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Evaluation of Muscat types and clones for the local market

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Summary

Ten European clones and selections of 'Muscat blanc' were investigated about their variability in genetic and enological behavior. The SSR profile of about 120 loci allowed to differentiate most of them. Nevertheless the differences within the variety are rare and do not reflect the heterogeneity of observed variability. One of the clones (B41/5) represents an earlier ripening type where the overall quality was independent from sugar content. A 27-1 clone was one of the clones with steady high rating concerning wine quality. An interesting alternative for early ripening areas could be the late ripening 'Goldmuskateller' due to the higher stability against Botrytis. One of the samples was not true to type and represented this cultivar.

K e y w o r d s : grapevine; *Vitis vinifera*; simple sequence repeats; genetic relationship; sensorial evaluation.

Introduction

The Muscat varieties belong to a huge and far spread family. Different cultivars with high diversity at the geno- and phenotypic level share the specific flavor of the Muscat aromas and were summarized as Muscats (Bronner 2003, Rusjan 2010). The region around the Caspian Sea is supposed to be the origin of the Muscat family (Ambrosi *et al*. 1994). Negrul (1946) classified them as *Proles caspica*. Phenicians and Greeks were responsible for the wide spread propagation of this variety (Hoffmann 1982). Furthermore the Romans produced Muscat wines and distributed material to their provinces. In the region around Frontignan since Roman times Muscat vines are established (Bassermann-Jordan 1975).

Plinius defined the cultivar as '*Uva apiana*'. It is an indication for the sensitivity of this variety to attacks from wasps and bees. Today´s name could stem from this sensitivity whereby it derived from musca or mouche (= fly). More realistic is that the very characteristic flavour of the berry designated as muscus or moscado (= moschus) is the reason for the name (Hoffmann 1982).

In Austrian viticulture Muscat varieties were frequently used as the most important contributor of flavor to wines mainly of mixed varieties or sometimes in blends. The ancient name was not identical with today's name and it could usually be detected behind the former synonyms of 'Weihrauch' and 'Schmeckender' (Goethe 1887). Other Muscats for instance the 'Grünmuskateller' have nothing to do with today 'Muscat blanc'. Due to the monovarietal plantings in cooler years the quality and finally the importance of the variety decreased. In several cases 'Muscat blanc' was replaced by 'Muscat Ottonel'. Due to global climate change nowadays 'Muscat Ottonel' is continuously replaced and 'Muscat blanc' is becoming more popular again. According to the last statistics in Austria 600 ha are available. That means a threefold multiplication within the last decade (www.weinausoesterreich.at).

The propagation and selection at different locations rendered numerous varieties, types, clones and especially synonyms. Ambrosi *et al.* (1994) mentioned that about 200 varieties of Muscat are described. On the other side many more synonyms exist for 'Muscat blanc' and other Muscat varieties than for any other cultivars. One of the reasons for synonyms was the tradition to add the name of the region where the grapes were grown to the wine. The following names which are still used demonstrate the false diversity: 'Muscat d'Alsace', 'Muscat de Frontignan', 'Moscato d'Asti', 'Muscat de Samos' etc. (Bronner 2003). All these accessions are corresponding with 'Muscat blanc á petits grains'.

A further reason for diversity in grapevines are the rather frequent mutations in berry color. Meanwhile the structure of different pigment colors as rose, grey and noir are well studied (Pelsy 2010). Even Muscat varieties with red berry color are showing the common main genetic profile of the white variants (Kerridge and Antcliff 1999). Despite intense efforts with ampelographical methods in the past, the relationship of Muscat varieties could be highlighted recently only by applying genetic markers (Crespan and Milani 2001, Costacurta *et al*. 2003).

In comparison to others the Muscat varieties contain higher amounts of primary flavor compounds. These substances are not modified during fermentation and are therefore available in the wine (Prillinger and Madner 1970). As main substances responsible for the Muscat flavor are mentioned the terpenes Linalool, Linalool oxide, alpha-Terpineol, Geraniol and Nerol. The occurrence of Linalool and Linalooloxide is not limited to Muscat varieties but can also be found in the flavor of Riesling, Traminer and Scheurebe (Rapp 1992). Due to the more or less identical flavor of different Muscat varieties it is realistic that only one origin for the specific trait of terpene formation exists (Battilana *et al*. 2011). The true Muscat varieties have inherited the locus from this single source. An easy estimation regarding inheritance of Muscat genetics could be performed at the frequently used locus Vrzag79. Within

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V. vinifera all true Muscats contain an allele with the length n +18 at the locus (Regner *et al*. 2004). Grando *et al*. (2003) could localize the trait of the terpene formation to the linkage group 5 and defined further SSR markers with a close position to the terpene relevant genes.

