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The Brazilian grapevine variety called 'Peverella' corresponds to the 'Boschera' variety

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Summary

Through fingerprinting analysis using reference microsatellite markers and comparing with the *Vitis* International Variety Catalogue (VIVC) database, it was possible to discover that in Brazil, the wine grape variety cultivated for over 70 years, called 'Peverella' (Variety number VIVC = 12963; National Register of Vine Varieties (Italy), code: 254) is actually the grape variety called 'Boschera' (Variety number VIVC = 1576; National Register of Vine Varieties (Italy), code: 326). This discovery makes wines produced in Brazil even rarer (with only approximately 10 hectares planted worldwide), presenting an additional means to promote and market this historic variety.

Keywords

Microsatellite markers, fingerprinting, genetic identification, Simple Sequence Repeat (SSR), pedigree, *Vitis vinifera* L.

Introduction

The history of grapevine species and varieties worldwide is closely tied to human civilization, with historical records and citations shedding light on their evolution and paths. Ampelography books like "A Practical Ampelography: Grapevine Identification" (Galet, 1979), were vital before molecular genetics, offering morphometric descriptions for identification (Chitwood *et al.*, 2014). However, subjective factors and environmental influences sometimes posed challenges in accurate identification (Margaryan *et al.*, 2021). Modern DNA analysis and comparison databases have revolutionized grapevine identification, enabling tracing timelines, defining crosses, and identifying origins (Barrias *et al.*, 2023; Biniari and Stavrakaki, 2019; D'Onofrio *et al.*, 2021; De Lorenzis *et al.*, 2013; Milišić *et al.*, 2021).

The largest Italian immigration to Brazil took place at the end of the 19th century and the beginning of the 20th century

(Demartini, 2006). Between 1875 and 1914, about 100,000 Italian immigrants arrived in southern Brazil, particularly in the region today known as *Serra Gaúcha*. They originated mainly from Veneto, Lombardy, Trentino, and Friuli (Mioranza and Frosi, 2009). However, the challenging geographical and environmental conditions of the new land posed obstacles to their traditional agricultural practices, especially viticulture. The introduced *Vitis vinifera* L. species struggled to adapt compared to American and hybrid varieties (De Majo and Moretto, 2021; Ortega and Jeziorny, 2011). In the mid-1900s, efforts to improve wine quality led to the reintroduction of *V. vinifera* varieties through seedling imports (Ortega and Jeziorny, 2011). The widespread adoption of renowned Italian cultivars, such as 'Trebiano', 'Vernaccia', 'Peverella', 'Bonarda', 'Barbera', 'Marzemino', among others, started in the 1950s (Camargo *et al.*, 2011; Cristaldi and Licata, 2015). Subsequently, varietal wines like 'Cabernet Franc', 'Merlot', and 'Riesling Italico' emerged. Yet, a shift in market preferences during the 1970s and 1980s led to the dominance of French-origin international grape varieties ('Cabernet Sauvignon', 'Tannat', 'Sémillon', 'Chardonnay', etc.) (Camargo *et al.*, 2011). However, some farmers preserved 'old' Italian varieties like 'Peverella' amidst this change.

The 'Peverella' variety is considered an ancient variety from the Trentino region (northern Italy). A survey of the historical varieties grown in Trentino, still present in old vineyards or located in marginal areas, led to the recovery of more than 100 genotypes, and among these, 18 different varieties were identified. Among these 18 varieties, DNA analysis of 'Peverella' accessions and the collection of ampelographic and ampelometric data allowed us to define that 'Peverella' in Trentino coincides with the 'Verdicchio Bianco' variety (Stefanini and Tomasi, 2010). From that moment onwards, the variety 'Peverella' became synonymous with the 'Verdicchio Bianco' variety.

A quick comparison of the ampelographic characteristics between the 'Verdicchio Bianco' variety in Italy and the 'Pe-



verella' variety found in Brazil reveals several distinguishing differences, particularly in terms of berry and branch characteristics. This led us first to the question if they are the same variety, followed by the hypothesis that 'Peperella' in Brazil is another variety. Through the fingerprinting analyses of the plant material found in Brazil, it was possible to identify the true-to-type variety, answer the question and confirm our hypothesis.

