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Microsatellite pedigree reconstruction provides evidence that 'Müller-Thurgau' is a grandson of 'Pinot' and 'Schiava Grossa'

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Summary

'Müller-Thurgau' has recently been proven to be a cross between 'Riesling' and 'Madeleine Royale', a 19th century cross whose parents are unknown. Parentage analysis based on 93 grape cultivars of central Europe genotyped at 57 microsatellites provides here evidence that 'Madeleine Royale' = 'Pinot' × 'Schiava Grossa'. Since 'Riesling' is known to be a progeny of 'Gouais Blanc', 'Müller-Thurgau' is therefore a grandchild of 'Pinot' (either 'Pinot' noir, gris or blanc), 'Schiava Grossa' (or 'Frankenthaler' or 'Trollinger') and 'Gouais Blanc' (or 'Heunisch Weiss').

K e y w o r d s : SSR, *Vitis vinifera*, grape, parentage, fingerprinting.

Introduction

'Müller-Thurgau' is a white-berried wine grape cultivar covering ca. 42'000 ha in the world, mainly in Germany (ca. 14,000 ha), in Hungary (ca. 8,000 ha), in Slovakia (ca. 5,300 ha) and in Austria (ca. 5,200 ha). It was obtained at Geisenheim research station by the Swiss breeder Hermann Müller in 1882 and it is today the world's most widespread deliberate cross. The crossing was initially registered by Hermann Müller as 'Riesling' × 'Silvaner', which explains the name 'Riesling × Silvaner' still in use for this grape in Switzerland (ca. 500 ha), and its synonym 'Rivaner' used in Germany and Austria. Since Müller originated from canton Thurgau in Switzerland, this crossing was later named 'Müller-Thurgau' by a German expert. However, the parentage of 'Müller-Thurgau' had always been the subject of discussion (see DETTWEILER et al. 2000 for details). Based on a mere 8 microsatellite molecular markers, which are co-dominantly inherited and commonly used in pedigree reconstruction (SEFC et al. 2001), REGNER (1996) initially suggested that 'Müller-Thurgau' was in fact a 'Riesling' \times 'Gutedel' (= 'Chasselas') crossing. Using 24 microsatellite makers, Sefc et al. (1997) showed that Regner's 'Gutedel' was in fact 'Chasselas de Courtiller', a table grape of minor importance bred in the 19th century by Courtiller in Saumur (France) and also called 'Admirable de Courtiller'. However, ampelographic and genetic analyses carried out by Dettweiler et al. (2000) proved the previous studies to be wrong by showing that the so-called 'Chasselas de Courtilier' was a mislabelling in the Austrian collection and that the true identity of the other parent of 'Müller-Thurgau' is 'Madeleine Royale' (or 'Königliche Magdalenentraube'), a widespread table grape obtained by Moreau-Robert in Angers (Loire, F) in 1845 (GALET 2000). Moreau-Robert did not indicate the parents of 'Madeleine Royale', but it is suspected to be an offspring of 'Chasselas'. French ampelographer Eugène Durand considered 'Madeleine Royale' as a variation of 'Pinot meunier', which was rejected by Mouillefert (1902) who suggested a link with 'Muscat Hâtif du Puy-de-Dôme' (= 'Muscat à Petits Grains Blancs').

Using 57 microsatellite markers, we provide strong evidence for the parentage of 'Madeleine Royale' and suggest an enhanced pedigree for 'Müller-Thurgau'.

Material and Methods

Dried leaves of 'Madeleine Royale', 'Müller-Thurgau', 'Schiava Grossa', 'Pinot' and 'Chasselas' were obtained from the collection at Agroscope Changins-Wädenswil, Domaine du Caudoz (Pully, Switzerland). Their true-totypeness was verified with published DNA profiles. DNA was extracted with the Qiagen DNEasy Plant Mini Kit and all cultivars were genotyped at 57 microsatellite loci (Tab. 1) as in VOUILLAMOZ *et al.* (2006).

