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"BLAUER PORTUGIESER", THE DISSEMINATION OF A GRAPEVINE

"BLAUER PORTUGIESER", A EXPANSÃO DE UMA CASTA

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SUMMARY

"Blauer Portugieser" is planted on more than 20,000 ha and therefore belongs to the top 60 cultivars for viticulture worldwide. Although its growing area decreases slightly, due to numerous off springs the gene pool of this variety is still growing and will further be used for practical viticulture.

In this study we used more than 30 SSR markers to investigate the origin of the cultivar. The DNA was prepared from fresh young leaves of plant material from the germplasm collections of Portugal and of Austria.

The analyses by microsatellites reveal at 32 SSR *loci* that the cultivar, "Blauer Portugieser" is identical to "Português Azul". Despite several common alleles two types of Moreto could not be recognized as closely or directly related . Furthermore two colour types (green and grey) of Blauer Portugieser with minor economic importance were analysed at all SSR *loci* and couldn`t be differentiated by SSR markers involved in this study.

Key words: SSR markers, microsatellites, *Vitis vinifera*
Palavras chave: Marcadores SSR, micro-satélites, *Vitis vinifera*

INTRODUCTION

"Blauer Portugieser" is a grapevine cultivar for the production of smooth light red wines. The early ripening of berries and quality of the wine convinced growers of temperate and cooler climate regions to cultivate this variety (Ambrosi *et al.,* 1994). According to Galet (1990) "Blauer Portugieser" was planted on more than 20,000 ha and therefore belonged to the top 60 cultivars for viticulture worldwide. The most important production is located in Germany (4,500 ha), Austria (3,000 ha), France, Croatia, Slovenia, Hungary, Czech Republic, Slovakia and Northern Italy (Ambrosi *et al.,* 1994).

In the literature there could be found different opinions about the origin of this cultivar. Recently the way of "Blauer Portugieser" from Portugal to Austria (Schumann, 1982) could be illuminated. The variety was imported by an Austrian embassador in 1772 and planted in the region of Vöslau. From there the new cultivar spread to several European vine growing areas. Several synonyms remind us about its way of dissemination. Some of the synonyms are "Voeslauer", "Badner", "Autrichien", "Feslauertraube", "Oporto" and "Kekoporto" (Goethe, 1887).

The sensitivity of the variety to winter frost is an indicator of the more southern origin of this cultivar (Ruckenbauer and Traxler, 1983). The earliness of ripening represents a valuable trait for grape breeding in temperate and cooler regions. Therefore the cultivar "Português Azul" was mainly kept in mountainous regions located at 1000 meter above sea level. Due to the favorable suitability of "Blauer Portugieser" for the Austrian viticulture the cultivar spread very quickly and even was used for numerous crossings. In the meantime an offspring of "Blauer Portugieser" x "Blaufränkisch" namely "Blauburger" has spread to some wine growing areas while recently bred cultivars "Roesler", "Seifert" and "Rathay" were classified and are going to be introduced for production. Therefore while the growing area for "Blauer Portugieser" was slightly reduced (Galet, 1990), the gene pool of this variety is still in use and will be disseminated by progenies of Portugieser.

The economic importance of Portugieser for Mid Europe intensified the wish to find out the origin of this cultivar. Furthermore it should be verified if "Moreto" shows genetic relationships to Portugieser. Once, the cultivar "Moreto" from Portugal was supposed as the origin of "Blauer Portugieser" (Goethe, 1887). This supposition was hardly questioned by ampelographical methods, but the identity should be verified by genetic analysis. The most appropriated tools for characterizing grapevine genotypes are nowadays microsatellite markers. We used more than 30 SSR (simple sequence repeats) markers to investigate the origin of the cultivar "Blauer Portugieser". Beside the comparison of the involved genotypes, data of more than 350 cultivars are available and have been taken into consideration by concluding this study.

MATERIAL AND METHODS

The plant material of "Moreto" and "Português Azul" was obtained from the germplasm collections of the *Instituto Nacional de Investigacão Agrária*, Quinta da Almoinha, Dois Portos, Portugal. Several "Portugieser Blau" clones and most of the grapevine material used in this study is kept by the *Höhere Bundeslehranstalt und Bundesamt für Wein- und Obstbau*, Klosterneuburg, Austria. Independent reference samples of cultivars were obtained from

grapevine collections of Freiburg (D), Geisenheim (D), Geilweilerhof (D), San Michele (I), Pecs (H), Montpellier (F) and Senkvice (Sk). More than 400 cultivars were analysed by genotyping with SSR markers. In table I an overview is given concerning cultivars, which could show some linkage to "Blauer Portugieser" due to their age and place of first appearance. However, all cultivars analysed so far were proved about their genetic similarities to "Blauer Portugieser".