Material and Methods

Plant Material

Leaf samples were collected from newly sprouted grapevines of the variety named 'Peperella' in the district of São Pedro, in the city of Bento Gonçalves – RS (Brazil), in three different vineyards spread over this territory (representing more than 70% of the area currently planted in Brazil). After collection, the samples were immediately transported to the laboratory for DNA extraction and further genetic analysis.

Genetic Analysis

Genomic DNA extraction from the plants was conducted in the Enology and Applied Microbiology Laboratory (LEMA) of the University of Caxias do Sul, Brazil. DNA extraction from young leaf was performed using the method described by Lodhi *et al.* (1994), with some modifications (Supplementary material). After DNA extraction the samples (10 ng μl^{-1}) were sent to Fondazione Edmund Mach (Italy) for fingerprinting analysis, namely the identification of the trueness-to-type. The latter was carried out optimizing the instructions reported in the Qiagen Multiplex SSR (Simple Sequence Repeats) kit (Qiagen, Germany). The overall molecular information is reported in Table S1 (Supplementary material). Amplicon capillary electrophoresis was carried out in an ABI 3130xl Genetic Analyzer (Life Technologies, CA) and the fragments (alleles) were sized with GeneMapper v4.0 in binning mode, using GeneScan 500LIZ (Life Technologies) size standard as an internal ladder. All analyses were compared with the *Vitis* In-

ternational Variety Catalogue (VIVC) database (<https://www.vivc.de>) (Maul *et al.*, 2023).

Results

True-to-type analysis

As shown in Table 1, the Brazilian 'Peperella' and the Italian 'Boschera' exhibit identical alleles for all the markers, except for VVMD28, which produced inconclusive bands. In contrast, when compared to 'Verdicchio Bianco' (a synonym for 'Peperella'), only one marker (VVMD7) shared the same alleles, while the remaining three markers displayed only one shared allele.

In summary, the results of the fingerprinting analyses showed that the variety planted in Brazil does not correspond to the actual 'Peperella' (Variety number VIVC = 12963; National Register of Vine Varieties (Italy), code: 254) of "Trentino origin" as was assumed; indeed, its genetic profile coincides to that of 'Boschera' (Variety number VIVC = 1576; National Register of Vine Varieties (Italy), code: 326) with probable origin in the Veneto region, where it is still grown today in the province of Treviso.

Bunch and berry characteristics of 'Verdicchio Bianco'/'Peperella' and 'Boschera' and characteristics of 'Peperella'/'Boschera' in Brazil

In Fig. 1-I it is possible to see some differences and similarities between the varieties. One of the most striking characteristics of 'Boschera' is the small brown spots around the berries (Fig. 1-I B and C). Conversely, 'Verdicchio Bianco' typically has smooth skin without these characteristic spots (Fig. 1-I A). Fig. 1-II shows different shapes of leaves and bunches of 'Peperella'/'Boschera' grapes from the vineyards in Brazil where the material for DNA analysis was collected. The leaves of 'Peperella'/'Boschera' grapes display a variety of shapes, as illustrated in Fig. 1-II. Some of the leaves may have an elongated, narrow shape, while others have a more rounded shape. In addition, grape bunches also vary in their structure

Table 1: Comparative Microsatellite profiles of 'Peperella'/'Boschera' (Brazil), 'Verdicchio Bianco' (Italy), and 'Boschera' (Italy).

SSR marker	'Peperella'/'Boschera' (Brazil) this paper	'Verdicchio' (Italy) VIVC 12963	'Boschera' (Italy) VIVC 1576
VVS2	135/135	133/155	135/135
VVMD5	234/248	230/242	234/248
VVMD7	239/247	239/247	239/247
VVMD25	255/255	241/241	255/255
VVMD27	190/195	180/186	190/195
VVMD28	md	236/258	236/236
VVMD32	262/264	252/256	262/264
VRZAG62	196/204	196/196	196/204
VRZAG79	249/251	249/257	249/251

