Garnacha and Garnacha Tintorera: Genetic Relationships and the Origin of Teinturier Varieties Cultivated in Spain

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Representative grapevine accessions *(Vitis vinifera)* cultivated in Spain under the names Garnacha and Garnacha Tintorera, as well as their synonyms, were analyzed to determine genetic diversity and relationships. Both varieties are characterized by high levels of intravarietal morphological variation. Results confirmed the monophyletic origin of the Garnacha variety, which is represented by a main genotype with several phenotypic variants, likely corresponding to somatic mutations. In contrast, Garnacha Tintorera was characterized as a genetically heterogeneous group, which included three different teinturier genotypes. Possible parentage relationships among the teinturier varieties were identified and further confirmed using microsatellites, showing that all are derived from crosses performed in the nineteenth century to improve color intensity of well-known red wine varieties.

Key words: Garnacha, Grenache, Garnacha Tintorera, Alicante Bouschet, teinturier, intravarietal diversity, AFLP, microsatellite, morphological variant

Garnacha is an ancient grapevine variety. It was first referenced in 1312, under the name Varnacie, in a legal document of the Paris parliament (Peñín et al. 1997). Garnacha is characterized by pentagonal three-lobed leaves and round, dark, red-violet berries with high sugar content. It is now the most widely grown red wine variety in the world, with more than 419,000 ha (Hidalgo 1999), of which almost half is located in Spain. It is also widely cultivated under the name Grenache in other countries, including France, the United States, and Australia. There are many synonyms for Garnacha, such as Alicante, Roussillon, Rivesaltes, Bois Jaune, and Carignane Rousse in France (Galet 2000), Cannonau and Tocai Rosso in Italy (Caló et al. 1990), and Garnacho, Aragonés, Lladoner, Tinta, and Alicante in Spain (Galet 2000). Garnacha identification is further complicated by the high level of morphological variation found among plants cultivated under this name. This variation has given rise to different morphotypes, which have been considered as different grape varieties when affecting important agronomic or ampelographic traits. This is the case of Garnacha Tinta (red), Garnacha Blanca (white), Garnacha Gris or Dorada (gray), or Garnacha Peluda (hairy). Furthermore, the word *Garnacha* is also used as homonym for other varieties. One of these well-known homonyms is Garnacha Tintorera, despite this variety being clearly distinguishable morphologically from Garnacha by the small pentagonal and five-lobed leaves and red-black berries with colored flesh. The Spanish word *tintorera* refers to the strongly colored flesh that characterizes all teinturier varieties. Garnacha Tintorera is a minor variety, with only 17,100 ha cultivated in Spain (Registros Viticolas 1999), and therefore of lesser economic importance. As with other teinturier varieties, it is not used to produce high-quality wines, but is blended in multivarietal wines to increase color intensity. Some authors consider Garnacha Tintorera a native Spanish variety (Hidalgo and Galet 1988, Peñín et al. 1997), while others consider it a synonym of the French variety Alicante Bouschet (Chirivella et al. 1995, Galet 2000). It can be found cultivated under different names. Most should be considered synonyms, such as Alicante, Negral, Tintorera, or Moratón (Rodriguez-Torres 2001), but homonyms, such as Garnacha, or false synonyms, such as Alicante (Garnacha is known as Alicante in France), have also been described. Adding to the confusion, high levels of morphological variation are also found among the teinturier plants grown as Garnacha Tintorera or under related names (Rodriguez-Torres 2001). Whereas some authors describe Garnacha Tintorera as a single variety (Hidalgo and Galet 1988), others suggest that more than one variety are cultivated under this name in Spain (García de los Salmones 1914; Martinez de Toda and Sancha 1996). Thus, this morphological variation could either represent somatic variants or indicate the presence of different homonym teinturier varieties mixed because of their colored flesh, a trait that differentiates them from most other grapevine varieties.

In order to understand the origin of the morphological variation observed in the Garnacha and Garnacha Tintorera

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varieties, we performed molecular characterization of representative accessions. Results indicate that Garnacha accessions are a single main genotype, and the studied morphological variants, even those considered as different varieties, are somatic variants that appear recurrently in the Garnacha genetic background. Analysis of Garnacha Tintorera accessions revealed the existence of three different teinturier genotypes. Genetic analysis based on amplified fragment length polymorphism (AFLP) and microsatellite markers showed that all accessions derive from documented crosses performed in the nineteenth century by Louis and Henri Bouschet (Viala and Vermorel 1909).

Materials and Methods

Plant material. Sixty-four accessions representative of the material cultivated in Spain under the names Garnacha and Garnacha Tintorera, as well as associated synonyms, were analyzed in this study. All had previously been characterized using ampelographic descriptors (Rodriguez-Torres 2001). The interspecific hybrid 110 Richter (*Vitis berlandieri* x *V. rupestris*) was also included as an outgroup sample. Local names, codes, and places of origin of the studied material are listed in Table 1. All accessions belong to the grape germplasm collection maintained at El Encín (Instituto Madrileño de Investigación Agraria y Alimentaria of Comunidad de Madrid, Alcalá de Henares, Spain) (Cabello 1995).

Molecular analysis. Total DNA was extracted from young leaves, which had been stored at -80°C, following the protocol described by Dellaporta et al. (1983). One percent polyvinylpyrrolidone was added to the extraction buffer to precipitate polyphenols (Lodhi et al. 1994).

