Vitis 33, 151-156 (1994)

Peroxidase isoenzyme patterns in Vitaceae

by

O. BACHMANN

Bundesanstalt für Züchtungsforschung an Kulturpflanzen, Institut für Rebenzüchtung Geilweilerhof, Siebeldingen, Deutschland

S u m m a r y : The isolation of active cytoplasmatic enzymes has been simplified and improved. Employing this technique 313 different cultivars and species of *Vitis* have been analyzed for peroxidase isoenzymes in the phloem of dormant canes. Using isoelectric focusing on polyacrylamid gel layers 38 different banding patterns were obtained. Bands with isoelectric points pH 3.5 to 3.8 (type A) were found to be characteristic for *Vitis vinifera* cultivars (211 samples). The frequency of type A in other *Vitis* species is 5 %. Only half of the interspecific cvs tested showed a type A pattern. Variability in neutral to basic peroxidases was useful to group *V. vinifera* and interspecific cultivars. The ploidy had no influence on the patterns whereas different colour variants exhibited differences. The single polymorphic isoenzyme peroxidase is sufficient to group cultivars and to discriminate between two samples.

K e y w o r d s: peroxidase isoenzyme, interspecific cultivars, Vitis species, ploidy.

Peroxidases (E.C.1.11.1.7) are enzymes involved in stress response after pathogen attack. They help to build up suberin barriers by crosslinking wall proteins. Suberization of the walls of wounded plant tissues in process of healing, involves deposition of polymeric material composed of aromatic and aliphatic components.

On the other hand, the isoenzymes can be used as biochemical character for the identification and differentiation of cultivars. Methods increasing confidence in the authenticity of cultivars are required. Although analysis of the genes instead of gene products will be more reliable to detect small differences within cultivars or even clones, the analysis of DNA is far from being simple or cheap. A first screening on the level of gene products (e.g. iso-enzymes) is easier to perform. This can be done by starchgel electrophoresis (SGE; ARULSEKAR and PARFITT 1986), polyacrylamid gelelectrophoresis (PAGE; BENIN *et al.*1988) or isoelectric focusing (IEF; CARGNELLO *et al.* 1988). The resolving principle for SGE and PAGE is based mainly on the different molecular weight of proteins, whereas in IEF resolution depends on different netto charge of proteins.

The components of samples applied to an IEF gel will move within a pH gradient to the pH where they lose their netto charge. At this point the proteins have a minimal solubility and precipitate into a sharp band. This is quite in contrast to SGE and PAGE where the resolution is wretched by diffusion resulting in more or less diffuse bands.

Gene expression is organ-specific, so only samples of the same age and organ can be compared. Organs with high protein content and low polyphenolics are suited best for this type of analysis. Storage proteins in seeds (LAMIKANRA 1993), pollen (CARGNELLO *et al.* 1988) and phloem (BACHMANN and BLAICH 1988) partially fulfill this requirements.

Storage proteins, however, are highly polymorphic. About 300 polypeptides can be separated in 2 D electrophoretic systems (LAMIKANRA 1993), which renders interpretation of those complicates polypeptide patterns very difficult. Polymorphic isoenzyme patterns with only few well separated bands can be more helpful. We use proteins respective enzymes isolated from the phloem of dormant canes, the isolation of which is simple and reliable.

Material and methods

Canes from the collection of the Institute for Grapevine Breeding Geilweilerhof were cut from November through March of the following year. The bark of the 3rd to 5th internodia of the cane was pealed off and the phloem was removed with a razor blade. About 2 g of this material was infiltrated at 0 °C with extraction buffer, modified after ARULSEKAR and PARFITT (1986) as follows:

0.05 M TRIS base (6.5 g/l); 0.007 M citric acid (1.5 g/l); 0.1 % cystein; HCl (1.0 g/l); 0.1 % ascorbic acid (1.0 g/l); 1.0 % polyethylene glycol (MW 4000) (10 g/l); 1 mM 2-mercaptoethanol (0.08 ml/l); 10 % sucrose (100 g/l).This buffer can be used for nearly 1 week if it is kept cold and in dark. The final pH will be about 8.0.

Air in the extracellular spaces was evacuated from the submerged material by placing the tube in a vacuum chamber at 200 Pa for 5 min. Upon return to atmospheric pressure the material is completely soaked with buffer. After 1 h of standing in ice the bufer is filtered off, the humid material is blotted dry with paper and then transferred in 0.75 ml Eppendorf tubes with a small hole in the bottom. These are put in a larger 1.5 ml Eppendorf tube, in order to collect the filtrate obtained by a 2 h centrifugation with 15 000 rpm at 0 °C. The extract (about 200-400 µl with high protein concentration) is stored on ice for further analysis. It can be used directly or diluted 1:1 with distilled water to lower the salt concentration to less than 100 mM.

Precast washed and dried polyacrylamid gels on my-

Dr. O. BACHMANN, Bundesanstalt für Züchtungsforschung an Kulturpflanzen, Institut für Rebenzüchtung Geilweilerhof, D-76833 Siebeldingen, Germany

lar films prepared according to WESTERMEIER (1990) are soaked for 1 h upside down in 10.5 ml of a solution of 0.245 ml Servalyt pH 2-11, 0.210 ml Servalyt pH 2-4, 0.295 ml Servalyt pH 9-11 in 10 % sorbitol.

Remaining solution is removed by a filter paper. The sensitive lower surface is covered by a clean piece of plastic foil. This "sandwich" is mounted on the cooling plate of a LKB 2117 Multiphor II chamber with 1 ml ethyleneglycol to enhance heat transfer. The Pt electrodes touch directly both edges of the plate in a distance of 100 mm. After prefocusing for 0.5 h to 500 V, 10 °C starting with 12 W to built up the pH gradient, 10-20 µl samples are applied to the acidic part of the plate with the help of a rubber band with sample holes. Horseradish peroxidase (HRP, Serva 31 943) is used to test the separation quality and to determine the isoelectric points. 1 h at low electric field (500 V/6 mA = 3.00 W) is necessary for the sample to penetrate the polyacrylamid layer. Then the sample holder is removed and during 1.5-2.0 h 2000 V is reached; focusing is finished at a minimal current of about 1.0 mA or less (depending on the salt concentration of the samples). The plate is washed with tap water at the backside to remove the heat transfer solution and incubated in a solution of 1 mM 3-amino-9-ethylcarbazol in 1 M Na acetat buffer (pH 5.6) and 5 µl hydrogenperoxid (30 %). Red coloured bands develop within 10-30 min at the sites of enzymatic activity. The reaction is stopped by washing the plate with tap and distilled water. All buffers and colouring substances are removed within 1 h. At the side of peroxidase activity a cherry-red pigment is deposited. Development is finished with a 10 min bath in 1.2 % glycerolThe gel is then air dried and preserved as a resistant film (Fig. 1).

