SHST
Horticulture and Forestry Society of Transylvania

David M *et al.* (2022)
Notulae Scientia Biologicae
Volume 14, Issue 2, Article number 11250
DOI:10.15835/nsb14211250
Research Article

NSB
Notulae Scientia Biologicae

Genetic differences as estimators of osmotic adjustment and source-sink balance in grapevine hybrid elites

Monica DAVID[a], Cristina-Magdalena CIOBOTEA[b], Mădălina F. BĂNUȚĂ[c], Gina NEDELEA, Ramona STAN, Andrei TIȚA*, Ionela D. SĂRDĂRESCU

*National Research and Development Institute for Biotechnology in Horticulture, Research Department, Ştefăneşti-Argeş, 37, Şoseaua Bucureşti-Piteşti, Argeş, 117715 Ştefăneşti, Romania; monicadavid12@yahoo.com; cioboteacristina76@yahoo.com; serban_madalina16@yahoo.com; nedelea_gina@yahoo.com; ramonastan89@gmail.com; tita_andrei10@yahoo.com (*corresponding author); ionela.toma93@yahoo.com*
[a,b,c] These authors contributed equally to the work

Abstract

This study deals with the best responses of a diverse collection of grapevine genotypes to osmotic stress associated with source-sink balance responses given by an estimator such as leaf area to fruit ratio. 'Centennial Seedless', a drought tolerant cultivar, was selected as control. The cultivars, 'Victoria' and 'Argessis', were chosen as a repetition from previous research dealing with pollen grain test, two years ago. Ten genotypes were hybrid elites in first and second hybrid generations. Three cultivars 'Victoria', 'Centennial Seedless', and 'Argessis' were grown under field conditions in containers, and in the soil under greenhouse conditions. Significant differences were found between genotypes for both responses to osmotic stress and source-sink balance. 'Centennial Seedless' and 'BP9' hybrid showed the best responses of induced osmotic adjustment; results confirmed the compensatory potassium uptake theory. 'Victoria' and 'Argessis' had almost the same average values as 'Centennial Seedless' osmotic estimator for induced osmotic adjustment. 'Victoria' and 'HR7' hybrid showed an increase in osmotic stress in the cell, after application of polyethylene glycol solutions without potassium cation and a lower source-sink ratio, which could be associated with higher photosynthesis rates. No correlations were identified between the mechanisms expressed by the analyzed estimators, indicating that they are activated and functional separately from each other, sometimes only compensatory.

Keywords: cellular level; potassium cation; translocation; turgor; water potential; water stress

Abbreviations: ANOVA- analysis of variance; CV-coefficient of variation; F_1-first hybrid generation; F_2-second hybrid generation; FW-fruit weight; LA-leaf area; LA:FW-leaf area to fruit weight; LSD-least significant differences; K^+-potassium cation; KCl-potassium chloride; KUP/HAK/KT-*Vitis vinifera* channel transporters; MS-mean square; OA-osmotic adjustment; P-probability; PEG-polyethylene glycol; SIRK-shaker channel; Stdev-standard deviation; ss-stressing solution; SS-sum of squares; se/cc-sieve element/companion cell; VvK1.2-*Vitis vinifera* gene

Received: 12 Apr 2021. Received in revised form: 22 Jun 2022. Accepted: 23 Jun 2022. Published online: 28 Jun 2022.
From Volume 13, Issue 1, 2021, Notulae Scientia Biologicae journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

Introduction

Pressure on agricultural use of water resources is rising (Simonneau *et al.*, 2017). Yield and berry quality depend on the vine's adaptability to drought (Lovisolo *et al.*, 2010). Adaptation to dry environments depends on the tradeoff between efficient conduits and low vulnerability to cavitation. Grapevines have long vessels, which is a common trait among liana species. Vessel sizes are dependent on the cultivar (Chouzouri and Schultz, 2005), giving room for genetic variation in drought response (Simonneau *et al.*, 2017).

Osmotic adjustment is a major response to drought, which allows the cells to maintain their water content and turgor, even when water potential decreases in their vicinity. When roots are exposed to a soil water deficit, root cells respond in terms of growth and differentiation (Lovisolo *et al.*, 2010), through acidification of the apoplast pumping protons from the cytoplasm. Some grapevine genotypes can be modulated by root-stocks with a higher K^+-uptake efficiency and transportation rate, leading to a decrease of vacuolar acidity (Pratelli *et al.*, 2002).

A shaker channel, SIRK, is expressed at low K^+ levels prior to veraison in the berry pericarp and is undetectable after onset of ripening (Gambale and Uozumi, 2006; Pratelli *et al.*, 2002; Rogiers *et al.*, 2017). The two KUP/HAK/KT transporters are also expressed highly in the skins of pre-veraison berries (Davies *et al.*, 2006; Rogiers *et al.*, 2017). The only K^+ uptake system identified thus far that is up-regulated at veraison is a shaker channel (VvK1.2) expressed in the plasma membrane of mesocarp and phloem tissues (Cuèllar *et al.*, 2013; Rogiers *et al.*, 2017). K^+ is the most abundant ion in the cell (Clarksont and Hanson, 1980) and is involved in a number of basic functions linked together at the genetic, cellular or the whole plant level, such as control of cell turgor (Hale, 1977; Delas *et al.*, 1989; Pratelli *et al.*, 2002).

The growth and maintenance of plant tissues are dependent on the translocation of newly fixed photo-assimilates from sources (the sites of synthesis, e.g., mature leaves) to the sinks (the sites of consumption or storage, e.g., roots and berries) (Rogiers *et. al.*, 2017). Growth after veraison is characterized by flesh cell expansion than cell division, allowing a rapid storage of sugar and amino acids. After veraison, the berry becomes a strong sink and depends on phloem sap flux, consisting of sugars, other organic molecules, ions and water, because xylem vessels are no longer functional (Düring *et al.*, 1987; Findlay *et al.*, 1987, Pratelli *et al.*, 2002).

