of table grapes on intra-vineyard and inter-vineyard variability ?

Classifiers

The classifier is an expert system able to make decisions for classification, that extracts from a group of known samples of relevant properties and on the basis of these attempts to recognize and classify unknown samples.

Why using a classifier?

- It is rapid
- It provides quantifiable performance
- It increases its performance with the number of samples

Classification of table grapes

Number of samples: 920 (from 102 vineyards)

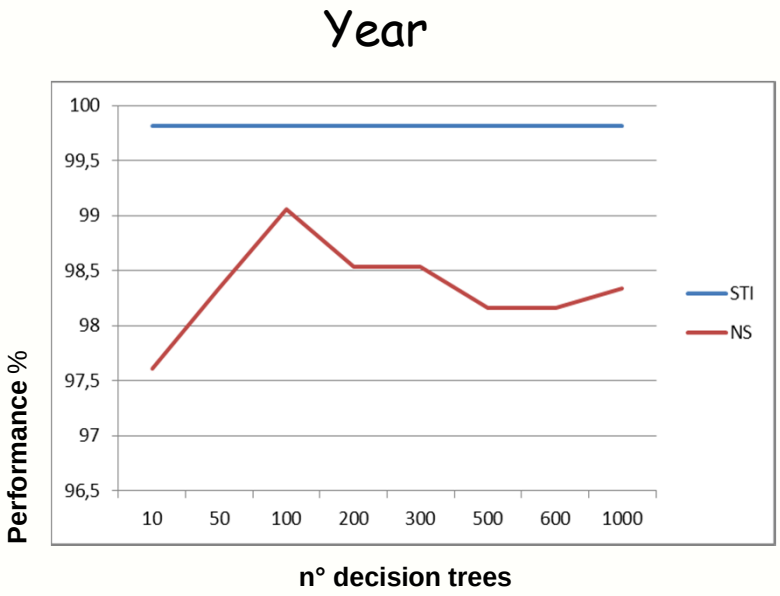
Year: 2009, 2010, 2011
Variety: Melissa, Italia, Crimson, Black Magic, Superior Seedless, Red Globe, Victoria, Apulia Rose, Calmeria, Palieri
Trunk girdling: yes/no
Plant growth regulators (PGR): GA_3, ABA, CPPU, Eth, Cytokinins

Algorithms	Description
J48	based on decision trees
Random Forest (RF)	based on decision trees
Error Back Propagation (EBP)	based on artificial neural networks (ANN)

V. Bevilacqua, M. Triggiani, V. Gallo, I. Cafagna, P. Mastrorilli, G. Ferrara
***Lecture Notes in Artificial Intelligence**, 2012*

Classification of table grapes

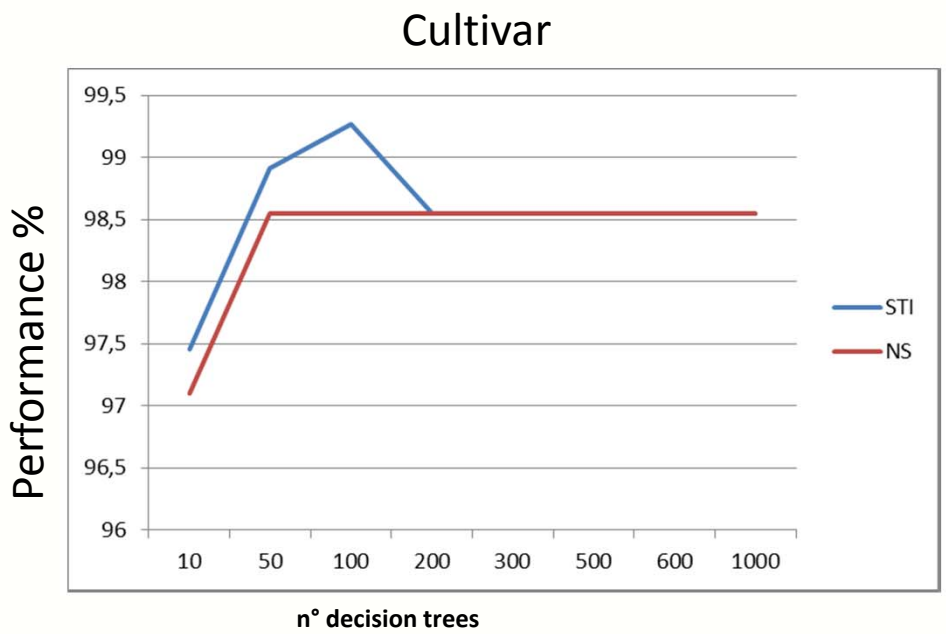
	Bucket width		Year	
	ppm		%	*Time (s)*
J48	0,01	STI	99,2	2
		NS	97,8	2
	0,04	STI	97,1	1
		NS	**99,3**	1
RF	0,01	STI	98,9	7
		NS	99,6	7
	0,04	STI	**99,7**	4
		NS	98,7	4
ANN	0,01	STI	98,0	3000
		NS	99,1	3000
	0,04	STI	**100,0**	800
		NS	99,8	800



