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'Cabernet Gernischt' is most likely to be 'Carmenère'

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Summary

Using a set of 32 microsatellite markers, 'Cabernet Gernischt' has been proven to most likely be 'Carmenère', an old grape cultivar from France, and the progeny of 'Cabernet Franc' and 'Gros Cabernet' has been confirmed. In addition, six 'Cabernet Gernischt' clones with different agronomic traits were identified.

Key words: microsatellites, Cabernet Gernischt, parentage, clone identification.

Introduction

In recent years, China has become one of the important countries in wine production and consumption in the world. According to a report from the organization of the international wine and spirit exhibition (VINEXPO), the production and marketing of Chinese wine was ranked seventh in the world in 2011. In 1892, the Changyu Wine Company was established in China, which strengthened the wine trade between China and other wine producing countries in the world and started a new age of Chinese wine industry. In addition, the Changyu Wine Company introduced a number of wine cultivars, one of which was named 'Cabernet Gernischt' by the Austrian winemaker von Babo of the Changyu Wine Company. However, the name 'Cabernet Gernischt' does not resemble to other languages, including German. Later, it was confirmed that the word "Gernischt" was actually the word "Gemischt", which means "mixture" in German. Therefore, 'Cabernet Gernischt' in fact, indicates a Cabernet cultivar of uncertain origin. 'Cabernet Gernischt' is called 'Cabernet Shelongzhu' in Chinese, and has become the most important red wine cultivar together with 'Cabernet Sauvignon' and 'Cabernet Franc'. The identify of 'Cabernet Gernischt' has recently gained great interest among grape researchers: (1), it was speculated to be 'Cabernet Franc' based on its morphological characters (LUO, 1999). (2), it has been found to be genetically close to 'Cabernet Franc' with RAPD and SSR markers (SONG *et al.* 2005, YAO *et al.* 2005). And (3), it was considered more likely to be the old variety 'Carmenère' employing RAPD molecular markers (LI *et al.* 2008). Therefore, to date, there has not been enough substantial evidence found to confirm the origin of 'Cabernet Gernischt'.

Additionally, a number of grape clones called 'Cabernet Gernischt' have very similar morphologies, but differ in some agronomic traits. Hence whether these clones are mutants of 'Cabernet Gernischt' that arose during its long-

term cultivation also needed to be determined. In addition, the importance of these clones in modern viticulture makes their identification crucial for wine grape nurseries and also for the wine industries (MONCADA *et al.* 2007).

In this work, the origin and parents of 'Cabernet Gernischt' were confirmed, and the genetic relationships between 'Cabernet Gernischt' and its possible mutants were analyzed by examining SSR markers, which are widely used for grape genealogical analyses (WÜNSCH and HORMAZA 2002, VOULLAMOZ *et al.* 2007, PELSUY *et al.* 2010).

Material and Methods

Plant materials: 'Cabernet Sauvignon', 'Cabernet Franc', 'Merlot', 'Carmenère', 'Gros Cabernet' and seven 'Cabernet Gernischt' clones were collected from the vineyards of different regions in China listed in the Table.

DNA isolation: DNA was extracted from young expanding leaves according to the method described by DOYLE and DOYLE (1990). The DNA quality was evaluated in a 0.8 % agarose gel stained with ethidium bromide, and the DNA concentration was measured with a NanoDrop2000 (USA).

Microsatellite (SSR) analysis: The analysis was based on 32 microsatellite markers (VCHR2a, VVMD7, VCHR3a, VCHR5a, UDV009, VCHR18d, UDV024, UDV038, UDV048, UDV052, UDV122, UDV067, UDV069, UDV073, UDV074, UDV100, UDV106, UDV112, UDV060, VCHR6a, VMC4H5, VMC4A1, VMC4A5, VMC4C6, VMC4D2, VMC4D4, UDV046, VMC4F3, VMC4G6, VMC4H6, VrZAB62, VrZAG79). PCR reactions were performed using a mixture (25 µl final volume) containing 12.5 µl of 2X BenchTop Taq Master Mix (Biomiga, USA), 4 ng of genomic DNA and 0.3 µM of each primer. The amplification conditions were adjusted to an initial denaturation step of 8 min at 95 °C, followed by 5 cycles of 40 s at 95 °C, 40 s at 48 °C and 90 s at 72 °C, and a final extension for 5 min at 72 °C before stopping the reaction.