The banding pattern is homologized with the help of a PC computer program (BLAICH 1994) so that different patterns can be easily compared (Fig. 2).

Results

321 Samples (Tab. 1) could be separated in four groups:
1. Cultivars of *V. vinifera* origin (211 cvs) -*vinifera*-
2. Cultivars of interspecific origin (61 cvs) -I.C.-
3. *Vitis* species (41 spp.) -*Vitis* spp.-
4. (Ampelopsis (7 spp.) and Parthenocissus (1 sp.) -non-*Vitis* spp.-

Two groups of peroxidase isoenzymes can be clearly distinguished: one (12 different types) in the acidic part of the plates and one in the neutral to basic part (12 different

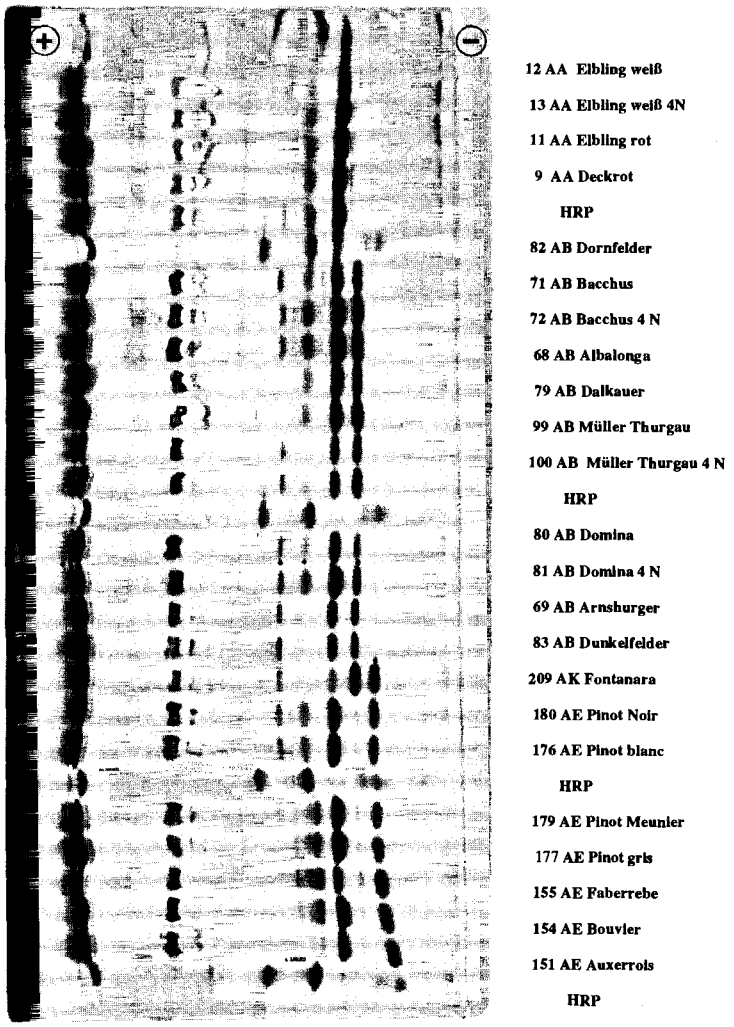

Fig. 1: Peroxidase patterns of some *V. vinifera*cultivars. It must be pointed out that this photo shows "single shot" patterns which do not always correspond to the final "synthetic" type which is based on the consistent absence of bands in repeated experiments.

types). In the presence of 8 M urea in the separating layer the acidic enzymes lose their activity in contrast to the neutral to basic enzymes (results not shown in this paper).

In *vinifera* cultivars the acidic part is without any variation. All samples belong to type A (Tab. 1, upper part). Different groups of bands are at the neutral to basic side of the plate. The acidic part in the I.C. cvs is more variable than in the *vinifera* group. a-C, a-D, a-E, a-F and a-K were found. In *Vitis* spp. there are only 2 samples (4.8 %) with the characteristic *vinifera* A pattern. The isoelectric points of the acidic grape peroxidases of type a-A are 3.55; 3.65 and 3.75.

