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New findings and actions in the recovery of old Mediterranean grapevine varieties

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Summary

In this work, we report new findings related to the recovery of grapevine diversity in the Comunitat Valenciana (Eastern Spain): accessions of old varieties at risk of disappearance, new genotypes, synonymies, homonymies, and some foreign old varieties. In addition, the *in vitro* establishment of some rescued varieties has been carried out as a complement to *ex-situ* conservation, as well as to provide material for biotechnological applications.

Keywords

‘Albillo Forastero’, ‘Forcallat Blanc’, ‘Moscatella’, ‘Nigheddu Polchinu’, ‘Cot de Cheragas’

Introduction

The recovery of old grapevine varieties has received increasing interest in recent years (*i.e.* Labagnara *et al.*, 2018; Jiménez *et al.*, 2019; Rahali *et al.*, 2019; García *et al.*, 2020; Mendoza *et al.*, 2022) to stop genetic erosion, to recover varieties that could be better adapted to climate changes, and to diversify wine products. In previous works, we rescued part of the rich biodiversity of vines in the Comunitat Valenciana, Eastern Spain, where more than 100 varieties were cultivated prior to the arrival of phylloxera (Gisbert *et al.*, 2018; Jiménez *et al.*, 2019; García *et al.*, 2020). Among the 203 accessions analyzed, more than 50 varieties were found, including some at great risk of disappearance like ‘Cor d’Angel’, ‘Morsí’, or ‘Raïm del Clotet’. In these works, previously unknown SSR profiles have been assigned to minor varieties (*i.e.* ‘Cor d’Angel’, ‘Gancha arroba’, ‘Esclafagerres’, ‘Macabeo negro’, ‘Mamella de Vaca’, ‘Montalbana’, ‘Morenillo de la Hoya’, ‘Raïm del Clotet’ and ‘Trepadell’). Additionally, hybrid direct producer grapevine varieties and unknown genotypes were located. Among the latter, NI-2 in Jiménez *et al.* (2019) was identified as ‘Siria’ after new information was added to the International *Vitis*

Catalogue, which is periodically updated by incorporating new varieties, synonymies, homonymies, and data of vine characterization. Research related to these cultivars and the preservation of the recovered germplasm are key activities in the rescue of grapevine biodiversity heritage.

Here, we report SSRs amplification results for vine nuclear DNAs of newfound grapevine accessions located in our latest prospections. These results gave us information that allowed us to assign names to unknown accessions, identify misnamed varieties, and detect new genotypes, homonymies, and synonymies. Unexpected germplasm of foreign origin and new accessions of the endangered varieties were also identified. In addition, *in vitro* establishment of some of the rescued germplasm was performed as a complementary tool for preservation and the application of *in vitro* culture techniques.

Material and Methods

Prospections were carried out in the three provinces of the Comunitat Valenciana, mainly in the province of Alicante (Fig. S1). A total of 100 accessions (S1-S16; U1-U84) were assessed. The supplementary data in Table S1 shows, for each accession, information about the name of the survey (when it is available), the location where each sample was found, the variety identified, its number in VIVC and the colour of berry skin corresponding to the variety identified. The identification was made by comparing SSRs profiles to our database and that of VIVC. Firstly, DNA was extracted using the commercial DNeasy Plant Mini Kit (Qiagen). Then, 15 nuclear SSR markers (VVS2, VVMD5, VVMD6, VVMD7, VVMD21, VVMD24, VVMD25, VVMD27, VVMD28, VVMD32, VrZAG62, VrZAG64, VrZAG79, VrZAG83, and VMC1b11) were amplified using two sets of multiplex PCR reactions as described by Peiró *et al.* (2018). *In vitro* establishment and culture were performed as in Gisbert (2011) and Gisbert *et al.* (2018). Plants were maintained in a growth chamber at 25 °C and 70 % humidity and with a photoperiod of 16 h with successive subcultures in aseptic conditions.



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Results and Discussion

In previous surveys, more than 50 grapevine varieties were found in old or neglected vineyards of the Comunitat Valenciana (Gisbert *et al.*, 2018; Jiménez *et al.*, 2019; García *et al.*, 2020), corroborating the rich varietal diversity of this area. New prospections were performed to locate others as well as to determine which variety corresponds to ‘Forcallat Blanc’. In some areas, the name ‘Forcallat Blanc’ is used as a synonym of ‘Airen’ (Hidalgo, 2002) but it also appeared as a synonym of ‘Luisa Blanca’ in the VIVC database (www.vivc.de; accessed 15/07/22). However, we thought that this old variety, which is also referred to as ‘Forcallat Blanca’ (Ministerio de Fomento, 1882; JCA [Junta Consultiva Agronómica], 1891), and as ‘Forcallada’ or ‘Forcallada Blanca’ in Valcárcel (1791) and García de los Salmones (1909), respectively, could be also present in old vineyards.

