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

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

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## 20 **Abstract**

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

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

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

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## 43 Introduction

44 In the last decades, a remarkable effort has been done in order to recover grapevine varieties  
45 whose cultivation had become marginal or that survived as single plants in multi-cultivar old  
46 vineyards. As a result of this effort, a significant number of nearly forgotten/neglected varieties  
47 have been recovered in most countries in southern Europe, as highlighted in the project “On-  
48 farm inventory of minor grape varieties in the European *Vitis* Database (GrapeOnFarm)”. As a  
49 general rule, prospecting missions initially focused on the oldest vineyards in well-known grape  
50 growing regions (Jung and Maul, 2004; Labagnara et al., 2018; Maigre et al., 1998; Mena et al.,  
51 2014; Urrestarazu et al., 2015; Yuste et al., 2019), and, only more recently, missions have been  
52 shifted towards areas where grape growing survived as a secondary or even marginal activity  
53 (Gago et al., 2011; Jiménez-Cantizano et al., 2020; Moreno-Sanz et al., 2011).

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

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

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

74 (ii) Rural depopulation: the loss of population in rural areas is a complex and long-term  
75 challenge worldwide (Labianca and Valverde, 2019; Li et al., 2019). In the last 150  
76 years, there have been several chained events that have gradually led to severe  
77 degrees of depopulation of some grape growing regions in Europe, with peasant  
78 population moving abroad, majorly to South and North America, to other European  
79 countries, or to urban areas within the origin country (Baines, 1995; Lasanta et al.,  
80 2017; Pinilla and Sáez, 2017), affecting more severely to semi-arid and mountain-  
81 areas (Lasanta et al., 2017). Depopulated grape growing areas in Spain can be found  
82 throughout the whole country, some examples being reported in detail for areas in  
83 Castilla y León (Franco-Jubete, 2007), Andalusia (Douglas et al., 1996; Douglas et  
84 al., 1994), and even in the now well-known Priorat area in Catalonia (Steevenson,  
85 2004). In France, depopulation can be behind the regression of viticulture in central  
86 areas such as the Massif Central and neighbouring southern Departments (André,  
87 1998; Legouy, 2014).

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

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

1 97 grapevine varieties in the peri-urban area of Pamplona, a medium-size town in northern Spain,  
2 98 where viticulture was relevant until the end of the 19<sup>th</sup> century, but suffered a continued  
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4 99 regression that led to near complete disappearance of vineyards (Astibia-Ayerra, 1992). In this  
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6 100 area, the reason for vineyard abandonment was a combination of cases (i) and (iii). Pamplona's  
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8 101 climate can be classified as a Huglin type I region, with relatively high pressure for fungal  
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10 102 diseases, where the population has increased from 35,000 inhabitants in 1900 up to > 350.000  
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12 103 inhabitants now. The current presence of vineyards in Navarra is nowadays restricted to some  
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14 104 vineyards in the southern part of the region, whereas in the mid and northern parts, where the  
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16 105 city of Pamplona is located, only approximately 5 very small (<0.3 ha) family-owned vineyards  
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18 106 remain, altogether with one commercial winery established in the 1980s to produce international  
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20 107 profile wines.

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24 108 The differential aspect of our approach is that, to our knowledge, this is the first work aimed at  
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26 109 recovering grapevine diversity in a non-viticultural area, which necessarily had to be performed  
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28 110 based on rescuing individual plants surviving at field margins or at non-cultivated pieces of  
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30 111 land. This is a very particular context, but that can probably be found at other regions in Europe,  
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32 112 which requires prospection to be based on the analysis of the available historical information as  
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34 113 open-access resources (orthophotos, land use maps and GIS applications).

## 35 36 37 38 39 114 **Materials and methods**

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42 115 This study was carried out in four well differentiated phases: (i) analysis of the available  
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44 116 historical information, (ii) prospecting works and collection of grapevine samples, (iii)  
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46 117 classification of the recovered plants in different categories, dating its time of origin based on  
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48 118 the last record of the presence of vineyards in the areas where they were found and, (iv) genetic  
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50 119 characterization for varietal identification.

### 51 52 53 54 120 **Analysis of the available historical information**

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56 121 With the aim of identifying the wine-growing zones of the peri-urban area of Pamplona in the  
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58 122 past, the layers "Límites catastrales de los municipios de Navarra actuales" ("Cadastral limits of

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

### 139 **Prospecting work and collection of grapevine samples**

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

### 149 **Classification of the recovered plants in different categories**

150 All the plants collected in the Pamplona peri-urban area were classified into four categories  
151 according to the lapse of time since the last record of vineyards in the specific location where  
152 each plant was found could be confirmed. For doing that, open access orthophotos of the years  
153 1956-1957, 1966-1971 and 2012 were used. The four categories proposed refer to:

- 154 • Category 1: Plants in probable areas of “old” vineyards as the last evidence of vineyards  
155 is reported in the orthophoto of 1956-1957.
- 156 • Category 2: Plants in probable areas of “non-modern” vineyards as the last evidence of  
157 vineyards is reported in the orthophoto of 1966-1971.
- 158 • Category 3: Plants in probable areas with a risk of “modern” presence of vineyards as  
159 the last evidence of vineyards is reported in the orthophoto of 2012.
- 160 • Category 4: Plants in riverside areas, unlikely to be a wine-growing area in the past.