TABLE I

List of 96 grapevine cultivars which were analysed by SSR markers and was supposed to could have genetic relationship to Portugieser Blau

Lista das 96 castas estudadas com marcadores SSR e que supostamente teriam relações genéticas com a "Blauer Portugieser"

Affenthaler N	Burgunder N/B/G	Fiano B	Monica B
Albana B	Cabernet franc N	Frankentaler B	Mondeuse B
Aligote B	Cabernet Sauv. N	Freisa N	Morillon B
Altesse B	Calitor B	Frühgipfler B	Moreto N
Amigne B	Canaiola N	Furmint B	Petit Meslier B
Ansonica B	Carignan N	Gamay N	Petit Verdot N
Aramon N	Catarrato B	Gordin B	Picolit B
Ardelanca B	Cesar N	Grenache N	Pineau d Àunis N
Aubun N	Chardonnay B	Heunisch B	Piquepol B
Auxerrois B	Chasselas B	Honigler B	Plant vert B
Barbaroux R	Chasselas musq. B	Humagne B	Poulsard N
Baroque B	Chasselas Court.B	Juracon B	Riesling N/RG
Basilicum B	Chenin B	Kauka N	Rousanne B
Bellevue B	Chicaud B	Köllner N	Sauvignon B
Bequinol N	Cirfandli R	Kurzstingler B	Savagnin B
Berbecel B	Clairette B	Lagrein N	Schwarzriesling N
Bermestia B	Colombard B	Laska N	Silvaner B/N
Bianchetta Tr.B	Cortese B	Madleine B	St. Laurent N
Arbst N	Courtillier musq.B	Malvasia N	Traminer G
Blaufraenkisch N	Croatina N	Marsanne B	Trollinger N
Bouquet B	Dolcetto N	Mauzac B	Teinturier N
Brachet G	Durella B	Melon B	Verdesse B
Brunello N	Elbling B	Merlot N	Veltliner Rot RG
Budai B	Erbaluce B	Molette B	Wildbacher N

The DNA was prepared from fresh young leaves using a shortened version of an established (Thomas *et al.*, 1993) purification method. The method is based on maceration of the tissue by liquid N2 and grinding in a mortar, then immediately DNA is extracted by addition of the lysis buffer. DNA of satisfying purity could be isolated from canes, roots, old leaves or other tissues by using the unshortened version (Thomas *et al.*, 1993).

PCR was performed at an OmniGene (Hybaid) thermal cycler using a two step protocol (Smith *et al.*, 1995) with the following procedure: 2 min at 94°C for denaturation and then 40 cycles of 30 sec. annealing temperature (45-55°C) and 30 sec 90°C denaturation.

Following SSR markers were used for genotyping: VVS (Thomas *et al.,* 1993), VVMD (Bowers *et al.*, 1996) VrZAG (Sefc *et al.*, 1999)

An aliquot of the reaction mixture was loaded on a 2% agarose gel to estimate the amount of allelic DNA. Separation of the alleles was performed at a 6% polyacrylamid gel with sequencing format. Detection of fragments was done by silver staining (Silver Sequence, Promega) of the gel and manual measuring of the length by using reference probes.

The morphological description of the studied biotypes is based on the "Descriptor list for grape vine varieties and *Vitis* species" (OIV, Edition 09. 1983), and we have used the 32 characters of "Primary Descriptor List for Grapevine Cultivars and Species (*Vitis* L.)" (EU-Project GENRES – 081, 1999), (Table II).

TABLE II

Ampelographic and phenological description of "Blauer Portugieser"

Descrição ampelográfica e fenológica da "Blauer Portugieser".

