

## Grapevine cultivar Müller-Thurgau and its true to type descent

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### Summary

**Ampelographic investigations supported by molecular marker analysis were used to reevaluate the progenitors of the grapevine cv. Müller-Thurgau. From these studies we conclude Müller-Thurgau to be a descendent of the offspring of a Riesling and Madeleine Royale (syn. Königliche Magdalentraube) hybridisation. The results reveal the importance of true to typeness of the grapevine varieties used for genotyping, parentage analyses, etc.**

### Introduction

The grapevine cv. Müller-Thurgau is known to be crossed at Geisenheim at the "Königliche Lehranstalt für Obst- und Weinbau" by Prof. Dr. Dr. h.c. HERMANN MÜLLER-THURGAU in 1882 (BECKER 1982). In 1891 MÜLLER-THURGAU became director of the "Eidgenössische Lehr- und Versuchsanstalt" at Wädenswil, Switzerland, and thus carried over cuttings of 150 preselected seedlings. One of these seedlings, breeding No. 58 (Riesling x Silvaner 1), was selected and propagated in 1897 for the first time. A few years later, in 1913, AUGUST DERN, a grapevine inspector in Franconia, brought cuttings of No. 58 which he named "Müller-Thurgau" back to Germany. Between 1920 and 1930 vines of Müller-Thurgau were grown on experimental plots in all German wine-growing regions. After World War II Germany needed an early ripening grape cultivar, easy to cultivate and with high yield stability. Elegant Müller-Thurgau wines were produced by reductive winemaking and the cultivar spread rapidly all over German vineyards. During the early seventies until the middle of the nineties the growing area of Müller-Thurgau exceeded that of Riesling. Müller-Thurgau still covers more than 20 % of today's vinegrowing area in Germany and is grown in several other countries, too (BECKER 1982).

Concerning the descent of Müller-Thurgau as a Riesling x Silvaner cross doubts raised early: MÜLLER-THURGAU himself replied in a letter to DERN that the cultivar brought to Franconia in 1913 was not the result of the first Riesling x Silvaner cross at Geisenheim (EICHELSBACHER 1957). Therefore there has been much speculation about the true parents (HILLEBRAND *et al.* 1997). Many specialists suggested that Müller-Thurgau was a selfing of Riesling. EICHELSBACHER (1957) indicated that Silvaner could not be a parent of Müller-Thurgau, comparing leaf and tip mor-

phology of Riesling, Silvaner, Müller-Thurgau and Rieslaner with those of selfings and backcrosses. In 1994, his hypothesis gained strong support by means of RAPD analysis, confirming (1) Riesling as one parent, (2) making the selfing hypothesis unlikely and (3) excluding Silvaner as the second parent (BÜSCHER *et al.* 1994). Analysis of microsatellite loci excluded that Riesling selfing generated Müller-Thurgau and confirmed that Silvaner is not a parent (THOMAS *et al.* 1994). In 1996, REGNER first proposed a cultivar of the Chasselas (Gutedel) family as the second gene donor. Microsatellites at 7 of 8 loci corresponded to the allele lengths of the Chasselas family (REGNER *et al.* 1996). A more detailed microsatellite analysis (SEFC *et al.* 1997) pointed to Admirable de Courtiller (syn. Chasselas de Courtiller), a table grape of minor importance, bred in the 19th century by Courtiller, director of the Botanical Garden in Saumur, France. In combination with the microsatellite pattern of Riesling the genotype of Müller-Thurgau could be explained at 24 different microsatellite loci (SEFC *et al.* 1997).

However, doubts about the proposed parentage still remained. According to the principles of heredity, characteristics of the parents are manifested in their offsprings. But from its phenotype Müller-Thurgau shows no similarities with Admirable de Courtiller though its genotype could be explained based on microsatellites. Since 5-10 % of the grape cultivars in grapevine collections are known to be not correctly annotated (DETTWEILER 1992), it might well be possible that a misnaming has occurred. This hypothesis has been addressed by ampelographic and by microsatellite analyses.