L i k e l i h o o d r a t i o s : The program Identity version 1.0 (WAGNER and SEFC 1999) was used to calculate the total probability of identity (PI) and the cumulative likelihood ratios (LRs) for the proposed parentage. Likelihood ratios were calculated as in VOUILLAMOZ *et al.* (2003). Allele frequencies were calculated from the profiles of 93 European cultivars genotyped at the same 57 microsatellite markers (data not shown).

Results and Discussion

Comparison with DNA profiles published in previous studies (DETTWEILER *et al.* 2000, SEFC *et al.* 2000) ascertained the true-to-typeness of 'Madeleine Royale', 'Müller-Thurgau', 'Schiava Grossa', 'Pinot' and 'Chasselas'. The total probability of identity (PI) among the 93 cultivars is extremely low: $1.3 \cdot 10^{-41}$. The supposed parentage between 'Madeleine Royale' and 'Chasselas' can be ruled out, because they do not share at least one allele at 9 out of 57 microsatellite loci (Tab. 1), as well as a parentage with 'Muscat à Petits Grains Blancs' (data not shown). On the opposite, 'Madeleine Royale' does share at least one allele at each marker with 'Pinot', an old variety from North-East-

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Table 1

Genotypes at 57 microsatellite markers. The proposed parentage 'Madeleine Royale' = 'Pinot' × 'Schiava Grossa' is consistent at all 57 markers analysed, whereas a parent-offspring relationship between 'Madeleine Royale' and 'Chasselas' can be ruled out (discrepancies in bold)

		N 11:	0.1.	
	Pinot	Madeleine	Schiava	Chasselas
	238-228	Royale	Grossa	226 228
VVMD5		236-228	238-236	236-228
VVMD6 VVMD7	205-205 243-239	214-205	214-211	212-205
		247-243	247-247	247-239
VVMD8	143-141	157-143	167-157	143-143
VVMD17	221-212	222-212	222-222	212-212
VVMD21	251-249	249-249	249-249	266-249
VVMD24	218-216	216-214	214-210	214-210
VVMD25	253-243	253-245	259-245	259-245
VVMD26	255-249	255-249	251-249	251-249
VVMD27	189-185	189-181	185-181	189-185
VVMD28	239-221	247-221	247-239	271-221
VVMD31	216-216	216-212	212-212	216-212
VVMD32	273-241	273-253	273-253	241-241
VVMD34	240-240	240-240	240-240	240-240
VVMD36	254-254	264-254	295-264	295-264
VrZAG21	206-200	206-202	206-202	206-200
VrZAG29	116-112	116-112	112-112	116-112
VrZAG62	195-189	195-189	195-193	205-195
VrZAG64	165-141	165-161	197-161	141-139
VrZAG79	245-239	259-245	259-239	259-251
VrZAG83	203-191	203-191	203-197	203-193
VrZAG93	189-189	189-189	189-189	199-189
VrZAG112	243-241	241-229	241-229	243-241
VVS1	190-183	190-183	190-181	190-183
VVS2	151-137	155-151	155-135	143-133
VVS4	173-168	173-168	168-167	168-168
VVS29	179-171	179-171	179-171	181-179
VMC1B11	172-166	172-166	172-172	174-172
VMC1C10	156-156	162-156	182-162	168-156
VMC1E8	230-226	230-226	230-208	230-228
VMC2A5	189-189	189-157	157-157	157-157
VMC2B11	180-178	180-170	182-170	182-176
VMC2B3	170-164	170-166	190-166	186-170
VMC2C3	198-170	198-170	198-198	198-192
VMC2E7	158-152	152-152	156-152	160-156
VMC2F10	115-93	93-93	93-93	99-93
VMC2H4	238-204	218-204	218-204	218-204
VMC3D12	222-205	222-205	205-205	222-205
VMC4C6	163-163	163-163	163-163	163-163
VMC4G6	122-122	138-122	138-138	138-132
VMC5A1	167-157	167-167	167-157	167-167
VMC5C1	175-147	153-147	153-153	147-147
VMC5C5	118-116	118-116	120-118	122-120
VMC5E9	221-217	227-221	227-199	221-207
VMC5G8	317-313	317-313	317-317	303-303
VMC5H2	209-194	209-194	209-194	194-194
VMC5H5	178-168	188-178	188-168	178-176
VMC6E1	165-151	161-151	165-161	165-141
VMC6E10	109-109	109-93	93-91	115-93
VMC6G1	178-170	188-170	188-178	178-170
VMC8D1	199-193	199-195	199-195	215-199
VMC8D11	132-122	136-122	136-132	140-136
VMC8F10	197-195	197-197	207-197	233-197
VMC8G6	169-147	155-147	155-147	169-147
VMC8G9	217-183	217-169	183-169	199-195
VMC9B5	248-244	248-244	244-244	244-242
VMC16F3	183-177	183-175	175-173	183-177

