Vitis **54** (Special Issue), 177–180 (2015)

Cluster architecture of old, neglected Croatian grapevine varieties (*Vitis vinifera* L.)

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Summary

Grape cluster architecture is an important trait affecting fruit composition and yield. Damage caused by gray mold (*Botrytis cinerea*) is directly related to cluster density and some other environmental factors. The aim of this study was to quantify characteristics of fully mature clusters of eleven old Croatian grapevine varieties (*Vitis vinifera* L.) which so far have not been phenotypically described in detail, and to examine *Botrytis cinerea* disease severity on their grapes under field conditions. Eight variables that describe the appearance of clusters were quantified in three consecutive years including cluster weight, length, width, volume, compactness, rachis weight, number of berries and single berry weight. Two-factor analysis of variance provided estimates of varietal and annual differences. Significant differences among eleven old Croatian varieties for all cluster traits were determined. Results suggest diverse cluster morphology within the old Croatian varieties revealing three variety groups by PCA analysis and their varying degrees of sensitivity to *Botrytis*. These phenotypic data on cluster structure represent a basis for future research, such as QTL analysis or assessment of productivity of these varieties.

K e y w o r d s : cluster morphology; *Botrytis*; ampelography; PCA analysis.

Introduction

Grape cluster architecture is an important trait affecting fruit composition and yield. At full maturity, there are clearly visible differences among cultivars in cluster shape, from very small to large, compact to very loose, and long to very short. Various cluster shapes and forms were likely one of the earliest criteria in human selection of grape cultivars, and later assisted in the morphological classification of grapevine cultivars into eco-geographical groups (Negrul 1938). Yield, one of the most important characteristics in viticulture, is influenced by numerous genetic loci and environmental factors. The cluster weight, berry size, and number of berries per cluster are essential components that significantly determine yield.

There are many cluster components, but cluster architecture is mainly determined by the total rachis length, weight, and length of lateral internodes (Shavrukov *et al.* 2004). Although cluster architecture is a very important trait for grape production, little is known about its genetic basis. Recently, 19 quantitative trait loci (QTL) and 50 genes were identified that are involved in genetic control of cluster architecture (Correa *et al.* 2014). This suggests a complex genetic base for grape cluster architecture.

Damage caused by gray mold (*Botrytis cinerea*) is directly related to cluster density and some other environmental factors. Cultivars with compact clusters are more sensitive to gray mold than those with loose clusters (Vail and Marois 1991, Mlikota Gabler *et al.* 2003). There are some viticultural techniques like cluster thinning or cluster division (Molitor *et al.* 2012), leaf removal (Poni *et al.* 2006), or anti-transpirants application (Palliotti *et al.* 2010) that can induce cluster loosening, but resistant cultivars are still intriguing for viticultural practis. The cluster compactness is usually estimated visually according to OIV system descriptors (OIV descriptor No. 204), and indirectly through several quantitative criteria that are calculated from cluster measurement variables (Tello and Ibáñez 2014).

The aim of this study was to quantify characteristics of fully mature clusters of eleven old Croatian grapevine varieties (*Vitis vinifera* L.), and to examine *Botrytis cinerea* disease severity on their grapes under field conditions. The varieties have been selected to represent different cluster structure and degree of cluster density.

Material and Methods

P l a n t m a t e r i a l : Eleven grapevine varieties (*Vitis vinifera* L.) previously identified by microsatellite markers (Zdunić *et al.* 2013) were selected for this study: 'Babić', 'Babica', 'Crljenak Viški', 'Glavinuša', 'Grk', 'Lasina', 'Plava Lovora', 'Plavac Mali Sivi', 'Pošip Bijeli', 'Svrdlovina', and 'Vrškajica'. They are very poorly represented in current grape production, but could be very important for future breeding programs and/or production revitalization. The clusters from each variety were collected from the grape germplasm repository of the Institute for Adriatic Crops and Karst Reclamation, which is located in Split (latitude, 43°30.335N; longitude, 016º29.855E; 14 m asl) in Croatia. The varieties were grown under identical cultivation and climatic conditions in a vineyard established in 2005. They are grafted onto 1103 Paulsen rootstocks and trained on the bilateral spur cordon system with spacing of 1.0 m among

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vines in a row and 2.0 m between rows. The soil in the vineyard is clay-sandy. The average annual temperature of the location was 16.1 °C; the average rainfall rate was 782.8 mm (average values for 1971 to 2000 recorded at the meteorological station in Split).