AFLP analysis was carried out following the protocol described by Vos et al. (1995), with slight modifications (Cervera et al. 1998). In order to compare the results obtained in different experiments, the AFLP primer combinations, as well as the sample used as outgroup, were the same as those ones used in previous studies. The primer combination used in the preamplification was EcoRI +A / MseI +C, while the two primer combinations used for selective radioactive amplification were 2 EcoRI (+ACC, +ACT) / MseI +CAT) and 2 EcoRI (+ACC, +ACT) / MseI +CTG. Radioactively labeled amplified fragments were separated in 4.5% acrylamide: bisacrylamide 19:1, 7 M urea, 1x TBE gels, and visualized after exposing the gels using Hyperfilm™ MP autoradiography films (Amersham Biosciences, Buckinghamshire, UK). Amplified fragments were separately scored by two persons and used to build a binary matrix of presence/absence. Only easily scorable bands, showing medium or high intensity, were considered for the analysis.

Allelic segregation at 21 microsatellite loci was also studied using radioactive labeled primers (VMC6B11, VMC6G8, VMC6E10, VMC6D12, VMC6C7, VMC6G10, VMC6C10 [Rosa Arroyo-García and José Miguel Martínez-Zapater, unpublished data]) or fluorescent labeled primers (VVMD5, VVMD7 [Bowers et al. 1996], VVMD27, VVMD28,

Table 1 Grapevine accessions analyzed, with teinturier indicated in bold. Local name, reference code at the germplasm bank of El Encín (Cabello 1995), and place of origin for each plant are indicated.

Reference code	Local name	Place of origin (Spain)
22-A-04	Garnacha	Álava
22-A-08	Garnacho Blanco	Alava
22-A-11	Garnacha Blanca	Logroño
22-A-39	Garnacha Tinta	Navarra
22-C-56	Moscatel Morisco	Málaga
22-D-06	Garnacha Negra	Huesca
22-D-07	Garnacha Basta	Huesca
22-D-21	Garnacha Gorda	Huesca
22-D-26	Bernacha Blanca	Teruel
22-D-30	Garnacha Fina	Teruel
22-D-34	Garnacha Negra	Teruel
22-D-36	Garnacha Peluda	Teruel
22-D-37	Garnacha Blanca	Teruel
22-D-48	Garnacha Francesa	Zaragoza
22-D-49	**Tintorera de Longares**	Zaragoza
22-D-50	Garnacha Negra	Zaragoza
22-E-36	Giró	Palma
22-F-32	**Garnacha**	Oviedo
22-F-42	**Tinto Madrid**	Cantabria
22-G-33	Garnacha Dorada	Barcelona
22-G-41	Garnacha Blanca	Gerona
22-G-43	Lladoner Negre	Gerona
22-G-49	Garnacha Tinta	Lérida
22-H-07	Garnacha Negra del País	Tarragona
22-H-19	Garnacha Peluda	Tarragona
22-H-29	Garnacha Blanca	Tarragona
22-H-34	Garnacha Negra	Tarragona
22-H-38	**Garnacha**	Albacete
22-H-42	**Garnacha Tintorera**	Albacete
22-H-45	**Tintorera**	Albacete
22-I-08	**Garnacha Tintorera**	Albacete
22-I-12	Tinto Navalcarnero	Ávila
22-I-13	Tinto de Aragón	Ávila
22-I-17	Garnacha	Avila
22-I-43	**Garnacha**	Cuenca
22-J-17	Garnacha	Madrid
22-J-30	**Negral**	Madrid
22-J-31	**Garnacha**	Madrid
22-J-33	**Garnacha Tintorera**	Toledo
22-J-34	Garnacha	Toledo
22-J-41	**Colorina**	Toledo
22-J-50	Tinto Navalcarnero	Burgos
22-J-51	Tinto Aragonés	Burgos
22-J-55	Aragón	Burgos
22-K-28	Garnacha	León
22-K-33	**Moratón**	León
22-K-37	Tinto Aragonés	Palencia
22-L-10	**Garnacha**	Soria
22-L-21	Garnacha	Valladolid
22-L-26	Garnacho Negro	Valladolid
22-L-30	**Garnacho**	Valladolid
22-L-39	Garnacho Rojo	Valladolid
22-L-60	Garnacha Tinta	Zaragoza
22-M-02	Navarro	Zamora
22-M-29	Garnacha C. de Rioja	Cáceres
22-M-45	**Alicante**	La Coruña
22-M-60	**Alicante**	Lugo
22-N-45	**Negrón de Aldán**	Pontevedra
22-O-04	**Tintorera**	Alicante
22-O-09	Garnacha	Castellon
22-O-38	**Tintorera de Liria**	Valencia
22-O-41	Garnacha	Valencia
22-O-49	**Alicante Bouschet**	Valencia
22-R-03	**Tintorero**	Alcanadre
	110 Richter	Commercial rootstock

Table 2 Morphological characteristics of each morphotype found among the Garnacha and teinturier (T1, T2, and T3) accessions studied. Values correspond to the mode of a total of six independent measurements (data collected by three different persons two consecutive years) for 49 ampelographic descriptors (OIV 1984).