* md = missing data

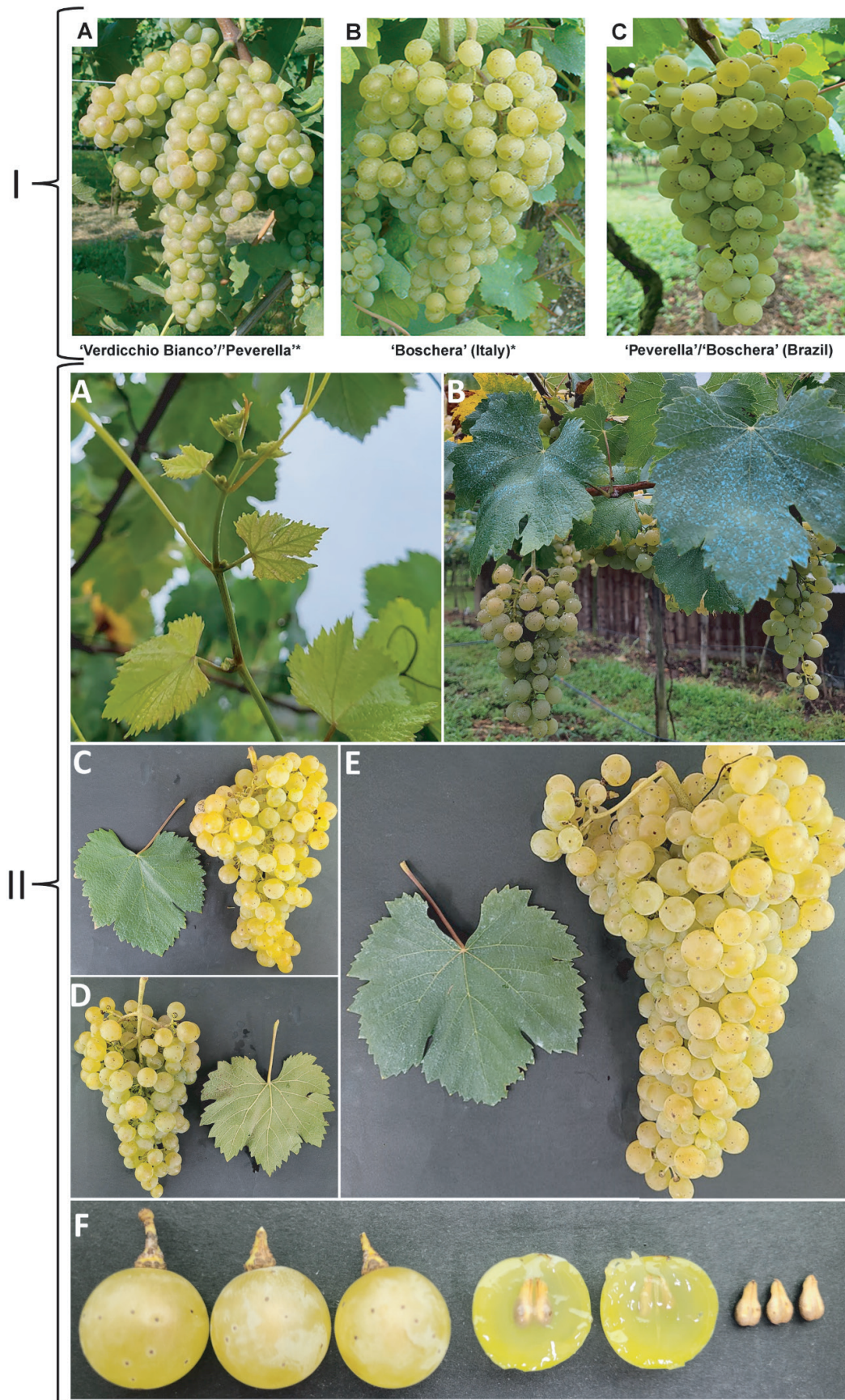


Fig. 1: I – Characteristics of the bunches and berries of varieties. * Photo was taken from the Italian website of the Ministry of Agriculture, Food Sovereignty and Forestry – National Catalogue of Vine Varieties (<http://catalogoviti.politicheagricole.it/result.php?codice=254>; <http://catalogoviti.politicheagricole.it/result.php?codice=326>). It is possible to view more detailed ampelographic details of the two varieties in the photo gallery on these websites. II – Leaf and bunches characteristics of 'Peverella'/'Boschera' grape samples collected in Brazil. Young vine branch (A); Vine leaves and bunches at the time of grape ripening (B); More rounded leaves and bunches with medium compactness (C); Loose bunch and lower side of leaf (D); Upper side of leaf and dense bunch (E); Details of berries and seeds (F).

and appearance. Some bunches can be compact and dense, with tightly clustered grapes, while others can have a looser shape, with spaces between the grapes. Fig. 1 -II F displays the berries detached from the bunch, where the brown dots on the berries can be seen more distinctly.