Determination of cluster structure: Ten representative clusters were collected from each variety at full maturity in three consecutive years (2008, 2009 and 2010) to determine cluster dimensions. Cluster weight (g), length (cm) and width (cm) were determined. The number of berries was counted for each cluster and berry weight determined. Rachis weight (g) was determined after manual removal of berries. The morphological volume of each cluster was calculated using the standard formula for a cone, $V_{cone} = 1/3\pi r^2 l$, where the radius r was taken as equivalent to half of the cluster width, and l = cluster length. Cluster compactness was evaluated using the index Cl-12 = cluster weight (g) / [cluster length (cm)]^2 (Tello and Ibanez 2014).

Assessment of Botrytis cinerea disease severity: The severity of *Botrytis cinerea* attack was assessed at full maturity by evaluating all clusters of ten vines from each variety using the EPPO guide for classifying visually observed symptoms of infection in seven classes: (0 %, 1 to 5 %, 6 to 10 %, 11 to 25 %, 26 to 50 %, 51 to 7 5%, 76 to 100 %).

Data analysis: Two-factor analysis of variance (ANOVA) for variety x year using the statistical software Statistica 8.0 (StatSoft, Inc., USA) provided estimates of varietal and annual differences. Standard error was determined using Fisher LSD with a 0.05 significance level. Principal component analysis (PCA) was used as a multivariate analytical tool to differentiate among varieties, using average values for eight grape cluster variables.

Results and Discussion

Significant differences among eleven old Croatian varieties for all cluster traits were determined during three consecutive vintages (Table). Clusters of 'Grk' were longest, with average length of 22.6 cm, while 'Babić' clusters were the widest (average 14.3 cm). Cluster weight varied from 176.0 to 453.0 g and the number of berries per cluster ranged from 68 to 298, depending on the variety. 'Grk' had the most berries per cluster but also the smallest berries; the average single berry weight was 1.3 g. 'Babić' had the largest single berry weight (3.1 g) and rachis weight (15.9 g), resulting also in the largest cluster volume (1200 cm^3). The lowest cluster volume observed was in 'Svrdlovina' (496 cm^3). The relative differences among varieties were not consistent among years, which resulted in a significant interaction between year and variety.

At full maturity, there were clearly visible differences between the compact and loose clusters. We quantified cluster compactness based on the Cl-12 index (ratio of cluster weight to length), which correlated significantly with the visual cluster compactness categories of OIV descriptor No. 204 proposed for rapid evaluation (Tello and Ibáñez 2014). Cluster compactness significantly varied among varieties and ranged from 0.41 for 'Plava Lovora' (very loose cluster) to 1.21 for 'Babica' (dense cluster). Differences among other varieties were significant and suggest a different degree of cluster compactness among them. Numerous studies have demonstrated that varieties with compact clusters result in high *Botrytis* infection while varieties with loose clusters are less susceptible (Vail and Marois 1991, Mlikota Gabler *et al.* 2003). Among our varieties, visual symptoms of *B. cinerea* infection on clusters ranged from 0 % to 50 % depending on variety.

Table

Average values and standard errors of cluster dimensions at harvest of eleven old Croatian varieties in 2008, 2009 and 2010