The effects of hydric stress on grapevine's photosynthesis influence berry development (Dry *et al.*, 2001). Development and composition are determined by severity and timing of water stress (Roby and Matthews, 2004), leading to a decrease of berry size and sugar accumulation due to an inhibition of photosynthesis (Williams and Matthews, 1990, Rogiers *et. al.*, 2017).

According to Münch's theory, an osmotic potential gradient is generated through loading of solutes into sieve tubes at the source and unloading at the sink, resulting in a water gradient and a mass flow up to 1 $m \cdot h^{-1}$ (Tyree and Fensom, 1970; Daudet *et al.*, 2002; Thompson and Hoolbrook, 2003; Rogiers *et al.*, 2017). Aquaporins in plasma membrane and tonoplast are highly expressed in expanding cells and up-regulated simultaneously with sugar transporters (Fouquet *et al.*, 2008; Rogiers *et al.*, 2017), but little is known how these transporters are interconnected with K^+'s role as an osmotic facilitator for phloem transport during ripening (Rogiers *et al.*, 2017). The mechanisms of phloem and K^+ transport in grapevine have been poorly investigated despite the evidence that this cation and its charge balancing anions contribute to the hydrostatic pressure gradient between collection and release phloem (Lang, 1983; Rogiers *et. al.*, 2017).

Following the previous researches obtained about connections between osmotic adjustment and source-sink balance in grapevine, the present study investigates the responses given by two estimators, one for osmotic adjustment (Patil and Ravikumar, 2011) and the other one for source-sink balance (Petrie *et al.*, 2000c; Parker, 2012) in grapevine hybrid elites. In addition, the relationship between the genotype responses obtained in this analysis for both estimators will be described.

Materials and Methods

Sampling design

a. Biological material used in pollen grain test

The experiment was conducted at N.R.D.I.B.H. vineyard (44°42' and 44°55'N), at Ștefănești, Argeș, Romania, under greenhouse and field conditions. A collection of thirteen grapevine genotypes were part of the study: three cultivars described as drought tolerant and ten hybrids. The drought tolerant genotypes were: 'Centennial Seedless', 'Victoria' and 'Argessis' (Glăman *et al.*, 2018). The hybrid elites were: 'CN21', 'APR1', 'BP9', 'CROM8', 'R10×V5', 'R10×V7', 'MI07V', 'CP76', 'HR7', 'R10×V8'.

'Centennial Seedless': a cross between 'Gold' × ('Emperor' × 'Pirovano75'); breeder: H.P. Olmo and A.T. Oyama, UC Davis, California, USA. It is cultivated in California, Chile, South Africa and Australia (Țârdea and Rotaru, 1996; VIVC, 2019).

'Victoria': a cross between 'Cardinal' × 'Afuz Ali'; breeder: Victoria Lepădatu, Viticultural Research Station, Drăgășani, Romania (VIVC). It has a good drought tolerance (Glăman *et al.*, 2018).

'Argessis': a cross between 'Moldova' × 'Augusta'; breeder Bădițescu Margareta and Popa Camelia at N.R.D.I.B.H. Ștefănești-Argeș. It has also a good drought tolerance (Glăman *et al.*, 2018).

All hybrid elites used in the analysis are F_1 breeds, except CP76, which is a F_2 generation. 'Victoria' and 'Argessis' were used as a repetition of a previous analysis, from 2020, where they were grown in containers, in the greenhouse.

In this study, 'Victoria' pollen grains were collected from the plants grown in the field. 'Argessis' pollen grains were collected from a plant cultivated in container, under greenhouse conditions. Pollen grains of 'CP76' hybrid were collected from one plant grown in container and another one, planted in the greenhouse soil, and then, analyzed separately for confirming the repetition of OA mechanisms.

Greenhouse conditions

Grapevine genotypes (*Vitis vinifera* L.) of different age were cultivated in containers (20 dm^3), in a soil:peat:sand mixture (1:1:1). The mean temperature was 24 °C, when flowers at anthesis were collected for pollen grain test proposed by Morgan (1999). The humidity was about 80%. Plants grown in containers were drip irrigated and those grown in the soil are watered.

Field conditions

In the field, the temperature was 25 °C and humidity 70%. 'Victoria' plant was 3 years old and was drip irrigated.

b. Biological material used in source-sink analysis

Plant material used in source-sink analysis was made up of: 'Centennial Seedless', 'Victoria', 'CN21', 'APR1', 'BP9', 'CROM8', 'R10×V7', 'MI07V', 'HR7', 'R10×V8'

Greenhouse conditions

The temperature and humidity were maintained at optimal conditions for grapevine growth.

Experimental procedures

c. Estimation of osmotic adjustment capacity of pollen grains

Pollen grains were collected in June. Before pollen test, the flowers were transported and rehydrated with water in PP-tubes for 30 minutes. The procedure of pollen test and estimations of OA expressions induced in pollen grains were the same as in a previous study in 2020 (Morgan, 1999; Patil and Ravikumar, 2011; David, 2020). The OA estimations expressed by projected cytoplasm area were obtained by increasing the number of pollen grains on each slide for each genotype, from 20 to 100 cells. Microscopic observations were made at a

magnification of 10X or 20X. Measurements of projected pollen grains cytoplasm were done using ImageJ, a program designed for cellular biology analysis, and recommended by Patil and Ravikumar (2011). All measurements of projected pollen grains cytoplasm were calculated for magnification of 10X in ImageJ.

d. Estimation of source-sink balance in grapevine genotypes

The measurements were made 90-100 days after buds burst (leaf maturity stage from N.R.D.I.B.H. greenhouse). Leaves from each hybrid were collected in plastic bags in order to avoid fading and water loss. Five mature leaves, collected from the main young shoot and between 9-12th node, were measured. Leaf area measurements were made following the formula of Kinskins' method:

LA= $\frac{\pi d^2}{4}$, where d represents distance between the top of the leaf blade and the farthermost point from the blade top (Dobrei *et al.*, 2008).

