Authors: Sara Crespo-Martínez*[1], Blanca Mayor*[1], Oihane Oneka[1], Maite Loidi[1], Ana Villa[1], Diana Marín[1, 2], Carlos Miranda[1, 2], Luis G. Santesteban[1, 2], Jorge Urrestarazu[c, 1, 2]

Recovery of ancient grapevine plant material in peri-urban areas. A case of success in Pamplona (Spain) leading to the recovery of cv. Berués

[1]Department of Agronomy, Biotechnology and Food Science, Public University of Navarre (UPNA), Campus Arrosadia 31006, Pamplona, Spain

[2]Institute for Multidisciplinary Research in Applied Biology (IMAB-UPNA), Public University of Navarre, Campus Arrosadia 31006 Pamplona, Spain

*These authors contributed equally to this work

Sara Crespo-Martínez: sara.crespo@unavarra.es
Blanca Mayor: blanca.mayor@unavarra.es
Oihane Oneka: oihane.oneka@unavarra.es
Maite Loidi: maite.loidi@unavarra.es
Ana Villa: ana.villa@unavarra.es
Diana Marín: diana.marin@unavarra.es
Carlos Miranda: carlos.miranda@unavarra.es
Luis G. Santesteban: gonzaga.santesteban@unavarra.es
Jorge Urrestarazu: Corresponding author (c) jorge.urrestarazu@unavarra.es

Author contributions

SCM: Data curation, formal analysis, writing – original draft, writing – review & editing
BM: Conceptualization, data curation, formal analysis, methodology, writing – original draft, writing – review & editing
OO: Data curation, formal analysis, writing – review & editing
ML: Data acquisition, writing – review & editing
AV: Data acquisition, writing – review & editing
DM: Data acquisition, writing – review & editing
CM: Funding acquisition, project administration, writing – review & editing
LGS: Conceptualization, funding acquisition, conceptualization, methodology, supervision Writing – review & editing
JU: Conceptualization, formal analysis, writing – original draft, writing – review & editing

Authors: Sara Crespo-Martínez*[1], Blanca Mayor*[1], Oihane Oneka[1], Maite Loidi[1], Ana Villa[1], Diana Marín[1, 2], Carlos Miranda[1, 2], Luis G. Santesteban[1, 2], Jorge Urrestarazu[c, 1, 2]

Recovery of ancient grapevine plant material in peri-urban areas. A case of success in Pamplona (Spain) leading to the recovery of cv. Berués

[1]Department of Agronomy, Biotechnology and Food Science, Public University of Navarre (UPNA), Campus Arrosadia 31006, Pamplona, Spain

[2]Institute for Multidisciplinary Research in Applied Biology (IMAB-UPNA), Public University of Navarre, Campus Arrosadia 31006 Pamplona, Spain

*These authors contributed equally to this work

Sara Crespo-Martínez: sara.crespo@unavarra.es
Blanca Mayor: blanca.mayor@unavarra.es
Oihane Oneka: oihane.oneka@unavarra.es
Maite Loidi: maite.loidi@unavarra.es
Ana Villa: ana.villa@unavarra.es
Diana Marín: diana.marin@unavarra.es
Carlos Miranda: carlos.miranda@unavarra.es
Luis G. Santesteban: gonzaga.santesteban@unavarra.es
Jorge Urrestarazu: Corresponding author (c) jorge.urrestarazu@unavarra.es

Abstract

Viticulture was relatively important in the peri-urban area of Pamplona till the end of the 19th century, but suffered a continued regression that has led to a nearly complete disappearance of vineyards. In this context, this work aims to evaluate the feasibility of recovering old grapevine germplasm in the peri-urban area of Pamplona. The lack of a conventional source for recovering plant material (i.e. absence of old vineyards in the area) implied the need of designing an alternative prospecting procedure. This method included the analysis of the available historical information as open-access resources (orthophotos, land use maps and GIS applications) aiming to identify the areas with the highest probability of finding vines surviving from the general abandonment and uprooting of vineyards that had occurred in the 20th century. Based on the

oldest on scale orthophoto available for Navarra, a vineyard land map of the peri-urban area of Pamplona in year 1956 was built, allowing prospecting efforts to be focused on specific areas of primarily interest. Following this strategy, a total of 120 plants were collected, which corresponded to 44 genotypes. The most prominent achievement of this prospecting mission was the recovery of 15 accessions of Berués, a very old variety with a remarkable importance in the region according to old historical records, and considered to be disappeared. The methodology proposed was effective in searching for the oldest standing-alone plants surviving in the peri-urban area of Pamplona, and may be adapted to assist the recovery of old grapevine germplasm in other currently non wine-growing regions/areas where viticulture was relevant some decades ago.

Keywords: *Vitis vinifera* L.; old varieties; varietal diversity; cartographic information; prospecting work; molecular markers

Introduction

In the last decades, a remarkable effort has been done in order to recover grapevine varieties whose cultivation had become marginal or that survived as single plants in multi-cultivar old vineyards. As a result of this effort, a significant number of nearly forgotten/neglected varieties have been recovered in most countries in southern Europe, as highlighted in the project "On-farm inventory of minor grape varieties in the European *Vitis* Database (GrapeOnFarm)". As a general rule, prospecting missions initially focused on the oldest vineyards in well-known grape growing regions (Jung and Maul, 2004; Labagnara et al., 2018; Maigre et al., 1998; Mena et al., 2014; Urrestarazu et al., 2015; Yuste et al., 2019), and, only more recently, missions have been shifted towards areas where grape growing survived as a secondary or even marginal activity (Gago et al., 2011; Jiménez-Cantizano et al., 2020; Moreno-Sanz et al., 2011).

There are mainly three reasons that, individually or in combination, have led the viticulture of some regions in Europe to be marginal or even practically abandoned in the last 150 years: (i) increased disease pressure, (ii) rural depopulation, and (iii) industrialization and urbanisation pressure. In the next paragraphs, the three aforementioned reasons for marginalization or abandonment of grape growing are exemplified. For brevity's sake, some cases in France and Spain are presented, though examples can probably be found throughout all the traditional grape growing countries.

(i) Increased disease pressure: the arrival to Europe of powdery and downy mildew implied an increase in the cost of growing grapes due to the necessity of including copper and sulphur treatments. In the cooler and rainier areas, where disease control is more difficult, diseases led to frequent crop losses and to an increase in cost that could not be easily assumed by growers (Azcárate-Luxán, 1996), causing a gradual abandonment of vineyards. This was the case of Rias Altas' vineyards in Spain (De Lemps, 1967), where grape growing became unsustainable, while in the neighbouring Rias Baixas, with milder climate, vineyards thrived through the crisis. A similar process occurred in most grape growing area in Asturias, Cantabria,

Bizkaia and Gipuzkoa (Piqueras-Haba, 2010). In France, this was also the case for vineyards in several departments in the Northwest, such as l'Ille-et-Vilaine, la Mayenne, l'Eure, l'Oise and Ardenne (Legouy, 2014), where vineyards nearly disappeared in the late 19th century.

(ii) Rural depopulation: the loss of population in rural areas is a complex and long-term challenge worldwide (Labianca and Valverde, 2019; Li et al., 2019). In the last 150 years, there have been several enchained events that have gradually led to severe degrees of depopulation of some grape growing regions in Europe, with peasant population moving abroad, majorly to South and North America, to other European countries, or to urban areas within the origin country (Baines, 1995; Lasanta et al., 2017; Pinilla and Sáez, 2017), affecting more severely to semi-arid and mountain-areas (Lasanta et al., 2017). Depopulated grape growing areas in Spain can be found throughout the whole country, some examples being reported in detail for areas in Castilla y León (Franco-Jubete, 2007), Andalusia (Douglas et al., 1996; Douglas et al., 1994), and even in the now well-known Priorat area in Catalonia (Steevenson, 2004). In France, depopulation can be behind the regression of viticulture in central areas such as the Massif Central and neighbouring southern Departments (André, 1998; Legouy, 2014).

(iii) Industrialisation and urbanisation pressure: grape growers in peri-urban areas of growing cities must face an additional source of pressure associated to city expansion. Although in some cases, such as Bordeaux area, where certain resistance due to the higher price value of the wine can be observed (Peres, 2009), the growth of cities usually leads to the disappearance of peri-urban vineyards. The epitomic case is that of Montpellier, where, from the 1960s, the city turned its back on its viticulture (Perrin et al., 2013), although now some efforts are being done to revert this trend.

The aim of this work was to evaluate the feasibility of recovering grapevine plant material of

grapevine varieties in the peri-urban area of Pamplona, a medium-size town in northern Spain, where viticulture was relevant until the end of the 19th century, but suffered a continued regression that led to near complete disappearance of vineyards (Astibia-Ayerra, 1992). In this area, the reason for vineyard abandonment was a combination of cases (i) and (iii). Pamplona's climate can be classified as a Huglin type I region, with relatively high pressure for fungal diseases, where the population has increased from 35,000 inhabitants in 1900 up to > 350.000 inhabitants now. The current presence of vineyards in Navarra is nowadays restricted to some vineyards in the southern part of the region, whereas in the mid and northern parts, where the city of Pamplona is located, only approximately 5 very small (<0.3 ha) family-owned vineyards remain, altogether with one commercial winery established in the 1980s to produce international profile wines.

The differential aspect of our approach is that, to our knowledge, this is the first work aimed at recovering grapevine diversity in a non-viticultural area, which necessarily had to be performed based on rescuing individual plants surviving at field margins or at non-cultivated pieces of land. This is a very particular context, but that can probably be found at other regions in Europe, which requires prospection to be based on the analysis of the available historical information as open-access resources (orthophotos, land use maps and GIS applications).

Materials and methods

This study was carried out in four well differentiated phases: (i) analysis of the available historical information, (ii) prospecting works and collection of grapevine samples, (iii) classification of the recovered plants in different categories, dating its time of origin based on the last record of the presence of vineyards in the areas where they were found and, (iv) genetic characterization for varietal identification.

Analysis of the available historical information

With the aim of identifying the wine-growing zones of the peri-urban area of Pamplona in the past, the layers "Límites catastrales de los municipios de Navarra actuales" ("Cadastral limits of

the current municipalities of Navarre") and “Mapa de Cultivos y Aprovechamientos de 1956” (“Agricultural soil and land use of 1956”), usually denoted as “MCA56”, were used; both layers are available on the website of the Spatial Data Infrastructure of Navarra (http://idena.navarra.es) (Gobierno de Navarra, n.d.). The layer "MCA56" provides a photointerpretation of soil and land uses that was implemented based on a series of orthophotos taken all around the Spanish territory by the United States Army in the years 1956-1957, being the oldest on scale orthophoto available for Navarra (1:50,000). This open-access resource includes a filter of 27 categories of crops and land uses, among them “vineyard”. To prepare the final map, the one that allows to spatially identify the existing wine-growing area of the peri-urban area of Pamplona in 1956, the two above mentioned layers (i.e., “Cadastral limits of the current municipalities of Navarre” and “MCA56”) were added to the QGIS software (QGIS Development Team, 2009). Thereafter, the layer "MCA56" was applied by selecting the option that allows representing exclusively on the map the existing wine-growing zones for the peri-urban area of Pamplona in 1956, while the layer " Cadastral limits of the current municipalities of Navarre " was used to delimit the searching area for municipalities surrounding Pamplona (i.e. its peri-urban area).

Prospecting work and collection of grapevine samples

When visiting the areas of potential interest, an initial visual exploration was conducted with special attention to patches as borders between plots and paths, ditches and streams or uncultivated lands, due to the higher probability of finding surviving grapevine plants. When a surviving plant was found, an identification code was assigned, a sample of 2-3 leaves of each plant was collected for the genetic analyses and their main ampelographic characteristics were described. Moreover, the plants were geolocated using the offline application CartoDroid (Instituto Tecnológico Agrario de Castilla y León - ITACyL) that allows exporting directly the vector layer of the location points of each plant to the QGIS software (QGIS Development Team, 2009).

Classification of the recovered plants in different categories

All the plants collected in the Pamplona peri-urban area were classified into four categories according to the lapse of time since the last record of vineyards in the specific location where each plant was found could be confirmed. For doing that, open access orthophotos of the years 1956-1957, 1966-1971 and 2012 were used. The four categories proposed refer to:

- Category 1: Plants in probable areas of "old" vineyards as the last evidence of vineyards is reported in the orthophoto of 1956-1957.
- Category 2: Plants in probable areas of "non-modern" vineyards as the last evidence of vineyards is reported in the orthophoto of 1966-1971.
- Category 3: Plants in probable areas with a risk of "modern" presence of vineyards as the last evidence of vineyards is reported in the orthophoto of 2012.
- Category 4: Plants in riverside areas, unlikely to be a wine-growing area in the past.