OIV descriptor[a]

Morphotype[b]	Accession type	001	002	003	004	007	008	011	012	005	016	017	051	053	067	068	070	072	074	075	076
Garnacha Tinta (r)	22-J-17	7	2	7	3	1	1	1	1	1	1	5	3	2	3	3	1	1	5	3	2
Garnacha Gris (g)	22-G-33	7	2	6	3	1	1	1	1	1	1	3	1	2	3	3	1	1	5	3	2
Garnacha Blanca (w)	22-G-41	7	1	3	3	1	1	1	1	1	1	3	1	2	3	3	1	2	5	3	2
Garnacha Peluda (h)	22-H-19	7	2	6	7	1	1	1	1	1	1	4	3	8	3	3	1	1	5	3	3
T1	22-O-49	7	2	8	6	2	2	1	1	4	1	3	4	7	4	5	1	1	4	3	2
T2	22-F-32	7	2	7	7	3	3	1	1	7	1	3	4	7	3	3	4	2	4	4	2
T3	22-L-30	7	1	3	8	2	2	1	1	2	1	5	3	9	3	3	3	2	5	6	3

Morphotype[b]	Accession type	079	080	81-1	81-2	082	83-1	83-2	084	087	090	091	102	202	203	204	206	207	208	209	220	221	223	225	230	236	241	244	301	503
Garnacha Tinta (r)	22-J-17	2	2	1	1	3	2	1	1	1	1	1	3	4	4	7	1	1	3	2	5	5	6	5	1	1	3	1	5	3
Garnacha Gris (g)	22-G-33	2	3	1	1	3	3	1	1	1	1	1	3	4	4	7	1	1	3	2	4	6	3	2	1	1	3	1	3	3
Garnacha Blanca (w)	22-G-41	2	2	1	1	3	3	1	1	1	1	1	3	4	4	7	1	5	3	2	5	5	3	1	1	1	3	1	3	3
Garnacha Peluda (h)	22-H-19	2	3	1	1	4	2	1	5	1	3	1	3	4	5	8	1	1	3	2	5	5	2	5	1	1	3	1	4	3
T1	22-O-49	2	3	1	1	3	3	1	5	3	2	1	3	3	3	7	2	1	3	2	5	6	2	6	2	1	3	1	1	3
T2	22-F-32	2	2	1	1	2	2	1	5	4	3	1	3	3	4	7	2	5	3	3	5	7	6	6	2	1	3	1	1	3
T3	22-L-30	3	2	1	1	4	2	4	6	3	5	1	3	5	5	7	1	5	3	3	5	6	2	6	2	1	3	1	3	3

[a]Marked columns refer to descriptors related with the variation identified between the different morphotypes and teinturier varieties: (004) young shoot: density of prostrate hairs of tip; (053) young leaf: density of prostrate hairs between veins at the lower side of leaf; (084) mature leaf: density of prostrate hairs on main veins (lower side); (090) mature leaf: density of prostrate hairs on petiole; (225) berry: color of skin; (230) berry: color of flesh.
[b]Red (r), gray (g), white (w), and hairy (h) morphotypes.

VVMD29 [Bowers et al. 1999], VVS2, VVS5 [Thomas and Scott 1993], ssrVrZAG29, ssrVrZAG47, ssrVrZAG62, ssrVrZAG67, ssrVrZAG79, ssrVrZAG83, ssrVrZAG112 [Sefc et al. 1999]). Radioactive reactions were carried out in a final volume of 20 µL containing 20 ng template DNA, 0.08 mM of each dNTP, 0.4 U of Taq DNA polymerase (Boehringer, Ingelheim, Germany), 10 mM Tris-HCl, 50 mM KCl, 2.5 mM $MgCl_2$, 4 ng of [ã33P]-forward primer, and 25 ng of reverse primer. Amplification was conducted in a Perkin-Elmer 9600 thermocycler (Boston, MA) with 5 min at 94°C initially, followed by 30 cycles each 94°C for 30 sec, 58°C for 30 sec, 72°C for 45 sec, and 72°C for 5 min. At the end of radioactive PCR, samples were denatured by adding an equal volume of formamide buffer (98% formamide, 10 mM EDTA pH 8.0, 0.05% bromophenol blue, and 0.05% xylene cyanol) and heated for 3 min at 94°C. Two microliters of each sample were loaded on 6% acrylamide/bisacrylamide 19:1, 7.5 M urea, and 1x TBE gels. For fluorescent based assays, PCR amplifications and fragment detection were performed as described in Garcia-Beneytez et al. (2002), using an ABI PRISM 310® Genetic Analyzer (Applied Biosystems, Foster City, CA). Allele binning of GeneScan values was carried out following the algorithm described by Ghosh et al. (1997).