The amplicons were separated and stained: 5 µl of the PCR product was first tested on a 1.2 % agarose gel. Then, the amplicons were denatured at 94 °C for 2 min in a buffer containing formamide and loaded into a sequencing gel (6 % polyacrylamide, 1X TBE, 7 M urea). The gel bands were revealed by silver staining, as indicated in CRESPIAN and MILANI (2001).

Data analysis: The coefficient of similarity was calculated using the SimQual procedure of the NTSYSpc V 2.0 program (ROHLF 1997). The genetic divergence was

Table

The tested grape cultivars/clones and their sources. 'Cabernet Gernischt' 1-6 indicate the six clones of Cabernet Gernischt. The clone characteristics were obtained by comparing 'Cabernet Gernischt' 1-6 with Cabernet Gernischt, respectively

Cultivar/clones	Clone characteristics	Source
Cabernet Gernischt	Standard agronomic traits	Beigou, Penglai city, Shandong Province, China
Cabernet Gernischt 1	High total soluble solids	Shangri-La, Penglai city, Shandong Province, China
Cabernet Gernischt 2	High acidity	Xingang, Penglai city, Shandong Province, China
Cabernet Gernischt 3	Small berry	Mozhikou, Penglai city, Shandong Province, China
Cabernet Gernischt 4	Large grape bunch	Zhaogezhuang, Penglai city, Shandong Province, China
Cabernet Gernischt 5	Large berry	Magezhuang, Penglai city, Shandong Province, China
Cabernet Gernischt 6	Strong growth vigor	Chateau State Guest, Penglai city, Shandong Province, China
Cabernet Sauvignon		Shangdong Agriculture University, Tai'an city, Shandong Province, China
Cabernet Franc		Shangdong Agriculture University, Tai'an city, Shandong Province, China
Merlot		Shangdong Agriculture University, Tai'an city, Shandong Province, China
Carmenère		Shangdong Agriculture University, Tai'an city, Shandong Province, China
Gros Cabernet		The Fruit Research Institute, Shanxi Academy of Agricultural Sciences, Taigu county, Shanxi Province, China

visualized using the unweighted-pair group-method average (UPGMA) clustering method (SNEATH *et al.* and SOKAL *et al.* 1973).

Results and Discussion

The origin of 'Cabernet Gernischt' has been a confusing topic since this cultivar appeared in China in the 1890s. Previous studies have indicated that 'Cabernet Gernischt' is genetically and morphologically similar to 'Carmenère' and 'Cabernet Franc' (LI *et al.* 2008, YAO *et al.* 2005). 32 SSR markers were screened from 54 markers for their potential to encompass the genetic diversity among 'Cabernet Sauvignon', 'Merlot', 'Cabernet Franc', 'Cabernet Gernischt', 'Carmenère' and 'Gros Cabernet' (data not shown). The genomic similarity among the above cultivars, *i.e.*, from 0.28 to 0.68, indicated the high capacity of these SSR markers to distinguish these cultivars. The 32 SSR markers produced completely identical allelic patterns for 'Cabernet Gernischt' and 'Carmenère', corresponding to 100 % similarity (Figure). In addition it was confirmed that 'Cabernet Gernischt' shares one allele with 'Cabernet Franc' and another with 'Gros Cabernet' in every locus analyzed except for

VMC4D4. The discrepancy at VMC4D4 may be explained by the possibility that a null allele exists at the VMC4D4 locus in 'Gros Cabernet'. Interestingly, Carmenère is sometimes confused with 'Cabernet Franc' in Italy (GALET 2000, PSZCZOLKOWSKI *et al.* 2000).