Genetic characterization for varietal identification of the recovered plants

Young leaves of each collected plant were ground to a fine powder in a microdismembrator (B. Braun Biotech International, Melsungen, Germany). Genomic DNA was isolated from 100 mg of this fine powder with Qiagen Dneasy Plant Mini kit (Qiagen, Hilden, Germany) according to the manufacturer´s instructions. DNA concentration of each sample was determined using a NanoDrop 2000 (Thermo Fischer Scientific, Wilmington, DE, USA), and DNA working dilutions of each sample were adjusted to 5 ng/µl.

A set of 25 SSR markers, distributed on the 19 grapevine linkage groups, were studied in three independent multiplex PCRs denoted as S, A and B, including each, respectively, 9, 6 and 10 markers. Amplification reactions were carried out in a 2720 thermal cycler (Applied Biosystems, Foster City, CA, USA), following the protocols described by Ibáñez et al., (2009), with several modifications for A and B multiplex PCRs as detailed in Urrestarazu et al., (2015). The separation of fragments was carried out in an ABI PRISM 3730 sequencer (Applied Biosystems, Foster City, CA, USA), using 500-LIZ as internal marker size and Peak Scanner Software version 1.0 (Applied Biosystems, Foster City, CA, USA) to size the fragments.

The SSR molecular profiles found were compared to the set of unique profiles from the *Vitis* International Variety Catalogue (www.vivc.de) (Maul et al., 2014) and to those in the European *Vitis* Database (www.eu-vitis.de/index.php) (Maul et al., 2012). Both databases include harmonized genetic characterization data from thousands of grapevine accessions with nine SSR markers, all of them used in this study. When SSR profiles obtained matched the profile reported for a specific variety in any of the two databases, their SSR profiles were also compared for the rest of the markers analysed with the published data obtained for these varieties in other studies (Jiménez-Cantizano, 2014; Mena, 2013; Mena et al., 2014).

Results and discussion

Historical map construction as the basis for prospecting works

Prospecting works of the present study were based on the constructed 1956 vineyard land map (see Material and methods section for details). According to the constructed vineyard map for the year 1956 (Figure 1A), 599 ha of wine-growing surface could be quantified, while the one corresponding to 2012 (Figure 1B) is nearly inexistent (i.e. 122 ha), associated mainly to the acreage of one single winery established in the 1980s to produce international profile wines. When comparing the maps of the years 1956 and 2012, abandoned vineyard lands in the peri-urban area of Pamplona since the second half of the 20th century became evident.

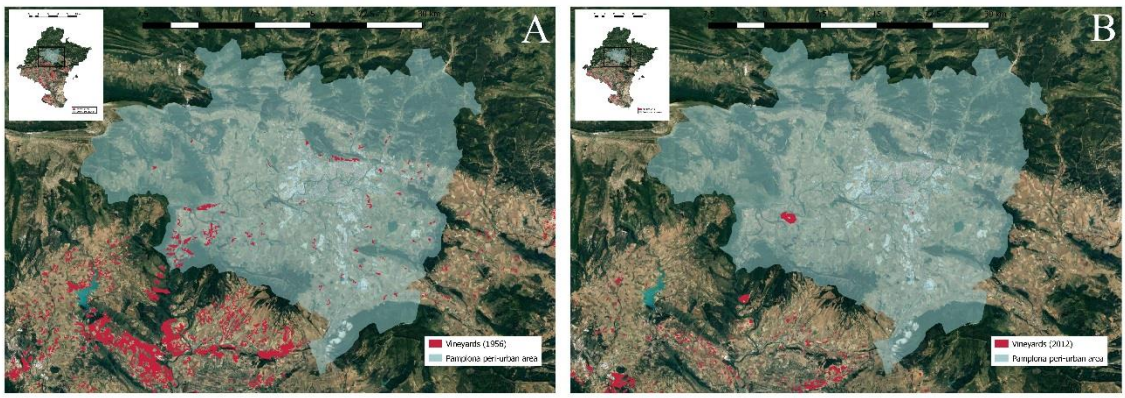

Figure 1. Map showing vineyard area in the peri-urban area of Pamplona in A) 1956 and B) 2012. Vineyard abandonment is clearly observed, the only relevant acreage in 2012 corresponding to the fields of a winery established in the 1980s to produce international profile wines.

The availability of the constructed 1956 vineyard land map of the peri-urban area of Pamplona enabled the identification of areas with potential interest as "old variety reservoirs", and consequently, allowed to focus the prospecting efforts on these specific areas in order to increase the chance of success at finding old vine plants. Based on this strategy, a total of 120 plants (hereafter referred to as accessions) were found in the peri-urban area of Pamplona (Figure 2). As it can be observed in Figure 1A, the location of most of the blue dots, representing the collected plants, happen to meet with the historical confirmed vineyard land that later were abandoned (highlighted in burgundy in the map).

Prospecting works have been traditionally carried out on still existing old vineyards. The criteria for selecting plants have been mainly based on field observations. Contrary, prospecting works on areas where grapevine has not been grown regularly for decades, as is the case of the peri-urban area of Pamplona, have a lack of on-site information. The lack of *in situ* information implied the need of implementing non-conventional methods. In this context, a methodology based on the overlay of historical cartography information onto a digital map was designed *ad hoc* for this specific case study.

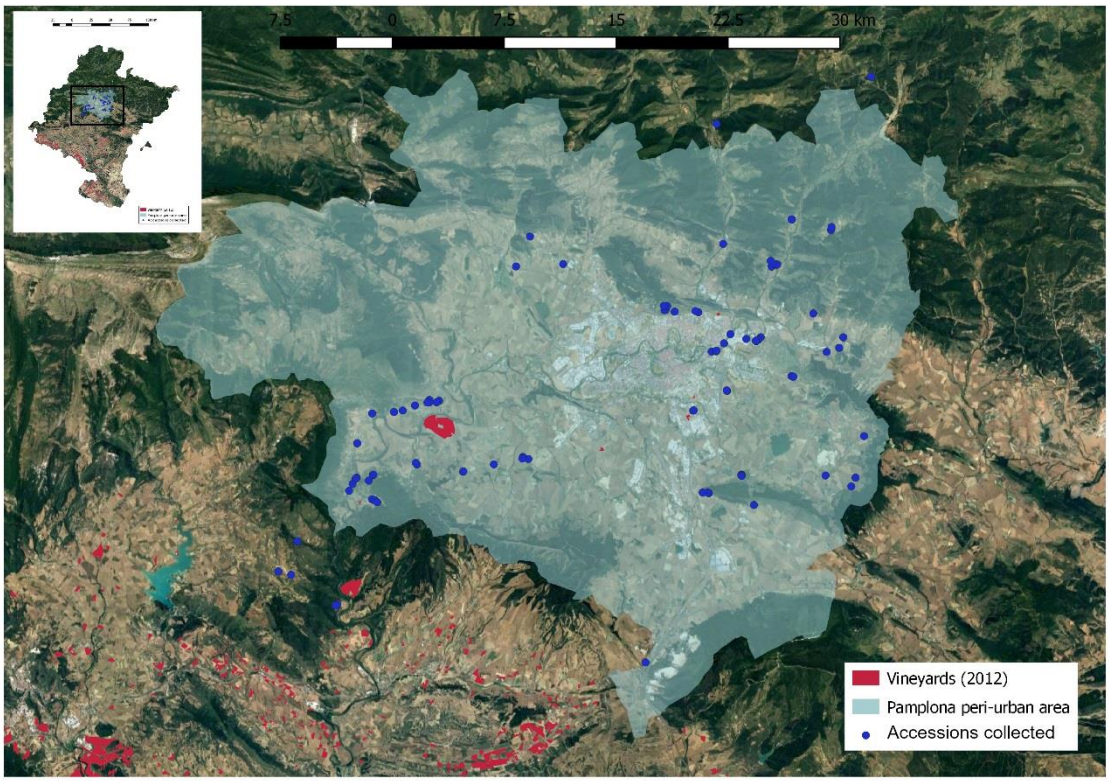

Figure 2. Satellite-based map of the peri-urban area of Pamplona. The current (2012) vineyards are marked in red, whereas Blue dots represent the location of the accessions included in this work, which location happen to meet with the historical confirmed vineyard land that later were abandoned.

Dating of plant material

After prospecting works were completed, cartographic information was further used to date each recovered plant, considering for that the plant locations and the last records of viticulture activity for each specific georeferenced position. The process followed is described in Figure 3. As observed, cartographic information from 1966-1971, 1998-2000 and 2012 of all referenced points were investigated to ascertain the presence of vineyards and to define the time-origin of each accession. The three blue dots marked by arrows in Figure 3, representing three of the accessions collected in this study, were located within plots that, according to the 1956 vineyard map, were confirmed as vineyards in that time. For the georeferenced point where the first

accession was collected, the one classified in Category 1 ("old" vineyards, being dated before 1956), no presence of vineyards was observed on it after 1956. By contrast, for the georeferenced points where the other two accessions were found, the ones corresponding to Category 2 ("non-modern" vineyards, being dated between 1966 and 1971) and Category 3 (presence of "modern" vineyards in 2012), presence of vineyards can be observed after 1956, between 1966-1971 and from 1988 to 2012, respectively.

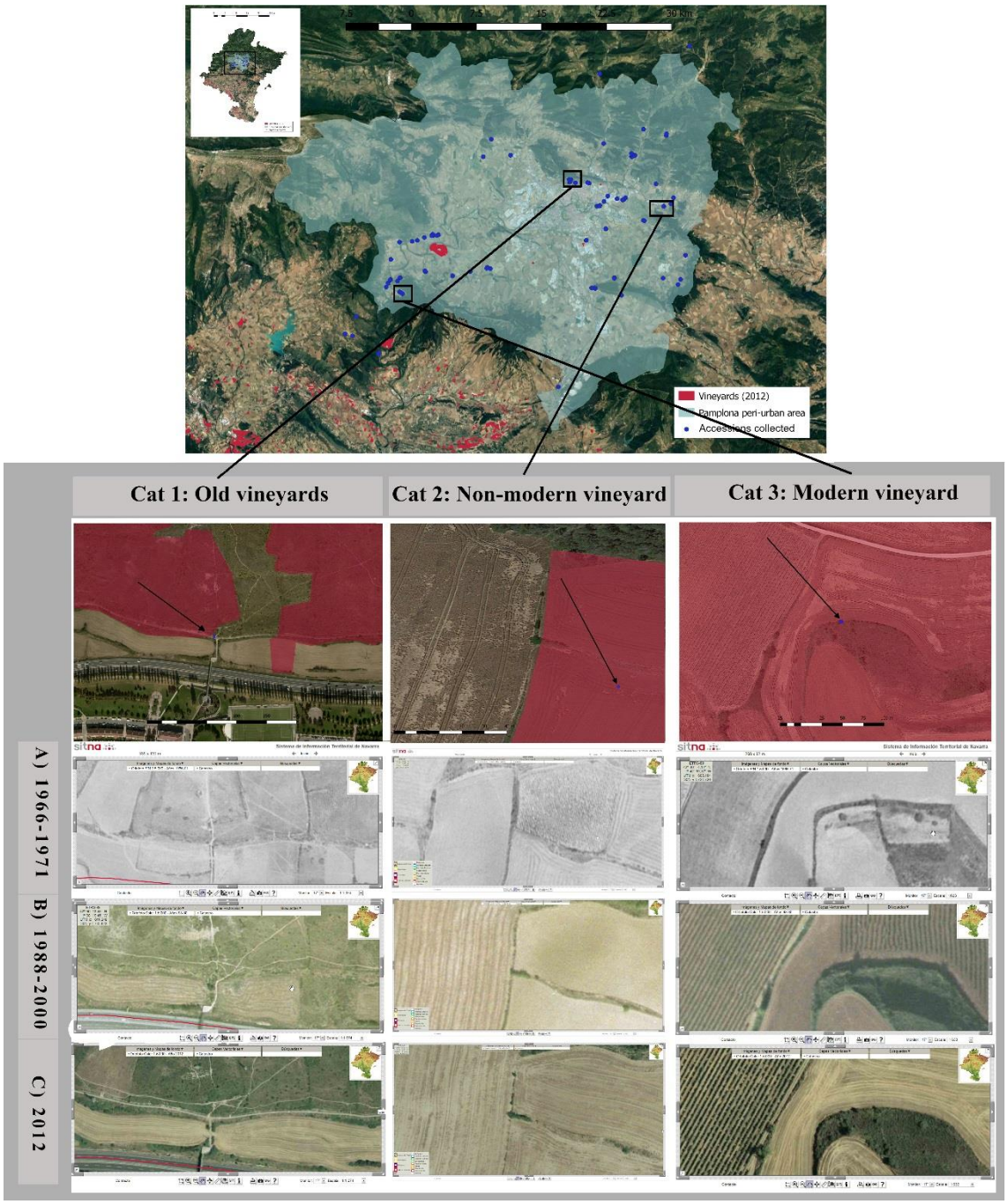

Figure 3. Maps used in the process for plant material dating. An example for the three different time points (Category 1: old vineyard, Category 2: Non-modern vineyard and Category 3: Modern vineyard) are displayed. Grapevine cultivation in every referenced accession (marked with arrows) was checked through cartographic pictures on A) 1966-71, B) 1998-2000 and C) 2012.