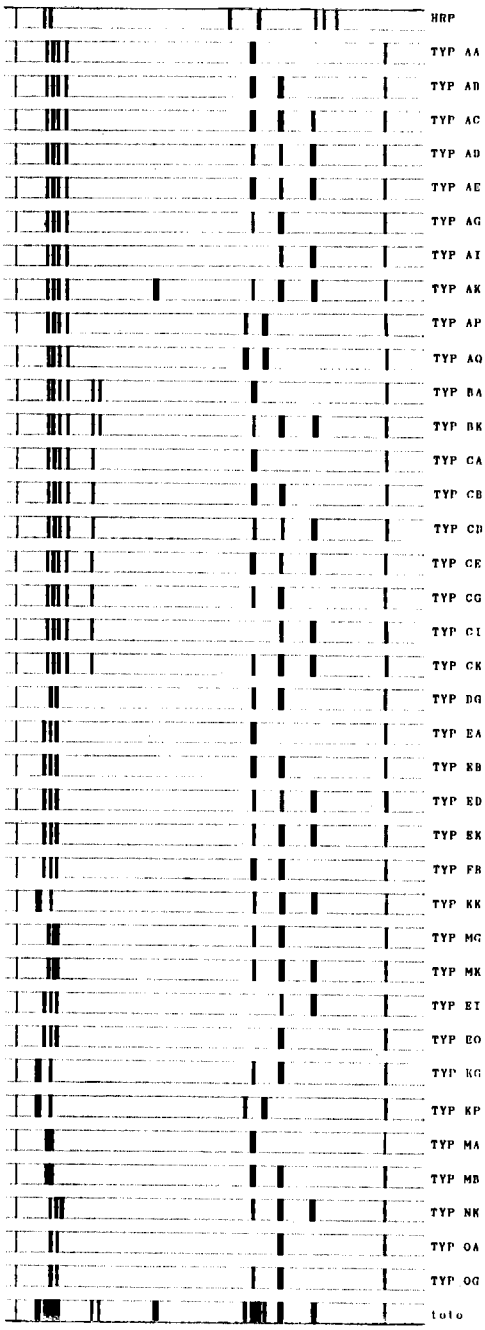

Fig. 2: Peroxidase patterns found by isoelectric analysis of 312 *Vitis* spp. and *Vitis* cvs; they are named by 2 capital letters (AA .. OG). The first one indicates the type of the acid subgroup (left side of the pattern; a-A .. a-O in Tab. 1). The second letter indicates the type of the neutral to basic subgroup (right side; nb-A .. nb-Q in Tab. 1). The strong band in the middle of pattern A belongs to the neutral to basic subgroup.

Table 1

Occurrence of different patterns of grapevine peroxidases in *Vitis vinifera*cvs, interspecific cvs and *Vitis* spp. The acidic subgroup of the enzymes and the neutral to basic subgroup each exhibit 12 different sub-patterns (a-A .. a-O and nb .. nb-Q, respectively; for further explanation see Figs. 1 and 2). From 144 possible combinations 37 have been found

pattern type	*V. vinifera* cvs	Interspec. cvs	*Vitis* spp.
acidic group			
a-A	211	28	2
a-B	0	2	0
a-C	0	13	7
a-D	0	2	0
a-E	0	11	21
a-F	0	1	0
a-G	0	0	0
a-I	0	0	0
a-K	0	2	3
a-M	0	2	2
a-N	0	0	4
a-O	0	0	2
	211	61	41 (313)
neutral to basic group			
nb-A	67	12	5
nb-B	67	10	7
nb-C	4	0	0
nb-D	12	6	0
nb-E	40	7	1
nb-G	15	5	10
nb-I	2	1	1
nb-K	4	13	14
nb-M	0	1	0
nb-O	0	0	2
nb-P	0	5	1
nb-Q	0	1	0
	211	61	41 (313)

In *Vitis* spp. and I.C. cvs, 11 different additional types (a-B to a-O) can be differentiated. In non-*Vitis* spp. there are 4 further types a-S; a-T; a-V; a-W (not shown). This group has no bands in common with *Vitis* spp., *Vitis* cvs and interspecific cvs.

Neutral to basic grouped isoenzyme bands (Tab.1, lower part) are separated in 12 differnt patterns (nb-A to nb-Q). In *vinifera* cvs only nb-A to nb-K are present. In I.C. cvs the *vinifera* types 1 to 8 are substituted by 3 further types (9, 11, and 12). *Vitis* spp. contain some further types (nb-O and nb-P) but are missing nb-C, nb-C, nb-M and nb-Q.

A band near the anode is common in case of all samples except non-*Vitis* and therefore it is not used for differentiation. In Tab. 2 all samples are grouped according to (1) *vinifera* cvs, I.C. cvs and *Vitis* spp.; (2) peroxidase isoenzyme pattern type; (3) alphabetical order of the name of analyzed sample. Within this order all samples carry a continuous number. In Tab. 3 all samples are arranged in alphabethical order. The band combinations found in all analyzed samples except the non-*Vitis* group are abbreviated in Tab. 2 by two capital letters. The first letter refers to the acidic group and the second one for the neutral to basic group (see also Fig. 2).

Table 2

Classification of 313 *Vitis* cvs and spp. into different groups (AA .. OG, according to Fig. 2). Different clones (including 2n/4n) of the same variety are written in *italics* (23 %), diferent colour forms are indicated by **bold** letters (7 %), an asterisk preceding the number indicates that a closely related form with differing pattern is present elsewhere in the Table. Listing within the 3 groups is in alphabetical order

Vitis vinifera cultivars (211)

1. AA (67/32%) 1. ABONDANT, 2. AFUS ALI, 3. BOUQUETRIESLING, 4. CHASSELAS IMPERIAL, 5. CHENIN BLANC, 6. *COMTESSA*, 7. *COMTESSA (4N)*, 8. COT, 9. DECKROT,10. EHRENFELSER, 11. **ELBLING ROT**, 12. ***ELBLING WEISS***, 13. *ELBLING WEISS (4N)*, 14. EMERALD RIESLING, 15. *FORTA*, 16. *FORTA (4N)*, 17. *HEROLDREBE*, 18. *HEROLDREBE (4N)*, 19. HEUNISCH BLAU, 20. HUXELREBE, 21. KNIPPERLE, 22. KORINTHIAKI, 23. LOUISETTE, 24. MUSCABONA, 25. MUSCAT ROUGE DE MADERE, 26. MUSKATRIESLING, 27. NIU NAI, 28. *NOBLESSA*, 29. *NOBLESSA (4N)*, 30. *OPTIMA* , 31. *OPTIMA (4N)*, 32. ORANIENSTEINER, 33. ORTEGA34. PERLE, 35. PIN ER PU TAO, 36. **PIQUEPOUL BLANC**, 37. **PIQUEPOUL NOIR**, *38. PORTUGIESER GRUEN, 39. RAEUSCHLING WEISS, 40. RAMISCO, 41. RIESLING BULGARSKI 42. **RIESLING ROT**, 43. *RIESLING WEISS (4N) CL.1*, *44. RIESLING WEISS (4N) CL.4*, 45. *RIESLING WEISS (4N) CL.90*, 46. *RIESLING WEISS (4N) CL.GLEISW.*, 47. ***RIESLING WEISS CL.4***, 48. *RIESLING WEISS CL.90*, 49. *RIESLING WEISS CL.239*, 50. *RIESLING WEISS CL.GLEISW.*, 51. ROTGIPFLER, 52. SAINT LAURENT, 53. SAUVIGNON BLANC (4N), 54. SCHEUREBE, 55. *SCHEUREBE (4N) CL.K.LAY*, 56. *SCHEUREBE CL.K.LAY*, 57. SEPTIMER 58. SÜSSROT, 59. SULTANINA, 60. SULTANINA, 61. SYRISCHER WEISS (4N), 62. ***TRAMINER ROT***, 63. *TRAMINER ROT (4N)*, 64. TRAMINER ROT (GEWÜRZTRAMINER), 65. **TRAMINER WEISS**, *66. VELTLINER GRUEN, 67. unknown