Bucketing:
STI = scaling to total intensity
NS = no scaling

Classification of table grapes

	Bucket width		Cultivar	
	ppm		%	Time (s)
J48	0,01	STI	93,8	3
		NS	92,7	3
	0,04	STI	**96,7**	1
		NS	94,2	1
RF	0,01	STI	97,5	8
		NS	98,1	8
	0,04	STI	**98,5**	5
		NS	98,5	5
ANN	0,01	STI	99,5	3400
		NS	41,0	3400
	0,04	STI	**99,6**	800
		NS	98,9	800

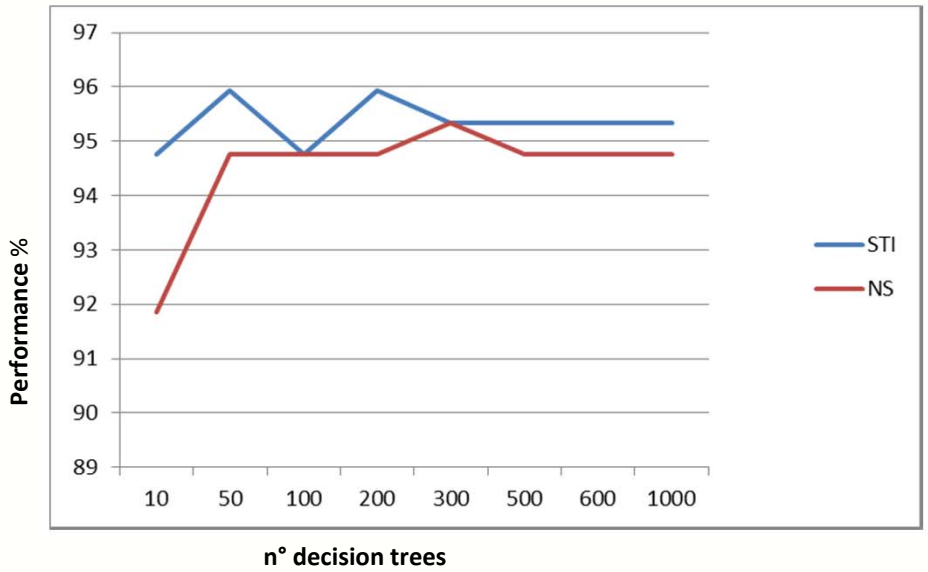


Bucketing:
STI = scaling to total intensity
NS = no scaling

Classification of table grapes

	Bucket width		Trunk girdling	
	ppm		%	*Time (s)*
J48	0,01	STI	90,6	2
		NS	88,9	2
	0,04	STI	90,1	1
		NS	**91,2**	1
RF	0,01	STI	93,0	5
		NS	94,1	5
	0,04	STI	**94,7**	3
		NS	91,8	3
ANN	0,01	STI	97,0	2400
		NS	98,1	2400
	0,04	STI	97,1	600
		NS	**98,2**	600