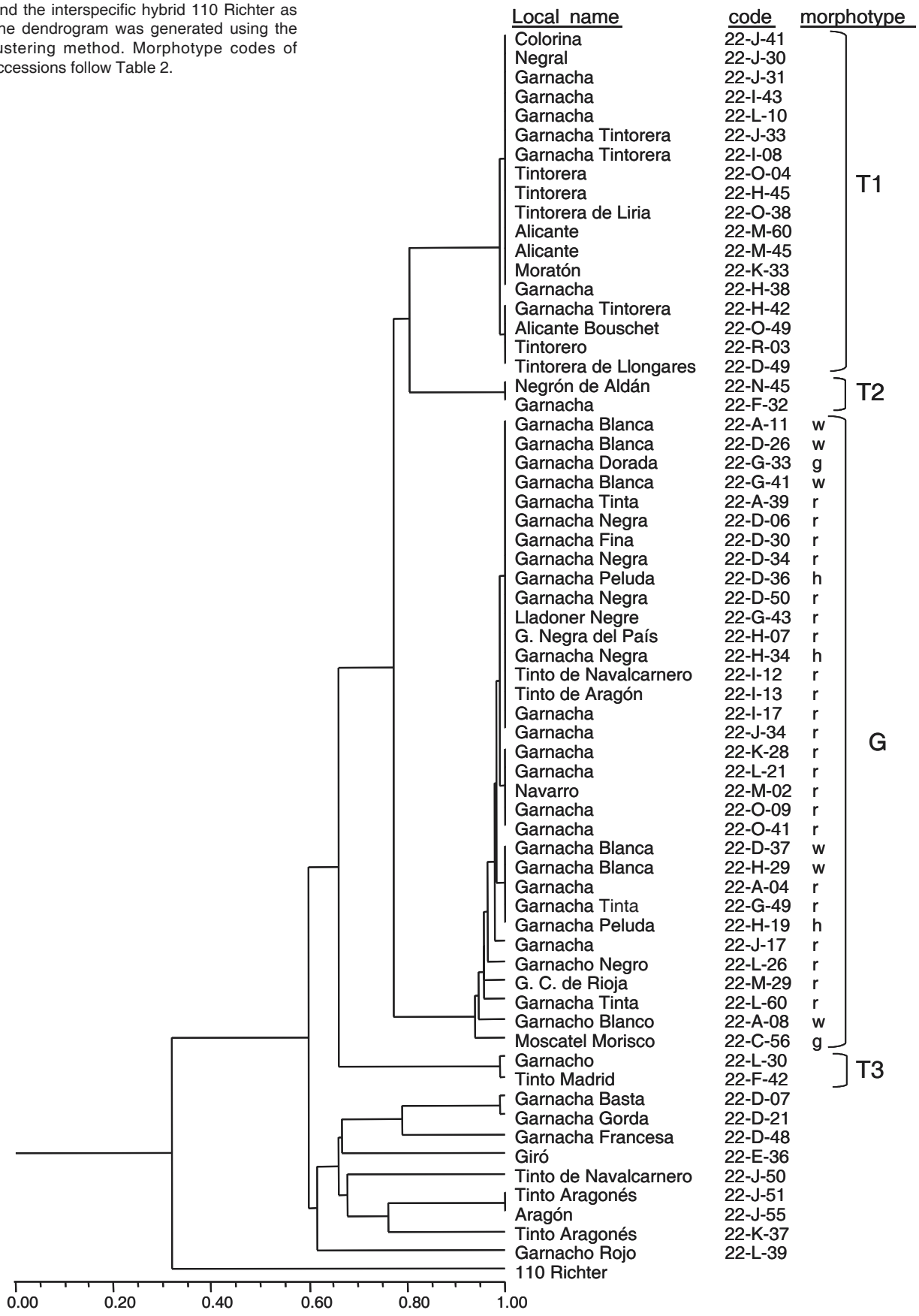
Statistical analysis. The AFLP binary matrix was analyzed with Numerical Taxonomy System software (NTsys version 2.02g, Exeter Software, Setauket, NY). A similarity matrix was generated using Dice coefficient (Sneath and Sokal 1973). Cluster analysis was performed using the unweighted pair-group method average (UPGMA) analysis and represented as a dendrogram. Cophenetic correlation between the similarity matrix and the cophenetic matrix was calculated to test well-fit of cluster analysis to the similarity matrix.

Allelic segregation at 21 microsatellite loci was studied for the parentage analysis. Allelic frequencies based on the analysis of 57 winegrape varieties were estimated for 12 loci (VVS2, VVS29, VVMD5, VVMD7, ssrVrZAG47, ssrVrZAG62, ssrVrZAG79, VMC6e10, VMC6b11, VMC6d12, VMC6c7, and VMC6g8). Allelic frequencies, and their 95% upper confidence limits, were used to estimate likelihood ratios, using the Identity software program (Centre for Applied Genetics, Vienna), to test the proposed parentage relationships by comparing the probability to obtain the observed genotypes with the proposed progenitors versus the probability of them being derived from other crosses (Bowers and Meredith 1997). The information about the nine remaining loci was used to further support these pedigrees.

Results

The 65 samples analyzed in this study are listed in Table 1, and the morphological descriptions of each morphotype found among the Garnacha accessions and the three teinturier genotypes studied (see below) are shown in Table 2. Molecular analysis of these samples with two AFLP primer combinations yielded a total of 267 bands: 126 for primer combination 2 EcoRI (+ACC, +ACT) / MseI +CTG and 141 for primer combination 2 EcoRI (+ACC, +ACT) / MseI +CAT). From

Figure 1 Graphic representation of genetic similarities among the analyzed accessions based on AFLP data. Genetic similarities were calculated using the Dice coefficient and the interspecific hybrid 110 Richter as outgroup. The dendrogram was generated using the UPGMA clustering method. Morphotype codes of Garnacha accessions follow Table 2.



these, 107 (40%) showed clear polymorphisms and were scored to build a binary matrix of presence/absence that was used to generate the matrix of genetic similarities (GS) among the pairs of analyzed accessions. A final dendrogram was built based upon the UPGMA analysis of the similarity matrix (Figure 1). The high value of cophenetic correlation between the similarity matrix and the cophenetic matrix (0.97, $p = 0.002$) showed the good fit of cluster analysis.

