

# Resolving the Parentage of the Apple Cultivar ‘Meran’

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**Abstract** In the mid-1970s, a new apple variety named ‘Meran’ was discovered in South Tyrol (northern Italy), which harbours the largest continuous apple growing area in Europe. The cultivar was registered for varietal protection and patented in several countries, and was declared to be a cross of the varieties ‘Golden Delicious’ and ‘Morgenduft’ (synonym ‘Rome Beauty’). The parentage of ‘Meran’ has, however, been questioned, and the present study aimed to assess the descent of this cultivar by the combined use of molecular genetic and bioinformatic tools. Five accessions of ‘Meran’ were collected from three different European germplasm collections and analysed at 14 variable microsatellite DNA loci. Subsequently, computer software was used to allocate the most likely parent pair from a set of cultivars representative for the apple growing area of South Tyrol in 1975. The molecular genetic data clearly excluded ‘Morgenduft’ as a gene donor to ‘Meran’ and provided strong evidence that ‘Meran’ is a cross of the cultivars ‘Golden Delicious’ and ‘Jonathan’, confirming previous assumptions based on morphological traits of the tree and fruit.

**Keywords** *Malus* × *domestica* · Apple cultivar · Meran · Breeding · Parentage allocation · Microsatellite DNA

## Klärung der Elternschaft der Apfelsorte ‘Meran’

**Zusammenfassung** Die Apfelsorte ‘Meran’ wurde Mitte der 1970er-Jahre in Südtirol (Nord-Italien), dem größten zusammenhängenden Obstbauggebiet Europas, entdeckt. Die Sorte wurde für den Sortenschutz registriert, in verschiedenen Ländern patentiert, und als Kreuzung von ‘Golden Delicious’ und ‘Morgenduft’ (Synonym ‘Rome Beauty’) deklariert. Die Elternschaft der Sorte ‘Meran’ wurde jedoch in Frage gestellt, sodass in der vorliegenden Arbeit die Abstammung dieser Sorte, mit Hilfe eines kombinierten molekulargenetischen und bioinformatischen Ansatzes, aufgeklärt werden soll. Fünf Akzessionen der Sorte ‘Meran’ aus drei verschiedenen europäischen Genbanken wurden an 14 variablen Mikrosatelliten-Loci untersucht. Das molekulargenetische Profil wurde in einer bioinformatischen Analyse einem Referenz-Daten-Set der 1975 in Südtirol am häufigsten angebauten Sorten gegenübergestellt, um das höchst wahrscheinlichste Elternpaar zu identifizieren. Die molekulargenetischen Daten schließen eine Elternschaft von ‘Morgenduft’ eindeutig aus, und es besteht eine klare Evidenz dafür, dass die Sorte ‘Meran’ eine Kreuzung von ‘Golden Delicious’ mit ‘Jonathan’ ist. Damit wird die, auf Grund der morphologischen Merkmale des Baumes und der Früchte angenommene Alternativhypothese zur Elternschaft, nun molekulargenetisch bestätigt.

**Schlüsselwörter** *Malus* × *domestica* · Apfelsorte · Meran · Züchtung · Abstammungsbestimmung · Mikrosatelliten DNA

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## Introduction

Apple (*Malus × domestica* Borkh) is one of the most important temperate fruit crops worldwide (Dalla Via and Baric 2012; Ellinger 2006), and while several thousand different varieties of the domesticated apple are documented, present day commercial apple production is dominated by only a small number of cultivars (Janick et al. 1996). Nevertheless, many efforts are being put into the breeding and selection of novel cultivars to be able to cope with future challenges of apple production, such as environmental changes, emergence of new pests and diseases or the altered demands of consumers (Lespinasse 2009).

South Tyrol (northern Italy) harbours the largest continuous European apple growing area, with more than 1.1 million of metric tons of annual produce on approximately 18,500 ha. In the mid-1970s, a new apple variety named ‘Meran’ was discovered in this region showing a number of interesting properties (Mantinger et al. 1986; Zanon 1988a). Compared to the cultivar ‘Golden Delicious’, ‘Meran’ was characterised by earlier onset of bearing, later harvesting time and better natural disease resistance. In addition, the medium-sized spherical fruit with a shining light red covering colour exhibited remarkable storage and shelf-life behaviour and received favourable ratings in tasting panels (Mantinger et al. 1986; Zanon 1988b). In 1982, ‘Meran’ was registered for varietal protection in Italy and the propagation license was granted to the Consortium of South Tyrolean Tree Nurseries (Mantinger et al. 1986), and in subsequent years, ‘Meran’ was entered into a variety of testing programmes in several European countries (Kellerhals 1987; Zanon 1988b).