ern France (mainly Burgundy), and 'Schiava Grossa', an old variety from Alto Adige/Süd Tyrol, also called 'Frankenthaler' or 'Trollinger', thus suggesting parent-offspring relationships. The putative parentage 'Madeleine Royale' = 'Pinot' × 'Schiava Grossa' is verified at all 57 microsatellites analysed (Tab. 1), and likelihood ratios (LRs) analysis strongly supports the parentage (Tab. 2). Indeed, LRs of the proposed parentage versus any other two parents is extremely high: $8.94 \cdot 10^{33}$, as well as LRs of the proposed parentage versus a cross between one of the parents and a relative of the other parent: $2.70 \cdot 10^6$ to $5.40 \cdot 10^7$.

Since 'Riesling' is a progeny of 'Gouais Blanc' or 'Heunisch Weiss' (REGNER *et al.* 1998, BOURSIQUOT *et al.* 2004), 'Müller-Thurgau' is therefore a grandchild of 'Pinot', 'Schiava Grossa' and 'Gouais Blanc'. Berry colour has recently been shown to be governed by the insertion of the *Gret1* retrotransposon in the gene *VvmybA1*, a transcriptional regulator of anthocyanin biosynthesis, berries being white when the variety is homozygous at *Gret1* (THIS *et al.* 2007). Since 'Madeleine Royale' has white berries, it can be assumed that both 'Pinot' and 'Schiava Grossa' possess at least one allele at the *VvmybA1* locus containing this large insertion.

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Table 2

Likelihood-ratio (LR) values for the proposed parentage 'Madeleine Royale' = 'Pinot' × 'Schiava Grossa' versus other possibilities. Relative allele frequencies were calculated from 93 cultivars at 57 microsatellites. Values in parentheses are the cumulative likelihood ratios calculated with the 95 % upper confidence limits for the allele frequencies

Proposed parents ^a of 'Madeleine Royale': (1) 'Pinot', (2) 'Schiava Grossa'							
Cumulative likelihood ratios of the proposed parentage (1) x (2) versus:							
$X\times Y^{\text{b}}$	$(1) \times X^{c}$	$(1) \times (2)$ relative ^d	$(2) \times X^{c}$	$(2) \times (1)$ relative ^d			
8.94 · 1033	$3.59 \cdot 10^{23}$	$5.40 \cdot 10^{7}$	$5.75 \cdot 10^{18}$	$2.70 \cdot 10^{6}$			
$(1.46 \cdot 10^{23})$	$(664 \cdot 10^{17})$	$(2.13 \cdot 10^6)$	$(4.80 \cdot 10^{13})$	$(1.02 \cdot 10^5)$			

^a The order of the parents does not indicate the actual direction

of the cross.

 $^{\rm b}{\rm X}$ and ${\rm Y}$ are random unrelated cultivars.

^e The identity of one of the suggested parents is assumed and the other parent is unknown.

^d The identity of one of the suggested parents is assumed and the other parent is a close relative to the other suggested parent.

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