The bunch weight (as FW) was calculating the mean value of all bunches collected from 10 young shoots at the stage of full maturity.

Statistical analysis

a. Statistical analysis used in pollen grain test

The statistical interpretation of the results was done with an ANOVA (Fischer-Snedecor test) with an EXCEL sheet, for each treatment and trait in six repetitions given by measurement of projected pollen grain cytoplasm area made on the images focused on six central locations of the slide observed at magnification of 10X. Analysis of variances homogeneity and effects of stressing solutions and morphological factors was made using the following equations:

1. Equation of squared omega (ω^2) proposed by Hays (1981) was used for interpretation of variances homogeneity analysis:

$\omega^2 = \frac{SS_{intragroup} - (k-1)\times MS_{intergroup}}{SS_{total} + MS_{intergroup}}$, where k represents the number of groups used in the analysis;

2. Equation of index of sizes effect (f) was used for interpretation of effects of stressing solutions and morphological factors on genotypes reaction under drought conditions:

$f = \frac{\sqrt{\frac{(k-1)\times(MS_{intragroup} - MS_{intergroup})}{N}}}{\sqrt{MS_{intergroup}}}$, where N represents total number of subjects that was used in the analysis. Interpretation of index of sizes effect was done using f values proposed by Cohen (1992b).

Standard deviations of cell cytoplasm area measurements and coefficients of variation (CV%) were calculated to estimate the stability and resolution of method used as cytometry analysis (Steen, 1990a). CV is the ratio between standard deviations of projected cytoplasm area exposed to stress solution and standard deviations of projected cytoplasm area exposed to control solution. The significance of results is obtained by the least significant differences test (Steel, 1977), at a significance level of 5% for all indicators used in the OA analysis. Relationships among traits given by OA expressions were examined through calculation of correlation coefficients (R).

b. Statistical analysis used in source-sink balance analysis

The statistical interpretation of the results was done using an analysis of variance (ANOVA) (Fischer-Snedecor test). ANOVA was performed for LA:FW estimator average obtained for each genotype. The significance of results was tested at a 5% level for this indicator. Relationships between estimators used in the OA analysis and LA:FW used in source-sink balance analysis were examined calculating correlation coefficients (R).

Results

Differences between genotypes for osmotic adjustment estimators

Significant differences were found between genotypes, both, for measurements of projected cytoplasm area of pollen grains and also, for osmotic adjustment estimators of pollen grains exposed to stressing solutions with and without K^+ (Figure 1; Figure 2). Differences between genotypes increased for expressions induced in stressing solutions with addition of KCl, indicating differential effects of K^+ obtained in OA for each genotype characterized by different ion capacity utilization (Figure 1a; Figure 1b). The values for measurements of projected cytoplasm area answer to a significant proportion from dispersion, having values between 58% and 83% (Figure 1a).

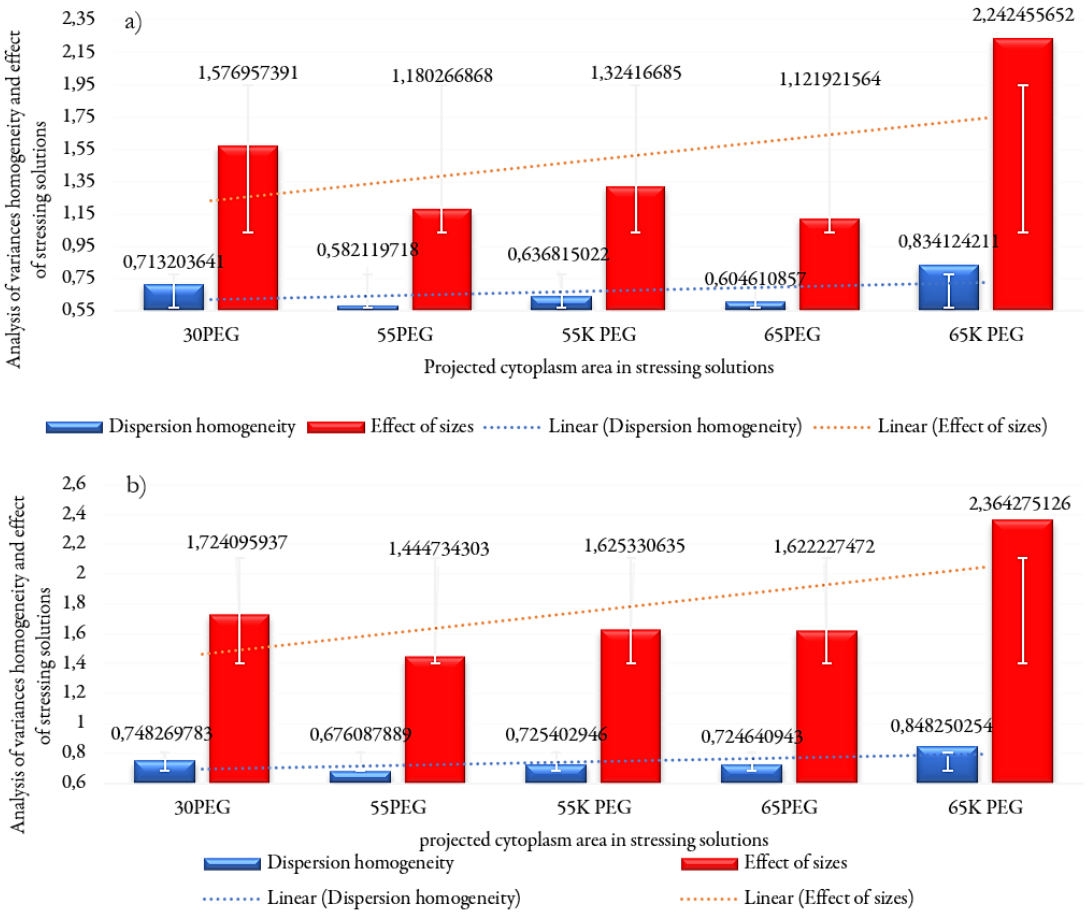


Figure 1. a. Analysis of variances homogeneity and effects of stressing solutions with and without K^+ on pollen projected cytoplasm area for 14 probes; b. The same analysis for 10 probes

