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On the origin of the grapevine variety Müller-Thurgau as investigated by the inheritance of random amplified polymorphic DNA (RAPD)

by

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S u m m a r y : Random amplified polymorphic DNA obtained with 10 different primers and peroxidase isoenzymes of Riesling, Silvaner and Müller-Thurgau were analyzed for genetic relationships between these grapevine varieties. It can be concluded that Müller-Thurgau is not a progenitor of a cross Riesling x Silvaner as generally assumed, but of Riesling and an unknown cultivar.

K e y w o r d s : grapevine variety, DNA, RAPD.

Introduction

The grapevine cultivar Müller-Thurgau (MT) is one of the most important varieties in central Europe, particularly in Germany, Austria and also Switzerland. There it is called Riesling x Silvaner (R x S) because these varieties are generally assumed to be the parents of MT. However, the validity of this assumption has been doubtful. BREIDER (1952) stated that the variety resulted from a selfing of R whereas the detailed morphometric analyses of EICHELSBACHER (1957) seemed to indicate that neither S nor probably R were among its parents. Peroxidase patterns published by BACHMANN and BLAICH (1988) showed a band in MT lacking in either of the supposed parents. Now, after three decades the techniques of DNA analysis (STRIEM *et al.* 1990; BOURQUIN *et al.* 1993; BOWERS *et al.* 1993; COLLINS and SYMONS 1993; GOGORCENA *et al.* 1993; JEAN-JACQUES *et al.* 1993) allow the reconsideration of this open question .

Material and methods

DNA from grapevine varieties grown in the living collection of our institute was prepared accoring to THOMAS *et al.* (1993) and analyzed by RAPD analysis with dekamer primers purchased from Operon Technologies as previously described (BÜSCHER *et al.* 1993). Temperature program: initial denaturation 5 min at 94 °C, followed by 45 cycles: 1 min denaturation at 94 °C, 1 min annealing at 36 °C, 1.5 min synthesis at 72 °C with a ramping of 2 s/°C from 36 to 72 °C. The DNA of 2-3 different plants was analyzed separately for each variety. More than 40 different varieties and 90 individuals of parents and seedling populations were analyzed with altogether 16 different primers. The most informative primers (Tab. 1) were selected for more detailed analyses of the cultivars Diana, Kerner, Müller-Thurgau, Riesling, Trollinger and Silvaner. With 9 primers up to 80 evaluable amplification products ("bands") per cultivar could be obtained. Similarity coefficients were calculated with the computer program of SCOTT *et al.* (1993).

In addition electrofocusing patterns of peroxidase isozymes in 27 grapevine varieties and strains, related to S or R (Tab. 2) were analyzed according to BACHMANN and BLAICH (1988).

T a b l e 1

Evaluation of bands in PCR patterns of some new varieties. OP-primers: M12, N06, N10, N15, U01, U08, U10, U14, U17. U10 and N06 were not used in cross 1 and 2, respectively.

Female parent Male parent Progenitor	Cross 1: Trollinger Riesling Kerner	Cross 2: Silvaner Müller-Th. Diana	Cross 3: Riesling Silvaner (?) Müller-Thurgau
Total number of bands in progeny patterns	72 (100 %)	54 (100 %)	62 (100 %)
Bands common to both parents and progenitor	38 (52.8%)	32 (59.3%)	46 (74.2 %)
Bands common to female parent and progenitor	19 (26.4 %)	16 (29.6 %)	9 (14.5 %)
Bands common to male parent and progenitor	13 (18.0 %)	5 (9.3 %)	1 *(1.6 %)*
Novel bands, present in progenitor only	2 (2.8 %)	1 (1.8%)	6 *(9.7 %)*
Total number of bands in patterns of female parent	67 (100 %)	66 (100 %)	66 (100 %)
Maternal bands not transmitted to progenitor	10 (14.9 %)	19 (28.8 %)	11 (16.7 %)
Total number of bands in patterns of male parent	60 (100 %)	53 (100 %)	80 (100 %)
Paternal Bands not transmitted to progenitor	9 (15.0 %)	17 (32.1 %)	33 *(41.25 %)*

Results and discussion

P C R - t e c h n i q u e : As already pointed out in BÜSCHER *et al.* (1993) the results of RAPD techniques with short primers are subject to a certain variability. Therefore

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Table 2

Similiarity between different cultivars grouped according to known genetic relationships.