2. AB (66/31%) 68. ALBALONGA, 69. ARNSBURGER, 70. AUGSTER BLAU, 71. *BACCHUS*, 72. *BACCHUS (4N)* 73. BAI XIANG JIAO, 74. *BLAUFRÄNKISCH*, 75. *BLAUFRÄNKISCH (4N)*, 76. CABERNET FRANC, 77. CARMENERE, 78. CHASSELAS VIOLET, 79. DALKAUER, 80. *DOMINA*, 81. *DOMINA (4N)*, 82. DORNFELDER, 83. DUNKELFELDER, 84. EARLY MUSCAT, 85. EGER 26, 86. FURMINT, 87. GEISDUTTE BLAU, 88. GUTENBORNER, 89. HIMBERTSCHA, 90. KANZLER 91. ***KERNER***, 92. *KERNER (4N)*, 93. **KERNLING**, 94. LAGREIN, 95. MARIENRIESLING 96. MARIENSTEINER, 97. MARKANT, 98. MAROC GROS, 99. *MÜLLER THURGAU*, 100. *MÜLLER THURGAU (4N)*, 101. *MÜLLER THURGAU (4N) CL.EBERTSH.*, 102. *MÜLLER THURGAU CL.EBERTSH.*, 103. MUSCAT DE MARSEILLE, 104. MUSCAT FLEUR D'ORANGER, 105. MUSCAT HAMBURG, 106. MUSCAT LIERVAL, 107. MUSCAT OTTONEL, 108. MUSCAT ST. LAURENT, *109. *PORTUGIESER BLAU*, 110. *PORTUGIESER BLAU (4N)*, 111. *PORTUGIESER BLAU (4N) CL.INGENHEIM*, 112. *PORTUGIESER BLAU CL.INGENHEIM*, 113. REGNER, 2. AB . 114. REICHENSTEINER, 115. RIESLING BLAU, 116. ROTBERGER, 117. SCHÖNBURGER, 118. *SHIRAZ*, 119. *SHIRAZ (4N)*, 120. *SIEGERREBE*, 121. *SIEGERREBE 4N*, 122. **TERRET GRIS** 123. **TERRET NOIR**, 124. *TOMPA MIHALY*, 125. *TOMPA MIHALY (4N)*, 126. VERDOT NOIR127. WELSCHRIESLING, 128. WüRZBURGER, 129. WÜRZER, 130. ZIERFANDLER ROT 131. ZIMMETTRAUBE, 132. ZWEIGELT BLAU, 133. unknown, 134. unknown

3. AC(4/2%) 135. EDELSTEINER, 136. *HELFENSTEINER*, 137. *HELFENSTEINER (4N)*, 138. NOBLING

4. AD(12/6%) 139. ARGANT, 140. *CHASSELAS BLANC*, 141. *CHASSELAS BLANC (4N)*, 142. FINDELKIND 143. FOSTER'S WHITE SEEDLING, 144. HONIGLER , 145. *KOSHU*, 146. *KOSHU (4N)*, 147. *MADELEINE ANGEVINE*, 148. *MADELEINE ANGEVINE (4N)*, 149. PERLE VON CSABA, 150. VERDOT GROS

5. AE (41/19%) 151. AUXERROIS, 152. AUXERROIS, 153. BLAUBURGER, 154. BOUVIER, 155. FABERREBE 156. FREISAMER, 157. GAMAY NOIR, 158. GAMAY TEINTURIER DE BOUZE, 159. GAMAY TEINTURIER DE CHAUDENAY, 160. GAMAY TEINTURIER FREAUX, 161. GLORIA, 162. HÄNGLING BLAU, 163. HEUNISCH ROT, 164. ITALIA, 165. KOLOR, 166. LONG YAN, 167. MALINGRE PRECOCE, 168. MERLOT NOIR , 169. *MORIO MUSKAT*, 170. *MORIO MUSKAT (4N)*, 171. MULTANER, 172. MUSCAT A PETITS GRAINES ROUGES, 173. MUSCAT BLANC, 174. MUSCAT PRECOCE DE SAUMUR, 175. OSTEINER, 176. **PINOT BLANC**, 177. **PINOT GRIS CL.DUNKELGRAU**,178. **PINOT GRIS CL.HELLGRAU**, 179. PINOT MEUNIER, 180. **PINOT NOIR**, 181. PINOT NOIR, 182. PUTZSCHEERE, 183. RIESLANER, 184. **SILVANER BLAU**, 185. ***SILVANER GRUEN (4N) CL. 75***, 186. *SILVANER GRUEN (4N) CL. BOSENHEIM*, 187. *SILVANER GRUEN CL. 75*, 188. *SILVANER GRUEN CL. BOSENHEIM*, 189. TEINTURIER, 190. WILDBACHER FRÜH BLAU

6. AG (17/8%) 191. AGOSTENGA, 192. BAI SHA SI LA, 193. CHASSELAS MUSQUE, 194. HEUNISCH WEISS 195. JUBILAEUMSREBE, 196. OSIRIS, 197. RABANER, 198. SCHIAVA GROSSA, 199. SCHIAVAGROSSA, 200. *SCHIAVA GROSSA*, 201. *SCHIAVA GROSSA (4N)*, 202. SISI, 203. TROLLINGER BLAU FRÜ DUFTIG, 204. **URBAN ROT**, 205. **URBAN BLAU** 206. **VELTLINER FRÜHROT**, 207. **VELTLINER ROT**

7. AK (4/2%) 208. DIANA (4N), 209. FONTANARA, 210. MERLOT BLANC, 211. SULMER,

Interspecific cultivars (61)

