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Analysis of phyllometric parameters efficiency in discrimination of Croatian native *V. vinifera* cultivars

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Summary

The aims of this study were to achieve correct cultivar classification of leaf samples by using phyllometric parameters to determine the performance of parameters applied as the discriminant criteria and to determine the minimal number of leaf parameters needed to accurately classify leaf samples within cultivars. Seventy-nine phyllometric parameters were measured/calculated on 360 leaf samples from eleven grapevine cultivars, gathered during several years, from different growing conditions and with some differences in the sampling methods applied. Stepwise discriminant analysis was used to rank the phyllometric parameters according to their efficiency in the discrimination of cultivars. A series of discriminant analyses was performed with successive introduction of new parameters as discriminatory elements until 100 % correct classification of leaf samples into the correct cultivar was achieved. This was achieved using only the seven highest ranking phyllometric parameters from the stepwise discriminant analysis. Additionally, canonical discriminant analysis was performed to evaluate the differences between cultivars in the same parameters. This study represents a model for analysis of the efficiency of different ampelometric parameters for discrimination of *V. vinifera* cultivars.

K e y w o r d s : ampelometry, discriminant analysis, cultivar identification.

Introduction

In last decades importance of preservation of the existing Croatian grapevine biodiversity was identified. For this reason, the ampelographic characteristics of Croatian native grapevine cultivars are the focus of this study. Genetic markers, as a superior tool for cultivar identification, however cannot replace ampelographic studies in cultivar evaluation. Ampelometric methods can also be a useful tool in cultivar identification, as they are less expensive, do not require special equipment and can give relevant results if managed properly and the resulting data are analyzed appropriately. For these reasons, they can still be the method of choice, especially for research groups without the resources or knowledge required for molecular analyses. Computer-aided survey systems for phyllometry, such as SuperAmpelo (Soldavini *et al.* 2006) and GRA.LE.D (Bodor *et al.* 2012) and some others, have been developed as solutions for the time-consuming limitations of phyllometric research. The aims of this study were to determine the performance of phyllometric parameters applied as discriminant criteria between eleven grapevine cultivars.

Material and Methods

In total, 360 leaf samples from eleven cultivars were used in this study. Croatian autochthonous cultivars were represented with different number of samples: 'Plavac mali' (76), 'Plavina' (34), 'Pošip' (52), 'Maraština' (11), 'Vugava' (29), 'Grk' (52), 'Žlahtina' (32) and 'Kraljevina' (9) as well as two international cultivars 'Pinot Noir' (16) and 'Chardonnay' (14). Leaf samples of all Croatian cultivars were taken from single vines selected during ongoing mass positive selection within commercial vineyards and from five vines (common sample) of their clone candidates. Leaf samples of 'Chardonnay' from eight and 'Pinot Noir' from two different clones planted in the same vineyard at the Faculty of Agriculture Zagreb. Leaf samples were collected between *verasion* and harvest. Ten adult leaves from the middle third of the shoot were used for all samples. Cultivars are this way represented with different number of samples, gathered from one or more locations and clones and from different years.

Using Superampelo software version 1.01. (Comunita Monastica SS. Pietro e Paulo, Germagno, Italy), 37 leaf parameters were measured on digital images of the leaf samples. These measurements were used for 42 new relative parameters design on the base of ratios between them (Table). Mean values from 10 mature leaves were determined for all measured and calculated parameters for all samples. For parameters measured on both sides of leaves, their average was calculated and used for further analyses. Discriminant analyses using PROC STEPDISC, PROC DISCRIM, and PROC CANDISC of SAS statistical Software, version 9.3 (SAS Institute, Cary, NC) were performed to evaluate the utility and importance of each leaf parameter by determining which added the most power for maximum discrimination between cultivars.