The AFLP-based dendrogram showed five main clusters (Figure 1). The largest one (coded as G) grouped most of the accessions under the name Garnacha, including accession 22-J-17, selected as representative of the Garnacha variety in the germplasm collection of El Encín. This cluster consisted of nine subgroups related at GS ≥ 0.95, and accessions belonging to each morphotype were scattered and mixed in the subgroups. Three clearly differentiated clusters (T1, T2, and T3) grouped all teinturier accessions. Most were included in cluster T1, which was identified as the Alicante Bouschet variety because of the presence of accession 22-O-49. A single polymorphism was detected among T1 accessions, which grouped them in two subclusters related at a GS value of 0.99. The second cluster of teinturier plants (T2) included two accessions showing the same genetic profile and an average GS value of 0.82 with T1. The third cluster (T3) grouped the two remaining teinturier accessions (GS = 0.99) at GS of 0.68 and 0.80 when compared to T1 and T2, respectively. The remaining accessions did not show significant genetic relationships with G, T1, T2, or T3 groups (0.50 < GS < 0.73), although some were more or less closely related among them. This was the case of Garnacha Basta (22-D-07) and Garnacha Gorda (22-D-21) (GS = 0.99) with Garnacha Francesa (22-D-48) (GS = 0.79), and of Tinto Aragonés (22-J-51) and Aragón (22-J-55) (GS = 1) with Tinto Aragonés (22-K-37) (GS = 0.76). Accessions such as Giró (22-E-36), Tinto Navalcalnero (22-J-50), and Garnacho Rojo (22-L-39) were not related to other analyzed accessions.

Previous AFLP studies with table grape varieties of known pedigrees established that accessions belonging to close related varieties, such as parents and offsprings or full siblings, showed GS values ranging from 0.8 to 0.9 (Cervera et al. 2000). The average GS value found between T1 and G accessions was 0.80, while that between cluster T1 and cluster T2 was 0.82. A detailed analysis of the GS matrix also revealed high GS values between T2 and T3 accessions (0.80), which were not clearly represented in the dendrogram. Since T1 corresponds to Alicante Bouschet, a variety derived from the controlled cross between Garnacha and Petit Bouschet (Viala and Vermorel 1909), a high GS value between groups G and T1 was expected. The lower GS value observed between T2 and G accessions (GS = 0.65), which rejects a possible parentage relationship between them, together with the high GS value observed between T1 and T2 (0.82), suggested that T2 could include two synonym accessions of the Petit Bouschet variety, the other recorded progenitor of Alicante Bouschet. Furthermore, the high GS value between T2 and T3 (GS = 0.80) indicated a possible parentage relationship, which did not exist between T3 and T1 (GS = 0.68) or between T3 and G (GS = 0.65).

Based on these results and the historical records about the crosses performed by Henri Bouschet between Petit Bouschet and different wine varieties (Viala and Vermorel 1909), we hypothesized that T2 could be Petit Bouschet and that T3 could also be a progeny variety derived from one of those crosses. To test these hypotheses we used AFLPs and microsatellites to analyze representative accessions of each teinturier group (T1, T2, and T3), Garnacha (G), and the most important red wine varieties cultivated at that time. If T1 is derived from a cross between T2 and G, and T3 from a cross between T2 and any other grapevine variety, then all the AFLP bands present in T1 and T3 should also be observed in, at least, one proposed progenitor.

AFLP pattern comparison of accessions belonging to these clusters supported these inferred relationships. As shown in Figure 2, the 41 bands observed in T1 (Alicante Bouschet) were all detected either in Garnacha and/or T2. Similarly, all amplified bands identified in T3 could be found in either T2 and/or a common and still-used Spanish wine variety: Graciano, also known as Morrastel (Figure 2). These results identified T2 and Graciano as the putative parents of teinturier varieties represented by cluster T3.

A useful tool to assess accurately parentage relationships is the study of allelic segregation of microsatellite markers based on their codominant-multiallelic nature (Bowers and Meredith 1997, Sefc et al. 1999, Regner et al. 2000). The allelic composition of representative accessions of each implicated variety was studied at 21 microsatellite loci. Parentage analysis based on 12 microsatellite loci (genotypes and allelic frequencies noted in Table 3) showed that T2 and Garnacha were the only compatible parents for Alicante Bouschet (T1) among the varieties studied. The likelihood ratio of the probability of the T1 genotype being obtained from the proposed parents versus the probability of this genotype being obtained from two random varieties was 1.1×10^9 (6.0×10^5 using the 95% upper confidence limits for the allelic frequencies). Moreover, comparative analysis between T2 and the representative accession of the Petit Bouschet variety (accession 14-I-03 from the germplasm collection of El Encín) showed identity at all tested microsatellites (data not shown). These results confirmed the T2 accessions as synonyms of the Petit Bouschet variety. Parentage analysis also confirmed T3 as the result of a crossbreeding between Graciano (Morrastel) and Petit Bouschet (T2) with a likelihood ratio of 8.6×10^{10} (1.1×10^7). The nine remaining microsatellite loci supported the parentage hypotheses described above, but they were not used for the statistical calculations because of the lack of allelic frequency data.