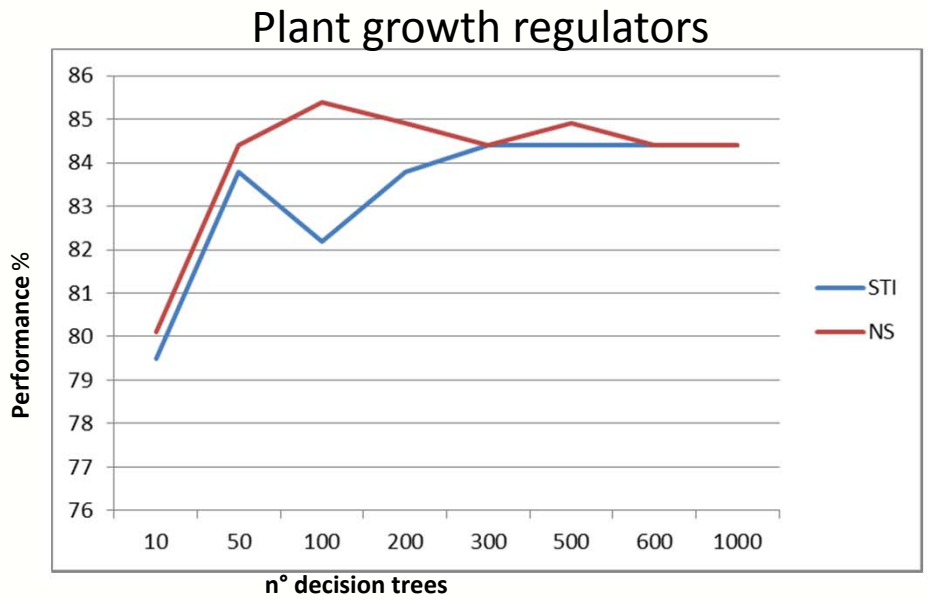
Trunk girdiling



Bucketing:
STI = scaling to total intensity
NS = no scaling

Classification of table grapes

	Bucket width		Plant growth regulators	
	ppm		%	*Time (s)*
J48	0,01	STI	80,1	3
		NS	63,2	3
	0,04	STI	79,0	1
		NS	74,7	1
RF	0,01	STI	83,8	12
		NS	83,8	12
	0,04	STI	**84,7**	6
		NS	84,9	6
ANN	0,01	STI	95,0	4000
		NS	67,2	4000
	0,04	STI	94,6	1200
		NS	**95,1**	1200



Bucketing:
STI = scaling to total intensity
NS = no scaling

Classification of table grapes

Bucket width: 0.04 ppm
Buckets scaled to total intensity

	Spectral region (ppm)	**Year**	**Variety**	**Trunk girdling**	**Plant growth regulators**
RF	***0-3***	*95,7*	***98,6***	*93,6*	***88,7***
	3-6	***100,0***	*97,5*	***96,8***	*83,3*
	6-10	*97,8*	*96,0*	*95,9*	*77,9*

	Spectral region (ppm)	**Year**	**Variety**	**Trunk girdling**	**Plant growth regulators**
ANN	***0-3***	*97,5*	***99,2***	*96,7*	***91,9***
	3-6	***100,0***	*98,6*	***98,2***	*85,4*
	6-10	*99,3*	*98,9*	*95,1*	*83,3*

Conclusions

The inter-vineyard prevails over intra-vineyard ⇨ The classification of grapes on the basis of agronomic practices is possible

The harvesting date influences the PCA outcome ⇨ The performance of the classification systems may be affected by different harvesting date

The possible problems arising from the harvesting date are not significant

The use of expert systems has led to excellent results ⇨ The value of the results is even more surprising if we consider how difficult is classification of fresh products (due to their high heterogeneity)

Application of expert classification systems may be a valuable support for valorization of table grapes

Conclusions

- Combination of NMR and HRMS techniques is an efficient tool for the valorization of food products by identification of their metabolic profiles

- NMR and HRMS are complementary methods and they offers a good strategy for the evaluation of both primary and secondary metabolites in the food products without any pre-treatment.

- NMR provides high reproducible data allowing stable performances of classifiers