Results and Discussion

Stepwise discriminant analysis (STEPDISC) of 79 (measured and calculated) leaf parameters ranked them according to their efficiency in differentiation of eleven eval-

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Table

Ranking of phyllometric parameters using Stepwise discriminant analysis base on Wilks' Lambda value on 360 leaves samples of eleven cultivars

Absolute parameters	Ranking*	Description	Relative parameters	Ranking*	Description
AL	2	angle between N1 and N2 measured at the first ramification	R11	1	O4N5/ON1
HN4	3	length of teeth N4	R29	4	LAM/DE
SPSP1	5	opening/overlapping of petiole sinus	R5	6	OP/ON1
OI	12	length petiole point to lower leaf sinus	R28	7	LAM/MU
FN2	13	length between tooth tip of N2 and tooth tip of the first secondary vein of N2	R30	8	MU/DE
ANGA	14	angle between shoot tip N3 and shoot tip N5 with centre in petiole point	R12	9	OO3/ON3
HN2	15	length of teeth N2	R3	10	HN2/BN2
N2N2‘	16	distance between tooth tip of N2 and N2‘	R9	11	ON3/ON1
O4N5	17	length of vein N5	R39	18	ON1/N2N2‘
PI	20	angle of opening/overlapping of petiole sinus with centre in petiole point	R40	19	ON3/ON2
GA	22	angle between N3 and N4	R32	21	AL/GA
LUPIC	26	length of leaf with petiole (mm)	R10	23	ON4/ON1
ON2	28	length of vein N2	R35	24	OO3/O3N4
O3ON4	29	length of vein N4	R42	25	ON4/ON2
Area	30	leaf total area (mm2)	R18	27	OI/OS
LUxLA	31	product of leaf LU and LA	R17	32	LUPIC/LU
N4N4‘	34	distance between tooth tip of N4 and N4‘	R1	33	LU/LA
BN2	37	width of teeth N2	R15	35	N4N4‘/N3N3‘
ON4	41	distance between petiole point and tooth tip N4	R6	36	OS/ON2
OO3	42	vein N3, length petiole sinus to vein N4	R7	38	OI/ON3
LAM	43	angle of S and S‘ with centre in shoot tip N1	R37	39	ON1/N4N4‘
ON3	44	length of vein N3	R8	40	ON2/ON1
LU	45	width of leaf (mm)	R34	47	O3N4/ON3
BN4	46	width of teeth N4	R2	48	AL+BE+GA/OS+OI
MU	53	angle of I and I‘ with centre in shoot tip N1	R33	49	BE/GA
OM	55	angle between N1 and N2 measured at tooth tip	R31	50	AL/BE
OS	59	length petiole point to upper leaf sinus	R24	51	GA/AL+BE+GA
DE	60	angle of N2 and N2‘ with centre in shoot tip N1	R23	52	BE/AL+BE
LA	63	length of leaf (mm)	R25	54	OM/OM+ET+TA
ALBEGA	64	summary of angles α + β + γ	R19	56	AL/OM
ALBE	65	summary of angles α + β	R16	57	N4N4‘/N2N2‘
OP		length of petiole	R22	61	AL/AL+BE
ON1		length of vein N1	R13	62	O3N4/ON4
N3N3‘		distance between tooth tip of N3 and N3‘	R41	66	ON4/ON3
BE		angle between N2 and N3 measured at the first ramification	R4		HN4/BN4
ET		angle between N2 and N3 measured at tooth tip	R14		N2N2‘/N3N3‘
TA		angle between N3 and N4 measured at tooth tip	R21		GA/TA
			R20		BE/ET
			R26		ET/OM+ET+TA
			R27		TA/OM+ET+TA
			R36		N4N4‘/N2N2‘
			R38		ON1/N3N3‘

*parameters with no ranking were excluded by the stepwise discriminant analysis

uated cultivars (Table). Using these rankings, a series of discriminant analyses were performed. Initially, only one parameter (R11) was used (highest ranking from STEPDISC), and in each new analysis, one new parameter was added as the differentiating factor among cultivars. This determined the cumulative efficiency of the parameters applied in the correct classification of 360 leaf samples within the correct cultivar. Using only four parameters in the discriminant analysis, 96.84 % of leaf samples were correctly classified, while for 100 % correct classification, only seven parameters were needed. Canonical discriminant analysis was performed based on this seven leaf parameters. All of the squared Mahalanobis distances determined this way were highly significant between all the cultivars. The first three canonical variables (CAN) explained 79.2 % of the total variability between cultivars, and the scatter plot (Figure) was plotted based on first two of them.