#### 161 **Genetic characterization for varietal identification of the recovered plants**

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

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



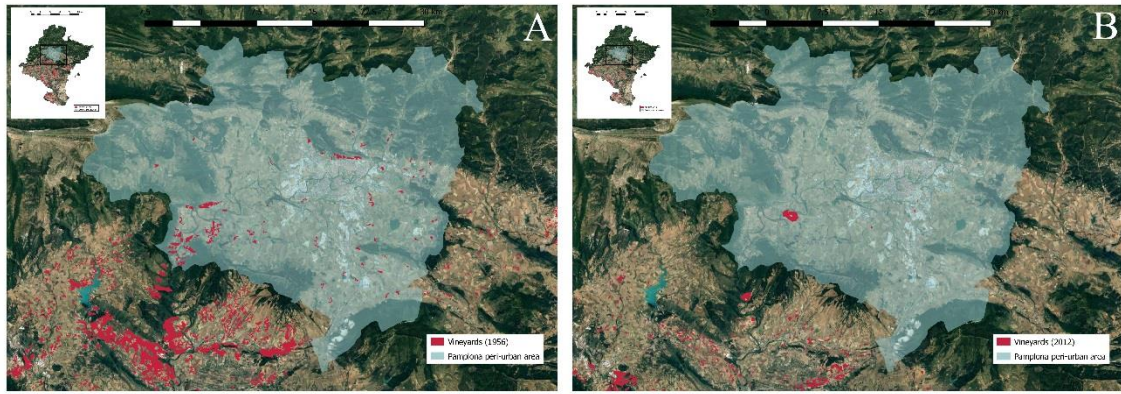
176 The SSR molecular profiles found were compared to the set of unique profiles from the *Vitis*  
177 International Variety Catalogue ([www.vivc.de](http://www.vivc.de)) (Maul et al., 2014) and to those in the European  
178 *Vitis* Database ([www.eu-vitis.de/index.php](http://www.eu-vitis.de/index.php))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

## 184 **Results and discussion**

### 185 **Historical map construction as the basis for prospecting works**

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

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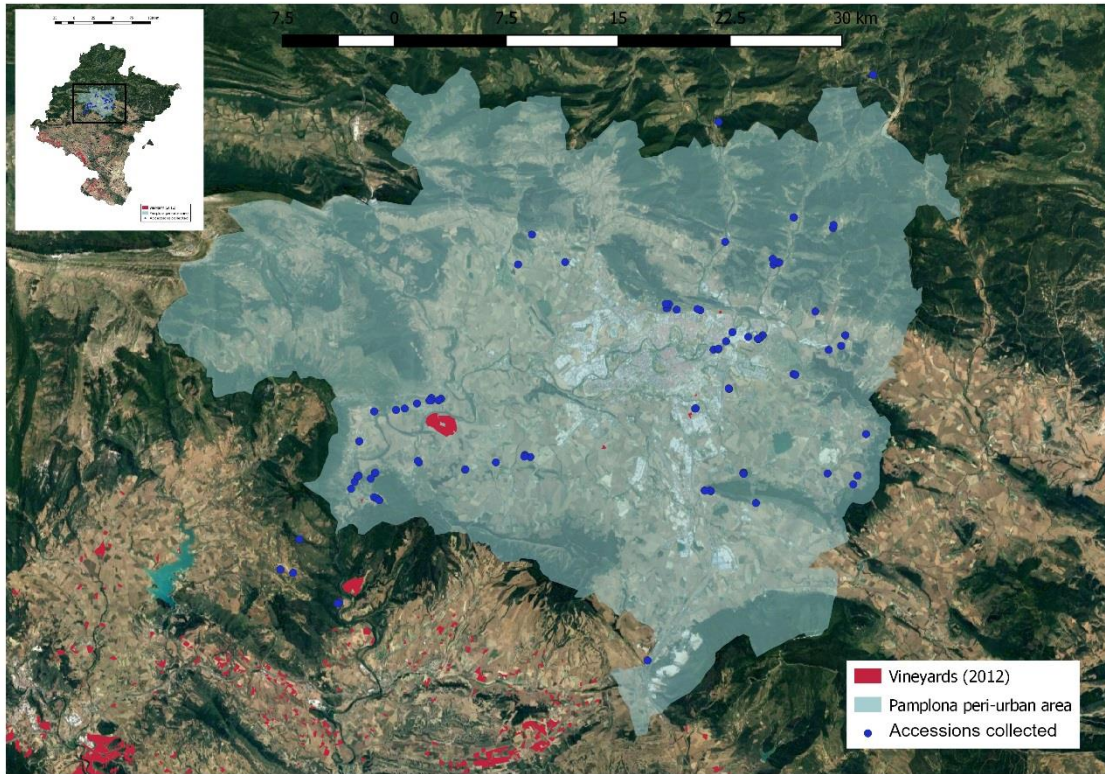
195 **Figure 1.** Map showing vineyard area in the peri-urban area of Pamplona in A) 1956 and B)  
 196 2012. Vineyard abandonment is clearly observed, the only relevant acreage in 2012  
 197 corresponding to the fields of a winery established in the 1980s to produce international profile  
 198 wines.