Young shoot:		
001	shape of the tip	opened
003	intensity of anthocyanin colouration of the prostrate hairs of the tip	absent
004	density of prostrate hairs of the tip	sparse
Shoot:		
007	colour of dorsal side of internodes	green with red stripes
008	colour of ventral side of internodes	green
015	anthocyan colouration of the scales of the buds	medium
Tendrils:		
016	dirtribution on the shoot	discontinuous
Young leaf:		
051	colour of the upper side (leaves 1-4)	green
053	density of prostrate hairs between veins (lower side, leaf 4)	very sparse
Mature leaf:		
067	shape of blade	pentagonal
068	number of lobes	three - five lobes
070	anthocyanin colouration of main veins of the upper side	absent
072	goffering of the blade	absent
074	profile	flat
075	blistering of the upper side	very weak
076	shape of teeth	both side convex
079	degree of petiole sinus opening	slightly overlapping
080	shape of base petiol sinus	V-shaped
081-1	presence of teeth on the margin of the petiole sinus	none
081-2	naked petiole sinus	none
083-2	presence of teeth at the base of the upper leaf sinus	none
084	density of prostrate hairs on the lower side	very weak
087	density of erect hairs on the lower side	weak
Inflorenscence:		
151	sex of flower	hermaphrodite
Bunch:		
208	shape	conical
209	presence of a wing	2 wings
Berry:		
223	shape	short elliptic
225	colour of skin	blue - black
231	colour of flesh	non coloured
236	particular flavour	none
241	presence of seeds	present

Shoot tip:nearly hairless, yellow green.
Mature leaf: large, light green, three to five lobes, thin, flat, teeth both side convex, base of petiol sinus V - shaped, slightly overlapping.
Bunch: coonical with 2 wings, medium to large.
Berry: short elliptic, blue - black, weakly bloom, thin skin, fruity sweet flavour.
Early budburst, early - middle early ripening, flowering medium, strong vegetative vigour, very high and continously crop (10,000 – 15,000 kg/ha).
This variety needs dry, calcerous, airness, rich on nutrition soil. Wet soil condition are not usefull.
Sensitiveness: winterfrost, springfrost, Oidium high sensitive, Plasmopara, Botrytis, wind, sensitive to pesticide with copper. Recommended rootstock: Kober 5 BB, Kober 125 AA.

This short version of ampelographic description was used for an efficient and faster preselection process. Only few genotypes showed high similarity and therefore these cultivars were involved in genetic analysis.

RESULTS AND DISCUSSION

According to the description (Table III) of the four comparated biotypes, we can verify some significative morphological differences between "Moreto" (Alentejo), "Moreto" (Dão) and "Blauer Portugieser" / "Português Azul". The latter have no significative morphological differences. In what concerns differences between the two "Moreto" and "Blauer Portugieser"/ "Português Azul" we can verify that both biotypes of "Moreto" present denser prostrate

TABLE III

Ampelographic description of "Blauer Portugieser", "Português Azul", "Moreto" (Alentejo) and "Moreto" (Dão).

Descrição ampelográfica da "Blauer Portugieser", "Português Azul", "Moreto" (Alentejo) e "Moreto" (Dão).

OIV Code	**"Blauer Portugieser"**	**"Português Azul"**	**"Moreto (Alentejo)"**	**"Moreto (Dão)"**
Young shoot:				
001 shape of the tip	7 - opened	7 - opened	7 - opened	7 - opened
003 intensity of anthocyanin coloration of the prostrate hairs of the tip	1 - absent	3 – weak	5 – medium	5 – medium
004 density of prostrate hairs of the tip	3 - sparse	3 - sparse	**7 – dense**	**5 – medium**
Shoot:				
007 colour of dorsal side of internodes	2 - green with red stripes	2 - green with red stripes	2 - green with red stripes	1 - green
008 colour of ventral side of internodes	1 - green	1 - green	2 - green with red stripes	1 - green
015 anthocyanin coloration of bud scales	5 - medium	5 - medium	**1 - absent**	**1 - absent**
Tendrils:				
016 dirtribution on the shoot	1 - discontinuous	1 - discontinuous	1 - discontinuous	1 - discontinuous
Young leaf:				
051 colour of the upper side (leaf 4)	1 - green	5 – copper yellow	5 – copper yellow	3 - yellow
053 density of prostrate hairs between veins (lower side, leaf 4)	1 - very sparse	1 - very sparse	**7 – dense**	**5 – medium**
Mature leaf:				
067 shape of blade	3 - pentagonal	3 - pentagonal	3 - pentagonal	3 - pentagonal
068 number of lobes	2/3 - three - five lobes	2/3 - three - five lobes	3 - five lobes	2 - three lobes
070 anthocyanin coloration of main veins on the upper side	1 - absent	1 - absent	1 - absent	1 – absent
072 goffering of the blade	1 - absent	1 - absent	**9 – present**	1 – absent
074 profile	1 - flat	1 - flat	**5 - rolled**	1 – flat
075 blistering of the upper side	1 - very weak	1 - very weak	5 – medium	**7 - strong**
076 shape of teeth	3 - both side convex	3 - both side convex	3 - both side convex	3 - both side convex
079 degree of petiole sinus opening	6 - slightly overlapping	6 - slightly overlapping	6 - slightly overlapping	**7 - lobes overlapping**
080 shape of base petiol sinus	2 - V-shaped	2 - V-shaped	2 - V-shaped	2 - V-shaped
081-1 presence of teeth on the margin of the petiole sinus	1 - none	1 - none	**2 – 1 tooth on the petiole sinus**	**2 – 1 tooth on the petiole sinus**
081-2 naked petiole sinus	1 - none	1 - none	1 - none	1 - none
083-2 presence of teeth at the base of the upper leaf sinus	1 - none	1 - none	1 - none	1 - none
084 density of prostrate hairs between the veins (lower side)	1 - very weak	1 - very weak	**5 – medium**	**5 – medium**
087 density of erect hairs on main veins (lower side)	3 - weak	1 – very weak	1 – very weak	1 – very weak
Inflorenscence:				
151 sex of flower	3 - hermaphrodite	3 - hermaphrodite	3 - hermaphrodite	3 - hermaphrodite
Bunch:				
208 shape	2 - conical	2 - conical	2 - conical	2 - conical
209 presence of a wing	2 - 2 wings	2 - 2 wings	2 - 2 wings	2 - 2 wings
OIV Code	**"Blauer Portugieser"**	**"Português Azul"**	**"Moreto (Alentejo)"**	**"Moreto (Dão)"**
Berry:				
223 shape	4 - short elliptic	3 - roundish	3 - roundish	7 – obovate
225 colour of skin	6 - blue - black	6 - blue - black	6 - blue - black	6 - blue - black
230 colour of flesh	1 - not colored	1 - not colored	1 - not colored	1 - not colored
236 particular flavour	1 - none	1 - none	1 - none	1 - none
241 presence of seeds	3 - present	3 - present	3 - present	3 - present