Variety	Cluster length (cm)	Cluster width (cm)	Cluster weight (g)	Rachis fresh weight (g)	Cluster volume (cm^3)	Cluster compactness index*	Number of berries	Single berry weight (g)
Babić	20.5 ± 0.6^{d}	14.3 ± 0.6^{e}	453.0 ± 34.6^{e}	15.9 ± 1.4^{e}	1200 ± 132.5^{f}	1.07 ± 0.06^{de}	153 ± 12.8^{ac}	3.1 ± 0.2^{e}
Babica	17.1 ± 0.5^{bc}	12.7 ± 0.5^{abc}	349.7 ± 25.1^{c}	14.4 ± 1.1^{bde}	769 ± 70.4^{abd}	1.21 ± 0.08^{f}	158 ± 16.3^{ad}	2.4 ± 0.1^{g}
Crljenak viški	18.1 ± 0.5^{c}	12.0 ± 0.5^{a}	271.8 ± 15.1^{a}	12.6 ± 0.7^{bc}	728 ± 70.6^{abd}	0.88 ± 0.07^{a}	209 ± 20.6^{e}	1.6 ± 0.2^{a}
Glavinuša	17.8 ± 0.5^{c}	12.3 ± 0.6^{ab}	209.8 ± 16.2^{bd}	9.1 ± 0.7^{a}	784 ± 99.4^{bd}	0.68 ± 0.05^{b}	122 ± 8.9^{b}	1.8 ± 0.1^{b}
Grk	22.6 ± 0.5^{e}	13.5 ± 0.4^{ce}	377.3 ± 17.3^{c}	13.6 ± 0.5^{bd}	1136 ± 81.9^{ef}	0.74 ± 0.03^{bc}	298 ± 21.6^{g}	1.3 ± 0.1^{f}
Lasina	16.8 ± 0.4^{abc}	12.0 ± 0.5^{a}	277.5 ± 16.1^{a}	13.1 ± 1.0^{bc}	700 ± 73.1^{abc}	0.99 ± 0.05^{ad}	147 ± 15.0^{abc}	2.1 ± 0.1^{d}
Plava Lovora	21.0 ± 0.8^{d}	11.7 ± 0.8^{ad}	176.0 ± 11.6^{b}	9.7 ± 0.9^{a}	927 ± 162.5^{d}	0.41 ± 0.03^{g}	133 ± 15.2^{abc}	1.5 ± 0.1^{a}
Plavac Mali Sivi	15.5 ± 0.3^{a}	12.3 ± 0.3^{abc}	280.5 ± 17.0^{a}	11.1 ± 0.6^{ac}	640 ± 39.7^{abc}	1.15 ± 0.05^{ef}	156 ± 11.8^{a}	1.9 ± 0.1^{c}
Pošip Bijeli	21.5 ± 0.6^{de}	13.3 ± 0.5^{bce}	383.6 ± 18.0^{c}	15.3 ± 1.2^{de}	1083 ± 119.1^{ef}	0.86 ± 0.05^{ac}	184 ± 9.7^{de}	2.1 ± 0.1^{d}
Svrdlovina	15.9 ± 0.3^{ab}	10.7 ± 0.4^{d}	231.6 ± 11.9^{ad}	10.2 ± 0.5^{a}	496 ± 35.5^{c}	0.93 ± 0.04^{a}	128 ± 8.1^{bc}	1.8 ± 0.1^{bc}
Vrškajica	16.4 ± 0.6^{ab}	10.7 ± 0.5^{d}	182.3 ± 11.9^{b}	9.2 ± 0.7^{a}	555 ± 66.9^{ac}	0.70 ± 0.04^{b}	68 ± 6.5^{f}	3.0 ± 0.2^{e}
Year								
2008	19.9 ± 0.4^{b}	14.0 ± 0.3^{b}	290.4 ± 11.2^{a}	14.2 ± 0.5^{b}	1115 ± 65.9^{b}	0.76 ± 0.02^{a}	209 ± 8.0^{c}	1.4 ± 0.0^{a}
2009	18.0 ± 0.3^{a}	11.3 ± 0.3^{a}	292.9 ± 14.4^{a}	11.3 ± 0.5^{a}	672 ± 40.6^{a}	0.89 ± 0.03^{b}	163 ± 10.4^{b}	2.0 ± 0.1^{b}
2010	17.5 ± 0.3^{a}	11.6 ± 0.2^{a}	287.5 ± 12.4^{a}	11.1 ± 0.5^{a}	672 ± 35.7^{a}	0.97 ± 0.03^{c}	106 ± 4.6^{a}	2.7 ± 0.1^{c}
Interaction Variety x Year	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Note: Values in columns followed by different letters are significantly different ($p < 0.05$).
*calculated according to cluster compactness index Cl-12 (Tello and Ibanez 2014).

'Crljenak Viški' was the most sensitive variety and berry damage was drastic in 2010 (50 % infected berries). 'Babić' and 'Lasina' showed very similar sensitivity to 'Crljenak Viški' (11 to 25 % infected berries over = three consecutive years), while 'Babica', 'Plavac Mali Sivi', and 'Svrdlovina' had medium sensitivity (6 to 10 % infected berries). A very small proportion of *Botrytis*-infected berries (1-5 %) were found in 'Glavinuša', 'Grk', and 'Vrškajica'. No symptoms of *Botrytis* infection were observed in 'Plava Lovora' in any year, which can be explained by its very open clusters. However, a more detailed understanding of the relationship between cluster compactness and *Botrytis* susceptibility would include several other factors related to the morphology, anatomy and physiology of berries, such as thickness and number of cell layers in the epidermis, number of lenticels and pores, the thickness of the cuticle and wax, and the chemical composition of berry skins (Mlikota Gabler *et al.* 2003).