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

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

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217

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

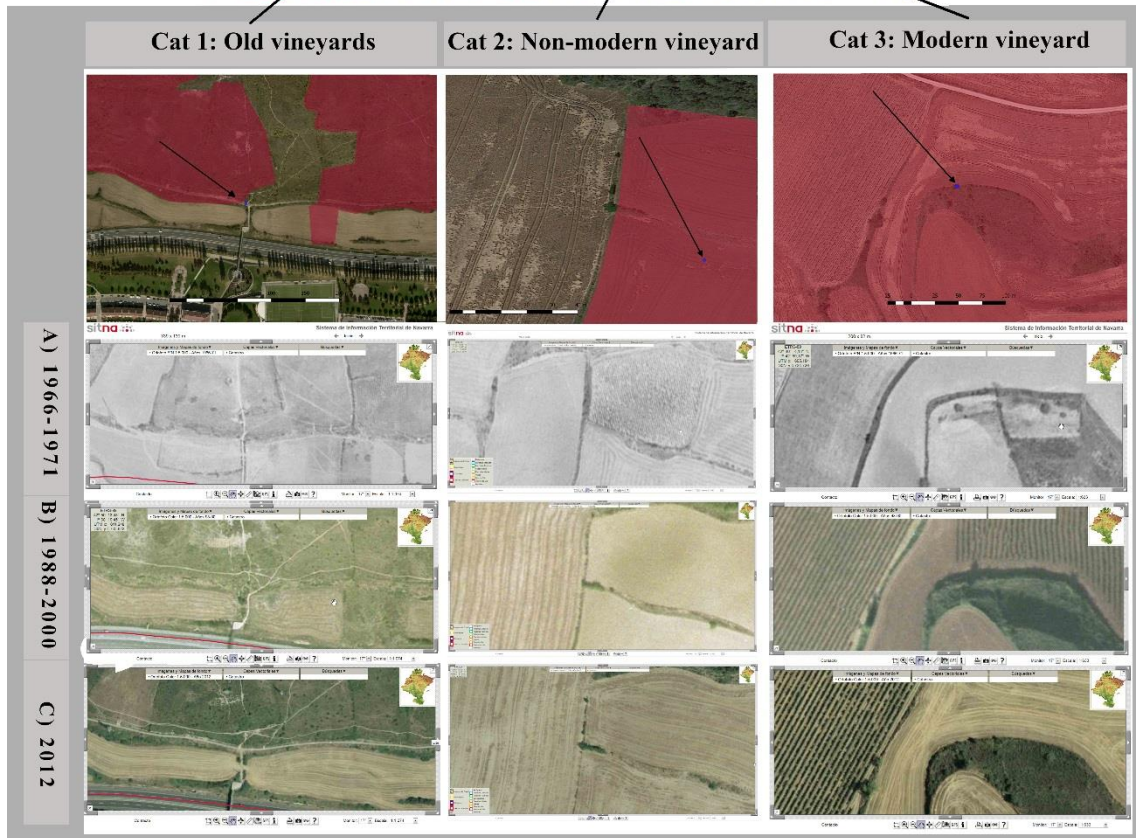
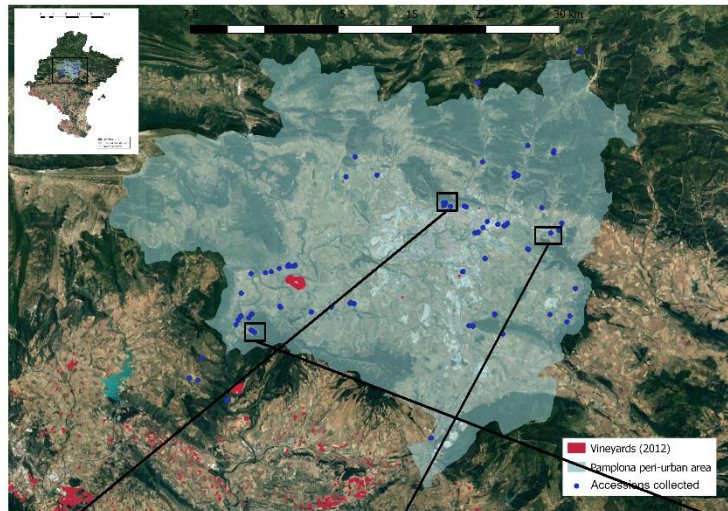
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### 223 **Dating of plant material**