Cultivars with no known relationship		Indirectly related cultivars		Parent and progenitor	
Kerner/ Silvaner	0.714	Kerner/ Diana	0.695	Müller-Th./ Diana	0.697
Riesling/ Silvaner	0.744	Kerner/ Müller-Th.	0.710	Riesling/ Kerner	0.767
Trollinger/ Diana	0.654	Riesling/ Diana	0.731	Silvaner/ Diana	0.790
Trollinger/ Müller-Th.	0.661			Trollinger/ Kerner	0.814
Trollinger/ Riesling	0.589				
Trollinger/ Silvaner	0.651				
Average of group	0.669		0.721		0.767

the presence or absence of bands was evaluated only if the pattern could be verified with DNA obtained from different individuals and in experimental repetitions.

RAPD - patterns: The analysis of control crosses with seedling populations revealed that most PCR bands follow Mendelian segregation as also described by BOWERS *et al.* (1993) for RFLP markers of grapevines. Thus bands occurring in F1 progeny can be derived from either of the two parents. This is to be expected, since recombination events between primer attaching sites should be extremely rare.

Newly observed bands in F1 patterns, lacking in the patterns of both parents, can be explained by recombination events. In all crosses observed not more than 2.8 % of the bands represent this novel type (Tab. 1, cross 1 and 2). MT, however, shows nearly 10 % of such novel bands (Tab. 1, cross 3, and Figure). This high percentage could be better explained by an unknown father rather than by a cross R x S: The "recombination" value fits well into the range 9.3 - 18.1 % for the number of "bands common to male parent and progenitor" of the other crosses.

In theory a band common to both parents should be present in 75 % of the progeny and may lack only if both parents are heterozygous for this character or after a recombination event. In MT "paternal" bands are lacking to a much higher percentage than in other crosses where this was seldom observed. Examples are given in Figure, b, however the data were not calculated since the genetic configuration of the parents (homo- *vs.* heterozygosity) cannot always be concluded from the intensity of the bands.

The average percentage of bands which are not inherited from one parent to a progenitor should be equal for both parents. This is true for cross 1 and 2 (Tab. 1) but not for cross 3 where only 16.7 % of the female parent's (R) bands cannot be found in the progenitor (MT) but 41.25 % of the assumed male parent's (S) bands are missing.

The calculation of similarity coefficients fits into this pattern. On the average the similarity between both par-