Principal Component Analysis (PCA) was used to group similar varieties based on eight cluster-relevant properties. The Figure shows the distribution of the varieties in the coordinate system defined by two latent factors of the PCA. PC1 and PC2 comprise 83.41 % of total variability, and are defined based on the real average values of eight cluster properties. PC1 accounts for 56.66 % of the total variability, while PC2 accounts for 26.75 %. The dispersion of varieties can be observed from their distribution in the coordinate system, which indicates the existence of at least three groups among varieties. Group I ('Babić', 'Pošip Bijeli', and 'Grk') had long, heavy (> 370 g), large volume (>1000 cm^3), and medium loose clusters. Group II ('Crljenak Viški', 'Glavinuša', and 'Plava Lovora') had medium length, low weight (~ 200 g), medium volume (700 to 900 cm^3), and loose clusters. Group III ('Babica', 'Plavac Mali Sivi', 'Lasina', 'Vrškajica', and 'Svrdlovina') had short to medium length, medium cluster weight (200 to 350 g), small cluster volume (500 to 700 cm^3), and compact clusters. PCA of the cluster data revealed clear differences among varieties.

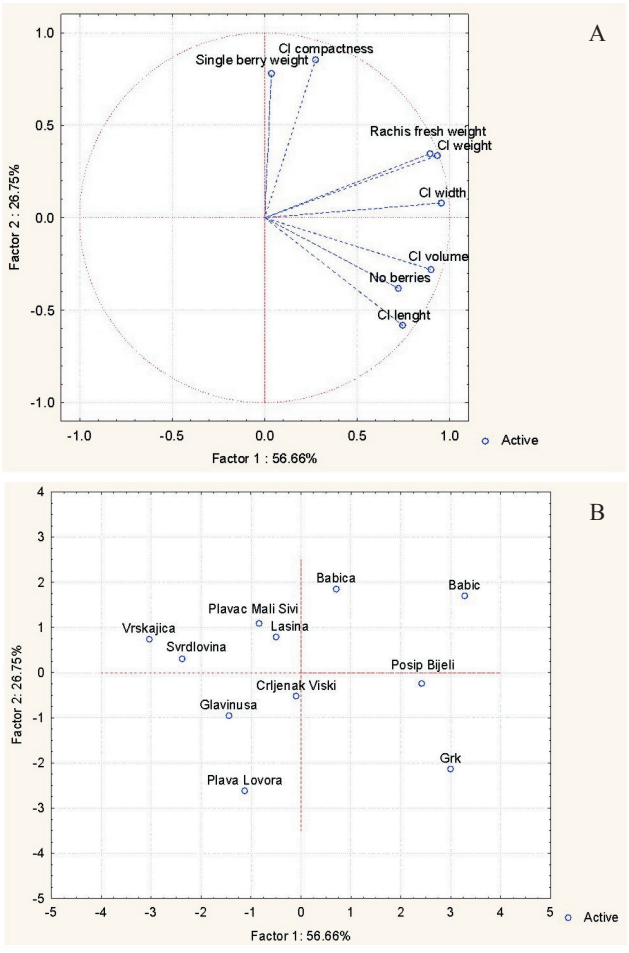

Figure: Projection of eight cluster characteristics on which the definition of latent PC factors 1 and 2 are based. PC factors 1 and 2 together comprise 83.41 % of total variability. B: Principal component analysis (PCA) of eleven varieties on the plane defined by the first two principal components.

Conclusion

The results of this study indicate different cluster structures among the old Croatian varieties, with different sensitivity to *Botrytis*. The significant variety by year difference in the magnitude of all characters expressed very sensitive variety reaction to annual variation in climatic condition that are closely related to the *Botrytis* attacks. PCA analysis revealed at least three different groups of varieties which were favored and selected for traditional wine growing in Croatia. These phenotypic data on cluster structure represent a basis for future research, such as QTL analysis or assessment of productivity of these varieties.

Acknowledgements

The authors thank the Croatian Ministry of Science, Education and Sports for financial supports (project ID: 091-0910468-0279). Joint publication of the COST Action FA1003 "East-West Collaboration for Grapevine Diversity Exploration and Mobilization of Adaptive Traits for Breeding".

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