The classification of the 120 accessions found according to their location is presented in Table 1. The majority of the accessions (76 out of 120) corresponded to Category 1 ("old" vineyards, being dated before 1956), followed by those belonging to Category 2 ("non-modern" vineyards, being dated between 1966 and 1971), 28 accessions. Only 2 accessions belonged to Category 3 (presence of "modern" vineyards in 2012), whereas the remaining 14 were included in Category 4 ("riverside areas"). This later class comprises accessions found in places unlikely to be a wine-growing area in the past, and could be considered as feral germplasm.

Table 1. Classification of the collected plants according to their origin. "Category 1: old vineyards", "Category 2: non-modern vineyards", "Category 3: modern presence of vineyards" and "Category 4: Plants in riverside areas". Cartographic information from 1966-1971, 1998-2000 and 2012 of all referenced points were investigated to ascertain the presence of vineyards and to define the time-origin of each accession.

Category	Date	Number of accessions	Percentage
1	1956 or before	75	62.50%
2	1966-1971	29	24.16%
3	1971-2012	2	1.67%
4	River sides	14	11.67%

The dating plant material approach based on georeferenced historical cartography has proven to be useful to classify the recovered plants into four time periods extending over the 20th century. Dating of plant material was useful to classify the collected plants within a specific period of time, but also allowed us to validate the effectiveness of the methodology designed *ad hoc* for this case study. Nearly 63% of the plants collected were assigned to Category 1, thus revealing that the proposed methodology allows both the finding and recovery of the oldest "still existing" standing-alone plants in the peri-urban area of Pamplona.

Varietal identification of the recovered plants using molecular markers

Within the 120 accessions collected in the peri-urban area of Pamplona, a total of 44 genotypes were obtained. Comparisons between the SSR profiles obtained to those found in international databases (the *Vitis* International Variety Catalogue and the European *Vitis* Database) have allowed the identification of 17 cultivars (Table 2).

Combining dating and varietal identification of the accessions found in the peri-urban area of Pamplona allowed to delineate an approximate idea of the varietal composition of the vineyards existing in 1956 in this area (Fig. 4). Among the oldest accessions (those comprised in Category 1), the most prevalent variety before 1956 in the "Pamplona Basin" was Garnacha (syn. Grenache) (33 accessions, 44% of those in Category 1), what is in agreement with the increasing popularity that this variety had from the mid-19th century in the region due to its lower susceptibility to powdery and downy mildew among the traditional varieties (Huetz de Lemps, 1993). The second most numerous variety is Berués (13 accessions, 17.33% of those included in Category 1), a traditional variety that is not cultivated nowadays. Additionally, within the remaining cultivars identified in Category 1, in four cases [Carignan (syn. Mazuelo), Tempranillo, Morate and Muscat à petit grains blancs (syn. Moscatel de Grano Menudo)] there is documentary evidence that they had at least some relevance in early 20th century in Navarre or in its neighbouring regions (Basque Country, La Rioja and Aragon) (Cabello et al., 2011; García De Los Salmones, 1914; Manso de Zúñiga, 1905). Concerning the other three cultivars identified in Category 1 (Castellana Blanca, Tinto de Navalcarnero and Muscat of Alexandria),

the cultivation of the two former ones was documented for regions far from Navarre or bordering regions (García De Los Salmones, 1914), while the latter is a widespread table grape variety and, thus, its appearance is not surprising.

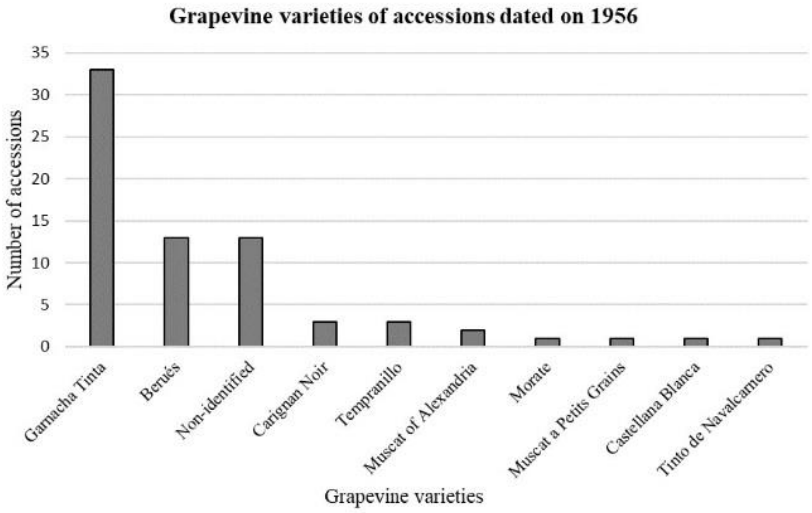

Figure 4. Varietal distribution of accessions classified as Category 1, dated before 1956. Number of accessions per variety is represented and varieties are ordered from more to less abundant.

Categories 2 and 3, the ones including, respectively, the plants collected in probable areas of "non-modern" and "modern" vineyards, grouped other eight cultivars: the Spanish cultivars Garnacha, Berués, Morate and Palomino Fino, the French cultivars Cabernet Sauvignon, Cabernet Franc and Cinsaut, and the interspecific hybrid (*Vitis aestivalis* × *Vitis vinifera*) Jacquez. The molecular profile of 28 accessions showed no coincidence with any of the genotypes included in the databases used for comparative purposes. Most of them are included in Category 4 (riverside areas), what reinforces the idea of their feral nature or their origin from unintentional cross-pollination.

According to the early work by García De Los Salmones (1914), a compilation of cultivars being grown at each Spanish region before the phylloxera outbreak, the cultivation of at least 27 different varieties was reported in the region of Navarre. Some initiatives have been carried out aiming to recover the varietal richness still existing in the oldest vineyards of the region in the last years (Cibriáin-Sabalza et al., 2016; Urrestarazu et al., 2015) resulting, as a whole, in more than 50 varieties identified, a significantly higher number than reported by García De Los Salmones (1914). In spite of the small agricultural surface covered by the prospecting mission performed in the peri-urban area of Pamplona, it is worth pointing out the remarkable grapevine variety reservoir that abandoned lands may still offer.

Table 2. List of varieties located in the prospecting works in the peri-urban area of Pamplona and classification of its accessions depending on date.

Prime name	Variety number VIVC	Number of accesions	Percentage	Category / Date			
				1. 1956 or before	2. 1966-1971	3. 1971-2012	4. River sides
Garnacha Tinta	4461	54	45.00%	33	21		
Berués de Huarte	1281	15	12.50%	13	2		
Carignan Noir	2098	3	2.50%	3			
Tempranillo Tinto	12350	3	2.50%	3			
Millardet et Grasset 41 B	7736	3	2.50%	2		1	
Muscat of Alexandria	8241	2	1.67%	2			
Morate	7981	2	1.67%	1	1		
Muscat a Petits Grains Blancs	8193	1	0.83%	1			
Castellana Blanca	26280	1	0.83%	1			
Tinto de Navalcarnero	26280	1	0.83%	1			
Rupestris du Lot	10389	1	0.83%	1			
Millardet et Grasset 420 A	7810	1	0.83%	1			
Cabernet Sauvignon	1929	1	0.83%		1		
Cabernet Franc	1927	1	0.83%		1		
Cinsaut	2672	1	0.83%		1		
Jacquez a Gros Grains[a]	5628	1	0.83%		1		
Palomino Fino	8888	1	0.83%		1		
Non-identified		28	23.33%	13		1	14
Total		120	99.97%	75	29	2	14

[a] Non true-to-type

Recovery of the variety Berués

Prospecting actions have been proved to be interesting not only to report the varietal richness at each specific region in the past, but also to recover emblematic cultivars preventing their loss. The major milestone underlying the prospecting mission of the peri-urban area of Pamplona has been the recovery of 15 accessions of Berués, a very old variety considered to be disappeared. The only plants of Berués preserved before this study was performed, where those collected probably in the same area at the beginning of 20th century by García de los Salmones (García De Los Salmones, 1914). This material is currently preserved at the grapevine collections of "El Encín" (IMIDRA, Madrid) and "El Rancho de la Merced" (IFAPA, Jerez de la Frontera), under the designation "Verués de Huarte", which has allowed the unequivocal identification of the accessions found.

The variety Berués has changed its varietal designation throughout the centuries, the synonyms Barbés, Barvés, Verués and Berués being the most frequent ones (Cibriáin-Sabalza et al., 2016). The cultivation of this variety was completely discontinued, and its name is not even recognized by most of the population nowadays. The historical information describing the agronomical-oenological features of Berués is very scarce, but centuries ago was preferred over other traditional varieties. It was reported to be ripen around two weeks earlier than Garnacha and Mazuelo (syn. Carignan), and described as sweet, delicate, with fragile skins and canes (Cibriaín Sabalza et al., 2016), citing (Valcárcel, 1767). According to historical bibliography, it is also known that Berués was recognized as a high-quality variety used in the production of wines and sparkling wines (Valcárcel, 1767).

Not much is known about the genetic origin of the Berués variety, except that is the result of a cross including Savagnin blanc (syn. Traminer Weiss) × an unknown cultivar. Several studies reported the major role of Savagnin blanc as a recurrent parent of many commercially important varieties such as, among many others, Pinot noir, Sauvignon blanc and Chenin blanc (Lacombe et al., 2013; Myles et al., 2011). Its role as a main founder variety is clearly linked to the fact that it is a very old variety. Genetic analyses of archaeological seeds found in France suggest

that Savagnin blanc or its direct relatives have been cultivated in France since the 1st century, as having a parent-offspring relationship with Savagnin blanc (Ramos-Madrigal et al., 2019) . In the same study, it could be confirmed that one seed dated to ~1100 CE found in Orléans was a genetic match to Savagnin blanc, providing evidence for at least 900 years of uninterrupted vegetative propagation. It seems that this variety could have played a relevant role in increasing the grapevine variability of Nortwestern Spain, as Savagnin blanc has been confirmed as a parent of emblematic varieties originated in this area, including Berués, but also Carrasquín, Maturana blanca, Parduca, Prieto picudo or Verdejo (Ramos-Madrigal et al., 2019). The geographical proximity and the existence of ancient commercial and pilgrim' routes between Spain and France could have favored the exchange of cultivars between regions from both countries since ancient times (Vidal et al., 1999).

Concluding remarks

Prospecting missions which take place in areas were viticulture is nowadays decadent usually rely on the information provided either by locals and owners of old vineyards, or from written records (Balda et al., 2014; D'Onofrio et al., 2016; Maraš et al., 2020; Santiago et al., 2008). Besides, historical cartography definitely offers a broad and reliable source of land use information, which has been deeply explored in some fields of study as, for instance, in land-use evolution studies. However, in the scope of viticulture, the use of cartographic information has been very scarce, mainly restricted to viticulture zoning and *terroirs* demarcation (Bois et al., 2008; Martínez and Gómez-Miguel, 2017; Vaudour and Shaw, 2005). To our knowledge, this is the first time that this type of information has been used to delineate potential areas of interest in terms of recovery actions. In this context, it is worth emphasizing the importance of public initiatives that cope cartography digitalization as open-source data.