For the local production 'Muscat blanc' requires favored areas protected from wind to improve the maturation development. In cooler seasons the wine is characterized by higher acidity and spicy aromas. Ideal soils for Muscat have a good drainage, warm up easily and have a not too fertile structure. Higher chalk content increases the risk of chlorosis. Sensitivity to Botrytis is closely linked to availability of water and therefore risk of berry burst. Vines are very sensitive to powdery and even sensitive to downy mildew (Hillebrand *et al*. 1997). Under specific circumstances the vines show symptoms of incompatibility to several products for plant protection. Aim of this study was to find Muscat types with high wine quality, typical Muscat aroma and better suitability for local production especially concerning lower sensitivity to fungal diseases.

Material and Methods

Plant material: For the comparison of different Muscat clones 11 different genotypes could be purchased from the local nurseries (Tab. 1). Three of them represent standard material as selections of individual nurseries. All other material meets the requirements of certified clones. One genotype was not true to type and could be identified during the analysis as a clone of 'Goldmuskateller'. Other Muscat types are maintained within the collection of the HBLA u BA Klosterneuburg.

Experimental field: The area where the clones were planted in a random way with 4 times 25 vines is located on a hill with a 15 % gradient oriented to the south close to Vienna. The soil is a disintegrated sandstone with a high content of clay and a medium absorption capacity. Dry summer conditions and a high pH value of the soil favored the use of Kober 5BB as rootstock. The space per vine was 3 x 1.2 m and therefore less than three thousand plants per ha. Canopy was built in the usual way with a trunk height of 90 cm and formation of one fruiting cane. During 6 years of evaluation no fertilizer was applied. Plant protection was done in an integrated way and the rows contained a cover crop. Other Muscat varieties were planted in the same location nearby but without the clonal experimental plant.

Evaluation of clones: The yield was measured and samples at harvest were analyzed for sugar and acidity content. Phytosanitary evaluation was performed two times per season by visual observations of ten vines per clone. Clonal wines were produced in the years 2008 to 2013 and tasted as young wines. The wines were rated using an unstructured scale and one value for all aspects of the quality. Each wine was tasted 4 times in a blind tasting together with 3 other clonal wines. The final result for a specific clone is the summary of 8 tasters with 4 repetitions per season, which means 32 single evaluations.

Genetic diversity: Genetic characterization of Muscat types and clones was performed with the specific 9 genetic markers selected during an EC project (This *et al*. 2004). The 11 Muscat accessions for the clonal comparison were analyzed using 120 SSR markers. The VVS markers were developed by Thomas and Scott (1993) and the VVMD markers by Bowers *et al*. (1996) as well as by Bowers *et al*. (1999). The VRZAG markers (Sefc *et al*. 1999) and VRG (Regner *et al*. 2006). were obtained from investigations into simple sequence repeats of *Vitis riparia.* UCH, VVIP, VVIV and other markers were obtained by the SSR consortium for grapevine and available at the genome database of www.ncbi.nlm.nih.gov.

DNA from the Muscat samples were extracted from young leaves by following the protocol described by Thomas *et al*. (1993) modified by Regner *et al*. (1998). The grapevine clones were evaluated according to the OIV descriptors and the data were compared for their pronounced morphology. The leaves were measured with the help of a digitizer tablet and the calculation of the parameters was performed with a self written software (data not shown). Phylogenetic relationship was estimated by calculating with SSPS cluster analysis and using the PhyQuest program for demonstration of the tree.

Results and Discussion

Phytosanitary evaluation reveals no significant differences within the clones in the sensitivity to powdery and downy mildew. Symptoms of the diseases have appeared in situations of higher infection pressure but no differences could be detected between the clones concerning mildew diseases. Damages or depletion due to deer, wasps and birds could be registered. In the case of birds the early ripening clone B 41/5 was more affected than the others. Due to dense bunches under bad weather conditions all clones were infected by Botrytis while the accession 'Goldmuskateller' was not infected. 'Goldmuskateller' shows loose clusters and ripens later than 'Muscat blanc'.

Density of must could be differentiated between the clones. Especially in years with late maturation (as 2010 and 2013) the clones differ significantly. Lower sugar content was analyzed in the selections Högl and Dreisiebner as well as clone 156 from INRA and 'Goldmuskateller'. As a later ripening genotype 'Goldmuskateller' did not reach sufficient maturity every year. Hence it cannot be recommended for cooler production areas. The clone 41/5 reached highest maturity but sometimes already lacked acidity. Consequently the quality of the wine is not as high as the sugar level would implicate. The highest acid content was found in 'Goldmuskateller' followed by the selections Dreisiebner and Slowenien and by the clone 453 from INRA. The differences in yield showed high variability and therefore it was not possible to get significant results. However, the selections Högl, Dreisiebner and Slowenien as well as the clone Fr 74 have a tendency to grow a smaller amount of grapes. They could not reach more than 2 kg per vine on average.

