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## Varietal diversity within grapevine accessions of cv. Tempranillo

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### Summary

**Intra-varietal diversity of 31 accessions named as Tempranillo or described as synonyms of this representative Spanish grapevine variety was assessed using 48 morphological descriptors and 95 AFLP markers obtained using 2 primer combinations. We could identify homonyms as well as accessions belonging to varieties which, depending on their regional origin, are incorrectly cited as Tempranillo synonyms. Genetic analysis revealed a high level of genetic similarity between Tempranillo accessions (SG >0.97), which could correspond to three highly related genotypes. This narrow genetic variation supports a monophyletic origin of Tempranillo, and could be the result of rapid multiplication and distribution of a very low number of clones.**

**Key words:** ampelography, grapevine genetic identification, monophyletic origin, intra-varietal variability, Tempranillo.

### Introduction

The Spanish word "temprano" means early, and therefore the name Tempranillo is logically related to the early ripening of this Spanish red wine variety. Contrary to most other Mediterranean varieties, Tempranillo has a short vegetative cycle, a characteristic of Northern varieties, and large hairy hepta-lobulated leaves as well as uniform fruit set and dark-blue berries with thick skin. Tempranillo is widely cultivated in northern and central Spain where it is the main variety in half of the Denominations of Origin (DOs). In various regions of Spain Tempranillo has different names *e.g.* Cencibel, Tinto Fino, Tinto del País, Tinta de Toro, Ull de Llebre, etc. Nowadays, original local names associated with Tempranillo have been given to other cultivars (homonymies) or different names have been given to clones of Tempranillo in different Spanish regions (synonymies) (RODRÍGUEZ-TORRES 2001). We have used ampelographic and AFLP molecular markers (VOS *et al.* 1995) to characterize the different genotypes sharing this common name as well as the proposed synonyms. The results show narrow intra-varietal genetic variation and allow to identify synonyms and to characterize false synonyms and homonyms.

### Material and Methods

Thirty-one accessions of *Vitis vinifera* described as Tempranillo or related synonyms were analyzed (Table). In addition the rootstock variety R-110 (*Vitis berlandieri* x *V. rupestris*) as a reference in the AFLP analysis is included (CERVERA *et al.* 1998, 2000, 2001). Representative individuals of these varieties are cultivated in the collection of El Encín, (Instituto Madrileño de Investigación Agraria y Alimentaria (IMIA) de la Comunidad de Madrid, Alcalá de Henares, Spain). Morphological characterization was performed following the descriptors of the O.I.V. (1984). This description was performed in two consecutive years by three ampelographers and modal values of the 6 observations were considered. Statistical analysis was carried out as described by CERVERA *et al.* (2001). Total genomic DNA extraction, AFLP and statistical analyses were performed according to CERVERA *et al.* (1998). Dendrograms were constructed using the UPGMA clustering method to the ampelographic data distance matrix calculated using the correlation distance index and Dice estimates of genetic similarities based on AFLP analysis, respectively.

### Results

A total of 48 descriptors (O.I.V. 1984) were used to characterize 31 accessions known as Tempranillo or Tempranillo synonyms. Phenetic distances between each pair of accessions are presented in Figure, a. The co-phenetic correlation was 0.98 ( $p=0.002$ ) indicating a good fit of the cluster analysis. Three different clusters were identified. The first cluster, from Tempranillo (VI) to Tempranillo (NA), included 26 accessions organized in 5 sub-clusters and grouped at a genetic similarity level (GS) of 0.89. The second cluster, from Tinta de Madrid (TO) to Tinto del País (BU), grouped 4 unrelated accessions with  $GS \leq 0.65$  and with Tempranillo accessions in cluster I. The third cluster, Tempranillo Temprano (LO), was even less related with the other accessions ( $GS \leq 0.32$ ). The results showed phenotypic heterogeneity among the samples, which may indicate the presence of more than one variety (clusters II and III). To unequivocally discriminate Tempranillo accessions and associated synonyms we analyzed these samples using high throughput molecular markers (AFLPs).