Here it is presented a novel method for the identification of old vine plants based on historical cartographic information. By means of this open-source tools, the identification of locations where vineyards were cultivated in the past becomes possible, easing both the selection of ancient germplasm and its dating. Based on this approach, we were able to recover the Berués

variety in the peri-urban area of Pamplona, a very old variety considered to be disappeared. Minor and neglected varieties recovered via prospections or from germplasm repositories gained reputation in the last decade. Wines from these varieties could be unique and hence niche products and provide a high added value. An in-depth agronomical-oenological study of Berués has been initiated, aiming to increase the knowledge on its potentiality in diversifying the market of this major geographical region.

Funding

This research received funding from the Department of Economic Development of the Navarre Government: Bono Tecnológico-2017 and the project BERUÉS (0011-1365-2019-000074).

Acknowledgments

The authors would like to thank Bodegas Otazu (http://www.otazu.com/), especially to José Luis Ruiz (alma mater of this project, and tragically passed away) and to Aitziber Larrea, as well as José Félix Cibriáin for his insightful comments on the relevance of Berués in the past.

Author contributions

SCM: Data curation, formal analysis, writing – original draft, writing – review & editing

BM: Conceptualization, data curation, formal analysis, methodology, writing – original draft, writing – review & editing

OO: Data curation, formal analysis, writing – review & editing

ML: Data acquisition, writing – review & editing

AV: Data acquisition, writing – review & editing

DM: Data acquisition, writing – review & editing

CM: Funding acquisition, project administration, writing – review & editing

LGS: Conceptualization, funding acquisition, conceptualization, methodology, supervision Writing – review & editing

JU: Conceptualization, formal analysis, writing – original draft, writing – review & editing

References

André, M.-F., 1998. Depopulation, land-use change and landscape transformation in the French Massif Central. Ambio 27, 351–353.

Astibia-Ayerra, H., 1992. Consideraciones en torno a un vino olvidado: el chacolí de Navarra. Cuad. Etnol. y Etnogr. Navarra 24, 39–54.

Azcárate-Luxán, I., 1996. Plagas agrícolas y forestales en España en los siglos XVIII y XIX. Ministerio de Agricultura, Pesca y Alimentación, Secretaría General Técnica.

Baines, D., 1995. Emigration from Europe 1815-1930. Cambridge University Press.

Balda, P., Ibáñez, J., Sancha, J.C., de Toda, F.M., 2014. Characterization and identification of minority red grape varieties recovered in Rioja, Spain. Am. J. Enol. Vitic. 65, 148–152.

Bois, B., Van Leeuwen, C., Pieri, P., Gaudillère, J.-P., Saur, E., Joly, D., Wald, L., Grimal, D., 2008. Viticultural agroclimatic cartography and zoning at mesoscale level using terrain information, remotely sensed data and weather station measurements. Case study of Bordeaux winegrowing area.

Cabello, F., Ortiz, J.M., Muñoz, G., Rodríguez, I., Benito, A., Rubio, C., García, S., Sáiz, R., 2011. Variedades de vid en España. Madrid, Comunidad Madrid Editor. Agrícola.

Cibriáin-Sabalza, J.F., Sagüés-Sarasa, A., Caminero-Lobera, L., Abad-Zamora, F.J., Rodríguez-Lorenzo, M., Múgica-Azpilicueta, L., Suberviola-Ripa, J., Giménez-Soro, R., Gogorcena-Aoiz, Y., 2016. Cepas Singulares de Navarra. Referencias históricas.

D'Onofrio, C., Fausto, C., Matarese, F., Materazzi, A., Scalabrelli, G., Fiorani, F., Poli, I., 2016. Genotyping of Grapevine Varieties from Garfagnana (Northern Tuscany): Evidence of a Historical Center of Diversity. Am. J. Enol. Vitic. 67, 120–126.

De Lemps, A.H., 1967. Vignobles et vins du Nord-Ouest de l'Espagne... Bellenef.

Douglas, T., Critchley, D., Park, G., 1996. The deintensification of terraced agricultural land near Trevelez, Sierra Nevada, Spain. Glob. Ecol. Biogeogr. Lett. 258–270. https://doi.org/https://doi.org/10.2307/2997794

Douglas, T.D., Kirkby, S.J., Critchley, R.W., Park, G.J., 1994. Agricultural terrace abandonment in the Alpujarra, Andalucia, Spain. L. Degrad. Dev. 5, 281–291. https://doi.org/https://doi.org/10.1002/ldr.3400050405

Franco-Jubete, F., 2007. Agricultura y despoblación rural en Palencia. Palencia, Publicaciones Inst. Tello Téllez Men. 78.

Gago, P., Santiago, J.-L., Boso, S., Alonso-Villaverde, V., Orriols, I., del Carmen Martínez, M.,

2011. Identity of three grapevine varieties from a rediscovered viticulture region in northwest Spain. OENO One 45, 245–254.

García De Los Salmones, N., 1914. Memoria general de las sesiones del congreso y ponencias presentadas. Imprenta Prov. Pamplona.

Gobierno de Navarra, n.d. Sistema de Información Territorial de Navarra (SITNA) [WWW Document]. 1956-2012. URL http://idena.navarra.es

Huetz de Lemps, A., 1993. Vignobles et vins d'Espagne. Bordeaux, Press. Univ. Bordeaux, coll.«Grappes millésimes.

Ibáñez, J., Vélez, M.D., de Andrés, M.T., Borrego, J., 2009. Molecular markers for establishing distinctness in vegetatively propagated crops: a case study in grapevine. Theor. Appl. Genet. 119, 1213.

Instituto Tecnológico Agrario de Castilla y León (ITACyL), n.d. Cartodroid.

Jiménez-Cantizano, A., 2014. Caracterización molecular del banco de germoplasma de vid del Rancho de la Merced.

Jiménez-Cantizano, A., Muñoz-Martín, A., Amores-Arrocha, A., Sancho-Galán, P., Palacios, V., 2020. Identification of Red Grapevine Cultivars (Vitis vinifera L.) Preserved in Ancient Vineyards in Axarquia (Andalusia, Spain). Plants 9, 1572.

Jung, A., Maul, E., 2004. Preservation of grapevine genetic resources in Germany, based on new findings in old historical vineyards. Bull. l'OIV-Office Int. la Vigne du Vin 77, 615–630.

Labagnara, T., Bergamini, C., Caputo, A.R., Cirigliano, P., 2018. Vitis vinifera L. germplasm diversity: a genetic and ampelometric study in ancient vineyards in the South of Basilicata region (Italy). Vitis 57, 1–8.

Labianca, M., Valverde, F.N., 2019. 8. Depopulation and aging in rural areas in the European Union: practices starting from the LEADER approach. Despoblación y Transform. sociodemográficas los Territ. Rural. los casos España, Ital. y Fr. 223.

Lacombe, T., Boursiquot, J.-M., Laucou, V., Di Vecchi-Staraz, M., Péros, J.-P., This, P., 2013. Large-scale parentage analysis in an extended set of grapevine cultivars (Vitis vinifera L.). Theor. Appl. Genet. 126, 401–414.

Lasanta, T., Arnáez, J., Pascual, N., Ruiz-Flaño, P., Errea, M.P., Lana-Renault, N., 2017. Space–time process and drivers of land abandonment in Europe. Catena 149, 810–823.

Legouy, F., 2014. La géohistoire de l'espace viticole français sur deux siècles (1808-2010): plusieurs cycles viticoles décryptés. Espac. [En ligne].

Li, Y., Westlund, H., Liu, Y., 2019. Why some rural areas decline while some others not: An overview of rural evolution in the world. J. Rural Stud. 68, 135–143.

Maigre, D., Brugger, J.-J., Gugerli, P., Pont, M., 1998. Safeguarding, conserving and valorizing the genetic diversity in Valais (Switzerland), in: VII International Symposium on Grapevine Genetics and Breeding 528. pp. 59–66.

Manso de Zúñiga, V.C., 1905. Memoria anual. Estación Enológica de Haro.

Maraš, V., Tello, J., Gazivoda, A., Mugoša, M., Perišić, M., Raičević, J., Štajner, N., Ocete, R., Božović, V., Popović, T., 2020. Population genetic analysis in old Montenegrin vineyards reveals ancient ways currently active to generate diversity in Vitis vinifera. Sci. Rep. 10, 1–13.

Martínez, Á., Gómez-Miguel, V.D., 2017. Vegetation index cartography as a methodology complement to terroir zoning for use in precision viticulture. Oeno One 51, 289–301. https://doi.org/10.20870/oeno-one.2017.51.4.1589

Maul, E., Sudharma, K.N., Ganesh, A., Hundemer, M., Kecke, S., Marx, G., Schreiber, T., Walk, M., vom Weg, S., Mahler-Ries, A., 2014. 30 Years VIVC-Vitis International Variety Catalogue (www. vivc. de).

Maul, E., Sudharma, K.N., Kecke, S., Marx, G., Müller, C., Audeguin, L., Boselli, M., Boursiquot, J.M., Bucchetti, B., Cabello, F., 2012. The European Vitis Database (www. eu-vitis. de): A technical innovation through an online uploading and interactive modification system.

Mena, A., 2013. Recuperación, caracterización y conservación de variedades de vid (Vitis vinifera L.) minoritarias de Castilla-la Mancha. Universidad de Castilla-la Mancha, Facultad de Ciencias Ambientales y Bioquimica, Departamento de Ciencias Ambientales, Toledo.

Mena, A., Martínez, J., Fernández-González, M., 2014. Recovery, identification and relationships by microsatellite analysis of ancient grapevine cultivars from Castilla-La Mancha: the largest wine growing region in the world. Genet. Resour. Crop Evol. 61, 625–637.

Moreno-Sanz, P., Loureiro, M.D., Suárez, B., 2011. Microsatellite characterization of grapevine (Vitis vinifera L.) genetic diversity in Asturias (Northern Spain). Sci. Hortic. (Amsterdam). 129, 433–440.

Myles, S., Boyko, A.R., Owens, C.L., Brown, P.J., Grassi, F., Aradhya, M.K., Prins, B., Reynolds, A., Chia, J.-M., Ware, D., 2011. Genetic structure and domestication history of the grape. Proc. Natl. Acad. Sci. 108, 3530–3535.

Peres, S., 2009. La résistance des espaces viticoles à l'extension urbaine Le cas du vignoble de Bordeaux. Rev. dEconomie Reg. Urbaine 155–177.

Perrin, C., Jarrige, F., Soulard, C.-T., 2013. L'espace et le temps des liens ville-agriculture: une présentation systémique du cas de Montpellier et sa région. Cah. Agric. 22, 552–558.

Pinilla, V., Sáez, L.A., 2017. La despoblación rural en España: génesis de un problema y políticas innovadoras. Inf. CEDDAR 2.

Piqueras-Haba, J., 2010. El oidium en España: la primera gran plaga americana del viñedo. Difusión y consecuencias 1850-1870. Scr. Nova. Rev. Electrónica Geogr. y Ciencias Soc. 14.

QGIS Development Team, 2009. QGIS Geographic Information System. Open Source Geospatial Foundation Project.

Ramos-Madrigal, J., Runge, A.K.W., Bouby, L., Lacombe, T., Castruita, J.A.S., Adam-Blondon, A.-F., Figueiral, I., Hallavant, C., Martínez-Zapater, J.M., Schaal, C., 2019. Palaeogenomic insights into the origins of French grapevine diversity. Nat. plants 5, 595–603.

Santiago, J.L., Boso, S., Gago, P., Alonso-Villaverde, V., Martínez, M.C., 2008. A contribution to the maintenance of grapevine diversity: The rescue of Tinta Castañal (Vitis vinifera L.), a variety on the edge of extinction. Sci. Hortic. (Amsterdam). 116, 199–204.

Steevenson, L.J., 2004. business of the institute: A Study of the Revival of Priorat and its Contemporary Challenges. J. Wine Res. 15, 65–84.

Urrestarazu, J., Miranda, C., Santesteban, L.G., Royo, J.B., 2015. Recovery and identification of grapevine varieties cultivated in old vineyards from Navarre (Northeastern Spain). Sci. Hortic. (Amsterdam). 191, 65–73.

Valcárcel, J.A., 1767. Agricultura general y gobierno de la casa de campo... en que por estenso se trata de todos lo bienes del campo: con los nuevos descubrimentos y metodos de cultivo para la multiplicacion de los granos, del aumento en la cria de ganados y en lo demas depend. por Joseph Estevan Donz.

Vaudour, E., Shaw, A.B., 2005. A worldwide perspective on viticultural zoning. South African J. Enol. Vitic. 26, 106–115.