1. AA (7/11%) 212. GEILWEILERHOF GA-48-12, 213. GEILWEILERHOF GA-50-34, 214. KYOHO, 215. NAUMBURG 5016-37, 216. ROU DING XIANG, 217. WALLIS GIANT (4N), 218. WATKINS
2. AB (5/9%) 219. BAILEY ALICANTE A, 220. EGER 1, 221. GEILWEILERHOF GA-58-14, 222. MARECHAL FOCH, 223. MUSCAT BAILEY A
4. AD (2/3%) 224. LEON MILLOT, 225. MARECHAL JOFFRE
5. AE (4//7%) 226. CAYUGA WHITE, 227. KUNBARAT, 228. KUNLEANY, 229. PHOENIX
6. AG (1/2%) 230. SIRIUS
7. AK (2/3%) 231. DIANA , 232. EGER 7
8. AM (1/2%) 233. REGENT
9. AP (4/7%) 234. AGAWAM , 235. *CATAWBA*, 237. *CATAWBA (4N)*, 237. NOAH, 238. ORION
10. AQ (1/2%) 239. BLUESTAR
11. BA (1/2%) 240. LUE NAI
12. BK (1/2%) 241. BETA
13. CA (2/3%) 242. *DR. DECKERREBE*, 243. *DR. DECKERREBE (4N)*
14. CB (1/2%) 244. HIRO HAMBURG
15. CD (3/5%) 245. *ARIS*, 246. *ARIS (4N)*, 247. CASTOR (4N)
16. CE (2/3%) 248. FREIBURG 3925-1, 249. OBERLIN 701
17. CG (1/2%) 250. POLLUX
18. CI (1/2%) 251. OBERLIN NOIR
19. CK (3/5%) 252. GEILWEILERHOF B-2-11, 253. GEILWEILERHOF B-2-11 (4N), 254. NAUMBURG 2-76-24
20. DG (2/3%) 255. TELEKI 125 AA, 256. TELEKI 5 C
21. EA (2/3%) 257. CHAMBOURCIN, 258. ISABELLA
22. EB (3/5%) 259. DELAWARE, 260. NAUMBURG 5004-51, 261. NAUMBURG 5028-670
23. ED (1/2%) 262. KOBER 5 BB
24. EF (1/2%) 263. SORI
25. EK (4/7%) 264. BINOVA, 265. GEISENHEIM 26, 266. *SELECTION OPPENHEIM 4*, 267. *SELECTION OPPENHEIM 4 (4N)*
26. FB (1/2%) 268. SIEGFRIEDREBE
27. KK (2/3%) 269. KOBER 127 BB, 270. TELEKI 5 A
28. MG (1/2%) 271. EVEX 13-3
29. MK (1/2%) 272. TELEKI 9 A

Vitis species (41)

1. AA (1/2%) 273. V. CINEREA ENGELMANN
6. AG (1/2%) 274. V. TRELEASEII MUNSON
13. CB (2/5%) 275. V. CHAMPINII PLANCHON 276. V. RIPARIA CL. BARRETT 75
16. CG (3/7%) 277. *CONCORD*, 278. *CONCORD (4N)*, 279. V. RIPARIA CL. PENETANGUISKENE
18. CK (2/5%) 280. V. RIPARIA CL. BARRETT 50, 281. V. SLAVINII REHDER
21. EA (2/5%) 282. V. LONGII PRINCE, 283. V. RUBRA MICHAUX
22. EB (5/30%) 284. V. DOANIANA MUNSON, 285. V. GIRDIANA MUNSON, 286. V. RIPARIA CL. BARRETT 5-6, 287. V. SIMPSONII MUNSON, 288. V. BETULIFOLIA DIELS ET GILG
30. EG (3/7%) 289. V. RIPARIA CL. BARRETT N 3-43, 290. V. RIPARIA MICHAUX, 291. V. SHUTTLEWORTHII HOUSE
31. EI (1/2%) 292. V. COIGNETIAE PULLIAT
32. EK (8/20%) 293. GEISENHEIM 1, 294. GOETHE 9, 295. RIPARIA GRAND GLABRE, 296. RIPARIA PORTALIS ROUGE, 297. RIPARIA PUBESCENT, 298. SALT CREEK, 299. V. RIPARIA CL. GOLDWATER, 300. V. RIPARIA CL. LAKE ST. GEORGES
33. EO (2/5%) 301. V. AESTIVALIS MICHAUX, 302. V. AESTIVALIS MICHAUX
34. KG (2/5%) 303. V. AMURENSIS RUPRECHT, 304. V. ANDERSONII REHDER
35. KP (1/2%) 305. V. LABRUSCA LINNE
36. MA (1/2%) 306. V. PIASEZKII MAXIMOWICZ
37. MB (1/2%) 307. V. BERLANDIERI PLANCHON
38. NK (4/10%) 308. *RUPESTRIS DU LOT*, . 309. *RUPESTRIS DU LOT (4N)*, 310. *V. RUPESTRIS SCHEELE*, 311. *V. RUPESTRIS SCHEELE (4N)*
39. OA (1/2%) 312. V. DAVIDII (ROM. DU CALL.) FOEX
40. OG (1/2%) 313. V. DAVIDII CL. B 1668 ??

Table 3

Analyzed cultivars in alphabetical order; more details may be found in Tab. 2 according to their leading numbers