T a b l e  
Grapevine accessions analyzed

| Code | Local name                | Author of synonym description | Origin           |
|------|---------------------------|-------------------------------|------------------|
| A09  | Tempranillo (VI)          |                               | Vitoria (VI)     |
| A17  | Tempranillo Temprano (LO) |                               | Logroño (LO)     |
| A23  | Tempranillo (LO)          |                               | Logroño (LO)     |
| A40  | Tempranillo (NA)          |                               | Navarra (NA)     |
| F42  | Tinto Madrid (S)          | PEÑÍN <i>et al.</i> (1997)    | Santander (S)    |
| G18  | Tempranillo (B)           |                               | Barcelona (B)    |
| H37  | Tinto Fino (AB)           | GARCÍA DE LOS SALMONES (1914) | Albacete (AB)    |
| I25  | Cencibel (CR-1)           | GARCÍA DE LOS SALMONES (1914) | Ciudad Real (CR) |
| I29  | Cencibel (CR-2)           | GARCÍA DE LOS SALMONES (1914) | Ciudad Real (CR) |
| I41  | Cencibel (CU)             | GARCÍA DE LOS SALMONES (1914) | Cuenca (CU)      |
| I55  | Cencibel (GU)             | GARCÍA DE LOS SALMONES (1914) | Guadalajara (GU) |
| J02  | Tinto Madrid (M)          | PEÑÍN <i>et al.</i> (1997)    | Madrid (M)       |
| J18  | Cencibel (M)              | GARCÍA DE LOS SALMONES (1914) | Madrid (M)       |
| J37  | Tinta de Madrid (TO)      | CLEMENTE (1807)               | Toledo (TO)      |
| J49  | Tinto Madrid (TO)         | PEÑÍN <i>et al.</i> (1997)    | Toledo (TO)      |
| J52  | Tempranillo de Rioja (BU) |                               | Burgos (BU)      |
| J53  | Tinto del País (BU)       | BORREGO <i>et al.</i> (1990)  | Burgos (BU)      |
| K32  | Tinta Madrid (LE)         | CLEMENTE (1807)               | León (LE)        |
| K47  | Tinto Madrid (SA)         | PEÑÍN <i>et al.</i> (1997)    | Salamanca (SA)   |
| L11  | Juan García (ZA)          | CLEMENTE (1807)               | Zamora (ZA)      |
| L12  | Tinto del País (SO)       | BORREGO <i>et al.</i> (1990)  | Soria (SO)       |
| L15  | Tinto Madrid (SO)         | PEÑÍN <i>et al.</i> (1997)    | Soria (SO)       |
| L16  | Tinto del País (VA-1)     | BORREGO <i>et al.</i> (1990)  | Valladolid (VA)  |
| L18  | Tinto Madrid (VA)         | PEÑÍN <i>et al.</i> (1997)    | Valladolid (VA)  |
| L29  | Tinto del País (VA-2)     | BORREGO <i>et al.</i> (1990)  | Valladolid (VA)  |
| L56  | Tinta de Madrid (ZA)      | CLEMENTE (1807)               | Zamora (ZA)      |
| L57  | Tempranillo (ZA)          |                               | Zamora (ZA)      |
| M04  | Tinta de Toro (ZA)        | GARCÍA DE LOS SALMONES (1914) | Zamora (ZA)      |
| M10  | Chinchillana (BA)         | PEÑÍN <i>et al.</i> (1997)    | Badajoz (BA)     |
| M11  | Escobera (BA)             | PEÑÍN <i>et al.</i> (1997)    | Badajoz (BA)     |
| M31  | Tempranillo (CC)          |                               | Cáceres (CC)     |

The analysis of 31 accessions of Tempranillo, proposed synonyms and the rootstock R-110 using two AFLP primer combinations yielded a total of 206 bands of which 95 polymorphic bands were scored. A dendrogram illustrates genetic similarity values obtained from AFLP data (Figure, b). The co-phenetic correlation was 0.99 ( $p=0.002$ ) indicating a good fit of the cluster analysis. The accessions showed GS levels between 0.53 and 1. Based on previous results (CERVERA *et al.* 1998), accessions showing similarities  $>0.90$  can be considered as belonging to the same variety while different varieties show similarities from 0.60 to 0.90. The dendrogram shows the existence of one main group of accessions including all Tempranillo samples with GS values  $\geq 0.97$  and excluding Tempranillo Temprano (LO), Tinto Madrid (S), Tinta de Madrid (TO), Tinto del País (BU) and Tinto Madrid (VA). No polymorphism at the DNA level was detected between Tempranillo (VI, LO, B, BU), Tinta de Toro (ZA), Tinto Fino (AB), Cencibel (GU), Tinto del País (VA-1, VA-2), and Tinto Madrid (M, SA, TO), as well as between Cencibel (CR-1, CR-2, CU, M), Tempranillo (CC, ZA),

Chinchillana (BA), Tinta de Madrid (ZA), Tinto Madrid (LE), Juan García (ZA), Tinto del País (SO), Escobera (BA), and Tinto Madrid (SO). These two sub-clusters were grouped at  $GS \geq 0.98$ , which is due to the existence of one polymorphism between them. Tempranillo (NA) showed  $GS \geq 0.98$  (one polymorphism) and 0.97 (two polymorphisms) respectively with the first and second group of Tempranillo.

The low genetic similarity observed when compared to other Tempranillo accessions ( $GS < 0.63$ ) identified Tempranillo Temprano (LO) as a different homonym variety. This analysis also allowed the identification of false synonyms such as Tinto Madrid (S), Tinta de Madrid (TO) as well as the related accessions Tinto del País (BU) and Tinto Madrid (VA), which should correspond to unrelated varieties.