The effect of stressing solutions with added K^+ on pollen projected cytoplasm area was higher than that without added K^+ on pollen projected cytoplasm area and, respectively, the effect of stressing solution 65% PEG with added K^+ was higher than that of the control solution (Figure 1a). The more genotype probe numbers were used, the lower effects of stressing solutions on pollen projected area were detected due to genetic variation given by each genotype (Figure 1a; Figure 1b).

The estimator values answer to a significant proportion from dispersion, having values from 45 to 58% for OA estimators (Figure 2). Effect of solutions with K^+, defined by genotype's K^+ accumulation capacity, was higher in projected cytoplasm area than the effect of solutions without K^+, indicating that the effect of induced OA has an important role in maintaining overall OA under osmotic stress conditions (0.94; 1.1) than intrinsic

OA (0.9; 0.98). But both effects were subordinated to the effect of overall OA (1.19; 1.34) (Figure 2; Figure 9).

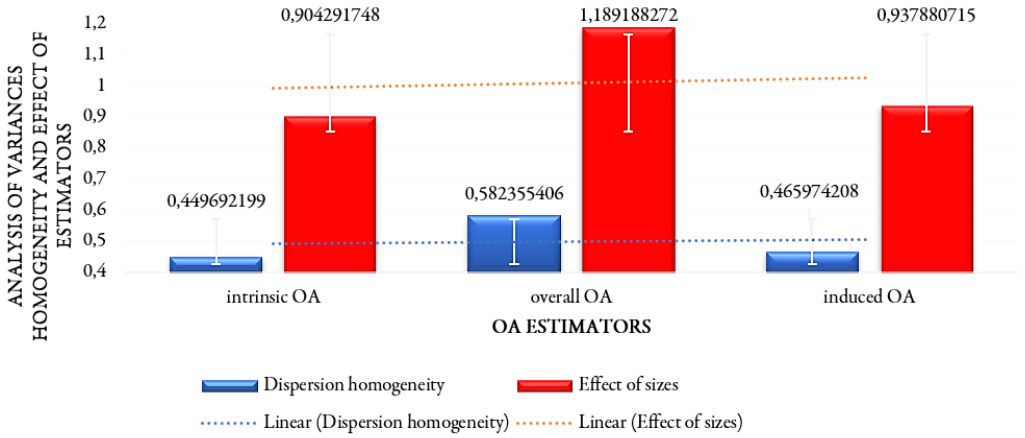
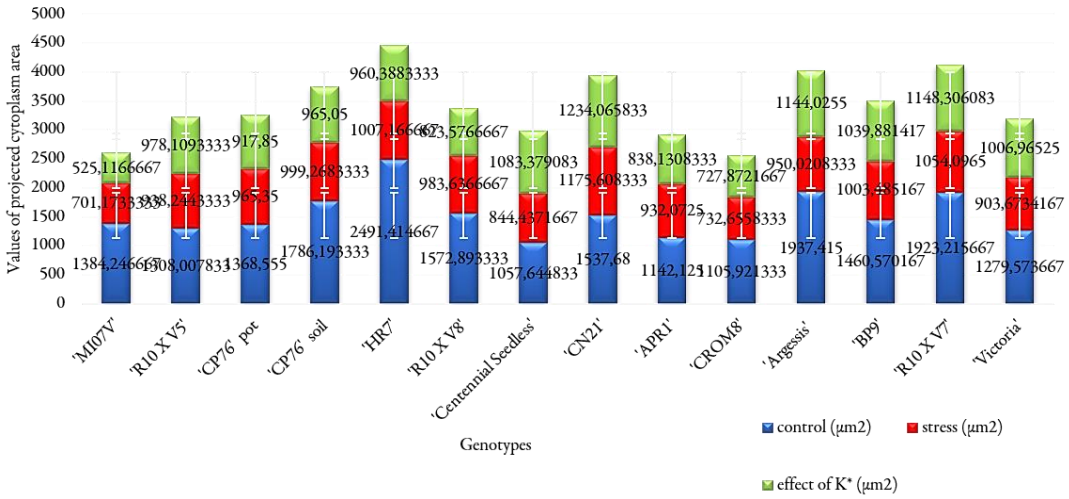


Figure 2. Analysis of variances homogeneity and effects of stressing solutions with and without K^+ in osmotic adjustment estimators for 14 probes



Stressing solutions	L.S.D. 5%
30% PEG	452.67
55% PEG	113.38
55K% PEG	150.84
65% PEG	60.86
65K% PEG	63.43

Figure 3. Genotype reactions for pollen projected cytoplasm area (μm^2) in non-stressing and stressing solutions (PEG 30%; 55%; 65%) as well as with and without K^+; L.S.D. indicate a significance level of 5% for 14 (a) and 10 (b) probes

'Centennial Seedless' (1.292 ± 0.134), 'Argessis' (1.206 ± 0.128), 'Victoria' (1.112 ± 0.067), 'R10xV7' (1.087 ± 0.095), 'CN21' (1.061 ± 0.060), 'R10xV5' (1.041 ± 0.084), and 'BP9' (1.037 ± 0.064) had the best values for induced OA (Table 1; Figure 4a). Pollen grains from two plants of the hybrid 'CP76', one cultivated in pot (0.952 ± 0.115) and the other one in the soil (0.963 ± 0.083) repeated the reactions in osmotic stress solutions with added K^+, with approximately the same average values, expressing the same effect of K^+ during OA (Table 1; Figure 4a).