The results of the identification (Tables S1-S3) showed that the four surveyed accessions with the name of ‘Forcallat Blanc’ correspond to three different varieties. One surveyed in Campo Arcís (Requena, Valencia) was ‘Airen’, and another from Alicante was ‘Folha de Figueira’ (syn. ‘Tortozón’), while the other two, from La Mata (Alicante), gave a new SSR profile that was also found in an unknown accession (U-31) located in Hondón de las Nieves (Alicante). Some ampelographic description is found for this variety in Valcárcel (1791) who described it as an early ripening variety of long bunches whose grapes achieved a golden colour when ripening and is adequate for wine making. These features matched those observed in plants grafted from the U-31 accession, which gave the new SSR profile that can be assigned to this variety (Fig. 1). In addition, the variety ‘Tortozón’ could be involved in the pedigree of ‘Forcallat Blanc’ since both share at least one allele per locus (Tables S2 and S3). We also looked for plants of ‘Franceset’ in La Llosa de Ranes, where it was economic value during the ‘golden period of Valencian viticulture’ (Piqueras Haba, 1985), locating this variety that was not found in previous surveys. The SSR profile for this sample (S5) and that for U-25 are close to that of ‘Chasselas Blanc’, a name that appears as a synonym of this variety in the VIVC. In old vines, it is common to find variability as consequence of the accumulation of mutations along the years.

Other varieties identified for the first time in the current prospections are: ‘Raïm de San Joan’, a variety with white berries and early ripening (around 24 June), which matched the variety ‘Madeleine Angevine Oberlin’, a ‘Madeleine Angevine’ × ‘Bouquettraube’ cross made at the Institut Viticole Oberlin (France) (www.vivc.de, accessed 12/07/22) in 1903; ‘Garnacha Roya’; ‘Palomino Fino’; ‘Cardeal’ (surveyed as ‘Ull de Llebre’); ‘Albillo Forastero’ (surveyed as ‘Malvasía de Canarias’); ‘Nigheddu polchinu’; and ‘Cot de Cheragás’ (Table S1). It was corroborated that the name ‘Ull de Llebre’ is used in the surroundings of Pinoso/La Algueña for the endangered variety ‘Cardeal’, but this name is also used in Catalonia for ‘Tempranillo’; therefore, a homonymy was detected. Regarding ‘Albillo Forastero’, it was probably introduced from the Canary Islands. Other varieties that could have been intro-

duced long ago are ‘Cot de Cheragás’ (U78) – also known as ‘Mourvedre d’Afrique’ (Galet, 2015) – resulting from the cross ‘Heben’ × ‘Monastrell’, and two accessions of the variety ‘Nigheddu polchinu’ (previously named Plant de Vic 98 n^o4; U14 and U26), which were growing in Campo Arcis (Requena, Valencia) and in a pre-phylloxera vineyard in the Guardamar dunes (Guardamar, Alicante).

This work also revealed a new synonymy for ‘Muscat d’Istanbul’ (Tables S1-S3), namely ‘Moscatella’, the location of one accession of the endangered variety ‘Cardeal’ (in La Algueña, Alicante) and one of ‘Cor d’Angel’ (in Monovar, Alicante), and a new accession of other scarce grapevine varieties like ‘Morenillo de la Hoya’, ‘Verdil’, ‘Esclafagerres’, and ‘Trepadell’. Two accessions, ‘Sensú’ (S16) and U40, remained unidentified.

Finally, accessions of ‘Arcos’, ‘Botó de Gall’, ‘Cor d’Angel’, ‘Esclafagerres’, ‘Grumer Moscatell’, ‘Morsí’, ‘Verdil’, and a variant of ‘Monastrell’ named ‘Veremeta’ (Gisbert *et al.*, 2022), were established *in vitro*, increasing the number of varieties that are preserved in our Institute under *in vitro* culture conditions (Fig. 2). *In vitro* plants represent a complementary tool for germplasm preservation and are a source of materials for the application of *in vitro* culture techniques, which are of interest in *Vitis* for virus sanitation, micropropagation, and/or biotechnological selection and breeding (San Pedro *et al.*, 2017a, b, Peiró *et al.*, 2020).