Sensorial evaluation of all 11 accessions resulted in clear differences of one vintage (Fig. 1). The recent wines from 2013 could be classified within four groups. Group 1 is formed by clone A 27-1, Fr 94, INRA 156 and WE H1 while in the second group B41/5, Sel. Dreisiebner, INRA 826 und 453 and 'Goldmuskateller' were defined as wine with medium quality. The selections Högl and Slowenien could not fulfill the demands for high quality and could be differentiated from all the others. In the years with hot temperature during the ripening process 'Goldmuskateller' could trump all other clones. The result is a confirmation that certified clones are able to produce the same high quality as standard selections. Several local growers express the opinion that monoclonal vines could not reach the same high quality as selections with a broader genetic base will do. In this evaluation process the selections failed to reach the same quantity or quality. Hence these results provide good arguments for the use of certified clones.

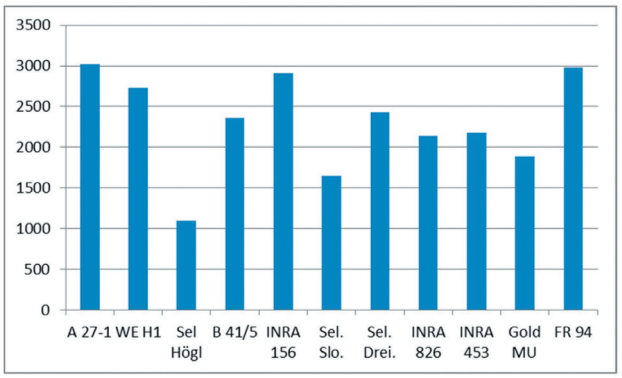

Fig. 1: Sensorial evaluation of 10 Muscat clones and 'Goldmuskateller' of the vintage 2013. The value represents the summary of 32 single evaluations performed with an unstructured scale.

All eleven accessions of Muscat (Tab. 1) were also analysed by genetic markers for detecting genetic polymorphism. As the reproducibility is perfect with SSR markers we used 120 loci to genotype the clonal material. The VVS, VVMD and VRZAG markers often used in varietal identification of grapevines could not detect any polymorphism. All ten Muscat clones showed the same profile by using these standard SSR loci. Nevertheless with this data the "trueness to type" could be confirmed. Other SSR loci as some of the VRG, UCH, VVIP and VVIV markers resulted in different allelic profiles (Tab. 2). Due to the long

Table 1

Muscat blanc clones, their origin and specific traits

Clones/Selection	Breeder/Origin	Source/Nursery	Traits / aim of selection
A 27-1	VÖR- Verband Österreichischer Rebveredler (A) assoc. of AT nurseries	Nursery Gangl, Klöch Styria(A)	Less density of prostate hairs at the shoot tip, little anthocyanin coloration of main veins, Higher density of erect hairs between veins; smaller bunches
B 41-5	Verein Burgenländischer Rebveredler (A)	Nursery Schellmann, Auersthal (A)	Short tendrils, U-shaped petiole sinus, smaller and looser bunches
WE H1	LVWO Weinsberg (D)	Scheiblhofer Vines, Andau (A)	More vigorous, larger and dense clusters, smaller berry size
FR 94	Staatliches Weinbauinstitut Freiburg (D)	Nursery Iby, Neckenmarkt (A)	Stability during blooming, looser clusters,
INRA 156	Institut National de Recherche Agronomique, Aveyron (F)	Nursery Tschida, Apetlon (A)	Smaller bunches, lower yield, lower sugar and terpene content of berries
INRA 453	Institut National de Recherche Agronomique, Drôme (F)	nursery Tschida, Apetlon (A)	Higher crop yield, smaller berry size less sensitivity to Botrytis, higher sugar and terpene content
INRA 826	Institut National de Recherche Agronomique, Pyrénées-Orientales (F)	nursery Tschida, Apetlon (A)	Very typical Muscat phenotype
Goldmuskateller	Südtirol	nursery Tschida, Apetlon (A)	Larger grapes with looser berries
Sel. Dreisiebner	winery Dreisiebner	Kober & Kohlfürst, Kottingbrunn (A)	No information available
Sel. Slowenien	no Information	nursery Hafner, Mönchhof (A)	No information available
Sel. Högl	Winery Högl	Nursery and winery Müller, Krustetten (A)	No information available

Table 2

Genetic differences of 10 accessions of 'Muscat blanc' and one 'Goldmuskateller' with SSR marker