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



232 accession was collected, the one classified in Category 1 (“old” vineyards, being dated before  
 233 1956), no presence of vineyards was observed on it after 1956. By contrast, for the  
 234 georeferenced points where the other two accessions were found, the ones corresponding to  
 235 Category 2 (“non-modern” vineyards, being dated between 1966 and 1971) and Category 3  
 236 (presence of “modern” vineyards in 2012), presence of vineyards can be observed after 1956,  
 237 between 1966-1971 and from 1988 to 2012, respectively.



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239 **Figure 3.** Maps used in the process for plant material dating. An example for the three different  
 240 time points (Category 1: old vineyard, Category 2: Non-modern vineyard and Category 3:  
 241 Modern vineyard) are displayed. Grapevine cultivation in every referenced accession (marked  
 242 with arrows) was checked through cartographic pictures on A) 1966-71, B) 1998-2000 and C)  
 243 2012.  
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245 The classification of the 120 accessions found according to their location is presented in Table  
 246 1. The majority of the accessions (76 out of 120) corresponded to Category 1 (“old” vineyards,  
 247 being dated before 1956), followed by those belonging to Category 2 (“non-modern” vineyards,  
 248 being 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  
 251 wine-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%

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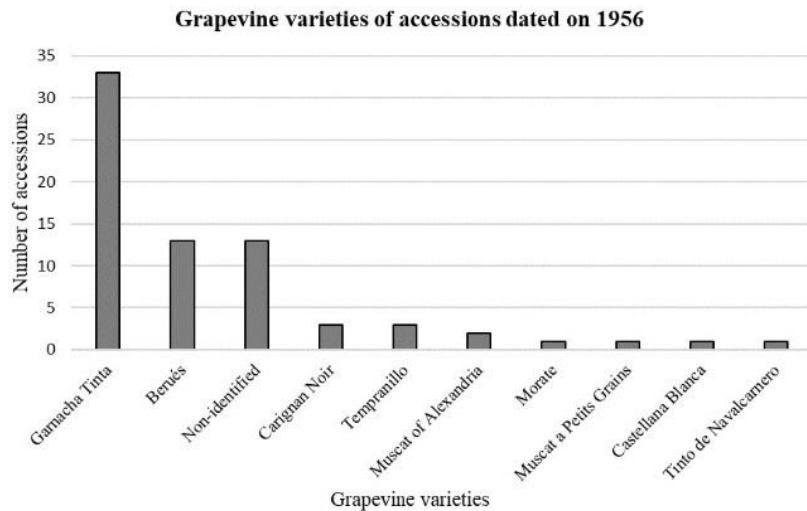
259 The dating plant material approach based on georeferenced historical cartography has proven to  
260 be useful to classify the recovered plants into four time periods extending over the 20<sup>th</sup> century.  
261 Dating of plant material was useful to classify the collected plants within a specific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

### 266 **Varietal identification of the recovered plants using molecular markers**

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

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

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



291  
 292 **Figure 4.** Varietal distribution of accessions classified as Category 1, dated before 1956.  
 293 Number of accessions per variety is represented and varieties are ordered from more to less  
 294 abundant.

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

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

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314 **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 accessions	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 <sup>a</sup>	5628	1	0.83%			1	
Palomino Fino	8888	1	0.83%			1	
Non-identified		28	23.33%	13		1	14
<b>Total</b>		<b>120</b>	<b>99.97%</b>	<b>75</b>	<b>29</b>	<b>2</b>	<b>14</b>

315 <sup>a</sup>Non true-to-type

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316 **Recovery of the variety Berués**

317 Prospecting actions have been proved to be interesting not only to report the varietal richness at  
318 each specific region in the past, but also to recover emblematic cultivars preventing their loss.

319 The major milestone underlying the prospecting mission of the peri-urban area of Pamplona has  
320 been the recovery of 15 accessions of Berués, a very old variety considered to be disappeared.

321 The only plants of Berués preserved before this study was performed, where those collected  
322 probably in the same area at the beginning of 20<sup>th</sup> century by García de los Salmones (García De  
323 Los Salmones, 1914). This material is currently preserved at the grapevine collections of “El  
324 Encín” (IMIDRA, Madrid) and “El Rancho de la Merced” (IFAPA, Jerez de la Frontera), under  
325 the designation “Verués de Huarte”, which has allowed the unequivocal identification of the  
326 accessions found.