1.	ABONDANT	# AA
2.	AFUS ALI	# AA
234.	AGAWAM	$ AP
191.	AGOSTENGA	# AG
68.	ALBALONGA	# AB
139.	ARGANT	# AD
245.	*ARIS*	*$ CD*
246.	*ARIS (4N)*	*$ CD*
69.	ARNSBURGER	# AB
70.	AUGSTER BLAU	# AB
151.	*AUXERROIS*	*# AE*
152.	*AUXERROIS*	*# AE*
71.	*BACCHUS*	*# AB*
72.	*BACCHUS (4N)*	*# AB*
192.	BAI SHA SI LA	# AG
73.	BAI XIANG JIAO	# AB
219.	BAILEY ALICANTE A	$ AB
241.	BETA	$ BK
264.	BINOVA	$ EK
153.	BLAUBURGER	# AE
74.	*BLAUFRÄNKISCH*	*# AB*
75.	*BLAUFRÄNKISCH(4N)*	*# AB*
239.	BLUESTAR	$ AQ
3.	BOUQUETRIESLING	# AA
154.	BOUVIER	# AE
76.	CABERNET FRANC	# AB
77.	CARMENERE	# AB
247.	CASTOR (4N)	$ CD
235.	CATAWBA	$ AP
236.	CATAWBA (4N)	$ AP
226.	CAYUGA WHITE	$ AE
257.	CHAMBOURCIN	$ EA
140.	*CHASSELAS BLANC*	*# AD*
141.	*CHASSELAS BLANC (4N)*	*# AD*
4.	CHASSELAS IMPERIAL	# AA
193.	CHASSELAS MUSQUE	# AG
78.	CHASSELAS VIOLET	# AB
5.	CHENIN BLANC	# AA
6.	*COMTESSA*	*# AA*
7.	*COMTESSA (4N)*	*# AA*
277.	*CONCORD*	*& CG*
278.	*CONCORD (4N)*	*& CG*
8.	COT	# AA
79.	DALKAUER	# AB
9.	DECKROT	# AA
259.	DELAWARE	$ EB
231.	*DIANA*	*$ AK*
208.	*DIANA (4N)*	*# AK*
80.	*DOMINA*	*# AB*
81.	*DOMINA (4N)*	*# AB*
82.	DORNFELDER	# AB
242.	*DR.DECKERREBE*	*$ CA*
243.	*DR.DECKERREBE (4N)*	*$ CA*
83.	DUNKELFELDER	# AB
84.	EARLY MUSCAT	# AB
135.	EDELSTEINER	# AC
220.	EGER 1	$ AB
85.	EGER 26	# AB
232.	EGER 7	$ AK
10.	EHRENFELSER	# AA
11.	**ELBLING ROT**	**# AA**
12.	**ELBLING WEISS**	**# AA**
13.	*ELBLING WEISS (4N)*	*# AA*
14.	**EMERALD RIESLING**	**# AA**
271.	EVEX 13-3	$ MG
155.	FABERREBE	# AE
142.	FINDELKIND	# AD
209.	FONTANARA	# AK
15.	*FORTA*	*# AA*
16.	*FORTA (4N)*	*# AA*
143.	FOSTER'S WHITE SEEDLING	# AD
248.	FREIBURG 3925-1	$ CE
156.	FREISAMER	# AE
86.	FURMINT	# AB
157.	GAMAY NOIR	# AE
158.	GAMAY TEINTURIER DE BOUZE	# AE
159.	GAMAY TEINT DE CHAUDENAY	# AE
160.	GAMAY TEINTURIER FREAUX	# AE
252.	*GEILWEILERHOF B-2-11*	*$ CK*
253.	*GEILWEILERHOF B-2-11 (4N)*	*$ CK*
212.	GEILWEILERHOF GA-48-12	$ AA
213.	GEILWEILERHOF GA-50-34	$ AA
221.	GEILWEILERHOF GA-58-14	$ AB
87.	GEISDUTTE BLAU	# AB
293.	GEISENHEIM 1	& EK
265.	GEISENHEIM 26	$ EK
161.	GLORIA	# AE
294.	GOETHE 9	& EK
88.	GUTENBORNER	# AB
162.	HÄNGLING BLAU	# AE
136.	*HELFENSTEINER*	*# AC*
137.	*HELFENSTEINER (4N)*	*# AC*
17.	*HEROLDREBE*	*# AA*
18.	*HEROLDREBE (4N)*	*# AA*
19.	HEUNISCH BLAU	# AA *
163.	HEUNISCH ROT	# AE *
194.	HEUNISCH WEISS	# AG *
89.	HIMBERTSCHA	# AB
244.	HIRO HAMBURG	$ CB
144.	HONIGLER	# AD
20.	HUXELREBE	# AA
258.	ISABELLA	$ EA
164.	ITALIA	# AE
195.	JUBILÄUMSREBE	# AG
90.	KANZLER	# AB
91.	***KERNER***	***# AB***
92.	*KERNER (4N)*	*# AB*
93.	**KERNLING**	**# AB**
21.	KNIPPERLE	# AA
269.	KOBER 127 BB	$ KK
262.	KOBER 5 BB	$ ED
165.	KOLOR	# AE
22.	KORINTHIAKI	# AA
145.	*KOSHU*	*# AD*
146.	*KOSHU (4N)*	*# AD*
227.	KUNBARAT	$ AE
228.	KUNLEANY	$ AE
214.	KYOHO	$ AA
94.	LAGREIN	# AB
224.	LEON MILLOT	$ AD
166.	LONG YAN	# AE
23.	LOUISETTE	# AA
240.	LUE NAI	$ BA
147.	*MADELEINE ANGEVINE*	*# AD*
148.	*MADELEINE ANGEVINE (4N)*	*# AD*
167.	MALINGRE PRECOCE	# AE
222.	MARECHAL FOCH	$ AB
225.	MARECHAL JOFFRE	$ AD
95.	MARIENRIESLING	# AB
96.	MARIENSTEINER	# AB
97.	MARKANT	# AB
98.	MAROC GROS	# AB
210.	MERLOT BLANC	# AK *
168.	MERLOT NOIR	# AE *
169.	*MORIO MUSKAT*	*# AE*
170.	*MORIO MUSKAT (4N)*	*# AE*
99.	*MÜLLER THURGAU*	*# AB*
100.	*MÜLLER THURGAU (4N)*	*# AB*
101.	*MÜLLER TH.(4N) CL.EBERTSH.*	*# AB*
102.	*MÜLLER TH.CL.EBERTSH.*	*# AB*
171.	MULTANER	# AE
24.	MUSCABONA	# AA
172.	MUSCAT A PET.GR. ROUGES	# AE
223.	MUSCAT BAILEY A	$ AB
173.	MUSCAT BLANC	# AE
103.	MUSCAT DE MARSEILLE	# AB
104.	MUSCAT FLEUR D'ORANGER	# AB
105.	MUSCAT HAMBURG	# AB
106.	MUSCAT LIERVAL	# AB
107.	MUSCAT OTTONEL	# AB
174.	MUSCAT PRECOCE DE SAUMUR	# AE
25.	MUSCAT ROUGE DE MADERE	# AA
108.	MUSCAT ST. LAURENT	# AB
26.	MUSKATRIESLING	# AA
254.	NAUMBURG 2-76-24	$ CK
260.	NAUMBURG 5004-51	$ EB
215.	NAUMBURG 5016-37	$ AA
261.	NAUMBURG 5028-670	$ EB
27.	NIU NAI	# AA
237.	NOAH	$ AP
28.	*NOBLESSA*	*# AA*
29.	*NOBLESSA (4N)*	*# AA*
138.	NOBLING	# AC
249.	OBERLIN 701	$ CE
251.	OBERLIN NOIR	$ CI
30.	*OPTIMA*	*# AA*
31.	*OPTIMA (4N)*	*# AA*
32.	ORANIENSTEINER	# AA
238.	ORION	$ AP
33.	ORTEGA	# AA
196.	OSIRIS	# AG
175.	OSTEINER	# AE