Genotype	VRG3	VRG15	UCH19	VVIP 36A	VVIV 35
Sel Slowenien	-	342	192/198/204	338	-
Sel Dreisiebner	215/240	-	192/198/204	-	-
Fr 94	215/240	142/149	192/198/204	338	-
INRA 826	215/240	139/149	192/198/204	338	-
INRA 453	215/240	231/342	192/198/204	338	105/137
INRA 156	215/240	342	192/206	338	105
B 41/5	215/240	-	192/198/204	338	105/137
A 27-1	215/240	342	192/198/204	338	105/137
Sel. Högl	215/240	342	192/198/204	338	105/137
WE H1	215/240	342	192/198/204	338	105/137
Goldmuskateller	240	342	194/204	338/348	-

period of using Muscat varieties it was estimated that one would find much more polymorphism comparable to other traditional varieties such as 'Traminer' or 'Pinot' (REGNER *et al* 2006), The 10 true to type 'Muscat blanc' could not completely be differentiated. The profiles of clone A27-1, We H1 and selection Högl were not discernible with involved SSR markers. All other clones or selections were characterized by an individual genetic profile.

The polymorphic alleles could be reproduced in a second trial. The genetic differences are not surprising however the possibility to find some deviations located in the range of an SSR marker enabled us to use them for identification. The occurrence of null alleles is one main source of polymorphic SSR loci. Mutations at the annealing side can easily inhibit the amplification of the allele. A null allele was accepted if the second trial results were the same. PCR protocol, however was not changed to achieve easier annealing conditions. The formation of new alleles is rare but could also be observed. When the size was out of the frame of the locus a larger rearrangement in the genome could have taken place. Few of them represented a third allele and could be created by crossing over or chimera.

All these deviations render possible an identification system of clones within the variety.

Polymorphism was used to calculate proximity and to form cluster of closer related clones (Fig. 2). The genetic relationship of the clones was expressed by a dendrogram. As the genetic polymorphism represents a deeper insight into the genome than the differences in morphology it is not surprising that the outcome is not identical.

The tool of genetic analysis to verify clonal variation is a perfect method for the breeding process. Most growers prefer not to cultivate the variety but clonal material of a traditional cultivar. In some countries with controlled production systems it is obligatory to use specific clones to have the wine accepted for the common (PDO or PGI) labelling.

Hence if the genetic analysis reveals that the genetic base of an individual clone differs from all other registered ones the individual character is shown. For any kind

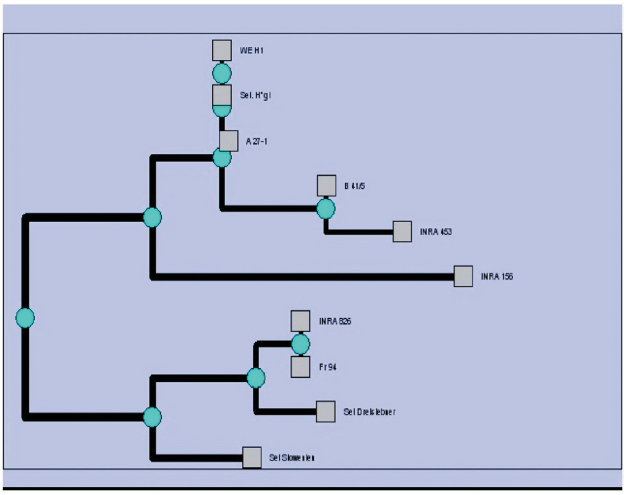

Fig. 2: Dendrogram of 10 Muscat clones calculated with SSPS hierarchical cluster analysis and constructed by program PhyQuest.

of protection additionally the uniformity and the stability have to be considered. In the past these criteria could not be evaluated for clones. Despite genetic or morphological differences the differentiation of clones is neglected by international law for plant protection. The genetic markers, however enable us to observe these parameters during propagation. At the moment the International Union for Protection of Varieties (UPOV) does not accept genetic differences as the only criteria for distinctness. The main argument is that the sequence as long as it is not relevant for the morphology will not be considered. Therefore this genetic information about a clone allows to prevent duplicates in clonal collections and make it possible to control the clonal identity. Whatever the grapevine community decided to do with the clonal identification it has never before been easier to identify clonal material.

As a conclusion of these field experiments we can deduct that ´Muscat blanc` needs careful plant protection and is not a variety useful for extensive viticulture. Neglecting

the appreciated wine quality it could not be recommended for new plantings. On the other side the market for fresh and fruity Muscat wines is still growing. An alternative for warmer areas and good terroirs could be ´Goldmuskateller` with better stability in the production, higher yield and quality. Recently additional Muscat varieties and clones were investigated. In the case that despite all obstacles 'Muscat blanc' will be chosen, certified clone A 27-1 will be a better choice than selections.

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