Vidal, J.R., Coarer, M., Defontaine, A., 1999. Genetic relationships among grapevine varieties grown in different French and Spanish regions based on RAPD markers. Euphytica 109, 161–172.

Yuste, A., Martínez-Gil, F., García-Martínez, S., López-Lluch, D., Peiró Barber, R.M., Jiménez,

C., Ruiz, J., Gisbert Domenech, M.C., 2019. Recovering ancient grapevine cultivars in the Spanish provinces of Alicante and Valencia. Acta Hortic. 43–46.

Authors: Sara Crespo-Martínez*[1], Blanca Mayor*[1], Oihane Oneka[1], Maite Loidi[1], Ana Villa[1], Diana Marín[1, 2], Carlos Miranda[1, 2], Luis G. Santesteban[1, 2], Jorge Urrestarazu[c, 1, 2]

Recovery of ancient grapevine plant material in peri-urban areas. A case of success in Pamplona (Spain) leading to the recovery of cv. Berués

[1]Department of Agronomy, Biotechnology and Food Science, Public University of Navarre (UPNA), Campus Arrosadia 31006, Pamplona, Spain

[2]Institute for Multidisciplinary Research in Applied Biology (IMAB-UPNA), Public University of Navarre, Campus Arrosadia 31006 Pamplona, Spain

*These authors contributed equally to this work

Sara Crespo-Martínez: sara.crespo@unavarra.es
Blanca Mayor: blanca.mayor@unavarra.es
Oihane Oneka: oihane.oneka@unavarra.es
Maite Loidi: maite.loidi@unavarra.es
Ana Villa: ana.villa@unavarra.es
Diana Marín: diana.marin@unavarra.es
Carlos Miranda: carlos.miranda@unavarra.es
Luis G. Santesteban: gonzaga.santesteban@unavarra.es
Jorge Urrestarazu: Corresponding author (c) jorge.urrestarazu@unavarra.es

Abstract

Viticulture was relatively important in the peri-urban area of Pamplona till the end of the 19th century, but suffered a continued regression that has led to a nearly complete disappearance of vineyards. In this context, this work aims to evaluate the feasibility of recovering old grapevine germplasm in the peri-urban area of Pamplona. The lack of a conventional source for recovering plant material (i.e. absence of old vineyards in the area) implied the need of designing an alternative prospecting procedure. This method included the analysis of the available historical information as open-access resources (orthophotos, land use maps and GIS applications) aiming to identify the areas with the highest probability of finding vines surviving from the general abandonment and uprooting of vineyards that had occurred in the 20th century. Based on the oldest

on scale orthophoto available for Navarra, a vineyard land map of the peri-urban area of Pamplona in year 1956 was built, allowing prospecting efforts to be focused on specific areas of primarily interest. Following this strategy, a total of 120 plants were collected, which corresponded to 44 genotypes. The most prominent achievement of this prospecting mission was the recovery of 15 accessions of Berués, a very old variety with a remarkable importance in the region according to old historical records, and considered to be disappeared. The methodology proposed was effective in searching for the oldest standing-alone plants surviving in the peri-urban area of Pamplona, and may be adapted to assist the recovery of old grapevine germplasm in other currently non wine-growing regions/areas where viticulture was relevant some decades ago.

Keywords: *Vitis vinifera* L.; old varieties; varietal diversity; cartographic information; prospecting work; molecular markers

Introduction

In the last ~~three~~ decades, a remarkable effort has been done in order to recover grapevine varieties whose cultivation had become marginal or that survived as single plants in multi-cultivar old vineyards. As a result of this effort, a significant number of nearly forgotten/neglected varieties have been recovered in most countries in southern Europe, as highlighted in the project "On-farm inventory of minor grape varieties in the European *Vitis* Database (GrapeOnFarm)" ~~developed by the *Vitis* group of the European Cooperative Programme for Plant Genetic Resources (ECPGR)~~. As a general rule, prospecting missions initially focused on the oldest vineyards in well-known grape growing regions (Jung and Maul, 2004; Labagnara et al., 2018; Maigre et al., 1998; Mena et al., 2014; Urrestarazu et al., 2015; Yuste et al., 2019), and, only more recently, missions have been shifted towards areas where grape growing survived as a secondary or even marginal activity (Gago et al., 2011; Jiménez-Cantizano et al., 2020; Moreno-Sanz et al., 2011). ~~The potential of these areas to harbour diverse grapevine germplasm is clear, since the decaying economical context of their viticulture, or even of the region itself, prevented modernisation processes involving the introduction of other varieties.~~

There are mainly three reasons that, individually or in combination, have led the viticulture of some regions in Europe to be marginal or even practically abandoned in the last 150 years: (i) increased disease pressure, (ii) rural depopulation, and (iii) industrialization and urbanisation pressure. ~~Competition with other crops due to changes in their profitability, such it has been the case in several Mediterranean areas between olive and grapes, usually reverts when market gets stabilized, and does not lead to vineyard abandonment.~~ In the next paragraphs, the three aforementioned reasons for marginalization or abandonment of grape growing are exemplified. For brevity's sake, some cases in France and Spain are presented, though examples can probably be found throughout all the traditional grape growing countries.

(i) Increased disease pressure: the arrival to Europe of powdery and downy mildew implied an increase in the cost of growing grapes due to the necessity of including copper and sulphur treatments. In the cooler and rainier areas, where disease control

is more difficult, diseases led to frequent crop losses and to an increase in cost that could not be easily assumed by growers (Azcárate-Luxán, 1996), causing a gradual abandonment of vineyards. This was the case of Rias Altas' vineyards in Spain (De Lemps, 1967), where grape growing became unsustainable, while in the neighbouring Rias Baixas, with milder climate, vineyards thrived through the crisis. A similar process occurred in most grape growing area in Asturias, Cantabria, Bizkaia and Gipuzkoa (Piqueras-Haba, 2010). In France, this was also the case for vineyards in several departments in the Northwest, such as l'Ille-et-Vilaine, la Mayenne, l'Eure, l'Oise and Ardenne (Legouy, 2014), where vineyards nearly disappeared in the late 19th century.

(ii) Rural depopulation: the loss of population in rural areas is a complex and long-term challenge worldwide (Labianca and Valverde, 2019; Li et al., 2019). In the last 150 years, there have been several enchained events that have gradually led to severe degrees of depopulation of some grape growing regions in Europe, with peasant population moving abroad, majorly to South and North America, to other European countries, or to urban areas within the origin country (Baines, 1995; Lasanta et al., 2017; Pinilla and Sáez, 2017), affecting more severely to semi-arid and mountain-areas (Lasanta et al., 2017). Depopulated grape growing areas in Spain can be found throughout the whole country, some examples being reported in detail for areas in Castilla y León (Franco-Jubete, 2007), Andalusia (Douglas et al., 1996; Douglas et al., 1994), and even in the now well-known Priorat area in Catalonia (Steevenson, 2004). In France, depopulation can be behind the regression of viticulture in central areas such as the Massif Central and neighbouring southern Departments (André, 1998; Legouy, 2014).

(iii) Industrialisation and urbanisation pressure: grape growers in peri-urban areas of growing cities must face an additional source of pressure associated to city expansion. Although in some cases, such as Bordeaux area, where certain resistance due to the

higher price value of the wine can be observed (Peres, 2009), the growth of cities usually leads to the disappearance of peri-urban vineyards. The epitomic case is that of Montpellier, where, from the 1960s, the city turned its back on its viticulture (Perrin et al., 2013), although now some efforts are being done to revert this trend.

The aim of this work was to evaluate the feasibility of recovering grapevine plant material of grapevine varieties in the peri-urban area of Pamplona, a medium-size town in northern Spain, where viticulture was relevant until the end of the 19th century, but suffered a continued regression that led to near complete disappearance of vineyards (Astibia-Ayerra, 1992). In this area, the reason for vineyard abandonment was a combination of cases (i) and (iii). Pamplona's climate can be classified as a Huglin type I region, with relatively high pressure for fungal diseases, where the population has increased from 35,000 inhabitants in 1900 up to > 350.000 inhabitants now. The current presence of vineyards in Navarra is nowadays restricted to some vineyards in the southern part of the region, whereas in the mid and northern parts, where the city of Pamplona is located, only approximately 5 very small (<0.3 ha) family-owned vineyards remain, altogether with one commercial winery established in the 1980s to produce international profile wines.

The differential aspect of our approach is that, to our knowledge, this is the first work aimed at recovering grapevine diversity in a non-viticultural area, which necessarily had to be performed based on rescuing individual plants surviving at field margins or at non-cultivated pieces of land. This is a very particular context, but that can probably be found at other regions in Europe, which requires prospection to be based on the analysis of the available historical information as open-access resources (orthophotos, land use maps and GIS applications).

Materials and methods

This study was carried out in four well differentiated phases: (i) analysis of the available historical information, (ii) prospecting works and collection of grapevine samples, (iii) classification of the recovered plants in different categories, dating its time of origin based on the last record of the presence of vineyards in the areas where they were found and, (iv) genetic characterization for

varietal identification.

Analysis of the available historical information

With the aim of identifying the wine-growing zones of the peri-urban area of Pamplona in the past, the layers “Límites catastrales de los municipios de Navarra actuales” ("Cadastral limits of the current municipalities of Navarre") and “Mapa de Cultivos y Aprovechamientos de 1956” (“Agricultural soil and land use of 1956”), usually denoted as “MCA56”, were used; both layers are available on the website of the Spatial Data Infrastructure of Navarra (http://idena.navarra.es) (Gobierno de Navarra, n.d.). The layer "MCA56" provides a photointerpretation of soil and land uses that was implemented based on a series of orthophotos taken all around the Spanish territory by the United States Army in the years 1956-1957, being the oldest on scale orthophoto available for Navarra (1:50,000). This open-access resource includes a filter of 27 categories of crops and land uses, among them “vineyard”. To prepare the final map, the one that allows to spatially identify the existing wine-growing area of the peri-urban area of Pamplona in 1956, the two above mentioned layers (i.e., “Cadastral limits of the current municipalities of Navarre” and “MCA56”) were added to the QGIS software (QGIS Development Team, 2009). Thereafter, the layer "MCA56" was applied by selecting the option that allows representing exclusively on the map the existing wine-growing zones for the peri-urban area of Pamplona in 1956, while the layer " Cadastral limits of the current municipalities of Navarre " was used to delimit the searching area for municipalities surrounding Pamplona (i.e. its peri-urban area).

Prospecting work and collection of grapevine samples

When visiting the areas of potential interest, an initial visual exploration was conducted with special attention to patches as borders between plots and paths, ditches and streams or uncultivated lands, due to the higher probability of finding surviving grapevine plants. When a surviving plant was found, an identification code was assigned, a sample of 2-3 leaves of each plant was collected for the genetic analyses and their main ampelographic characteristics were described. Moreover, the plants were geolocated using the offline application CartoDroid (Instituto Tecnológico Agrario de Castilla y León - ITACyL) that allows exporting directly the

vector layer of the location points of each plant to the QGIS software (QGIS Development Team, 2009).

Classification of the recovered plants in different categories

All the plants collected in the Pamplona peri-urban area were classified into four categories according to the lapse of time since the last record of vineyards in the specific location where each plant was found could be confirmed. For doing that, open access orthophotos of the years 1956-1957, 1966-1971 and 2012 were used. The four categories proposed refer to:

- Category 1: Plants in probable areas of "old" vineyards as the last evidence of vineyards is reported in the orthophoto of 1956-1957.
- Category 2: Plants in probable areas of "non-modern" vineyards as the last evidence of vineyards is reported in the orthophoto of 1966-1971.
- Category 3: Plants in probable areas with a risk of "modern" presence of vineyards as the last evidence of vineyards is reported in the orthophoto of 2012.
- Category 4: Plants in riverside areas, unlikely to be a wine-growing area in the past.

Genetic characterization for varietal identification of the recovered plants

Young leaves of each collected plant were ground to a fine powder in a microdismembrator (B. Braun Biotech International, Melsungen, Germany). Genomic DNA was isolated from 100 mg of this fine powder with Qiagen Dneasy Plant Mini kit (Qiagen, Hilden, Germany) according to the manufacturer´s instructions. DNA concentration of each sample was determined using a NanoDrop 2000 (Thermo Fischer Scientific, Wilmington, DE, USA), and DNA working dilutions of each sample were adjusted to 5 ng/µl.