'Carmenère' originated from France and was once widely cultivated in southwest France. It has been classified together with 'Cabernet Sauvignon', 'Merlot', 'Petit Verdot' and 'Fer' into the Carmenet elite group, one of the 13 sortotypes (or ecogeographical groups) distinguished in France according to morphological traits (BISSON 2009). However, 'Carmenère' was almost lost after the Phylloxera crisis and also because of low yields caused by coulure in France (VIALA and VERMOREL 1901-1910, GALET 2000). 'Carmenère' has spread into other countries (MONCADA *et al.* 2007). There were 8,826.7 ha cultivated in Chile according to the "Servicio Agrícola y Ganadero (Chile), Catastro vitícola nacional 2009". Since it was ascertained that 'Cabernet Gernischt' is most likely 'Carmenère', China, became another important area of 'Carmenère' cultivation. Twenty-six accessions of 'Carmenère' from Chile, France and Italy were found and clustered into three groups using SSR and AFLP (MONCADA *et al.* 2007). Similarly, several possible 'Cabernet Gernischt' clones, which differed

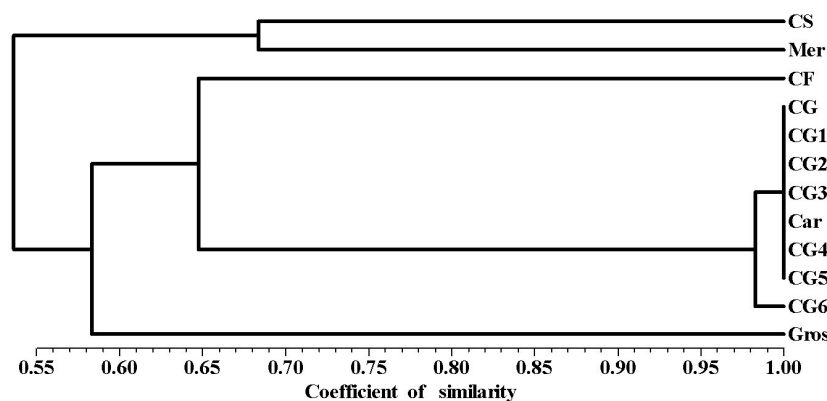


Figure: Dendrogram representing the genetic similarities among 'Carmenère' (Car), 'Cabernet Franc' (CF), 'Gros Cabernet' (Gros), 'Cabernet Gernischt' (CG) and six 'Cabernet Gernischt' clones (CG1-6). 'Cabernet Sauvignon' (CS) and 'Merlot' (Mer) are used as the control genotypes.

in terms of their agronomic performances, were found in different vineyards of the Peng Lai region, Shandong Province, China, in this study (Table).

To verify their true to type identify these clones, 'Cabernet Gernischt', used as a standard genotype, and the six possible 'Cabernet Gernischt' clones ('Cabernet Gernischt' 1-6), were compared by the same 32 SSR markers, 'Cabernet Gernischt' and 'Cabernet Gernischt' 1-5 shared 100 % similarity at all 32 SSR loci (data not shown, Figure). In contrast, 'Cabernet Gernischt' 6 had identical (100 % similarity) SSR loci with 'Cabernet Gernischt' and the other clones, except at VMC4H5 locus, for which four alleles were detected. Thus 'Cabernet Gernischt' 6 shared 98.3 % similarity to 'Cabernet Gernischt' and the other clones. Taken together, the results indicate that 'Cabernet Gernischt' 1-6 are all 'Cabernet Gernischt' and their morphological differences probably result from spontaneous somatic mutations, which occurred during their long-term cultivation (PELSY *et al.* 2010). VMC4H5 was the only SSR marker able to differentiate 'Cabernet Gernischt' 6 from the other clones. Hence, VMC4H5 can be used for identification of 'Cabernet Gernischt' 6.

Conclusion

In this paper, screening of 32 highly polymorphic SSR markers strongly supports the likelihood that 'Cabernet Gernischt' from China is the French grape cultivar 'Carmenère' and that its progeny of 'Cabernet Franc' and 'Gros Cabernet' was confirmed. In addition, 'Cabernet Gernischt' clones with distinct agronomic traits have been generated during the long-term cultivation of 'Cabernet Gernischt'.

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