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## Research Note

## Genetic and ampelographic identification of different grape varieties known as 'Tempranillo' in Rioja (Spain)

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**Key words:** minority varieties, 'Tempranillo', microsatellite markers, ampelographic descriptors.

**Introduction:** Spanish viticulture possessed great genetic resources which were in danger of extinction, but nowadays they are being recovered and studied through several research programmes. During previous studies carried out in Rioja appellation (MARTÍNEZ DE TODA *et al.* 2004), 76 vine accessions were collected and planted in a germplasm bank in the farm of Rioja's Government, called "La Grajera".

'Tempranillo' is the red variety with higher number of hectares grown in the Iberic Peninsula, in Spain and in Rioja appellation. In the germplasm bank of "La Grajera" there are 14 accessions whose local name includes 'Tempranillo': 'Tempranillo', 'Tempranillo Blanco', 'Tempranillo Royo', 'Tempranillo del Barón' and 'Tempranillo Temprano'. The objective of this work is to identify all these accessions containing the word 'Tempranillo' in the local name.

**Material and Methods:** The study focused on the accessions of the germplasm bank of Rioja's Government, which during a previous ampelographic and ampelometric characterization (MARTÍNEZ DE TODA *et al.* 2004) were grouped as 'Tempranillo' (accessions R-30, R-31, R-32, A-34, AR-40, I-58, NA-104 y NA-105), 'Tempranillo Blanco' (accession CI-101) and 'Tempranillo Royo' (accession B-47) and those whose local designation had the word "Tempranillo" inside, as 'Tempranillo del Barón' (accession N-23) and 'Tempranillo Temprano' (accessions A-15, AR-39 y AR-41).

According to the OIV Official List (O.I.V. 2001) 44 ampelographic characters proposed by RODRÍGUEZ *et al.* (2003) were studied during the years 2008-2010. The value of each character corresponds to the mode of the observations done in the vineyard and in the laboratory by a previously formed independent panel of three people.

The molecular analyses were made according to the methodology described by IBAÑEZ *et al.* (2003) in the IMIDRA laboratories (El Encín farm, Madrid). Nine loci microsatellites were studied in all the accessions: VVS2 (THOMAS and SCOTT 1993); VVMD5, VVMD27, VVMD28

(BOWERS *et al.* 1996 and 1999); VrZAG62, VrZAG29, VrZAG67, VrZAG83, VrZAG112 (SEFC *et al.* 1999). The microsatellite sizes were compared to several data bases of Spanish varieties (IBAÑEZ *et al.* 2003) and other European data bases (SEFC *et al.* 2000) in order to identify each accession.

The statistical analysis was carried out with the help of the statistical software INFOSAT (Professional edition 2007, Córdoba, Argentina). For ampelographic data, a distance matrix was constructed from the correlation distance index. The matrix was subjected to cluster analysis by the unweighted pair-group method analysis (UPGMA), standardized values and Pearson's coefficient.

**Results and Discussion:** Ampelographic characterization: As a consequence of the study of the 44 ampelographic characters observed in 14 accessions, a dendrogram was obtained (Figure). There are three different groups. The accession N-23, known locally as 'Tempranillo del Barón', differs most from the others.

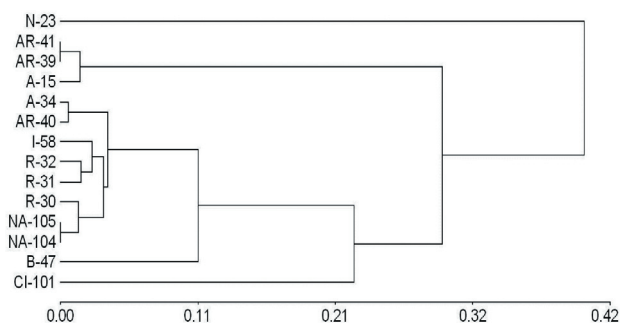


Figure: Dendrogram constructed from the 44 ampelographic descriptors data distance matrix using the UPGMA clustering method and Pearson coefficient, representing morphological relationships among the 14 studied accessions.

The second group of varieties is formed by three different accessions: AR-41, AR-39 and A-15. All of them were named locally as 'Tempranillo Temprano' and show similar characteristics in the dendrogram. They could be the same variety, but it has to be confirmed by the molecular markers analysis. The accession A-15 differs from the accessions AR-41 and AR-39, which show the same result (Figure).

The last group is bigger and more complex. It is formed by all the accessions locally known as 'Tempranillo', 'Tempranillo Royo' and 'Tempranillo Blanco'. The accession CI-101 corresponding to 'Tempranillo Blanco' differs significantly from the others accessions of this group. The accession B-47, known as 'Tempranillo Royo' is closer to 'Tempranillo Blanco' than the other accessions known as 'Tempranillo'. Finally, the sub-group of the accessions known locally as 'Tempranillo' is formed by 8 nearby accessions and they could be the same variety: A-34, AR-40, I-58, R-32, R-31, R-30, NA-104 y NA-105.