A set of 25 SSR markers, distributed on the 19 grapevine linkage groups, were studied in three independent multiplex PCRs denoted as S, A and B, including each, respectively, 9, 6 and 10 markers. Amplification reactions were carried out in a 2720 thermal cycler (Applied Biosystems, Foster City, CA, USA), following the protocols described by Ibáñez et al., (2009), with several

modifications for A and B multiplex PCRs as detailed in Urrestarazu et al., (2015). The separation of fragments was carried out in an ABI PRISM 3730 sequencer (Applied Biosystems, Foster City, CA, USA), using 500-LIZ as internal marker size and Peak Scanner Software version 1.0 (Applied Biosystems, Foster City, CA, USA) to size the fragments.

The SSR molecular profiles found were compared to the set of unique profiles from the *Vitis* International Variety Catalogue (www.vivc.de) (Maul et al., 2014) and to those in the European *Vitis* Database (www.eu-vitis.de/index.php) (Maul et al., 2012). Both databases include harmonized genetic characterization data from thousands of grapevine accessions with nine SSR markers, all of them used in this study. When SSR profiles obtained matched the profile reported for a specific variety in any of the two databases, their SSR profiles were also compared for the rest of the markers analysed with the published data obtained for these varieties in other studies (Jiménez-Cantizano, 2014; Mena, 2013; Mena et al., 2014).

Results and discussion

Historical map construction as the basis for prospecting works

Prospecting works of the present study were based on the constructed 1956 vineyard land map (see Material and methods section for details). According to the constructed vineyard map for the year 1956 (Figure 1A), 599 ha of wine-growing surface could be quantified, while the one corresponding to 2012 (Figure 1B) is nearly inexistent (i.e. 122 ha), associated mainly to the acreage of one single winery established in the 1980s to produce international profile wines. When comparing the maps of the years 1956 and 2012, abandoned vineyard lands in the peri-urban area of Pamplona since the second half of the 20th century became evident.

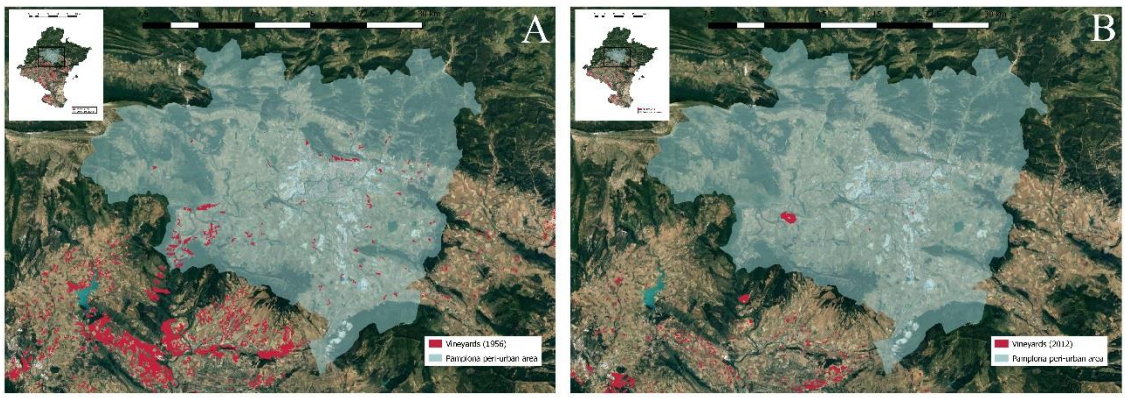

Figure 1. Map showing vineyard area in the peri-urban area of Pamplona in A) 1956 and B) 2012. Vineyard abandonment is clearly observed, the only relevant acreage in 2012 corresponding to the fields of a winery established in the 1980s to produce international profile wines.

The availability of the constructed 1956 vineyard land map of the peri-urban area of Pamplona enabled the identification of areas with potential interest as "old variety reservoirs", and consequently, allowed to focus the prospecting efforts on these specific areas in order to increase the chance of success at finding old vine plants. Based on this strategy, a total of 120 plants (hereafter referred to as accessions) were found in the peri-urban area of Pamplona (Figure 2). As it can be observed in Figure 1A, the location of most of the blue dots, representing the collected plants, happen to meet with the historical confirmed vineyard land that later were abandoned (highlighted in burgundy in the map).

Prospecting works have been traditionally carried out on still existing old vineyards. The criteria for selecting plants have been mainly based on field observations. Contrary, prospecting works on areas where grapevine has not been grown regularly for decades, as is the case of the peri-urban area of Pamplona, have a lack of on-site information. The lack of *in situ* information implied the need of implementing non-conventional methods. In this context, a methodology based on the overlay of historical cartography information onto a digital map was designed *ad hoc* for this specific case study.

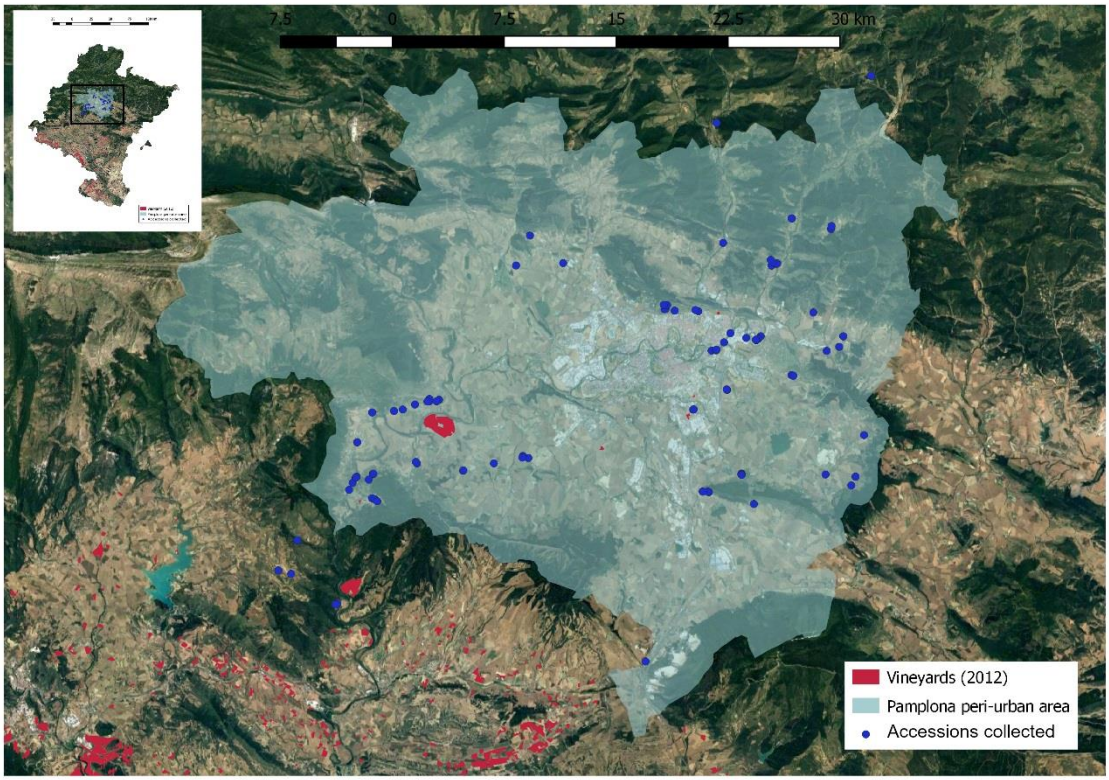

Figure 2. Satellite-based map of the peri-urban area of Pamplona. The current (2012) vineyards are marked in red, whereas Blue dots represent the location of the accessions included in this work, which location happen to meet with the historical confirmed vineyard land that later were abandoned.

Dating of plant material

After prospecting works were completed, cartographic information was further used to date each recovered plant, considering for that the plant locations and the last records of viticulture activity for each specific georeferenced position. The process followed is described in Figure 3. As observed, cartographic information from 1966-1971, 1998-2000 and 2012 of all referenced points were investigated to ascertain the presence of vineyards and to define the time-origin of each accession. The three blue dots marked by arrows in Figure 3, representing three of the accessions collected in this study, were located within plots that, according to the 1956 vineyard map, were confirmed as vineyards in that time. For the georeferenced point where the first accession was collected, the one classified in Category 1 ("old" vineyards, being dated before 1956), no presence of vineyards was observed on it after 1956. By contrast, for the georeferenced points where the

other two accessions were found, the ones corresponding to Category 2 ("non-modern" vineyards, being dated between 1966 and 1971) and Category 3 (presence of "modern" vineyards in 2012), presence of vineyards can be observed after 1956, between 1966-1971 and from 1988 to 2012, respectively.

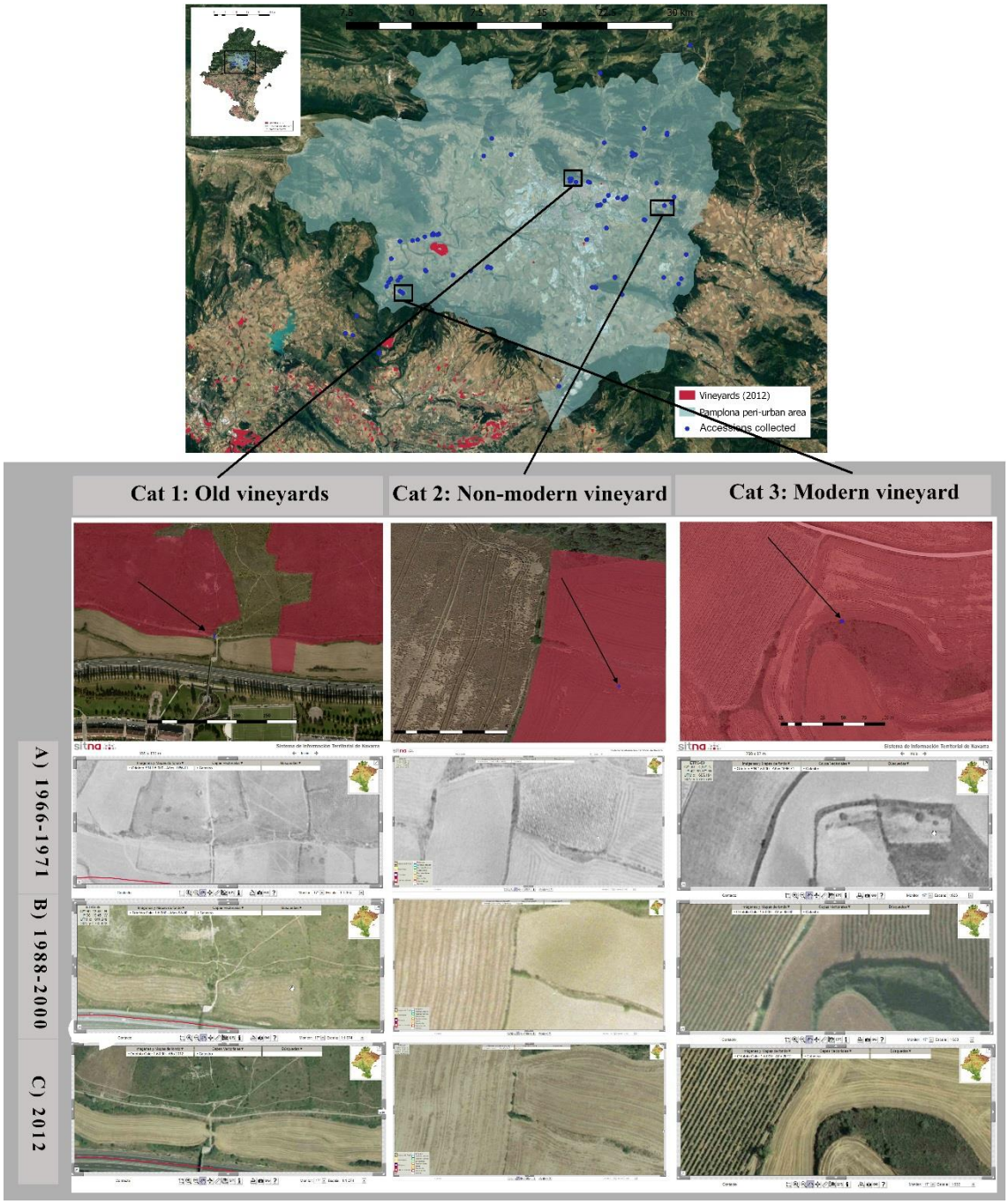

Figure 3. Maps used in the process for plant material dating. An example for the three different time points (Category 1: old vineyard, Category 2: Non-modern vineyard and Category 3: Modern

242 vineyard) are displayed. Grapevine cultivation in every referenced accession (marked with
243 arrows) was checked through cartographic pictures on A) 1966-71, B) 1998-2000 and C) 2012.