Table 1. Means ± standard deviations for osmotic adjustment estimators of grapevine pollen grains

Genotype	Expression of:		
	'Intrinsic' OA estimator	'Overall' OA estimator	'Induced' OA estimator
'MI07V'	0.507 ± 0.122 S	0.379 ± 0.080 S	0.748 ± 0.102 MS
R10 X V5'	0.717 ± 0.095 MS	0.748 ± 0.091 MS	1.041 ± 0.084 R
'CP76' pot	0.705 ± 0.070 MS	0.671 ± 0.096 MS	0.952 ± 0.115 MR
'CP76' soil	0.559 ± 0.088 S	0.540 ± 0.064 S	0.963 ± 0.083 MR
'HR7'	0.404 ± 0.031 S	0.385 ± 0.049 S	0.955 ± 0.098 MR
'R10 X V8'	0.625 ± 0.098 MS	0.524 ± 0.092 S	0.832 ± 0.069 MS
'Centennial Seedless'	0.798 ± 0.090 MS	1.024 ± 0.154 R	1.292 ± 0.134 R
'CN21'	0.765 ± 0.091 MS	0.803 ± 0.104 MS	1.061 ± 0.060 R
'APR1'	0.816 ± 0.038 MS	0.734 ± 0.038 MS	0.905 ± 0.050 MR
'CROM8'	0.662 ± 0.040 MS	0.658 ± 0.044 MS	0.994 ± 0.076 MR
'Argessis'	0.490 ± 0.047 S	0.591 ± 0.043 S	1.206 ± 0.128 R
'BP9'	0.687 ± 0.058 MS	0.712 ± 0.083 MS	1.037 ± 0.064 R
'R10 X V7'	0.548 ± 0.040 S	0.597 ± 0.024 S	1.087 ± 0.095 R
'Victoria'	0.706 ± 0.026 MS	0.787 ± 0.050 MS	1.112 ± 0.067 R
Cultivars susceptible	S	S	S
Cultivars resistant	R	R	R
Cultivars medium susceptible	MS	MS	MS
Cultivars medium resistant	MR	MR	MR

Coefficients of variation (CV) ranged between 0.87-1.14 for cell reactions in stressing solution without added K^+ and 0.92-1.15 for cell reactions in stressing solutions with K^+ (Table 2). Thus, a result with a CV of 1.5% is considered as excellent for a cytometry analysis (Figure 4b; Table 2).

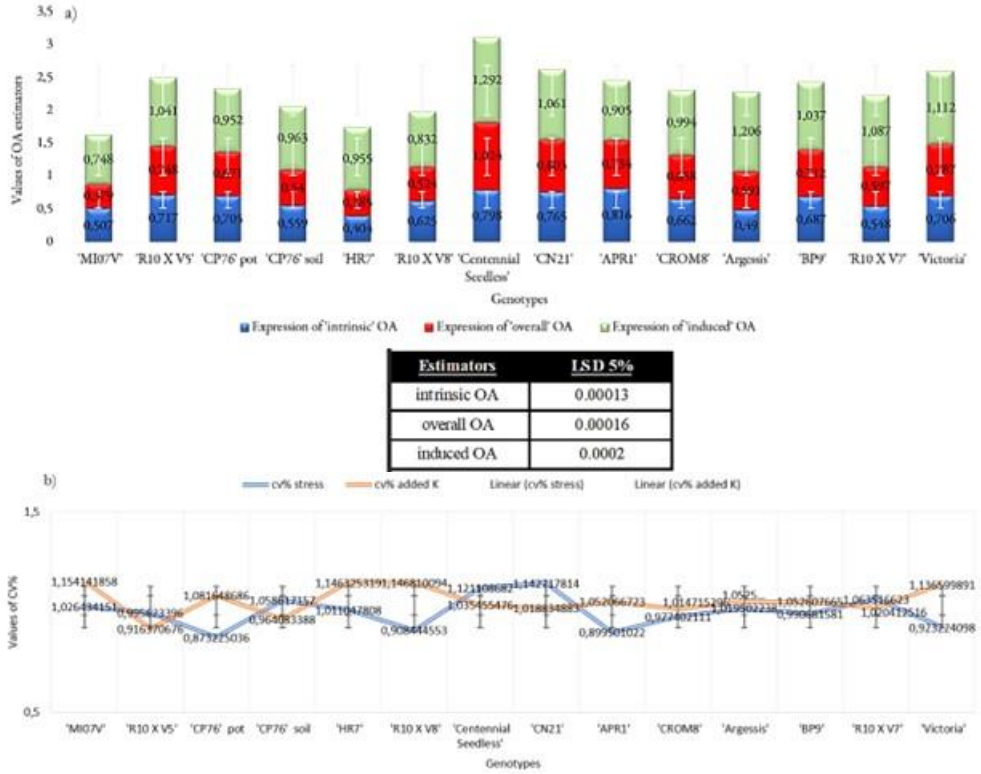


Figure 4. Genotype reactions for osmotic adjustment estimators; L.S.D. values indicate a significance level of 5% for 14 probes (Up); Distribution for coefficients of variation (CV%) on cell reactions in stressing solutions with and without K (Down)

Table 2. Means for standard deviations of projected cell cytoplasm area measurements (μm²) and CV% (coefficient of variation) values expressed in stressing solutions without and with addition of KCl