### Discussion

Tempranillo has long been considered as an example of so-called variety-populations, consisting of plants of differ-

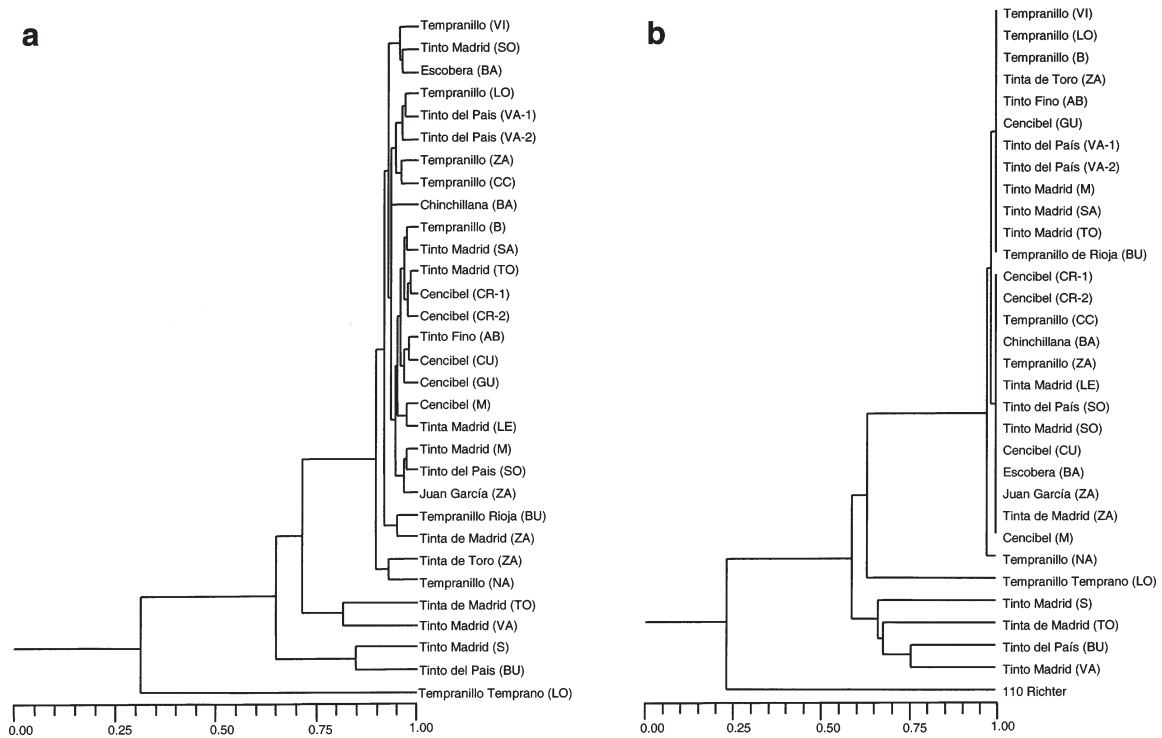


Figure: Dendrograms representing morphological (a) and genetic (b) relationships among Tempranillo accessions and proposed synonyms.

ent genetic origin and sharing morphological characteristics (CABELLO and DE LA FUENTE 1993). However, genetic characterization of accessions based on AFLP markers indicated that the variety Tempranillo consists of various clones with a higher level of genetic similarity ( $SG > 0.97$ ) than observed within other Spanish varieties such as Albillo (0.92), Turruntés (0.87) and Malvasía del Bierzo (0.91), when analyzed using the same AFLP primer combinations (CERVERA *et al.* 2001). This result strongly supports a monophyletic origin for Tempranillo. The narrow intra-varietal genetic diversity of Tempranillo may be explained either by a lower somatic variation rate as compared to other varieties, or a reduction of the genetic variability caused by vegetative propagation of low numbers of genotypes. The 26 Tempranillo accessions analyzed may derive from three closely related genotypes. The first group of Tempranillos grouped 12 accessions (from Tempranillo (VI) to Tempranillo de Rioja (BU), Figure, b), including a pre-phyloxeric accession collected in 1893 (Tempranillo (LO)). The second group, with 13 accessions (from Cencibel (CR-1) to Cencibel (M)), includes three identical accessions collected from post-phyloxeric plantations in the first half of the 20th century (Cencibel (M), Chinchillana (BA) and Escobera (BA)). Finally, the third group is represented by a single pre-phyloxeric accession collected in Navarra and recently added to the grapevine collection (Tempranillo (NA)). If the old accessions of the three groups are representatives of the original clones which were used to re-plant younger vineyards, then clones grown nowadays under the name Tempranillo, as well as related synonyms, may only represent a part of the original variability of this ancient Spanish

variety, while other local ancient clones may have been wiped out and with them most of the intra-varietal diversity.