244

245 The classification of the 120 accessions found according to their location is presented in Table 1.
246 The majority of the accessions (76 out of 120) corresponded to Category 1 ("old" vineyards, being
247 dated before 1956), followed by those belonging to Category 2 ("non-modern" vineyards, being
248 dated between 1966 and 1971), 28 accessions. Only 2 accessions belonged to Category 3
249 (presence of "modern" vineyards in 2012), whereas the remaining 14 were included in Category
250 4 ("riverside areas"). This later class comprises accessions found in places unlikely to be a wine-
251 growing area in the past, and could be considered as feral germplasm.

252

253 **Table 1**. Classification of the collected plants according to their origin. "Category 1: old
254 vineyards", "Category 2: non-modern vineyards", "Category 3: modern presence of vineyards"
255 and "Category 4: Plants in riverside areas". Cartographic information from 1966-1971, 1998-
256 2000 and 2012 of all referenced points were investigated to ascertain the presence of vineyards
257 and to define the time-origin of each accession.

Category	Date	Number of accessions	Percentage
1	1956 or before	75	62.50%
2	1966-1971	29	24.16%
3	1971-2012	2	1.67%
4	River sides	14	11.67%

258

259 The dating plant material approach based on georeferenced historical cartography has proven to
260 be useful to classify the recovered plants into four time periods extending over the 20th century.
261 Dating of plant material was useful to classify the collected plants within a specific period of time,

but also allowed us to validate the effectiveness of the methodology designed *ad hoc* for this case study. Nearly 63% of the plants collected were assigned to Category 1, thus revealing that the proposed methodology allows both the finding and recovery of the oldest "still existing" standing-alone plants in the peri-urban area of Pamplona.

Varietal identification of the recovered plants using molecular markers

Within the 120 accessions collected in the peri-urban area of Pamplona, a total of 44 genotypes were obtained. Comparisons between the SSR profiles obtained to those found in international databases (the *Vitis* International Variety Catalogue and the European *Vitis* Database) have allowed the identification of 17 cultivars (Table 2).

Combining dating and varietal identification of the accessions found in the peri-urban area of Pamplona allowed to delineate an approximate idea of the varietal composition of the vineyards existing in 1956 in this area (Fig. 4). Among the oldest accessions (those comprised in Category 1), the most prevalent variety before 1956 in the "Pamplona Basin" was Garnacha (syn. Grenache) (33 accessions, 44% of those in Category 1), what is in agreement with the increasing popularity that this variety had from the mid-19th century in the region due to its lower susceptibility to powdery and downy mildew among the traditional varieties (Huetz de Lemps, 1993). The second most numerous variety is Berués (13 accessions, 17.33% of those included in Category 1), a traditional variety that is not cultivated nowadays. Additionally, within the remaining cultivars identified in Category 1, in four cases [Carignan (syn. Mazuelo), Tempranillo, Morate and Muscat à petit grains blancs (syn. Moscatel de Grano Menudo)] there is documentary evidence that they had at least some relevance in early 20th century in Navarre or in its neighbouring regions (Basque Country, La Rioja and Aragon) (Cabello et al., 2011; García De Los Salmones, 1914; Manso de Zúñiga, 1905). Concerning the other three cultivars identified in Category 1 (Castellana Blanca, Tinto de Navalcarnero and Muscat of Alexandria), the cultivation of the two former ones was documented for regions far from Navarre or bordering regions (García De Los Salmones, 1914), while the latter is a widespread table grape variety and, thus, its appearance is not surprising.

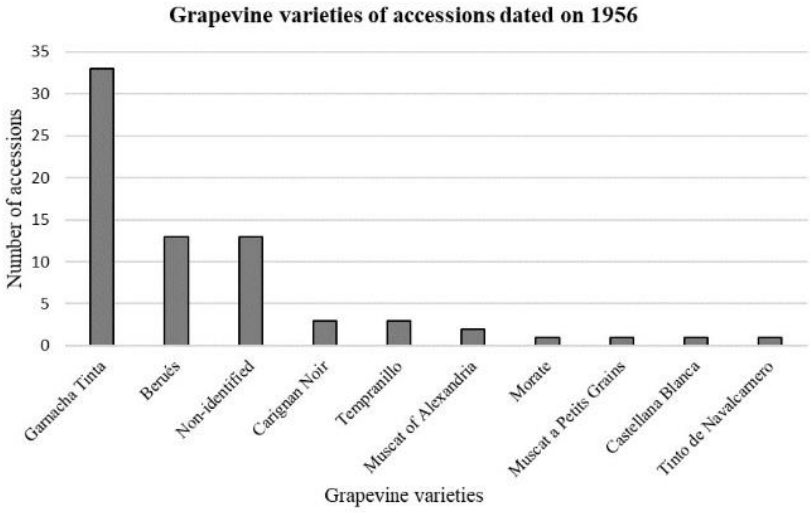

Figure 4. Varietal distribution of accessions classified as Category 1, dated before 1956. Number of accessions per variety is represented and varieties are ordered from more to less abundant.

Categories 2 and 3, the ones including, respectively, the plants collected in probable areas of "non-modern" and "modern" vineyards, grouped other eight cultivars: the Spanish cultivars Garnacha, Berués, Morate and Palomino Fino, the French cultivars Cabernet Sauvignon, Cabernet Franc and Cinsaut, and the interspecific hybrid (*Vitis aestivalis* × *Vitis vinifera*) Jacquez. The molecular profile of 28 accessions showed no coincidence with any of the genotypes included in the databases used for comparative purposes. Most of them are included in Category 4 (riverside areas), what reinforces the idea of their feral nature or their origin from unintentional cross-pollination.

According to the early work by García De Los Salmones (1914), a compilation of cultivars being grown at each Spanish region before the phylloxera outbreak, the cultivation of at least 27 different varieties was reported in the region of Navarre. Some initiatives have been carried out aiming to recover the varietal richness still existing in the oldest vineyards of the region in the last years (Cibriáin-Sabalza et al., 2016; Urrestarazu et al., 2015) resulting, as a whole, in more than 50 varieties identified, a significantly higher number than reported by García De Los Salmones (1914). In spite of the small agricultural surface covered by the prospecting mission

309 performed in the peri-urban area of Pamplona, it is worth pointing out the remarkable grapevine
310 variety reservoir that abandoned lands may still offer.
311

312 **Table 2**. List of varieties located in the prospecting works in the peri-urban area of Pamplona and classification of its accessions depending on date.

Prime name	Variety number VIVC	Number of accesions	Percentage	Category / Date			
				1. 1956 or before	2. 1966-1971	3. 1971-2012	4. River sides
Garnacha Tinta	4461	54	45.00%	33	21		
Berués de Huarte	1281	15	12.50%	13	2		
Carignan Noir	2098	3	2.50%	3			
Tempranillo Tinto	12350	3	2.50%	3			
Millardet et Grasset 41 B	7736	3	2.50%	2		1	
Muscat of Alexandria	8241	2	1.67%	2			
Morate	7981	2	1.67%	1	1		
Muscat a Petits Grains Blancs	8193	1	0.83%	1			
Castellana Blanca	26280	1	0.83%	1			
Tinto de Navalcarnero	26280	1	0.83%	1			
Rupestris du Lot	10389	1	0.83%	1			
Millardet et Grasset 420 A	7810	1	0.83%	1			
Cabernet Sauvignon	1929	1	0.83%		1		
Cabernet Franc	1927	1	0.83%		1		
Cinsaut	2672	1	0.83%		1		
Jacquez a Gros Grains[a]	5628	1	0.83%		1		
Palomino Fino	8888	1	0.83%		1		
Non-identified		28	23.33%	13		1	14
Total		120	99.97%	75	29	2	14

313 [a] Non true-to-type

Recovery of the variety Berués

Prospecting actions have been proved to be interesting not only to report the varietal richness at each specific region in the past, but also to recover emblematic cultivars preventing their loss. The major milestone underlying the prospecting mission of the peri-urban area of Pamplona has been the recovery of 15 accessions of Berués, a very old variety considered to be disappeared. The only plants of Berués preserved before this study was performed, where those collected probably in the same area at the beginning of 20th century by García de los Salmones (García De Los Salmones, 1914). This material is currently preserved at the grapevine collections of "El Encín" (IMIDRA, Madrid) and "El Rancho de la Merced" (IFAPA, Jerez de la Frontera), under the designation "Verués de Huarte", which has allowed the unequivocal identification of the accessions found.

The variety Berués has changed its varietal designation throughout the centuries, the synonyms Barbés, Barvés, Verués and Berués being the most frequent ones (Cibriáin-Sabalza et al., 2016). The cultivation of this variety was completely discontinued, and its name is not even recognized by most of the population nowadays. The historical information describing the agronomical-oenological features of Berués is very scarce, but centuries ago was preferred over other traditional varieties. It was reported to be ripen around two weeks earlier than Garnacha and Mazuelo (syn. Carignan), and described as sweet, delicate, with fragile skins and canes (Cibriaín Sabalza et al., 2016), citing (Valcárcel, 1767). According to historical bibliography, it is also known that Berués was recognized as a high-quality variety used in the production of wines and sparkling wines (Valcárcel, 1767).

Not much is known about the genetic origin of the Berués variety, except that is the result of a cross including Savagnin blanc (syn. Traminer Weiss) × an unknown cultivar. Several studies reported the major role of Savagnin blanc as a recurrent parent of many commercially important varieties such as, among many others, Pinot noir, Sauvignon blanc and Chenin blanc (Lacombe et al., 2013; Myles et al., 2011). Its role as a main founder variety is clearly linked to the fact that it is a very old variety. Genetic analyses of archaeological seeds found in France suggest that

Savagnin blanc or its direct relatives have been cultivated in France since the 1st century, as having a parent-offspring relationship with Savagnin blanc (Ramos-Madrigal et al., 2019) . In the same study, it could be confirmed that one seed dated to ~1100 CE found in Orléans was a genetic match to Savagnin blanc, providing evidence for at least 900 years of uninterrupted vegetative propagation. It seems that this variety could have played a relevant role in increasing the grapevine variability of Nortwestern Spain, as Savagnin blanc has been confirmed as a parent of emblematic varieties originated in this area, including Berués, but also Carrasquín, Maturana blanca, Parduca, Prieto picudo or Verdejo (Ramos-Madrigal et al., 2019). The geographical proximity and the existence of ancient commercial and pilgrim' routes between Spain and France could have favored the exchange of cultivars between regions from both countries since ancient times (Vidal et al., 1999).

Concluding remarks

Prospecting missions which take place in areas were viticulture is nowadays decadent usually rely on the information provided either by locals and owners of old vineyards, or from written records (Balda et al., 2014; D'Onofrio et al., 2016; Maraš et al., 2020; Santiago et al., 2008). Besides, historical cartography definitely offers a broad and reliable source of land use information, which has been deeply explored in some fields of study as, for instance, in land-use evolution studies. However, in the scope of viticulture, the use of cartographic information has been very scarce, mainly restricted to viticulture zoning and *terroirs* demarcation (Bois et al., 2008; Martínez and Gómez-Miguel, 2017; Vaudour and Shaw, 2005). To our knowledge, this is the first time that this type of information has been used to delineate potential areas of interest in terms of recovery actions. In this context, it is worth emphasizing the importance of public initiatives that cope cartography digitalization as open-source data.

Here it is presented a novel method for the identification of old vine plants based on historical cartographic information. By means of this open-source tools, the identification of locations where vineyards were cultivated in the past becomes possible, easing both the selection of ancient germplasm and its dating. Based on this approach, we were able to recover the Berués variety in

the peri-urban area of Pamplona, a very old variety considered to be disappeared. Minor and neglected varieties recovered via prospections or from germplasm repositories gained reputation in the last decade. Wines from these varieties could be unique and hence niche products and provide a high added value. An in-depth agronomical-oenological study of Berués has been initiated, aiming to increase the knowledge on its potentiality in diversifying the market of this major geographical region.

Funding

This research received funding from the Department of Economic Development of the Navarre Government: Bono Tecnológico-2017 and the project BERUÉS (0011-1365-2019-000074).

Acknowledgments

The authors would like to thank Bodegas Otazu (http://www.otazu.com/), especially to José Luis Ruiz (alma mater of this project, and tragically passed away) and to Aitziber Larrea, as well as José Félix Cibriáin for his insightful comments on the relevance of Berués in the past.