Discussion

Genetic relationships within Garnacha accessions. In a previous study, 49 ampelographic descriptors were used to morphologically characterize a representative collection of

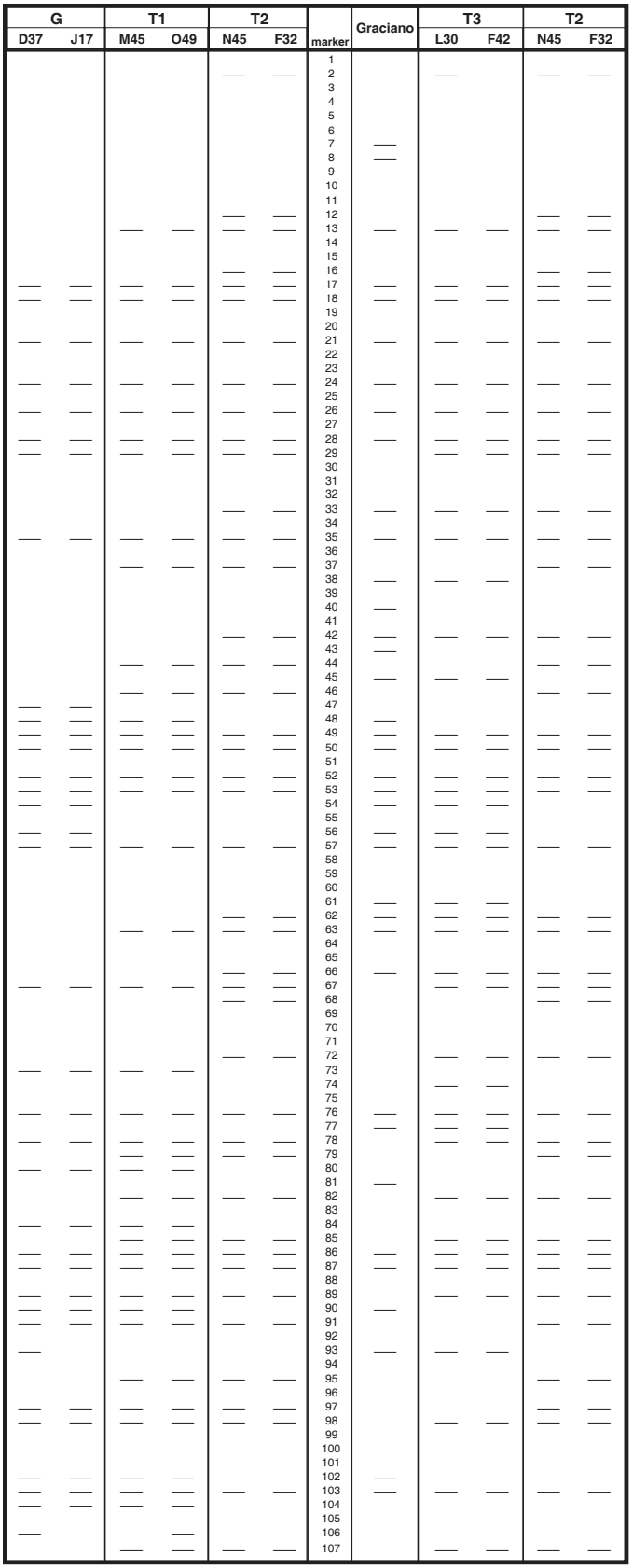


Figure 2 Schematic representations of AFLP profiles of representative accessions involved in the proposed parentage relationships. The figure represents the AFLP profiles generated with both analyzed primer combinations. Bands numbered from 1 to 49 refer to primer combination 2 EcoRI (+ACC, +ACT) / MseI +CAT), and bands from 50 to 107 refer to primer combination 2 EcoRI (+ACC, +ACT) / MseI +CTG.

wine grapevines cultivated in Spain (Rodriguez-Torres 2001). This analysis highlighted the high phenotypic variation found among grapevines cultivated under the name Garnacha or historically related synonyms when compared to other winegrape varieties such as Tempranillo and Parellada. This variation could be due either to a polyclonal origin (different genotypes grown under the same name) or to somatic variation giving rise to specific morphological variants within the same basic genotype. Previous AFLP-based analysis using the same primer combinations and outgroup sample established that the GS values found among *Vitis vinifera* accessions range from 0.6 to 1.0 (Cervera et al. 1998). In general, GS values higher than 0.9 are found between accessions belonging to the same variety, whereas GS values ranging from 0.6 to 0.9 correspond to accessions belonging to different varieties (Cervera et al. 1998, 2000).

AFLP characterization of Garnacha accessions identified a main Garnacha genotype that grouped all morphotypes described in Garnacha and many synonyms, such as Garnacho Blanco, Garnacho Negro, Navarro, Lladoner, Tinto de Aragón (22-I-13), and Tinto de Navalcarnero (22-I-12). This study also allowed the identification of several homonym accessions that, despite having the same name, belonged to different genotypes, such as Garnacha Tintorera, Garnacha Francesa, Garnacha Basta, and Garnacha Gorda. Although Garnacha Basta and Garnacha Gorda accessions were classified based on their ampelographic descriptors as belonging to the Garnacha variety (Rodriguez-Torres 2001), molecular and phenologic data from original growing areas suggest that these accessions may be Vidadillo. Furthermore, AFLP characterization revealed Giró as a false synonym, and names such as Tinto de Navalcalnero and Tinto de Aragón are used as homonyms for Garnacha in some Spanish regions but as synonyms in other regions. High GS values were found between some of these homonyms, such as between Garnacha Basta and Garnacha Gorda with Garnacha Francesa (GS = 0.79), as well as Tinto Aragonés (22-J-51) and Aragón with Tinto Aragonés (22-K-37) (GS = 0.76), suggesting the possibility of parentage relationships among them. High GS values were also found between Garnacha and the teinturier accessions grouped in cluster T1 and will be further described when discussing the origin of the identified varieties.