In contrast, ampelographic diversity within Tempranillo was apparently higher than the genetic variability estimated by AFLP. This result however may be due to the combined use of unstable or subjective descriptors (such as density of petiole hairs) and descriptors, such as berry shape, which are not measured as quantitative traits. In this way, slight variation may turn into highly variable values overestimated in the statistical analysis. In any case, no statistically significant differences were found if ampelographic descriptions of accessions belonging to the three Tempranillo genotypes were compared and thus, no discriminatory descriptors were identified.

The ampelographic and genetic analysis also allowed the identification of Tempranillo synonyms. In addition to Cencibel, Tinto Fino, Tinta de Madrid, Tinta Madrid, Tinto Madrid, Tinto del País, Tinta de Toro, Chinchillana, and Escobera we also identified Juan García as a new synonym (Table). It is important to note that all clones belonging to Tinta de Madrid, Tinta Madrid, Tinto Madrid and Tinto del País can not be considered to be Tempranillo. These varieties are heterogeneous at the genetic level grouping Tempranillo accessions (Tinto Madrid (M, SA, TO, SO), Tinta Madrid (LE), Tinta de Madrid (ZA) and Tinto del País (VA-1, VA-2, SO)) together with clones belonging to other varieties (Tinta de Madrid (TO), Tinto Madrid (S, VA) and Tinto del País (BU)) of the same regions. Based on ampelographic descriptions and AFLP analysis, some of the false synonyms and the homonym were assigned to other varieties. The homonym Tempranillo Temprano (LO), was identified

as an accession of Moristel ( $GS \geq 0.93$ ), an autochthonous and ancient variety of northeastern Spain. The false synonyms Tinta de Madrid (TO) and Tinto del País (BU) were identified as Garnacha ( $GS \geq 0.96$ ) and Bobal ( $GS \geq 0.93$ ), respectively.

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### References

- CABELLO, F.; DE LA FUENTE, J. M.; 1993: Caracterización de la variedad "Tempranillo" y sus sinonimias Españolas. XV Jornadas de Viticultura y Enología de Tierra de Barros, Dirección General de Comercio e Industrias Agrarias de la Consejería de Agricultura y Comercio de la Junta de Extremadura.
- CERVERA, M. T.; CABEZAS, J. A.; SANCHA, J. C.; MARTÍNEZ DE TODA, F.; MARTÍNEZ-ZAPATER, J. M.; 1998: Application of AFLPs to the characterization of grapevine *Vitis vinifera* L. genetic resources. A case study with accessions from Rioja (Spain). *Theor. Appl. Genet.* **97**, 51-59.
- ; - ; SÁNCHEZ-ESCRIBANO, E.; CENIS, J. L.; MARTÍNEZ-ZAPATER, J. M.; 2000: Characterization of genetic variation within table grape varieties (*Vitis vinifera* L.) based on AFLP markers. *Vitis* **39**, 109-114.
- ; RODRÍGUEZ, I.; CABEZAS, J. A.; CHÁVEZ, J.; MARTÍNEZ-ZAPATER, J. M.; CABELLO, F.; 2001: Morphological and molecular characterization of grapevine accessions known as Albillo, one of the oldest varieties grown in Spain. *Am. J. Enol. Vitic.* **52**, 127-135.
- CLEMENTE, S.; 1807: Ensayo Sobre las Variedades de la Vid Común que Vegetan en Andalucía, con un Índice Etimológico y tres Listas de Plantas en que se Caracterizan Varias Especies Nuevas. Poulet, Madrid.
- GARCÍA DE LOS SALMONES, N.; 1914: Colección de Plantas de los Campos Oficiales. Recopilación. Instituto Nacional de Investigaciones Agronómicas, Madrid.
- O.I.V.; 1984: Codes des Caractères Descriptifs des Variétés et Espèces de *Vitis*. A. Dedon, Paris.
- PEÑÍN, J.; CERVERA, A.; CABELLO, F.; DIEZ, R.; PEÑÍN, P.; 1997: Cepas del Mundo. Ediciones PI y Erre, Madrid.
- RODRÍGUEZ-TORRES, I.; 2001: Caracterización de variedades de vid por métodos ampelográficos y bioquímicos. Resolución de homonimias y sinonimias. Thesis E.T.S.I. Agrónomos. Universidad Politécnica de Madrid.
- VOS, P.; HOGERS, R.; BLEEKER, M.; REIJANS, M.; VAN DE LEE, T.; HORNES, M.; FRIJTERS, A.; POT, J.; PELEMAN, J.; KUIPER, M.; ZABEAU, M.; 1995: AFLP: A new technique for DNA fingerprinting. *Nucl. Acids Res.* **23**, 4407-4414.

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