**Genetic characterization:** The results of the 9 microsatellite markers analyzed for each accession are shown in the Table. Three different groups were observed. According to the DNA analysis, it can be affirmed that there are only three different groups in the fourteen studied accessions, as it follows: Group A: accession N-23;

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Table

Size of the alleles for the nine microsatellite loci in the different accessions

Accession	Variety	VrZAG67	VVMD27	VVMD5	VrZAG29	VrZAG62	VrZAG112	VVS2	VrZAG83	VVMD28									
A-34	Tempranillo	124	148	181	181	233	233	111	111	196	200	228	237	142	144	195	195	257	257
AR-40	Tempranillo	124	148	181	181	233	233	111	111	196	200	228	237	142	144	195	195	257	257
B-47	Tempranillo	124	148	181	181	233	233	111	111	196	200	228	237	142	144	195	195	257	257
CI-101	Tempranillo	124	148	181	181	233	233	111	111	196	200	228	237	142	144	195	195	257	257
I-58	Tempranillo	124	148	181	181	233	233	111	111	196	200	228	237	142	144	195	195	257	257
NA-104	Tempranillo	124	148	181	181	233	233	111	111	196	200	228	237	142	144	195	195	257	257
NA-105	Tempranillo	124	148	181	181	233	233	111	111	196	200	228	237	142	144	195	195	257	257
R-30	Tempranillo	124	148	181	181	233	233	111	111	196	200	228	237	142	144	195	195	257	257
R-31	Tempranillo	124	148	181	181	233	233	111	111	196	200	228	237	142	144	195	195	257	257
R-32	Tempranillo	124	148	181	181	233	233	111	111	196	200	228	237	142	144	195	195	257	257
A-15	Portugais Bleu	124	130	179	192	223	229	113	115	188	204	228	239	142	151	191	195	228	259
AR-39	Portugais Bleu	124	130	179	192	223	229	113	115	188	204	228	239	142	151	191	195	228	259
AR-41	Portugais Bleu	124	130	179	192	223	229	113	115	188	204	228	239	142	151	191	195	228	259
N-23	Trepát	138	150	177	192	223	231	111	111	188	204	228	233	131	142	195	201	257	257

Group B: accessions A-34, AR-40, B-47, CI-101, I-58, R-32, R-31, R-30, NA-104 and NA-105; Group C: accessions AR-41, AR-39 and A-15.

By comparison of the results obtained for the microsatellite markers size in the group A against the microsatellite markers data base, it is proved that the accession known locally as 'Tempranillo del Barón' corresponds to the Spanish variety 'Trepát'. Previous studies showed (MARTÍNEZ DE TODA and SANCHA 1997, MARTÍNEZ DE TODA *et al.* 2004) that this accession was a homonym of 'Tempranillo', which means that it was considered a different variety, but the identification was not performed until now.

Using the same methodology for the group B, it is concluded that all the accessions show the same microsatellite markers profile as the Spanish variety 'Tempranillo'. Due to the ampelographic characterization of this study, it is known that, despite of having the same microsatellite markers result, the accessions B-47 and CI-101 are different from 'Tempranillo Tinto' and they correspond to 'Tempranillo Royo' and 'Tempranillo Blanco' respectively.

Finally, in the group C it is proved that all of the accessions correspond to the Austrian variety 'Blauer Portugieser' or 'Portugais Bleu'. Previously, the three accessions were considered a homonym of 'Tempranillo', because they showed remarkable differences from Tempranillo in the ampelographic analysis (MARTÍNEZ DE TODA *et al.*, 2004).

It is proved that according to genetic and ampelographic analysis, there are five different varieties in all the studied accessions: 'Trepát', 'Tempranillo Blanco', 'Tempranillo Royo', 'Tempranillo' and 'Portugais Bleu' or 'Blauer Portugieser'.

**Conclusions:** While molecular markers permitted the identification of three different varieties ('Tempranillo', 'Trepát' and 'Portugais Bleu'), ampelographic characterization, according to DNA analysis, played a decisive role in order to explain the differentiation of three different varie-

ties inside the 'Tempranillo' group: 'Tempranillo Blanco', 'Tempranillo Royo' and 'Tempranillo Tinto'. As a consequence of both studies, five varieties were detected, with special interest for 'Tempranillo Royo' that had never before been cited. Besides, two new synonymies were established: 'Tempranillo del Barón' as 'Trepát' and 'Tempranillo Temprano' as 'Portugais Bleu'.

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