Author contributions

SCM: Data curation, formal analysis, writing – original draft, writing – review & editing

BM: Conceptualization, data curation, formal analysis, methodology, writing – original draft, writing – review & editing

OO: Data curation, formal analysis, writing – review & editing

ML: Data acquisition, writing – review & editing

AV: Data acquisition, writing – review & editing

DM: Data acquisition, writing – review & editing

CM: Funding acquisition, project administration, writing – review & editing

LGS: Conceptualization, funding acquisition, conceptualization, methodology, supervision Writing – review & editing

JU: Conceptualization, formal analysis, writing – original draft, writing – review & editing

393 **References**

394 André, M.-F., 1998. Depopulation, land-use change and landscape transformation in the French
395 Massif Central. Ambio 27, 351–353.

396 Astibia-Ayerra, H., 1992. Consideraciones en torno a un vino olvidado: el chacolí de Navarra.
397 Cuad. Etnol. y Etnogr. Navarra 24, 39–54.

398 Azcárate-Luxán, I., 1996. Plagas agrícolas y forestales en España en los siglos XVIII y XIX.
399 Ministerio de Agricultura, Pesca y Alimentación, Secretaría General Técnica.

400 Baines, D., 1995. Emigration from Europe 1815-1930. Cambridge University Press.

401 Balda, P., Ibáñez, J., Sancha, J.C., de Toda, F.M., 2014. Characterization and identification of
402 minority red grape varieties recovered in Rioja, Spain. Am. J. Enol. Vitic. 65, 148–152.

403 Bois, B., Van Leeuwen, C., Pieri, P., Gaudillère, J.-P., Saur, E., Joly, D., Wald, L., Grimal, D.,
404 2008. Viticultural agroclimatic cartography and zoning at mesoscale level using terrain
405 information, remotely sensed data and weather station measurements. Case study of
406 Bordeaux winegrowing area.

407 Cabello, F., Ortiz, J.M., Muñoz, G., Rodríguez, I., Benito, A., Rubio, C., García, S., Sáiz, R.,
408 2011. Variedades de vid en España. Madrid, Comunidad Madrid Editor. Agrícola.

409 Cibriáin-Sabalza, J.F., Sagüés-Sarasa, A., Caminero-Lobera, L., Abad-Zamora, F.J., Rodríguez-
410 Lorenzo, M., Múgica-Azpilicueta, L., Suberviola-Ripa, J., Giménez-Soro, R., Gogorcena-
411 Aoiz, Y., 2016. Cepas Singulares de Navarra. Referencias históricas.

412 D'Onofrio, C., Fausto, C., Matarese, F., Materazzi, A., Scalabrelli, G., Fiorani, F., Poli, I., 2016.
413 Genotyping of Grapevine Varieties from Garfagnana (Northern Tuscany): Evidence of a
414 Historical Center of Diversity. Am. J. Enol. Vitic. 67, 120–126.

415 De Lemps, A.H., 1967. Vignobles et vins du Nord-Ouest de l'Espagne... Bellenef.

416 Douglas, T., Critchley, D., Park, G., 1996. The deintensification of terraced agricultural land
417 near Trevelez, Sierra Nevada, Spain. Glob. Ecol. Biogeogr. Lett. 258–270.
418 https://doi.org/https://doi.org/10.2307/2997794

419 Douglas, T.D., Kirkby, S.J., Critchley, R.W., Park, G.J., 1994. Agricultural terrace
420 abandonment in the Alpujarra, Andalucia, Spain. L. Degrad. Dev. 5, 281–291.
421 https://doi.org/https://doi.org/10.1002/ldr.3400050405

422 Franco-Jubete, F., 2007. Agricultura y despoblación rural en Palencia. Palencia, Publicaciones
423 Inst. Tello Téllez Men. 78.

424 Gago, P., Santiago, J.-L., Boso, S., Alonso-Villaverde, V., Orriols, I., del Carmen Martínez, M.,

2011. Identity of three grapevine varieties from a rediscovered viticulture region in northwest Spain. OENO One 45, 245–254.

García De Los Salmones, N., 1914. Memoria general de las sesiones del congreso y ponencias presentadas. Imprenta Prov. Pamplona.

Gobierno de Navarra, n.d. Sistema de Información Territorial de Navarra (SITNA) [WWW Document]. 1956-2012. URL http://idena.navarra.es

Huetz de Lemps, A., 1993. Vignobles et vins d'Espagne. Bordeaux, Press. Univ. Bordeaux, coll.«Grappes millésimes.

Ibáñez, J., Vélez, M.D., de Andrés, M.T., Borrego, J., 2009. Molecular markers for establishing distinctness in vegetatively propagated crops: a case study in grapevine. Theor. Appl. Genet. 119, 1213.

Instituto Tecnológico Agrario de Castilla y León (ITACyL), n.d. Cartodroid.

Jiménez-Cantizano, A., 2014. Caracterización molecular del banco de germoplasma de vid del Rancho de la Merced.

Jiménez-Cantizano, A., Muñoz-Martín, A., Amores-Arrocha, A., Sancho-Galán, P., Palacios, V., 2020. Identification of Red Grapevine Cultivars (Vitis vinifera L.) Preserved in Ancient Vineyards in Axarquia (Andalusia, Spain). Plants 9, 1572.

Jung, A., Maul, E., 2004. Preservation of grapevine genetic resources in Germany, based on new findings in old historical vineyards. Bull. l'OIV-Office Int. la Vigne du Vin 77, 615–630.

Labagnara, T., Bergamini, C., Caputo, A.R., Cirigliano, P., 2018. Vitis vinifera L. germplasm diversity: a genetic and ampelometric study in ancient vineyards in the South of Basilicata region (Italy). Vitis 57, 1–8.

Labianca, M., Valverde, F.N., 2019. 8. Depopulation and aging in rural areas in the European Union: practices starting from the LEADER approach. Despoblación y Transform. sociodemográficas los Territ. Rural. los casos España, Ital. y Fr. 223.

Lacombe, T., Boursiquot, J.-M., Laucou, V., Di Vecchi-Staraz, M., Péros, J.-P., This, P., 2013. Large-scale parentage analysis in an extended set of grapevine cultivars (Vitis vinifera L.). Theor. Appl. Genet. 126, 401–414.

Lasanta, T., Arnáez, J., Pascual, N., Ruiz-Flaño, P., Errea, M.P., Lana-Renault, N., 2017. Space–time process and drivers of land abandonment in Europe. Catena 149, 810–823.

Legouy, F., 2014. La géohistoire de l'espace viticole français sur deux siècles (1808-2010): plusieurs cycles viticoles décryptés. Espac. [En ligne].

Li, Y., Westlund, H., Liu, Y., 2019. Why some rural areas decline while some others not: An overview of rural evolution in the world. J. Rural Stud. 68, 135–143.

Maigre, D., Brugger, J.-J., Gugerli, P., Pont, M., 1998. Safeguarding, conserving and valorizing the genetic diversity in Valais (Switzerland), in: VII International Symposium on Grapevine Genetics and Breeding 528. pp. 59–66.

Manso de Zúñiga, V.C., 1905. Memoria anual. Estación Enológica de Haro.

Maraš, V., Tello, J., Gazivoda, A., Mugoša, M., Perišić, M., Raičević, J., Štajner, N., Ocete, R., Božović, V., Popović, T., 2020. Population genetic analysis in old Montenegrin vineyards reveals ancient ways currently active to generate diversity in Vitis vinifera. Sci. Rep. 10, 1–13.

Martínez, Á., Gómez-Miguel, V.D., 2017. Vegetation index cartography as a methodology complement to terroir zoning for use in precision viticulture. Oeno One 51, 289–301. https://doi.org/10.20870/oeno-one.2017.51.4.1589

Maul, E., Sudharma, K.N., Ganesh, A., Hundemer, M., Kecke, S., Marx, G., Schreiber, T., Walk, M., vom Weg, S., Mahler-Ries, A., 2014. 30 Years VIVC-Vitis International Variety Catalogue (www. vivc. de).

Maul, E., Sudharma, K.N., Kecke, S., Marx, G., Müller, C., Audeguin, L., Boselli, M., Boursiquot, J.M., Bucchetti, B., Cabello, F., 2012. The European Vitis Database (www. eu-vitis. de): A technical innovation through an online uploading and interactive modification system.

Mena, A., 2013. Recuperación, caracterización y conservación de variedades de vid (Vitis vinifera L.) minoritarias de Castilla-la Mancha. Universidad de Castilla-la Mancha, Facultad de Ciencias Ambientales y Bioquimica, Departamento de Ciencias Ambientales, Toledo.

Mena, A., Martínez, J., Fernández-González, M., 2014. Recovery, identification and relationships by microsatellite analysis of ancient grapevine cultivars from Castilla-La Mancha: the largest wine growing region in the world. Genet. Resour. Crop Evol. 61, 625–637.

Moreno-Sanz, P., Loureiro, M.D., Suárez, B., 2011. Microsatellite characterization of grapevine (Vitis vinifera L.) genetic diversity in Asturias (Northern Spain). Sci. Hortic. (Amsterdam). 129, 433–440.

Myles, S., Boyko, A.R., Owens, C.L., Brown, P.J., Grassi, F., Aradhya, M.K., Prins, B., Reynolds, A., Chia, J.-M., Ware, D., 2011. Genetic structure and domestication history of the grape. Proc. Natl. Acad. Sci. 108, 3530–3535.

492 Peres, S., 2009. La résistance des espaces viticoles à l'extension urbaine Le cas du vignoble de
493 Bordeaux. Rev. dEconomie Reg. Urbaine 155–177.

494 Perrin, C., Jarrige, F., Soulard, C.-T., 2013. L'espace et le temps des liens ville-agriculture: une
495 présentation systémique du cas de Montpellier et sa région. Cah. Agric. 22, 552–558.

496 Pinilla, V., Sáez, L.A., 2017. La despoblación rural en España: génesis de un problema y
497 políticas innovadoras. Inf. CEDDAR 2.

498 Piqueras-Haba, J., 2010. El oidium en España: la primera gran plaga americana del viñedo.
499 Difusión y consecuencias 1850-1870. Scr. Nova. Rev. Electrónica Geogr. y Ciencias Soc.
500 14.

501 QGIS Development Team, 2009. QGIS Geographic Information System. Open Source
502 Geospatial Foundation Project.

503 Ramos-Madrigal, J., Runge, A.K.W., Bouby, L., Lacombe, T., Castruita, J.A.S., Adam-
504 Blondon, A.-F., Figueiral, I., Hallavant, C., Martínez-Zapater, J.M., Schaal, C., 2019.
505 Palaeogenomic insights into the origins of French grapevine diversity. Nat. plants 5, 595–
506 603.

507 Santiago, J.L., Boso, S., Gago, P., Alonso-Villaverde, V., Martínez, M.C., 2008. A contribution
508 to the maintenance of grapevine diversity: The rescue of Tinta Castañal (Vitis vinifera L.),
509 a variety on the edge of extinction. Sci. Hortic. (Amsterdam). 116, 199–204.

510 Steevenson, L.J., 2004. business of the institute: A Study of the Revival of Priorat and its
511 Contemporary Challenges. J. Wine Res. 15, 65–84.

512 Urrestarazu, J., Miranda, C., Santesteban, L.G., Royo, J.B., 2015. Recovery and identification of
513 grapevine varieties cultivated in old vineyards from Navarre (Northeastern Spain). Sci.
514 Hortic. (Amsterdam). 191, 65–73.

515 Valcárcel, J.A., 1767. Agricultura general y gobierno de la casa de campo... en que por estenso
516 se trata de todos lo bienes del campo: con los nuevos descubrimentos y metodos de cultivo
517 para la multiplicacion de los granos, del aumento en la cria de ganados y en lo demas
518 depend. por Joseph Estevan Donz.

519 Vaudour, E., Shaw, A.B., 2005. A worldwide perspective on viticultural zoning. South African
520 J. Enol. Vitic. 26, 106–115.

521 Vidal, J.R., Coarer, M., Defontaine, A., 1999. Genetic relationships among grapevine varieties
522 grown in different French and Spanish regions based on RAPD markers. Euphytica 109,
523 161–172.

524 Yuste, A., Martínez-Gil, F., García-Martínez, S., López-Lluch, D., Peiró Barber, R.M., Jiménez,

C., Ruiz, J., Gisbert Domenech, M.C., 2019. Recovering ancient grapevine cultivars in the Spanish provinces of Alicante and Valencia. Acta Hortic. 43–46.