The review of the available literature on ‘Meran’, however, raises a number of questions regarding its origin. Several sources state that the farmer Franz Waldner discovered the apple variety as a chance seedling in a field on the outskirts of the town of Meran (Drahorad 1992; Kellerhals 1987; Mantinger et al. 1986; Mantinger 1987; Votteler 2005). Other sources, in turn, reveal that the apple variety was registered for patent in the United States as “A new and distinct variety of apple created by crossing” by the ‘inventors’ Karl Zanon and Franz Waldner (USPTO 1985). Moreover, the patent even specified that “‘Meran’ is a cross of the varieties ‘Golden Delicious’ and ‘Morgenduft’”. This parentage, however, was questioned by some experts based on morphological traits of the tree and fruit (Drahorad et al. 1993). The present study thus aimed to assess the parentage of ‘Meran’ by the combined use of molecular genetic and bioinformatic tools in order to shed some light on the descent of this cultivar.

## Material and Methods

Leaf material was collected from five different accessions of the apple cultivar ‘Meran’ in three distinct cultivar collections; three accessions derived from the Research Centre Laimburg, one accession from the Höhere Bundeslehranstalt und Bundesamt für Wein- und Obstbau Klosterneuburg (Austria) and one from the National Fruit Collection Brogdale (UK). DNA was isolated from leaf discs using the “NucleoSpin Plant Mini Kit” (Macherey-Nagel, Düren, Germany). Each DNA sample was genotyped at 14 microsatellite loci (CH01c06, CH01d08, CH01f02, CH01f07a, CH02b10, CH02c02a, CH02c09, CH02c11, CH02d08, CH02d12, CH02h11a, CH03a04, COL, CH01h01; Liebhard et al. 2002) and analysed at a CEQ 8000 Genetic Analysis System (Beckman Coulter, Fullerton, CA, USA) (Baric et al. 2008). Allele sizes were assigned using the Fragment Analysis Software Version 8.0 from the same manufacturer.

The computer program PAPA version 2.0 (Duchesne et al. 2002) was employed so as to assess the potential parents of the cultivar ‘Meran’. The genotype obtained for this cultivar was inserted into the ‘offspring file’, while the ‘collected parents file’ contained the molecular genetic profiles of ten apple cultivars from the database of the Research Centre Laimburg, which were genotyped at the same set of 14 microsatellite loci (Baric et al. 2009). The ‘collected parents’ dataset included the most commonly grown apple cultivars in 1975, which according to Stainer (2000) comprise ‘Golden Delicious’, ‘Jonathan’, ‘Morgenduft’ (synonym ‘Rome Beauty’), ‘Red Delicious’ and ‘Granny Smith’ (the triploids ‘Gravensteiner’ and ‘Winesap’ were omitted). Other locally grown old apple cultivars ‘Champagner Renette’, ‘Kalterer Böhmer’, ‘Steinpepping’, ‘Goldparmäne’ and ‘Tiroler Spitzleederer’ were also included in the dataset. The allocation procedure for non-sexed specimens, choosing the *No Error* model that assumes perfect scoring, was run to allocate the most likely parental pair for ‘Meran’. In order to assess the allocation accuracy, parental simulations using the same error model as above were run. The number of iterations was set to 10 and the number of pseudo-offspring generated at each iteration was set to 45.

## Results

The same genotype was found for all the five ‘Meran’ accessions analysed (Table 1), confirming that the true-to-type genotype is maintained in all the three cultivar collections sampled. The parentage analysis performed with PAPA 2.0 resulted in the most likely allocation of the offspring ‘Meran’ to the parental pair ‘Golden Delicious’ and ‘Jonathan’. The statistical assessment of the allocation accuracy using the dataset of 14 loci resulted in the highest possible rate of cor-

**Table 1** Molecular genetic profiles at 14 microsatellite loci of the offspring ‘Meran’ and the putative parental cultivars