Genotype	stdev	stdev;	stdev;	stdev; cv% means	stdev;	stdev;
	30% ss	cv% 55% ss	cv% 65% ss		cv% 55K% ss	cv% 65K% ss
'MI07V'	± 6.67	± 7.40;	± 6.98;	± 7.19;	± 9.89;	± 8.81;
		1.11	0.94	1.03	1.42	0.89
'R10 X V5'	± 7.46	± 8.31;	± 7.30;	± 7.80;	± 6.05;	± 6.07;
		1.11	0.88	1	0.83	1
'CP76' pot	± 6.90	± 5.33;	± 5.19;	± 5.26;	± 6.46;	± 5.94;
		0.77	0.97	0.87	1.24	0.92
'CP76' soil	± 5.96	± 6.15;	± 6.67;	± 6.41;	± 6.72;	± 6.19;
		1.03	1.08	1.06	1.01	0.92
'HR7'	± 6.07	± 7.17;	± 6.03;	± 6.60;	± 8.46;	± 7.52;
		1.18	0.84	1.01	1.4	0.89
'R10 X V8'	± 8.96	± 8.13;	± 7.39;	± 7.76;	± 7.78;	± 9.66;
		0.91	0.91	0.91	1.05	1.24
'Centennial Seedless'	± 5.63	± 8.09;	± 6.51;	± 7.30;	± 7.13;	± 6.96;
		1.44	0.8	1.12	1.1	0.98
'CN21'	± 6.64	± 8.54;	± 8.53;	± 8.54;	± 8.50;	± 8.85;
		1.29	1	1.14	1	1.04
'APR1'	± 7.91	± 7.37;	± 6.39;	± 6.88;	± 6.82;	± 7.08;
		0.93	0.87	0.9	1.07	1.04
'CROM8'	± 8.16	± 9.13;	± 7.63;	± 8.38;	± 9.27;	± 7.54;
		1.12	0.84	0.98	1.22	0.81
'Argessis'	± 6.39	± 7.63;	± 6.44;	± 7.04;	± 6.66;	± 7.14;
		1.19	0.84	1.02	1.03	1.07
'BP9'	± 6.83	± 6.52;	± 6.69;	± 6.61;	± 5.63;	± 7.12;
		0.96	1.03	0.99	0.84	1.26
'R10 X V7'	± 7.29	± 7.68;	± 8.24;	± 7.96;	± 8.64;	± 8.57;
		1.05	1.07	1.06	1.05	0.99
'Victoria'	± 7.02	± 6.31;	± 5.98;	± 6.15;	± 4.31;	± 6.69;
		0.9	0.95	0.92	0.72	1.55
L.S.D.	0.01	0.01	0.01	0.01	0.01	0.01