### Material and Methods

Herbarized leaves designated as Admirable de Courtiller were obtained from Klosterneuburg, Austria. For visual diagnosis and comparison leaves of true to type cultivars in the herbarium at Geilweilerhof were used and ampelographic literature with ampelographic descriptions and images of the cultivar typus was consulted (GALET 1964, 1990).

For molecular analysis young leaves of 5 cultivars, Madeleine Royale, Riesling White, Müller-Thurgau, Admirable de Courtiller and Silvaner White (all true to type) were collected from the Geilweilerhof collection. DNA samples of each cultivar were prepared according to the protocol of THOMAS *et al.* (1993). Microsatellite analysis

was performed at 6 microsatellite loci VVS2 (THOMAS and SCOTT 1993), VVMD5, VVMD7, VVMD27 (BOWERS *et al.* 1996), and *ssrVrZAG62*, *ssrVrZAG79* (SEFC *et al.* 1999). Three-step PCR was performed in 25  $\mu$ l of reaction volumes using a Gene Amp PCR System 9600 (Perkin Elmer) following the protocol of CRESPIAN *et al.* (1999). The PCR reaction mixtures contained 20–40 ng of template DNA, 1 U Taq DNA Polymerase (Roche Molecular Biochemicals, Mannheim, Germany), buffer (final concentration: 10 mM Tris-HCl, 1.5 mM MgCl<sub>2</sub>, 50 mM KCl, pH 8.3 (20 °C)), 200  $\mu$ M of each dNTP, 10 pmoles of each primer for the *ssrVrZAG* loci and 20 pmoles for the VVMD and VVS2 loci, respectively. For the electrophoresis, 2.0  $\mu$ l of each PCR reaction were denatured at 94 °C for 3 min, loaded on a 8 % polyacrylamide/urea sequencing gel and visualized by silver staining according to Promega and modified according to ECHT *et al.* (1996). Allele sizes were determined using defined size markers.

### Results and Discussion

It is a well known phenomenon that plant genetic resource collections suffer to a certain degree from misnamings. Worldwide grapevine collections previously analyzed showed 90–95 % of correct namings (DETTWEILER 1992). Therefore it is a continuous task to eliminate mistakes in order to maintain reliable collections of genetic resources.

During the course of reviewing herbarized grapevine leaves from various sources, leaves from Klosterneuburg designated as Admirable de Courtiller came across. Ampelographic studies on this material, however, showed no similarity with leaves of the true to type Admirable de Courtiller from Geilweilerhof (Fig. 1). According to its leaf characteristics (strong goffering of the blade around



ADMIRABLE DE COURTILLER

Fig. 1: Herbarized leaf of the true to type accession of Admirable de Courtiller from Geilweilerhof.

the petiole sinus, 5-lobed, overlapping of the petiole sinus opening, overlapping of the upper leaf sinus, angular tooth shape) it became evident that the designated Admirable de Courtiller was in fact Madeleine Royale (syn. Königliche Magdalenentraube), a widespread table grape bred by a French, Moreau-Robert, in 1845 (AMBROSI *et al.* 1998). The result of the visual inspection according to ampelographic descriptions (GALET 1964, 1990) was supported by microsatellite analysis, using DNA of Madeleine Royale grown in the collection at Geilweilerhof. Thus, the plant

material obtained and designated as Admirable de Courtiller in fact turned out to be the table grape cultivar Madeleine Royale.

This finding results in a new description of the parentage of the grapevine cv. Müller-Thurgau. Despite the fact that the molecular analyses of REGNER *et al.* (1996) and SEFC *et al.* (1997) which have been confirmed by GRANDO *et al.* (1998) are correct, due to a misnaming Müller-Thurgau must be addressed as a cross between Riesling and Madeleine Royale (Fig. 2). This conclusion from ampelographic studies is supported by the allelic profile of Müller-Thurgau obtained at 6 loci (Table). The allele lengths found in Riesling and Madeleine Royale can explain all alleles of Müller-Thurgau, while the differences in both allele lengths at the loci VVS2, VVMD7, VVMD27 and *ssrVrZAG79* definitely exclude the true to type Admirable de Courtiller as gene donor for Müller-Thurgau. Although the allele lengths of Madeleine Royale are not identical with the published allele lengths for the misnamed Admirable de Courtiller from Klosterneuburg (SEFC *et al.* 1997, GRANDO *et al.* 1998) the data presented are consistent within the experimental variation. It is obvious that the allelic profile of Madeleine Royale corresponds to that of Admirable de Courtiller from Klosterneuburg because only a shift of relative distances, caused by different methods of fragment length determination, is observed between the allelic patterns. This problem is frequently encountered in the interpretation of multiple bands (*e.g.* stuttering bands) and due to the varying techniques for allele sizing in different laboratories.