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

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

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

## 354 **Concluding remarks**

355 Prospecting missions which take place in areas where viticulture is nowadays decadent usually  
356 rely on the information provided either by locals and owners of old vineyards, or from written  
357 records (Balda et al., 2014; D'Onofrio et al., 2016; Maraš et al., 2020; Santiago et al., 2008).  
358 Besides, historical cartography definitely offers a broad and reliable source of land use  
359 information, which has been deeply explored in some fields of study as, for instance, in land-use  
360 evolution studies. However, in the scope of viticulture, the use of cartographic information has  
361 been very scarce, mainly restricted to viticulture zoning and *terroirs* demarcation (Bois et al.,  
362 2008; Martínez and Gómez-Miguel, 2017; Vaudour and Shaw, 2005). To our knowledge, this is  
363 the first time that this type of information has been used to delineate potential areas of interest in  
364 terms of recovery actions. In this context, it is worth emphasizing the importance of public  
365 initiatives that cope cartography digitalization as open-source data.

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

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2 370 variety in the peri-urban area of Pamplona, a very old variety considered to be disappeared.  
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4 371 Minor and neglected varieties recovered via prospections or from germplasm repositories  
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6 372 gained reputation in the last decade. Wines from these varieties could be unique and hence niche  
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8 373 products and provide a high added value. An in-depth agronomical-oenological study of Berués  
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10 374 has been initiated, aiming to increase the knowledge on its potentiality in diversifying the  
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12 375 market of this major geographical region.

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## 27 383 **Author contributions**

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29 384 SCM: Data curation, formal analysis, writing – original draft, writing – review & editing

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31 385 BM: Conceptualization, data curation, formal analysis, methodology, writing – original draft,  
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37 388 ML: Data acquisition, writing – review & editing

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39 389 AV: Data acquisition, writing – review & editing

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41 390 DM: Data acquisition, writing – review & editing

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43 391 CM: Funding acquisition, project administration, writing – review & editing

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45 392 LGS: Conceptualization, funding acquisition, conceptualization, methodology, supervision  
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49 394 JU: Conceptualization, formal analysis, writing – original draft, writing – review & editing

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

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19

## 20 **Abstract**

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

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

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

41

## 42 Introduction

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

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

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

69 is more difficult, diseases led to frequent crop losses and to an increase in cost that  
70 could not be easily assumed by growers (Azcárate-Luxán, 1996), causing a gradual  
71 abandonment of vineyards. This was the case of Rias Altas' vineyards in Spain (De  
72 Lemps, 1967), where grape growing became unsustainable, while in the neighbouring  
73 Rias Baixas, with milder climate, vineyards thrived through the crisis. A similar  
74 process occurred in most grape growing area in Asturias, Cantabria, Bizkaia and  
75 Gipuzkoa (Piqueras-Haba, 2010). In France, this was also the case for vineyards in  
76 several departments in the Northwest, such as l'Ille-et-Vilaine, la Mayenne, l'Eure,  
77 l'Oise and Ardenne (Legouy, 2014), where vineyards nearly disappeared in the late  
78 19<sup>th</sup> century.

79 (ii) Rural depopulation: the loss of population in rural areas is a complex and long-term  
80 challenge worldwide (Labianca and Valverde, 2019; Li et al., 2019). In the last 150  
81 years, there have been several enchainned events that have gradually led to severe  
82 degrees of depopulation of some grape growing regions in Europe, with peasant  
83 population moving abroad, majorly to South and North America, to other European  
84 countries, or to urban areas within the origin country (Baines, 1995; Lasanta et al.,  
85 2017; Pinilla and Sáez, 2017), affecting more severely to semi-arid and mountain-  
86 areas (Lasanta et al., 2017). Depopulated grape growing areas in Spain can be found  
87 throughout the whole country, some examples being reported in detail for areas in  
88 Castilla y León (Franco-Jubete, 2007), Andalusia (Douglas et al., 1996; Douglas et  
89 al., 1994), and even in the now well-known Priorat area in Catalonia (Steevenson,  
90 2004). In France, depopulation can be behind the regression of viticulture in central  
91 areas such as the Massif Central and neighbouring southern Departments (André,  
92 1998; Legouy, 2014).

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

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

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

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

## 117 **Materials and methods**

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

122 varietal identification.

### 123 **Analysis of the available historical information**

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

### 141 **Prospecting work and collection of grapevine samples**

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



149 vector layer of the location points of each plant to the QGIS software (QGIS Development Team,  
150 2009).