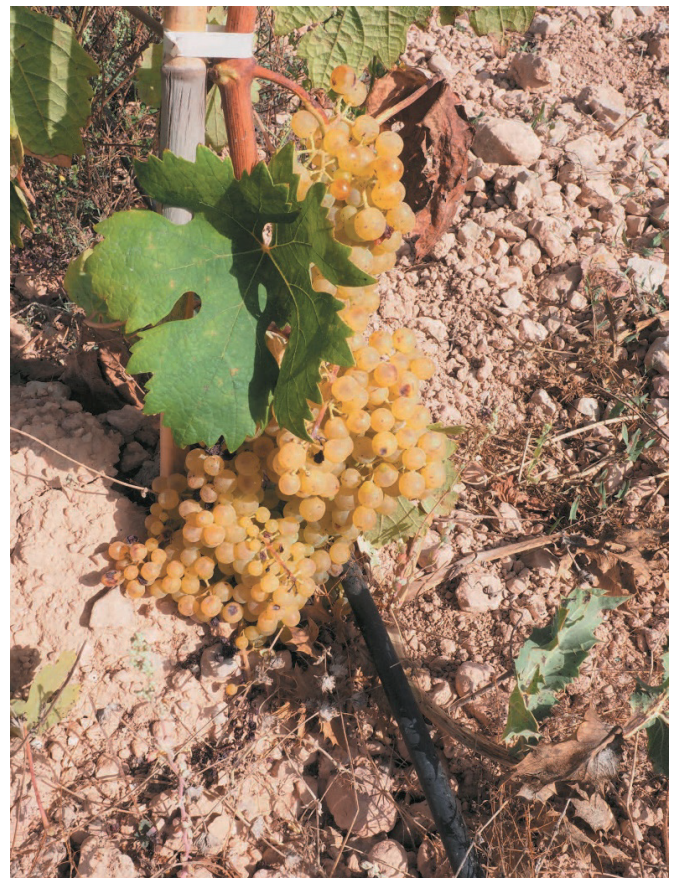


Figure 1. Detail of leaf and bunches of a Forcallat Blanc plant (grafted from the sample U-31 which has the SSR profile of this variety).

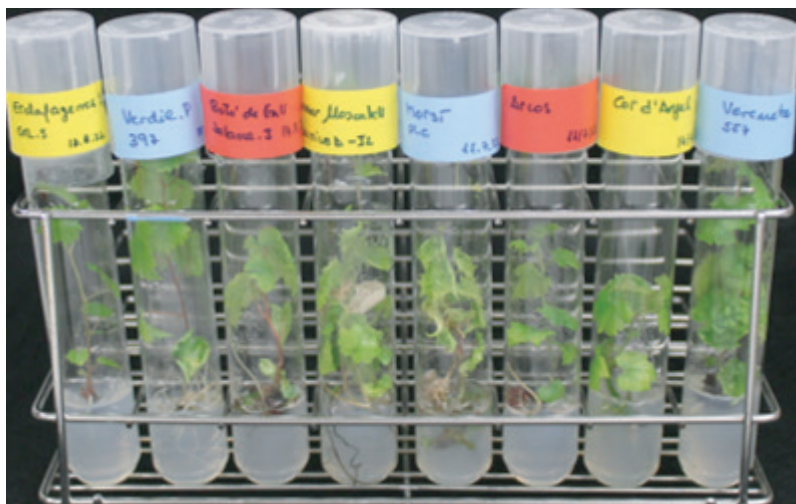


Figure 2. Grapevine varieties growing under *in vitro* culture in MW medium (San Pedro et al. 2017a): ‘Esclafagerres’, ‘Verdill’, ‘Botó de Gall’ (prime name ‘Ahmeur bou Ahmeur’), ‘Grumer Moscatell’ (prime name ‘Muscat d’Istanbul’), ‘Arcos’, ‘Cor d’Angel’, ‘Morsí’ and ‘Vermeta’ (a variant of the ‘Monastrell’ variety; Gisbert et al. 2022).

Acknowledgements

The prospectations and varietal identification were funded by Conselleria de Agricultura, Desarrollo Rural, Emergencia Climática y Transición Ecológica (Generalitat Valenciana) under grant agreement S8788000. We acknowledge the collaboration of Carles Jiménez (GVA), Winery Oriol Romeu, Hilarion (Project ‘Sopla Levante’), Santiago García (UMH), and Francisco Martínez-Gil (UV), who participated in some surveys. We thank the UPV (PAID-11-21) for funding for the *in vitro* preservation of grapevines.

Conflicts of interest

The authors declare that they do not have any conflicts of interest.