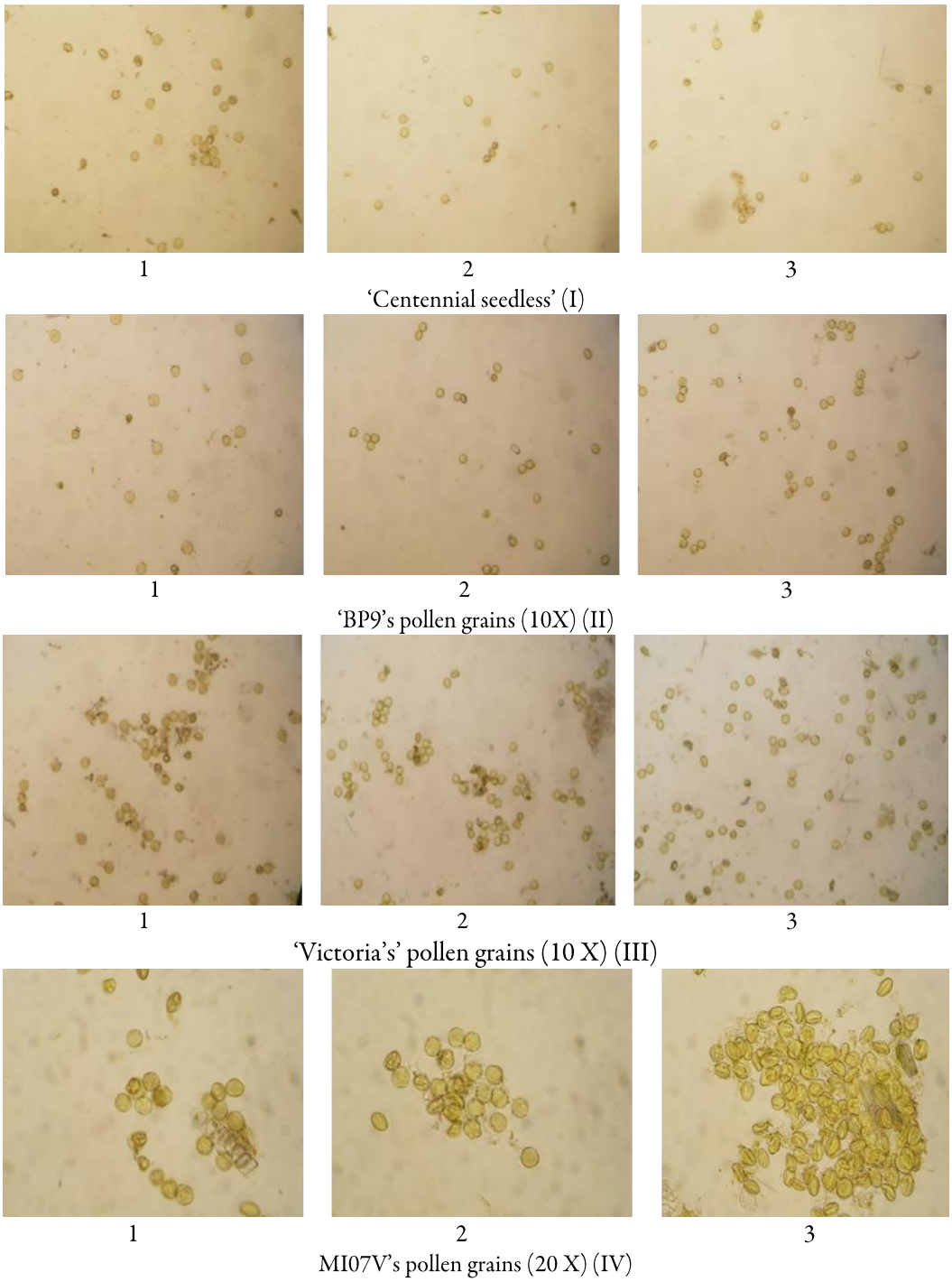


Figure 5. Examples of resistant (I, II, III – 1: pollen grains reaction in control solution; 2: pollen grains reaction in stressing solution 65% PEG; 3: pollen grains reaction in stressing solution 65% PEG with added K^+) and susceptible (IV – 1: pollen grains reaction in control solution; 2: pollen grains reaction in stressing solution 65% PEG; 3: pollen grains reaction in stressing solution 65% PEG with added K^+) genotypes under osmotic stress conditions

Relationships between expressions of osmotic adjustment

Expressions of intrinsic OA had a positive significant correlation of $R^2 = 0.74^{**}$ with overall OA (Figure 6). Correlation between genotype expressions of overall OA and induced OA was also significant with $R^2 = 0.49^{*}$ (Figure 7), while a correlation between intrinsic OA and induced OA wasn't found (Figure 8). These

results probably indicate an interaction between K^+ and organic osmolytes in overall OA, used for maintaining the cytosolic osmolarity, but each OA mechanism is activated separately, one by other. The overall analysis of correlations for all fourteen probes underlined several genotypes with a very good capacity for OA, which are above regression lines (Figure 6; Figure 7), such as 'Centennial Seedless', 'Argessis', 'Victoria' and 'BP9' hybrid. Almost all genotypes are quite narrow arranged along the regression line (Figure 6; Figure 7).

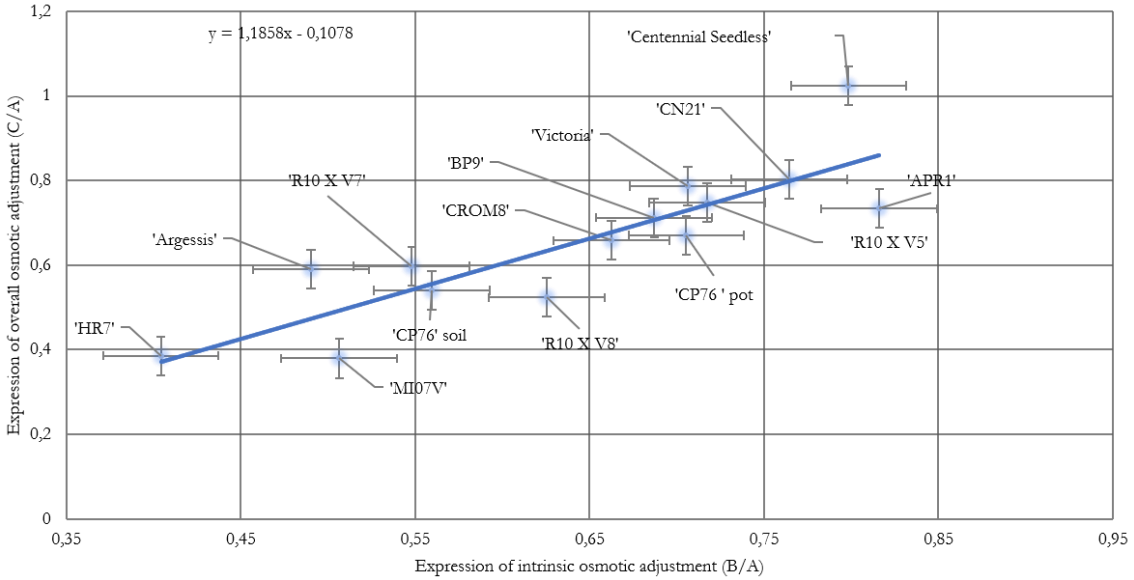


Figure 6. Relationship between intrinsic osmotic adjustment (B/A) and overall osmotic adjustment (C/A)
Black line shows the global linear regression for all fourteen probes. $R^2 = 0.74^{**}$; $P < 0.01$