### 151 **Classification of the recovered plants in different categories**

152 All the plants collected in the Pamplona peri-urban area were classified into four categories  
153 according to the lapse of time since the last record of vineyards in the specific location where  
154 each plant was found could be confirmed. For doing that, open access orthophotos of the years  
155 1956-1957, 1966-1971 and 2012 were used. The four categories proposed refer to:

- 156 • Category 1: Plants in probable areas of “old” vineyards as the last evidence of vineyards  
157 is reported in the orthophoto of 1956-1957.
- 158 • Category 2: Plants in probable areas of “non-modern” vineyards as the last evidence of  
159 vineyards is reported in the orthophoto of 1966-1971.
- 160 • Category 3: Plants in probable areas with a risk of “modern” presence of vineyards as the  
161 last evidence of vineyards is reported in the orthophoto of 2012.
- 162 • Category 4: Plants in riverside areas, unlikely to be a wine-growing area in the past.

### 163 **Genetic characterization for varietal identification of the recovered plants**

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

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

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

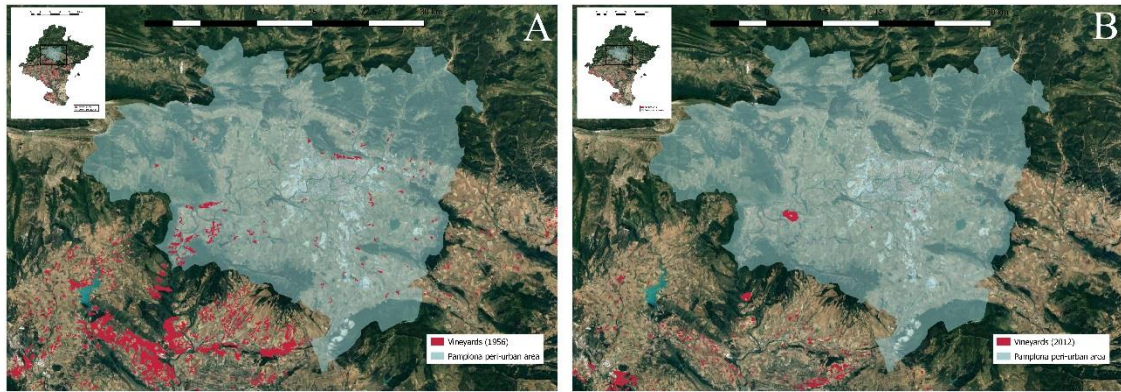
178 The SSR molecular profiles found were compared to the set of unique profiles from the *Vitis*  
179 International Variety Catalogue ([www.vivc.de](http://www.vivc.de)) (Maul et al., 2014) and to those in the European  
180 *Vitis* Database ([www.eu-vitis.de/index.php](http://www.eu-vitis.de/index.php))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