Discussion

The results of fingerprint analysis with microsatellite markers performed on the samples collected in Brazil (Table 1), revealed that the genetic profile found is equivalent to that of 'Boschera' (Variety number VIVC=1576) microsatellite by profile (vivc.de). 'Boschera' (Variety number VIVC = 1576; National Register of Vine Varieties (Italy), code: 326) has no official registered synonym (Asperti, 2021; BOSCHERA (vivc.de); Registro Nazionale delle Varietà di Vite (politicheagricole.it)). However, in the Veneto region (the possible origin of this grape) one of the many informal synonyms used to name this variety was 'Pevarella' (Calò et al., 1996). This may have caused the confusion with the ancient Trentino variety called 'Peperella'. Furthermore, in the book *Ampelografia generale della provincia di Treviso* (1870) (Costacurta and Michelet, 2021), one of the synonyms mentioned of the 'Boschera' grape is 'Peperella moscatella', which may also contributed to the spreading of the name 'Peperella' when this grape arrived in Brazil. In a book published in 2013 about grape varieties for wine making (Robinson et al., 2013), the authors put 'Boschera' as being a synonym of 'Verdicchio Bianco', but nowadays it is known that they are not the same variety.

Historical and genetic studies indicate that the grape 'Boschera' is a grape of Veneto origin (northern Italian region), more precisely from the province of Treviso, as the cities of Vittorio Veneto, Conegliano and surroundings, have ampelographic reports describing this variety since 1870 (Costacurta and Michelet, 2021). According to a study on varietal relatives of Italian varieties (D'Onofrio et al., 2021), this history begins in the southwest of the Italian peninsula with 'Visparola', which is the genitor of a multitude of native Italian varieties. This variety then migrated to the north of the country along the eastern side, giving rise to several varieties, including 'Vulpea'. In turn, 'Vulpea' already in the territory of north-eastern Italy gives rise to several other varieties, including 'Boschera' and 'Glera' (Prosecco) for example (D'Onofrio et al., 2021).

The name 'Boschera' is derived from the Italian word *bosque*, meaning grove, alluding to its robust growth near forests. This variety demonstrates tolerance to fungal infections like *Peronospora* and *Botrytis* (Costacurta and Michelet, 2021). Despite the limited presence of vineyards in Italy (approximately 6 hectares in 2010) (Asperti, 2021), 'Boschera' is significant in Treviso (Veneto), being a key component, along with 'Glera' and 'Verdiso', in the production of a *passito* wine (dessert wine made from dehydrated grapes) known as Torchiato di Fregona (Colli di Conegliano DOCG). 'Boschera' stands out for its thick skin, which makes it easier to dehydrate, and its high acidity allows it to produce sparkling wines (Asperti, 2021; Costacurta and Michelet, 2021). In Brazil, this variety plays a significant historical and cultural role, and despite the current limited cultivation area of approximately 3 hectares (Mello et al., 2017), historical records indicate that this variety played a

pivotal role in producing the initial white wines from *V. vinifera* grapes in Brazil (Giovannini and Vitor, 2009). The discovery that the variety known as 'Peperella' in Brazil is 'Boschera', not 'Verdicchio Bianco', presents numerous opportunities for Brazilian producers. This revelation allows them to enhance the value of their products even more, considering 'Boschera' is exceptionally rare in the world of wine.

Conclusion

Through DNA analysis and visual comparisons of grape varieties, it was determined that the grapevine variety known as 'Peperella', found in the southern region of Brazil, is the variety known as 'Boschera' (Variety number VIVC = 1576; National Register of Vine Varieties (Italy), code: 326). This significant discovery holds immense potential for grape producers in Brazil, particularly considering the historical significance of this grape in the region and the relatively limited number of hectares still cultivated worldwide.

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Conflicts of interest

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

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