References

- García, J., Peiró, R., Martínez-Gil, F., Soler, J.X., Jiménez, C., Yuste, A., Xirivella, C., Gisbert, C., 2020: Recovering old grapevine varieties. *Vitis* 59, 101-103, DOI: 10.5073/vitis.2020.59.101-103
- García de los Salmones, N., 1909: Organización del Servicio Agrícola Provincial de la Diputación de Navarra. Imprenta Provincial Pamplona. Pamplona, Spain.
- Gisbert, C., 2011: Establecimiento de cultivos *in vitro* a partir de semillas o de material de campo. Colección Artículos docentes ETSIAMN-UPV. Publicado en RIUNET el repositorio institucional de la UPV. <http://hdl.handle.net/10251/9798>.
- Gisbert, C., Peiró, R., San Pedro, T., Olmos, A., Jiménez, C., García, J., 2018: Recovering ancient grapevines varieties: from genetic variability to *In vitro* conservation. In: A.M. Joao, F. Cosme (Eds): *InTechOpen*, 4 – 21. London, UK. <http://dx.doi.org/10.5772/intechopen.71133>
- Gisbert, C., Soler, J.X., Fos, M., Intrigliolo, D., Yuste, A., Pico, B., Torrent, D., Peiró, R., 2022: Characterization of local Mediterranean grapevine varieties for their resilience to semi-arid conditions under a rain fed regime. *Agronomy* 12, 2234, DOI: 10.3390/agronomy12092234
- Hidalgo, L., 2002: La Viticultura Levantina. In: *Agricultura: Revista agropecuaria y ganadera* 846, 826-833.
- Jiménez, C., Peiró, R., Yuste, A., García, J., Martínez-Gil, F., Gisbert, C., 2019: Looking for old grapevine varieties. *Vitis* 58, 59-60, DOI: 10.5073/vitis.2019.58.59-60
- JCA [Junta Consultiva Agronómica], 1891: Avance estadístico sobre cultivo y producción de vid en España. Ed. Tipoligrafía de L. Péant é hijos. Madrid, Spain.
- 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, DOI: 10.5073/vitis.2018.57.1-8
- Mendoza, K., Aliquó, G., Prieto, J.A., Blas, R., Flores, J., Casas, A., Grados, M., Aybar, L., Torres, M.R., 2022: Prospection and identification of traditional-heritage Peruvian grapevine cultivars (*Vitis vinifera* L.) from Ica and Cañete valleys. *Vitis* 61, 47–51, DOI: 10.5073/vitis.2022.61.47-51
- Ministerio de Fomento, 1882: Congreso nacional de agricultura de Valencia. Expedición a Sagunto. Gaceta agrícola del Ministerio de Fomento. pp. 479-484.
- Peiró, R., Crespo, A., Soler, J., Jiménez, C., Cabello, F., Gisbert, C., 2018: Genetic variability assessment in ‘Muscat’ grapevines including ‘Muscat of Alexandria’ clones from selection programs. *Spanish Journal of Agricultural Research*. 2: e0702, DOI: 10.5424/sjar/2018162-12537
- Peiró, R., Jiménez, C., Perpiñà, G., Soler, J.X., Gisbert, C., 2020: Evaluation of the genetic diversity and root architecture under osmotic stress of common grapevine rootstocks and clones. *Scientia Horticulturae* 266, 109283, DOI: 10.1016/j.scienta.2020.109283
- Piqueras Haba, J., 1985: La agricultura valenciana de exportación y su formación histórica. Instituto de Estudios Agrarios, Pesqueros y Alimentarios. Madrid. Spain.
- Rahali, M., Migliaro, D., Laiadi, Z., Bertazzon, N., Angelini, E., Crespan, M., 2019: Genetic identification, origin and sanitary status of grapevine cultivars (*Vitis vinifera* L.) grown in Babar, Algeria. *Vitis* 58, 153-158, DOI: 10.5073/vitis.2019.58.153-158

San Pedro, T., Peiró, R., Vilanova, J., Olmos, A., Gisbert, C., 2017a: In vitro propagation of the *Vitis vinifera* L. cv. ‘Monastrell’. *Electronic Journal of Biotechnology* 27, 80-83, DOI: 10.1016/j.ejbt.2017.03.006

San Pedro, T., Gammoudi, N., Peiró, R., Olmos, A., Gisbert, C., 2017b: Somatic embryogenesis from seeds in a broad sense of *Vitis vinifera* L. varieties: Rescue of true-to-type virus-free plants. *BMC Plant Biology* 17,226, DOI: 10.1186/s12870-017-1159-3