34.	PERLE	# AA
149.	PERLE VON CSABA	# AD
229	PHOENIX	$ AE
35.	PIN ER PU TAO	# AA
176.	*PINOT BLANC*	*# AE*
177.	*PINOT GRIS CL.DUNKELGRAU*	*# AE*
178.	*PINOT GRIS CL.HELLGRAU*	*# AE*
179.	*PINOT MEUNIER*	*# AE*
180.	*PINOT NOIR*	*# AE*
181.	*PINOT NOIR*	*# AE*
36.	**PIQUEPOUL BLANC**	**# AA**
37.	**PIQUEPOUL NOIR**	**# AA**
250.	POLLUX	$ CG
109.	*PORTUGIESER BLAU*	*# AB* *
110.	*PORTUGIESER BLAU (4N)*	*# AB*
111.	*PORT.BLAU (4N) CL.INGENHEIM*	*# AB*
112.	*PORT.BLAU CL.INGENHEIM*	*# AB*
38.	PORTUGIESER GRUEN	# AA *
182.	PUTZSCHEERE	# AE
197.	RABANER	# AG
39.	RÄUSCHLING WEISS	# AA
40.	RAMISCO	# AA
233.	REGENT	$ AM
113.	REGNER	# AB
114.	REICHENSTEINER	# AB
183.	RIESLANER	# AE
115.	RIESLING BLAU	# AB *
41.	RIESLING BULGARSKI	# AA
42.	**RIESLING ROT**	**# AA**
43.	***RIESLING WEISS (4N) CL.1***	***# AA***
44.	*RIESLING WEISS (4N) CL.4*	*# AA*
45.	*RIESLING WEISS (4N) CL.90*	*# AA*
46.	*RIESLING WEISS (4N) CL.GLEIS.*	*# AA*
49.	*RIESLING WEISS CL.239*	*# AA*
47.	*RIESLING WEISS CL.4*	*# AA*
48.	*RIESLING WEISS CL.90*	*# AA*
50.	*RIESLING WEISS CL.GLEIS.*	*# AA*
295.	RIPARIA GRAND GLABRE	& EK
296.	RIPARIA PORTALIS ROUGE	& EK
297.	RIPARIA PUBESCENT	& EK
116.	ROTBERGER	# AB
51.	ROTGIPFLER	# AA
216.	ROU DING XIANG	$ AA
308.	*RUPESTRIS DU LOT*	*& NK*
309.	*RUPESTRIS DU LOT (4N)*	*& NK*
52.	SAINT LAURENT	# AA
298.	SALT CREEK	& EK
53.	SAUVIGNON BLANC (4N)	# AA
54.	*SCHEUREBE*	*# AA*
55.	*SCHEUREBE (4N) CL.K.LAY*	*# AA*
56.	*SCHEUREBE CL.K.LAY*	*# AA*
200.	*SCHIAVA GROSSA*	*# AG*
198.	*SCHIAVA GROSSA*	*# AG*
199.	*SCHIAVA GROSSA*	*# AG*
201.	*SCHIAVA GROSSA (4N)*	*# AG*
117.	SCHÖNBURGER	# AB
266.	*SELECTION OPPENHEIM 4*	*$ EK*
267.	*SELECTION OPPENHEIM 4 (4N)*	*$ EK*
57	SEPTIMER	# AA
118.	*SHIRAZ*	*# AB*
119.	*SHIRAZ (4N)*	*# AB*
120.	*SIEGERREBE*	*# AB*
121.	*SIEGERREBE 4N*	*# AB*
268.	SIEGFRIEDREBE	$ FB
184.	**SILVANER BLAU**	**# AE**
185.	***SILVANER GRUEN (4N) CL. 75***	***# AE***
186.	*SILV.GRUEN (4N) CL. BOSENHEIM*	*# AE*
187.	*SILVANER GRUEN CL. 75*	*# AE*
188.	*SILVANER GRUEN CL. BOSENH.*	*# AE*
230.	SIRIUS	$ AG
202.	SISI	# AG
263.	SORI	$ EF
58.	SÜSSROT	# AA
211.	SULMER	# AK
59.	*SULTANINA*	*# AA*
60.	*SULTANINA*	*# AA*
61.	SYRISCHER WEISS (4N)	# AA
189.	TEINTURIER	# AE
255.	TELEKI 125 AA	$ DG
270.	TELEKI 5 A	$ KK
256.	TELEKI 5 C	$ DG
272.	TELEKI 9 A	$ MK
122.	**TERRET GRIS**	**# AB**
123.	**TERRET NOIR**	**# AB**
124.	*TOMPA MIHALY*	*# AB*
125.	*TOMPA MIHALY (4N)*	*# AB*
62.	***TRAMINER ROT***	***# AA***
63.	*TRAMINER ROT (4N)*	*# AA*
64.	*TRAM.ROT (GEWÜRZTRAMINER)*	*# AA*
65.	TRAMINER WEISS	# AA
203.	TROLLINGER BL.FRUEHDUFTIG	# AG
204.	**URBAN ROT**	**# AG**
205.	**URBAN BLAU**	**# AG**
302.	*V. AESTIVALIS MICHAUX*	*& EO*
301.	*V. AESTIVALIS MICHAUX*	*& EO*
303.	V. AMURENSIS RUPRECHT	& KG
304.	V. ANDERSONII REHDER	& KG
307.	V. BERLANDIERI PLANCHON	& MB
288.	V. BETULIFOLIA DIELS ET GILG	& EE
275.	V. CHAMPINII PLANCHON	& CB
273.	V. CINEREA ENGELMANN	& AA
292.	V. COIGNETIAE PULLIAT	& EI
312.	V. DAVIDII (ROM. DU CALL.) FOEX	& OA
313.	V. DAVIDII CL. B 1668	& OG
284.	V. DOANIANA MUNSON	& EB
285.	V. GIRDIANA MUNSON	& EB
305.	V. LABRUSCA LINNE	& KP
282.	V. LONGII PRINCE	& EA
306.	V. PIASEZKII MAXIMOWICZ	& MA
286.	V. RIPARIA CL. BARRETT 5-6	& EB
280.	V. RIPARIA CL. BARRETT 50	& CK
276.	V. RIPARIA CL. BARRETT 75	& CB
289.	V. RIPARIA CL. BARRETT N 3-43	& EG
299.	V. RIPARIA CL. GOLDWATER	& EK
300.	V. RIPARIA CL. LAKE ST. GEORG.	& EK
279.	V. RIPARIA CL. PENETANG.	& CG
290.	V. RIPARIA MICHAUX	& EG
283.	V. RUBRA MICHAUX	& EA
310.	*V. RUPESTRIS SCHEELE*	*& NK*
311.	*V. RUPESTRIS SCHEELE (4N)*	*& NK*
291.	V. SHUTTLEWORTHII HOUSE	& EG
287.	V. SIMPSONII MUNSON	& EB
281.	V. SLAVINII REHDER	& CK
274.	V. TRELEASEII MUNSON	& AG
206.	**VELTLINER FRÜHROT**	**# AI ***
66.	VELTLINER GRÜN	# AA *
207.	**VELTLINER ROT**	**# AI**
150.	VERDOT GROS	# AD *
126.	VERDOT NOIR	# AB *
217.	WALLIS GIANT (4N)	$ AA
218.	WATKINS	$ AA
127.	WELSCHRIESLING	# AB
190.	WILDBACHER FRÜH BLAU	# AE
128.	WÜRZBURGER	# AB
129.	WÜRZER	# AB
130.	ZIERFANDLER ROT	# AB
131.	ZIMMETTRAUBE	# AB
132.	ZWEIGELT BLAU	# AB
67.	unknown	# AA
133.	unknown	# AB
134.	unknown	# AB