hairs on the tip of the young shoot and on the lower side of the young and mature leaf. Generally, they also have one tooth on the margin of the petiole sinus. On the other hand, the "Moreto" (Alentejo) has a rolled profile, with goffering of the blade and the "Moreto" (Dão) present overlapping lobes of the petiole sinus.

According to the shown descriptors "Blauer Portugieser" offers the possibility to be identical with the cultivar "Português Azul". Nevertheless as "Moreto" was supposed to have a linkage to "Blauer Portugieser", two different "Moreto" (Alentejo and Dão) types were involved in genotyping.

"Português Azul" is cultivated in the demarcated region of Dão and Douro and early ripening is appreciated for production in higher areas. "Português

Azul" is authorized for the production of the regional wine of "Beiras" (Portaria nº 158/93), namely wines with the designation of "Beira Alta". The ampelographical similarities reveal that "Blauer Portugieser" and "Português Azul" could be related very close or could even be identical.

The analysis by microsatellites confirmed that "Blauer Portugieser" is identical to "Português Azul" (Table IV). At all 32 SSR loci investigated in this study the allele length was of the same size. As there could neither be recognized

Table IV

Allele length of the cultivars "Português Azul", "Portugieser Blau", "Moreto" (Dão) and "Moreto" (Alentejo) at different SSR loci.

Alelos das cultivares "Português Azul", "Portugieser Blau", "Moreto" (Dão) and "Moreto" (Alentejo) em diferentes SSR loci.

Locus	"Português Azul"	"Blauer Portugieser"	"Moreto Alentejo"	"Moreto Dão"
VVS 1	179:180	179:180	180:189	180:189
VVS 2	142:150	142:150	146:150	136:150
VVS 3	218:218	218:218	212:218	212:218
VVS 4	167:174	167:174	167	167
VVS 29	168	168	168	168
VVMD 5	224:230	224:230	224:234	224:234
VVMD 6	189:206	189:206	199:206	199:206
VVMD 7	240:252	240:252	240:254	246:252
VVMD 8	140	140	138:144	140:144
VVMD 14	238:244	238:244	236:244	236:244
VVMD 17	221:222	221:222	220:221	220:221
VVMD 21	248:257	248:257	248:255	257
VVMD 24	207:215	207:215	207	207:209
VVMD 25	250:250	250:250	242:250	250:256
VVMD 26	251	251	251	251
VVMD 27	180:193	180:193	180:188	180:188
VVMD 28	228:260	228:260	236:246	236:246
VVMD 31	203:209	203:209	211	211
VVMD 32	251:271	251:271	251:255	253:255
VVMD 36	262:274	262:274	252:262	252:262
VRZAG 7	155	155	155	155
VRZAG 15	165	165	165	165
VRZAG 21	200:206	200:206	202:204	202:204
VRZAG 25	225:236	225:236	236	236
VRZAG 29	114:116	114:116	112:116	112:116
VRZAG 30	149	149	149	149
VRZAG 47	159:172	159:172	159:167	159:167
VRZAG 62	187:203	187:203	187	195:203
VRZAG 64	139:163	139:163	143:163	139:141
VRZAG 67	126:132	126:132	126	126:132
VRZAG 79	248:258	248:258	242:248	248:250
VRZAG 83	190:194	190:194	194:198	196:200
VRZAG 112	229:240	229:240	238:240	229:240