Our results are in correspondence with the earlier researches which showed that leaf size dependent parameters can vary greatly as a result of different environmental conditions (Gomez-del-Campo *et al.* 2002) and pruning

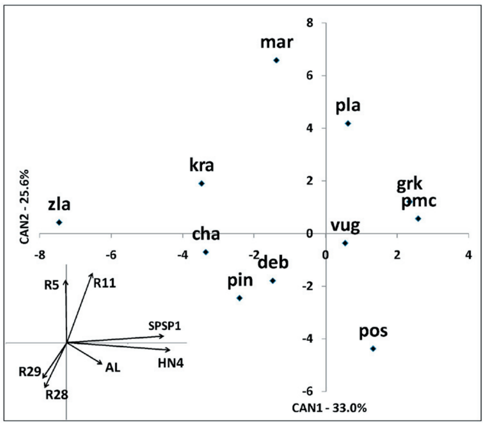

Figure: Canonical discriminant analysis of eleven cultivars based on the seven leaf parameters showing the greatest discrimination power. Pin – Pinot noir, pos – Posip, vug – Vugava, grk – Grk, pla – Plavina, mar – Marastina, deb – debit, zla – Zlahtina, kra – Kraljevina, pmc – Plavac mali and cha – Chardonnay. The inserted vector diagram indicates the direction and intensity of seven variables (see Tables 2 and 3 for abbreviations) in the space defined by CAN1 and CAN2.

level/bud load (Bodor *et al.* 2013) while angles between veins and especially the ratios between measured parameters (distances and/or angles) are considered stable within cultivars (Tomažić and Korošec-Koruza 2003, Santiago *et al.* 2005). The fact that some parameters are not affected by different environmental factors is often used as the main criterion for their utilization in cultivar identification (Tomažić and Korošec-Koruza 2003, Santiago *et al.* 2005). Based on our results it is clear that a stable parameter need not necessarily have good efficiency in cultivar differentiation and this is in correspondence with results obtained by Dettweiler (1987) which also shoved that parameter choice depends on the ampelometric characteristics of cultivars employed. For this reason in case of cultivar differentiation using phyllometry, results of previous studies on parameters efficiency, must be re-evaluated on a set of cultivars used.

The ranking of the leaf parameters in cultivar distinguishing efficiency based on STEPDISC analysis used in this study, and different types of ranking used by other authors (Weihl and Dettweiler 2000, Tomažić and Korošec-Koruza 2003, Harbi-Ben Slimane *et al.* 2010), does not give information on the minimal number of leaf parameters needed to performed effective or even 100 % exact classification of leaf samples within the correct cultivars. This is possible using a series of discriminant analyses (PROC DISCRIM) with the successive introduction of new parameters as discriminatory elements based on STEPDISC ranking. It was sufficient to use only the four most efficient parameters to achieve more than 95 % correct classification of leaf samples to the appropriate cultivar and to achieve 100 % exact classification of all 360 leaf samples, seven parameters were needed. Introducing the additional parameters, with lower STEPDISC ranking, in some cases resulted in decrease of the classification efficiency.

Conclusion

This study reports on the discrimination efficiency of certain phyllometric parameters previously applied, while also providing a first report on some new and highly efficient phyllometric parameters. It also represents a model for the analysis of discriminant efficiency of different ampelometric parameters for the discrimination of *V. vinifera* cultivars.

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