Locus	Apple cultivar			
	‘Meran’	‘Golden Delicious’	‘Morgenduft’	‘Jonathan’
CH01c06	<i>156:188</i>	<i>156:162</i>	<i>158:188</i>	<i>188</i>
CH01d08	<i>240:250</i>	<i>250:272</i>	<i>250<sup>a</sup>:254<sup>b</sup></i>	<i>240:272</i>
CH01f02	<i>170:208</i>	<i>170:180</i>	<i>208</i>	<i>208</i>
CH01f07a	<i>193:197</i>	<i>177:197</i>	<i>177<sup>b</sup>:195<sup>b</sup></i>	<i>193:195</i>
CH02b10	<i>125:133</i>	<i>121:125</i>	<i>119<sup>b</sup>:155<sup>b</sup></i>	<i>125:133</i>
CH02c02a	<i>173:177</i>	<i>177:183</i>	<i>129<sup>b</sup>:181<sup>b</sup></i>	<i>173:175</i>
CH02c09	<i>245:251</i>	<i>245:259</i>	<i>245<sup>a</sup>:257<sup>b</sup></i>	<i>251:259</i>
CH02c11	<i>222:238</i>	<i>222:236</i>	<i>230<sup>b</sup>:236<sup>b</sup></i>	<i>232:238</i>
CH02d08	<i>226:232</i>	<i>226:228</i>	<i>258<sup>b</sup></i>	<i>232:258</i>
CH02d12	<i>191:195</i>	<i>195:199</i>	<i>191:199</i>	<i>191:199</i>
CH02h11a	<i>98:122</i>	<i>122:124</i>	<i>116<sup>b</sup>:122<sup>a</sup></i>	<i>98:114</i>
CH03a04	<i>98:120</i>	<i>120</i>	<i>116<sup>b</sup>:118<sup>b</sup></i>	<i>98:112</i>
COL	<i>219:231</i>	<i>219:231</i>	<i>219<sup>a</sup>:229<sup>b</sup></i>	<i>229:231</i>
CH01h01	<i>118:122</i>	<i>120:122</i>	<i>116<sup>b</sup>:124<sup>b</sup></i>	<i>118:134</i>

Alleles are listed only once if a cultivar was homozygous at a particular locus

Identical allele sizes are represented in italics letters

<sup>a</sup>Allele sizes denote mismatches with the offspring when ‘Golden Delicious’ is considered to represent the second parent

<sup>b</sup>Allele sizes denote mismatches with the offspring when ‘Morgenduft’ is considered alone

rectness among allocated pseudo-offspring (= 1) as well as the highest possible number of correct allocations over the number of offspring (= 1).

The molecular genetic data clearly disproved the parentage for the cultivar ‘Morgenduft’. Seven of the 14 microsatellite loci did not match with the ‘Meran’ genotype when ‘Morgenduft’ was considered alone and 11 did not match when ‘Golden Delicious’ was considered to represent the second parent (Table 1). The true-to-typeness of the molecular genetic profiles of ‘Golden Delicious’, ‘Morgenduft’ and ‘Jonathan’ was previously verified by the analysis of distinct accessions of these cultivars from at least three different European germplasm collections (Baric et al. 2009, 2011).

## Discussion

The present study provides strong evidence that the South Tyrolean apple cultivar ‘Meran’ is not a cross of the cultivars ‘Golden Delicious’ and ‘Morgenduft’ but of ‘Golden Delicious’ and ‘Jonathan’. Should ‘Meran’ have been created by controlled breeding, as was suggested in the United States Patent document number P5406 (USPTO 1985), then the molecular data indicate that ‘Golden Delicious’ was pollinated by a different gene donor and the breeding records

should be revised. In fact, the descent of ‘Meran’ has already been disputed and some experts suspected a closer relatedness to ‘Jonathan’ (Drahorad et al. 1993). Drahorad et al. (1993) pointed to the slightly smaller size of the fruit with an aromatic and acidic pulp, the particular form of the leaves and fruit-bearing branches as well as the low scab susceptibility as the arguments in favour of a progenitor/offspring relationship between ‘Jonathan’ and ‘Meran’.

The cultivar ‘Meran’ has initially aroused great interest within the apple growing industry due to some of its advantageous features, in particular its remarkable storage and shelf-life performance (Drahorad 1992; Kellerhals 1987; Mantinger et al. 1986; Rolff 2001; Zanon 1988a, b). However, ‘Meran’ also exhibited some shortcomings, such as a tendency towards excessive fruit setting and the need for extensive thinning to prevent development of undersized and discoloured fruit. Furthermore, the trees required particularly favourable climatic conditions in order to bear high quality fruit (Drahorad 1992; Kellerhals 1987; Mantinger 1986). These might have been the reasons why ‘Meran’ has never become a widely-grown cultivar and has made its way from experimental plots to germplasm collections.

Molecular markers such as microsatellites have proven to be useful tools for cultivar identification, relationship analyses, and estimates of genetic diversity within and among germplasm collections (Baric et al. 2009; Guarino et al. 2006; Guilford et al. 1997; Hokanson et al. 1998; Wünsch and Hormaza 2002) but also for the monitoring of breeding programmes and parentage allocation (Cabe et al. 2005; Evans et al. 2011). In this study, molecular genetic data have contributed to a critical revision of the parentage of the only registered apple variety originating from South Tyrol up to date.

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