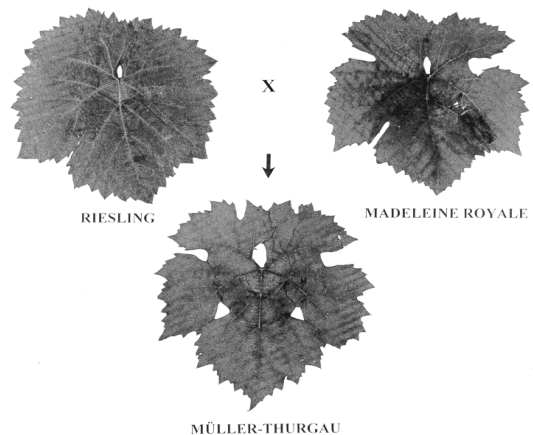


Fig. 2: Herbarized leaves of Müller-Thurgau and its parents, Riesling and Madeleine Royale.

Several phenotypical and phenological similarities between Müller-Thurgau and Madeleine Royale indicate their close relationship, the most prominent being: early ripening, moderate resistance to winter frost, high sensitivity to *Plasmopara viticola* and *Botrytis cinerea*, similarities of leaf anatomy (see above), roundish to slightly-elliptic berry shape. From our studies including molecular markers it can thus be stated that Riesling and Madeleine Royale are the parents of Müller-Thurgau. As a conclusion, there is no doubt that the confirmation of true to

Table

Parentage analysis at six microsatellite loci for Müller-Thurgau and its presumed parents

|  | VVS2    | VVMD5   | VVMD7   | VVMD27  | ssrVrZAG 62 | ssrVrZAG79 | Ref. |
|--|---------|---------|---------|---------|-------------|------------|------|
| Müller-Thurgau   | 142:150 | 224:226 | 244:254 |         | 193:193     | 242:244    | (1)  |
|  | 139:148 | 222:224 | 245:255 |         |             |            | (2)  |
|  | 143:151 | 228:230 | 247:257 | 182:182 | 194:194     | 243:245    | (3)  |
| Riesling White   | 142:150 | 224:232 | 246:254 |         | 193:203     | 242:244    | (1)  |
|  | 139:148 | 222:230 | 247:255 |         |             |            | (2)  |
|  | 143:151 | 228:236 | 249:257 | 182:190 | 194:204     | 243:245    | (3)  |
| Madeleine Royale   | 151:155 | 230:238 | 243:247 | 182:190 | 188:194     | 245:259    | (3)  |
| Admirable de Courtiller<br>(Klosterneuburg)                | 150:154 | 226:234 | 240:244 |         | 187:193     | 244:258    | (1)  |
|  | 148:152 | 224:232 | 241:245 |         |             |            | (2)  |
| Admirable de Courtiller<br>(true to type at Geilweilerhof) | 133:137 | 228:238 | 239:243 | 186:195 | 188:194     | 251:257    | (3)  |
| Silvaner White   | 150:152 | 224:230 | 240:244 |         | 187:203     | 248:250    | (1)  |
|  | 148:150 | 222:228 | 241:245 |         |             |            | (2)  |
|  | 151:153 | 228:234 | 243:247 | 190:195 | 188:204     | 249:251    | (3)  |

(1) SEFC *et al.* (1997): Analysis on ALF Express, 2-step PCR(2) GRANDO *et al.* (1998): Analysis on ABI 310, 3-step PCR

(3) this publication: Analysis using silver staining, 3-step PCR

typeness of the cultivars in the international grapevine collections remains a serious problem and needs to be considered as a prerequisite for all marker analyses and parentage studies.

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Received March 20, 2000