Morphological and molecular variation within Garnacha. Morphological variation within Garnacha mainly affects two traits: density of prostrated hairs in shoot tips and leaves and berry skin color. This variability allows the distinction of several morphotypes (Table 2), such as Garnacha (red and nude), Garnacha Peluda (red and hairy), Garnacha Gris (gray and nude), and Garnacha Blanca (white and nude). Although some of these morphotypes are considered different varieties by Spanish Denominations of Origin, many studies using molecular markers have failed to identify genotypic differences among them, including Royo et al. (1989) using isozymes, Moreno et al. (1998) using ISSRs, and Ibáñez et al. (2003) using microsatellites.

The presence and density of prostrated hairs in tips and leaves is a trait scored by four of the ampelographic descriptors

Table 3 Genotypes and allelic frequencies (in parentheses) of representative accession implicated in the proposed parentage relationships.

	G (22-J-17)	T1 (22-H-42)	T2 (22-N-45)	T3 (22-L-30)	Graciano (22-A-05)	Allelic frequencies (%)				
VVS2	134:142	129:142	129:149	136:149	136:149	129 (20.18)	134 (9.65)	136 (3.51)	142 (24.56)	149 (14.04)
VVS29	168:168	168:177	168:177	168:177	168:177	168 (82.46)	177 (10.53)			
VVMD5	222:237	222:235	231:235	222:231	222:235	222 (20.18)	231 (15.79)	235 (9.65)	237 (12.28)	
VVMD7	238:241	238:241	238:241	238:241	238:238	238 (49.12)	241 (19.30)			
VrZAG47	171:171	157:171	157:165	157:159	155:159	155 (13.16)	157 (24.56)	159 (12.28)	165 (18.42)	171 (19.30)
VrZAG62	188:188	188:188	188:195	188:188	186:188	186 (15.79)	188 (38.60)	195 (11.40)		
VrZAG79	255:255	241:255	241:241	241:257	257:257	241 (11.40)	255 (19.30)	257 (7.89)		
VMC6e10	95:110	95:95	95:116	95:113	110:113	95 (11.82)	110 (25.45)	113 (1.82)	116 (10.00)	
VMC6b11	109:92	85:92	85:92	83:85	83:83	83 (3.51)	85 (3.51)	92 (40.35)	109 (4.39)	
VMC6d12	150:160	160:160	160:160	130:160	130:130	130 (9.38)	150 (9.38)	160 (48.96)		
VMC6c7	138:157	138:157	138:157	138:138	138:157	138 (45.54)	157 (49.11)			
VMC6G8	95:101	89:95	89:101	101:101	95:101	89 (5.36)	95 (6.25)	101 (45.54)		

commonly used to classify grapevine varieties (OIV 1984), as shown in Table 2. Differentiation due to these descriptors is significant enough in the ampelographic classification to group all the hairy accessions (Garnacha Peluda) in an independent cluster, more related with the teinturier ones, also hairy varieties, than with the other Garnacha accessions (Rodriguez-Torres 2001). A color gradient in berry skin color, from red (Garnacha or Garnacha Tinta) to gray (Garnacha Gris, Dorada, or Rosa) and white (Garnacha Blanca), can also be found in Garnacha. However, genetic classification based on AFLP data places those accessions within a single Garnacha cluster (G in Figure 1). Although different subclusters can be distinguished within the Garnacha cluster, they are not associated with a specific hairy or color morphotype, suggesting that those morphotypes appeared recurrently by somatic mutation during clonal propagation. These results agree with those obtained studying the Pinot group, where molecular analysis did not allow the differential grouping of color morphotypes using SSRs and RAPDs (Regner et al. 2000) or AFLPs (Astrid Fornek 2002, personal communication). Garnacha plants showing branches with different berry skin colors are frequently observed. Color mutants are detected with a high frequency not only in Garnacha but also in other classical varieties, which may indicate that some genotypic combinations are more susceptible to undergoing this type of mutations. For example, Pinot Meunier is a variety belonging to the Pinot group in which leaves sometimes had sectors lacking their normal hairy phenotype, and this phenotypic effect has been related with its chimerical nature (Franks et al. 2002, Boss and Thomas 2002).

Origin of teinturier varieties cultivated in Spain. Most teinturier varieties now cultivated worldwide were developed by Louis and Henri Bouschet during the nineteenth century. They are hybrids derived from controlled crosses which were designed to increase color intensity of well-known, high-quality red wine varieties cultivated at that time. Alicante Bouschet, described as an F_1 progeny of the cross between Garnacha and Petit Bouschet (an Aramon x Teinturier du Cher hybrid developed by Louis Bouschet in 1828) (Viala and Vermorel 1909), was the most successful of those progeny and today is the most widely grown red-fleshed teinturier in the world with 35,000 ha (Hidalgo 1999). Garnacha Tintorera has been described as an autochthonous variety and has long been considered as the only teinturier cultivated in Spain. It is still considered by some authors as a true Spanish variety, different from Alicante Bouschet (Hidalgo and Galet 1988, Peñín et al. 1997), whereas other authors consider them as synonyms (Chirivella et al. 1995, Galet 2000).