Discussion

In a first paper (BACHMANN and BLAICH 1988) a small collective of 71 different varieties were compared for their peroxidase pattern of phloem internodes. Since at that time the acidic proteins were not separated, the characteristic acidic A type of *vinifera* and some I.C. cvs was not detected. With the new technique we obtain both the previously described patterns for the neutral to basic isoperoxidases of *vinfera* cvs, and in addition a further separation of the acidic isoperoxidases.

Within *vinifera* only the basic peroxidases could be used for differentiation, since acid type A is characteristic for all *viniferas* with 211 cultivars belonging to this group. In contrast, only half of the I.C. cvs (28 of 61) and only 2 of 41 *Vitis* spp. are of type A. The relative frequencies of the isoenzymes in *vinifera* cvs are AA 32 %,AB 32 %, AE 19 %, AG 7 %, AD 6 %, AC 2 %, AK 2 %. In I.C. cvs the frequency of type AA is 11 %, AB 8 %, AD 3 %AE 6 %, AG 2 %, AK 3 %, AM 2 %, AP 6 %, AQ 2 %.

Rootstocks which are mostly of type D*, E* (* indicates any type of basic enzymes), KK and MK are different from *vinifera* and I.C. cvs New I.C. breedings with "accepted wine quality" usually belong to the *vinifera* type A*.

V. riparia selections very often have the pattern type EK (7), EG (2), CB (1) and EB (1). Characteristic for the amerasian *Vitis* species is a high variability in both peroxidase groups. The basic C and D types could not be found in *Vitis* species,where the most frequent type is K (34 %), which is exhibited with 21 % of the I.C. and only 2 % in *V. vinifera* cvs.

Since all peroxidase bands in non-*Vitis* spp. are different from the rest of the analyzed material, these samples were not evaluated. There is no enzymatic interrelationship for peroxidase isoenzymes between *Vitis* on the one hand and *Ampelopsis* and *Parthenocissus* on the other, although the latter have some identical bands. Cultivars of the same genetic origin like colour mutants in the burgundy group or Pinot family (Pinot blanc: 176, Pinot gris: 177/178, Pinot noir: 180, and Pinot Meunier: 179) have the same pattern (AE). This group contains also cultivars like Auxerrois: 151-152 and Gamay: 157-160. This supports cv. Auxerrois originating from the burgundy family.

No variation within polyploid forms and clones could be detected although 23 % of 313 samples are forms of different ploidy types (marked with *italics* in Tab. 2 and 3.)

Colour forms of Elbling (red: 11, white: 12-13), Piquepoul (blanc: 37, noir: 38) Riesling (red: 42, white: 43-50), Silvaner (blue: 184, green: 185-188), Traminer (white: 65, red: 62-63), 122. Teret (gris: < 122, noir: 123), Urban (red: 204, blue: 205) could not be distinguished by their pattern. However, Riesling (blue: 15 = AB, white: 41- 50 = AA), Portugieser (blue: 109-112 = AB, green: 38 = AA), Merlot (noir: 68 = AE, blanc: 210 = AK), two clones of Veltliner (red: 206,207 = AI, green: 66 = AA) are different. Heunisch (blue:19 = AA, red: 163 = AE, white: 194 = AG) is represented by 3 different patterns. These samples are marked with asterisks in Tab. 2 and 3.

Colour varieties exhibiting different patterns will be tested with more material from other collections. Genetic differences should then be corroborated with molecular genetic methods (BÜSCHER *et al.* 1994).

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Received July 11, 1994