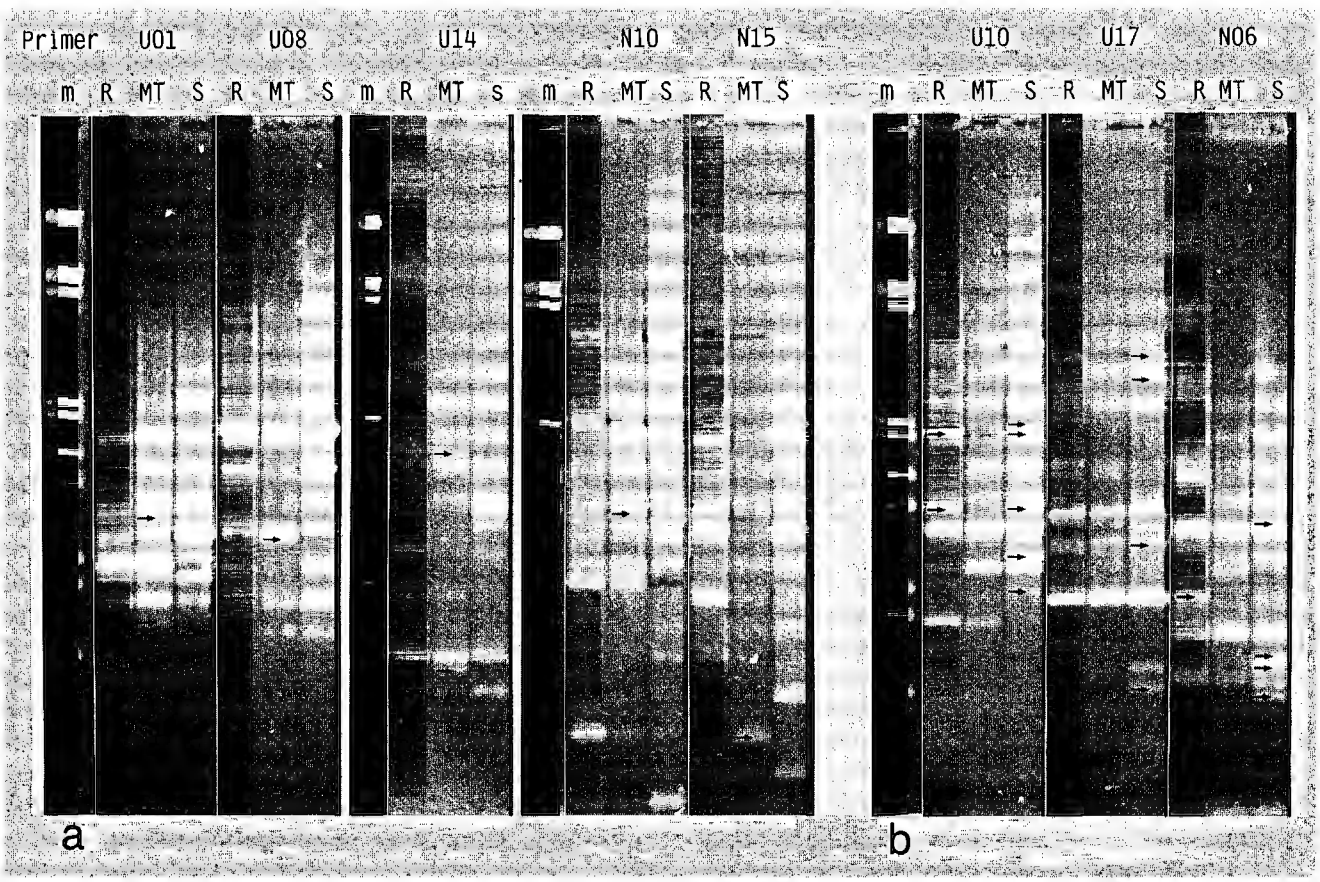

Figure: RAPD patterns obtained with different OP primers of the wine grape cultivars Riesling (R), Müller-Thurgau (MT) and Silvaner (S); m marker: λDNA cut with EcoRI and HindIII. (a) Bands in MT which are not present in either R or S are indicated by arrow heads; (b) bands of R and S are indicated which are not present in MT.

ents should be lower than between parents and progeny. This can be demonstrated by comparisons between related and unrelated cultivars (Tab. 2 and 3). From Tab. 3 it is evident that Silvaner is even more similar to Riesling than to its supposed progenitor Müller-Thurgau.

Table 3

Similarity coefficients between the PCR band patterns of parents and progenitor of 3 different crosses of V. vinifera varieties including the assumed ancestry of Müller-Thurgau.

Female parent Male parent Progenitor	Trollinger Riesling Kerner	Silvaner Müller-Thurgau Diana	Riesling Silvaner (?) Müller-Thurgau
between:	Similarity coefficients		
Female parent and progenitor	0.814	0.790	0.852
Male parent and progenitor	0.767	0.697	***0.662***
Both parents	0.589	0.648	***0.744***

Isoenzymes: The molecular basis of enzyme patterns of grapevine peroxidases is unknown: many enzyme bands may be due to epigenetic modifications of one gene product rather than caused by separate genes. Although they are therefore not very valuable as a stand-alone genetic character they may yield additional informations: The patterns obtained from different related varieties could be classified into 4 groups (Tab. 4). Also in this type of analysis all progeny analyzed, except MT, fits into the groups of either S or R.

Table 4

Some peroxidase isoenzymes of different clones of Riesling and Silvaner and of some related new varieties. The names (P2, P3, P4) of the enzyme bands obtained by isoelectric focusing correspond to Bachmann and Blaich (1988).

Variety	Parents	P4	P3	P2
Riesling rot		X	-	-
Riesling weiß (Cl.4, Cl.90, Cl.239, Cl.Glw.)		X	-	-
Riesling weiß (4N) (Cl.1, Cl.4, Cl.90, Cl.Glw.)		X	-	-
Silvaner grün (Cl.Bosenheim, Cl.75)		X	-	X
Silvaner grün 4N (Cl.Bosenheim, Cl.75)		X	-	X
Silvaner blau		X	-	X
Ehrenfelser	R X S	X	-	-
Oranienstelner	R X S	X	-	-
Scheurebe	S X R	X	-	-
Scheurebe Cl.K.Lay	S X R	X	-	-
Scheurebe 4N Cl.K.Lay	S X R	X	-	-
Riesling bulgarski	Dimyat X R	X	-	-
Multaner	R X S	X	-	X
Osteiner	R X S	X	-	X
Rieslaner	S X R	X	-	X
Müller-Thurgau	R X S (?)	X	X	-
Müller-Thurgau Cl.Ebertsh.	R X S (?)	X	X	-
Müller-Thurgau (4N)	R X S (?)	X	X	-
Müller-Thurgau (4N) Cl.Ebertsh.	R X S (?)	X	X	-

Conclusions

From these observations it can be concluded that MT is not derived from Riesling x Silvaner. This is not surprising since Eichelsbacher (1957) excluded both varieties as parents. His data show, however, that Müller-Thurgau is in some respects similar to Riesling but not to Silvaner and the high similarity of the RAPD PCR patterns (85 %) indicates too that Riesling is one of the parents of Müller-Thurgau. In view of the breeding techniques applied to grapevines it is more reasonable to assume an inadvertent cross-fertilization by an unknown pollen grain rather than the carry-over of an unknown grapeseed.

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