The molecular analysis of the Spanish teinturier accessions showed the presence of three different genotypes (clusters T1, T2, and T3 in Figure 1). One of these varieties (T1) has been ampelographically and genetically identified as the hybrid variety Alicante Bouschet. Furthermore, GS values based on the AFLP analysis identified Garnacha (G) and T2 as putative progenitors of Alicante Bouschet (T1) (Figure 3). Therefore, T2 accessions should be synonyms of Petit Bouschet. This hypothesis was supported by comparison of AFLP fingerprints of accessions belonging to these three groups and further confirmed by studying the allelic segregation at 21 microsatellite loci. Following the same approach, the high GS

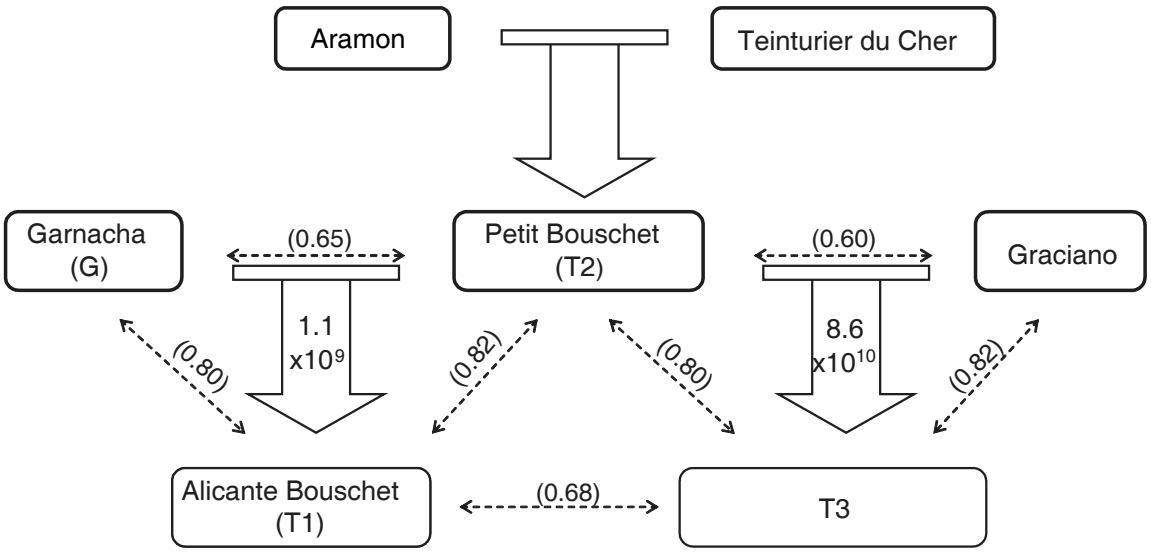


Figure 3 Graphic representation of the origin of teinturier varieties cultivated in Spain based on AFLP, microsatellite, and bibliographic data. Numbers in parentheses indicate GS values based on AFLP data. Numbers inside the arrows indicate the likelihood, based on the frequencies of their alleles at 12 microsatellite loci, for the proposed progenitors to be correct when compared with two other random varieties.

values found between T3 and T2 accessions identified T3 as one of the F_1 hybrids generated by Henri Bouschet using Petit Bouschet (T2) as a progenitor (Viala and Vermorel 1909). The comparison of AFLP profiles of T2, T3, and the red wine varieties most widely used during nineteenth century allowed the identification of the other progenitor as Graciano (also known as Morrastel). This hypothesis was also confirmed by the analysis of allele combination at 21 microsatellite loci. Therefore, the genotype represented by cluster T3 corresponds to one of the hybrids generated by Henri Bouschet in 1855 by pollinating Graciano vines with Petit Bouschet pollen (Viala and Vermorel 1909). Many progeny were obtained from this controlled cross, but only one showed an improved performance: Morrastel Bouschet à Gros Grains. Accessions belonging to this variety, as well as those derived from other F_1 plants resulting from this cross, such as Morrastel Bouschet à Sarments Eriges, Morrastel Bouschet à Feuilles Lascinées, Morrastel Bouschet à Petit Grain, and Carignan Bouschet, should be analyzed to confirm the identity of the teinturier hybrid variety represented by T3 accessions. Thus, all teinturier varieties studied belong to the Petit Bouschet genotype or derive from it, and no additional autochthonous variety was identified.

Conclusion

We have analyzed representative accessions of grapevines cultivated in Spain under the names Garnacha, Garnacha Tintorera, and associated synonyms, with the aim of understanding the origin of the morphological variation observed in these varieties. Our results indicate that all Garnacha morphotypes studied—Garnacha Tinta (red), Garnacha Gris (gray), Garnacha Blanca (white), and Garnacha Peluda (hairy)—correspond to the same genotype and likely represent somaclonal variants that appear recurrently. In contrast, the study of Garnacha Tintorera accessions and synonyms demonstrated the presence of three different teinturier genotypes and revealed the existence of parentage relationships among them. Further experiments identified the first genotype as the French variety Alicante Bouschet, supporting the already proposed synonymy between Garnacha Tintorera and Alicante Bouschet, the second as Petit Bouschet (one of the parents of Alicante Bouschet), and the third as a hybrid variety derived from the cross between Petit Bouschet and Graciano.

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