## 186 **Results and discussion**

### 187 **Historical map construction as the basis for prospecting works**

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

195



196

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

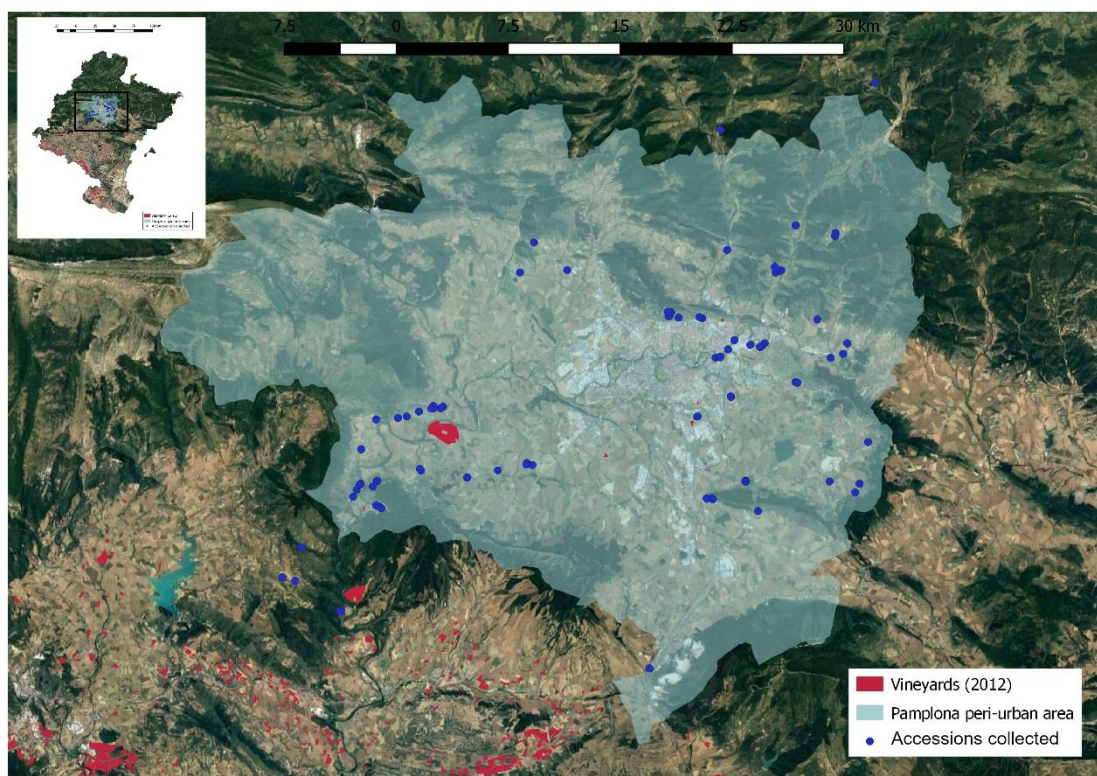
200

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

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

216

217



218

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

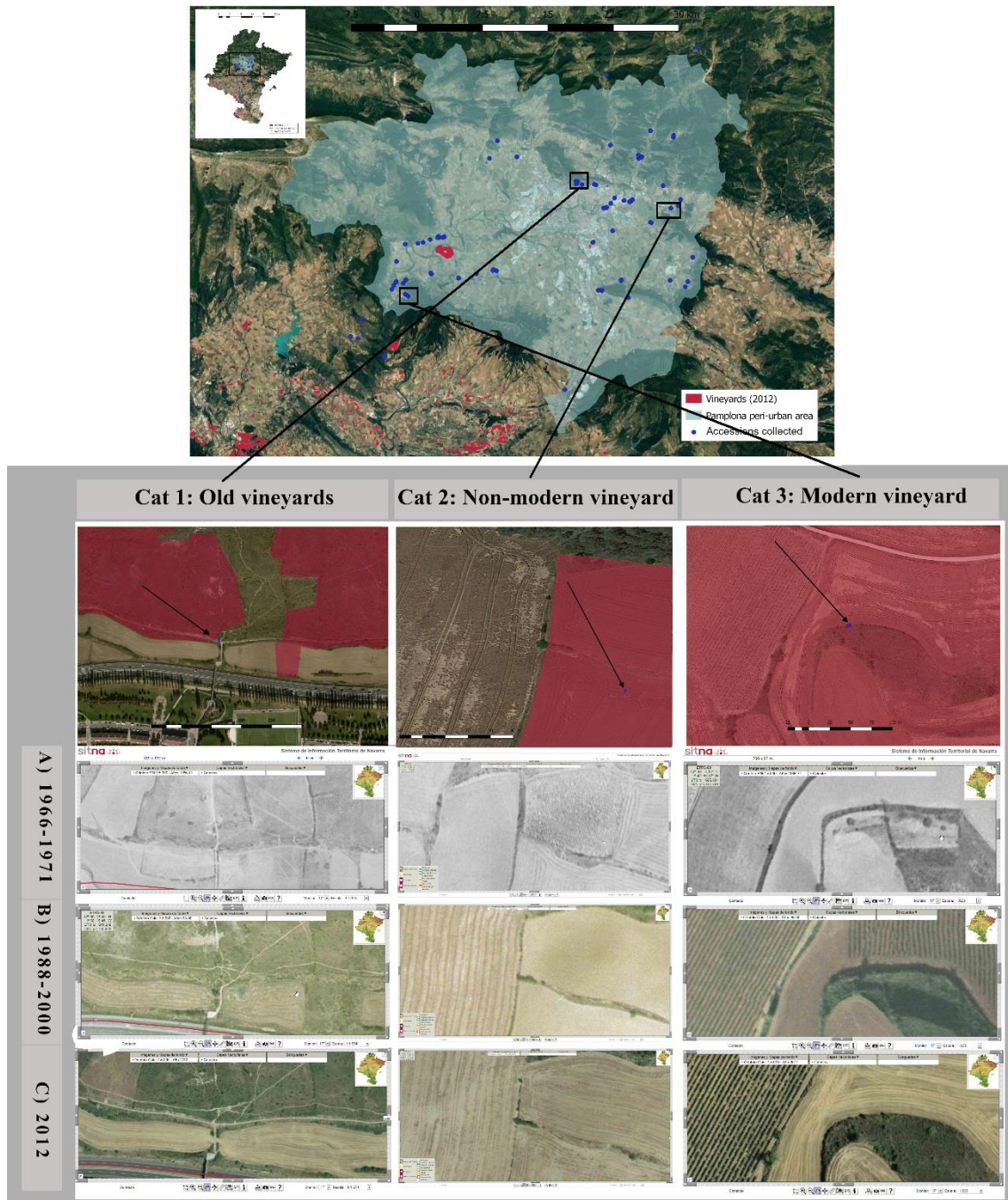
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#### 224 **Dating of plant material**

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



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



239

240 **Figure 3.** Maps used in the process for plant material dating. An example for the three different  
 241 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 20<sup>th</sup> century.