any deviation in ampelographic nor in genetic profile it is evident that both genotypes represent one cultivar. Despite more than 200 years of separate propagation the genetic divergence seems to be insignificant. This fact is in contradiction to the occurrence of Portugieser types with different berry color as available with "*Portugieser Grau*" and "*Portugieser Grün*". As we know already (Regner *et al.,* 1998) from the Pinot cultivars berry color is not representative for the genetic stability of the cultivar. Therefore the appearance of grey and green berry color of Portugieser has not indicated genetic instability. Moreover the two color types (green and grey) of "Blauer Portugieser" were analysed at all SSR *loci* and couldn't be differentiated by any SSR markers involved in this study. However these types of Portugieser can easily be differentiated by their berry color and therefore are regarded as individual cultivars. Otherwise these cultivars obtained only minor economic importance.

In former times (1887) Goethe supposed that Portugieser was derived or even should be identical to the cultivar "Moreto". The genetic profile of two types (Alentejo and Dão) of "Moreto" differs (Table IV) and therefore more divergence of this cultivar can be assumed. However, the deviations of alleles by comparing them with "Blauer Portugieser" (Table III) are sufficient to exclude a direct genetic relationship of Moreto. Portugieser shared several alleles with Moreto and therefore a common ancestor cultivar cannot be excluded.

Considering these results and previous information we can conclude that "Blauer Portugieser" was originated from Portugal and that this cultivar is identical to "Português Azul". Further propagation was done at several Austrian nurseries. Therefore other synonyms of Portugieser irregularily were created for this cultivar. Despite intensive vegetative propagation no genetic changes could be found using SSR markers.

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RESUMO

"Blauer Portugieser", a expansão de uma casta

A "Blauer Portugieser" ocupa mais de 20.000 ha de vinha, pertencendo ao grupo das 60 culti-

vares mais plantadas no Mundo. Embora a área de cultura desta casta esteja em regressão, o seu património genético ainda é muito importante e continuará a sê-lo devido à disseminação dos cruzamentos de que é progenitora.

Neste estudo usaram-se mais de 30 marcadores SSR (de Sequências Simples Repetidas) para determinar a origem da cultivar "Blauer Portugieser", sendo o ADN obtido de folhas jovens provenientes de plantas existentes em colecções de germoplasma de Portugal e Áustria.

A análise dos micro-satélites revelou que a "Blauer Portugieser" é, em todos os 32 *loci* das SSR estudadas, idêntica à "Português Azul". Além disso, analisaram-se para todos os *loci* das SSR, duas mutações de côr de bago da "Blauer Portugieser" (branco e rosado) com menor importância económica, não tendo sido possível diferenciá-las pelos marcadores SSR envolvidos neste estudo.

RÉSUMÉ

"Blauer Portugieser", la dissemination d'un cépage

"Blauer Portugieser" est cultivé sur plus de 20.000 ha de vignes et, pour cela, il appartient au groupe des 60 variétés plus importants du monde. Quand même, son surface de culture est en regression, son patrimoine génétique est et restera trés important dû à la dissemination des croisements duquels il est ancêtre.

Dans cette étude nous avons utilisé plus de 30 marqueurs SSR (simple sequence repeats) pour determiner l'origine du cépage "Blauer Portugieser". L'ADN a été obtenu de jeune feuilles prouvenant de plants existants en collections de germoplasm du Portugal et d'Autriche.

L'analyse des microsatellites a révélé que le "Blauer Portugieser" est, pour tous les 32 *loci* SSR étudiés, identique a le "Português Azul". D'autre part, deux types de "Blauer Portugieser" avec moindre importance economique (avec baies blancs et baies gris), on été analysés pour tous les *loci* SSR et il n'y a été possible les différencier avec les marqueurs SSR utilisés dans cette étude.

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