261 Dating of plant material was useful to classify the collected plants within a specific period of time,

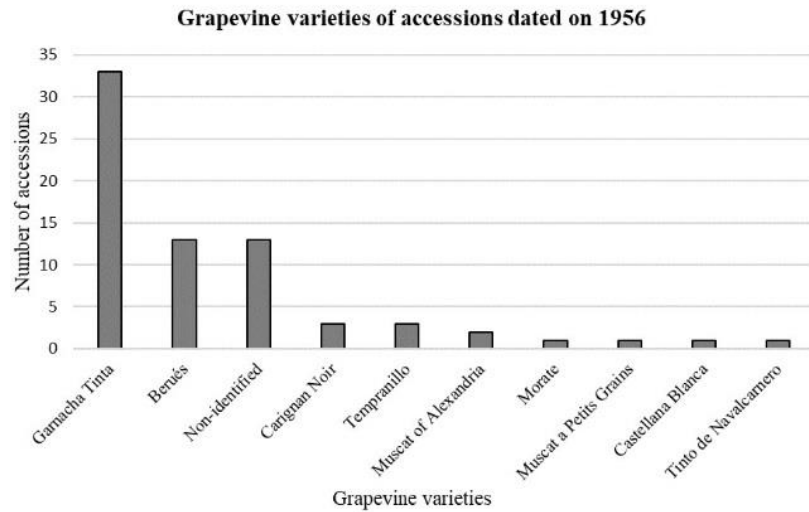
262 but also allowed us to validate the effectiveness of the methodology designed *ad hoc* for this case  
263 study. Nearly 63% of the plants collected were assigned to Category 1, thus revealing that the  
264 proposed methodology allows both the finding and recovery of the oldest “still existing”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

#### 266 **Varietal identification of the recovered plants using molecular markers**

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

271 Combining dating and varietal identification of the accessions found in the peri-urban area of  
272 Pamplona allowed to delineate an approximate idea of the varietal composition of the vineyards  
273 existing in 1956 in this area (Fig. 4). Among the oldest accessions (those comprised in Category  
274 1), the most prevalent variety before 1956 in the “Pamplona Basin” was Garnacha (syn. Grenache)  
275 (33 accessions, 44% of those in Category 1), what is in agreement with the increasing popularity  
276 that this variety had from the mid-19<sup>th</sup> century in the region due to its lower susceptibility to  
277 powdery and downy mildew among the traditional varieties (Huetz de Lemps, 1993). The second  
278 most numerous variety is Berués (13 accessions, 17.33% of those included in Category 1), a  
279 traditional variety that is not cultivated nowadays. Additionally, within the remaining cultivars  
280 identified in Category 1, in four cases [Carignan (syn. Mazuelo), Tempranillo, Morate and Muscat  
281 à petit grains blancs (syn. Moscatel de Grano Menudo)] there is documentary evidence that they  
282 had at least some relevance in early 20<sup>th</sup> century in Navarre or in its neighbouring regions (Basque  
283 Country, La Rioja and Aragon) (Cabello et al., 2011; García De Los Salmones, 1914; Manso de  
284 Zúñiga, 1905). Concerning the other three cultivars identified in Category 1 (Castellana Blanca,  
285 Tinto de Navalcarnero and Muscat of Alexandria), the cultivation of the two former ones was  
286 documented for regions far from Navarre or bordering regions (García De Los Salmones, 1914),  
287 while the latter is a widespread table grape variety and, thus, its appearance is not surprising.

288



290

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

293

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

302 According to the early work by García De Los Salmones (1914), a compilation of cultivars being  
 303 grown at each Spanish region before the phylloxera outbreak, the cultivation of at least 27  
 304 different varieties was reported in the region of Navarre. Some initiatives have been carried out  
 305 aiming to recover the varietal richness still existing in the oldest vineyards of the region in the  
 306 last years (Cibriáin-Sabalza et al., 2016; Urrestarazu et al., 2015) resulting, as a whole, in more  
 307 than 50 varieties identified, a significantly higher number than reported by García De Los  
 308 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 accessions	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 Navacarnero	26280	1	0.83%	1			
Rupestres 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 <sup>a</sup>	5628	1	0.83%			1	
Palomino Fino	8888	1	0.83%			1	
Non-identified		28	23.33%	13		1	14
<b>Total</b>		<b>120</b>	<b>99.97%</b>	<b>75</b>	<b>29</b>	<b>2</b>	<b>14</b>

313 <sup>a</sup>Non true-to-type

### 314 **Recovery of the variety Berués**

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

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

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

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

## 352 **Concluding remarks**

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

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

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

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384 writing – review & editing

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386 ML: Data acquisition, writing – review & editing

387 AV: Data acquisition, writing – review & editing

388 DM: Data acquisition, writing – review & editing

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