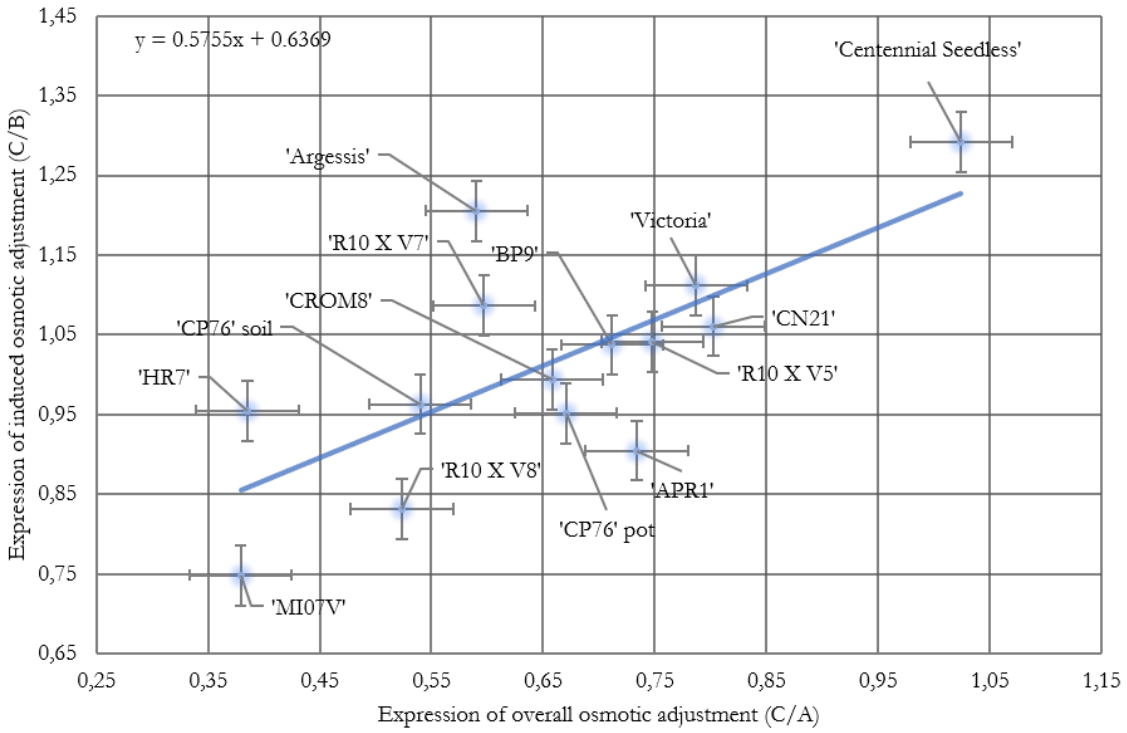


Figure 7. Relationship between overall osmotic adjustment (C/A) and induced osmotic adjustment (C/B)
Black line shows the global linear regression for all fourteen probes. $R^2 = 0.49^{*}$; $P < 0.05$

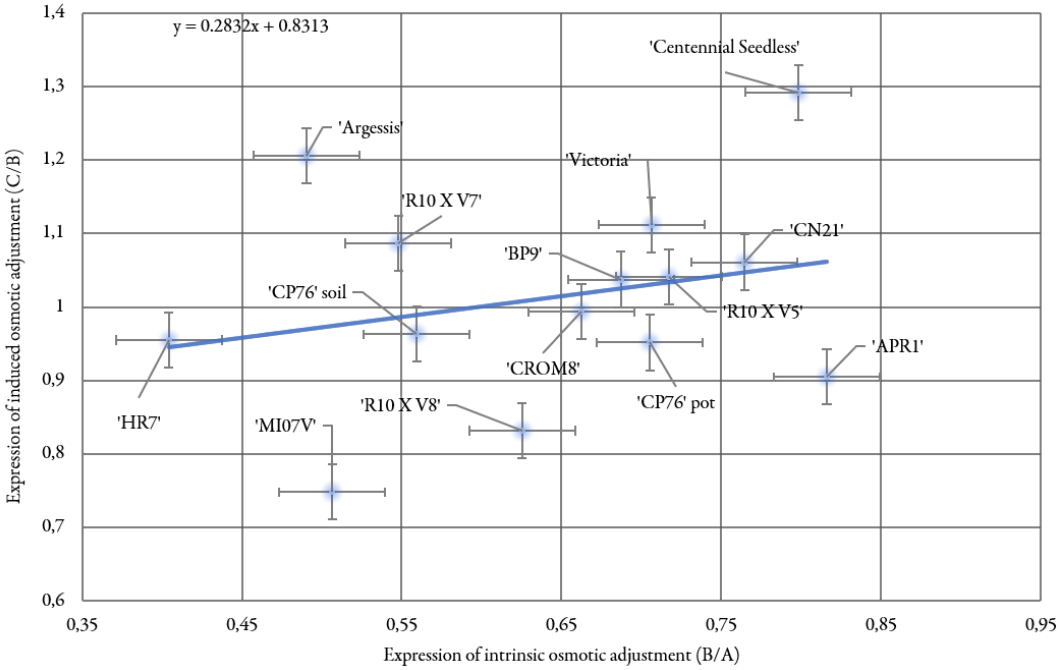
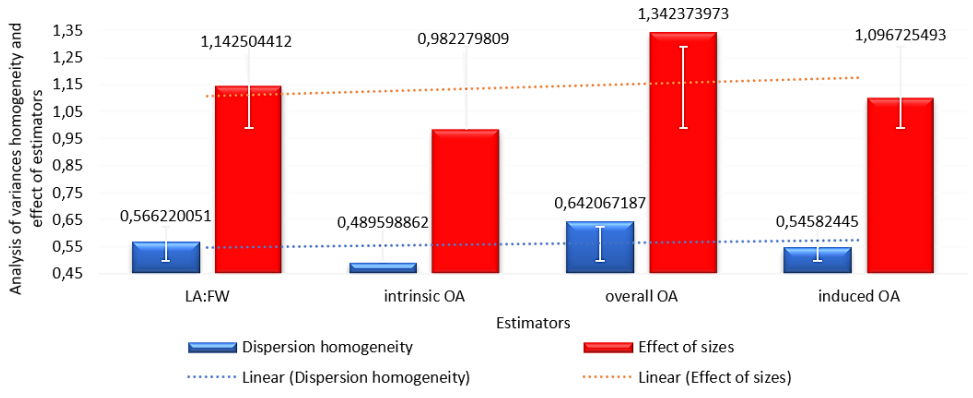


Figure 8. Relationship between intrinsic osmotic adjustment (B/A) and induced osmotic adjustment (C/B)
Black line shows the global linear regression for all fourteen probes. $R^2 = 0.062$ (**n.s.**)

Differences between genotypes for source-sink balance estimator

Significant differences were found both, in LA and FW indicators, and respectively LA/FW estimator ($F = 8.25$; $MS_{intergroup} = 0.6$). The estimator values answer to a significant proportion from dispersion, having values from 49 to 64% for OA estimators and 57% for source-balance sink estimator respectively. Effect of source-sink balance estimators for 10 grapevine genotypes was about 1.14, with a high influence of morphological factors in source-sink balance estimator (Figure 9).

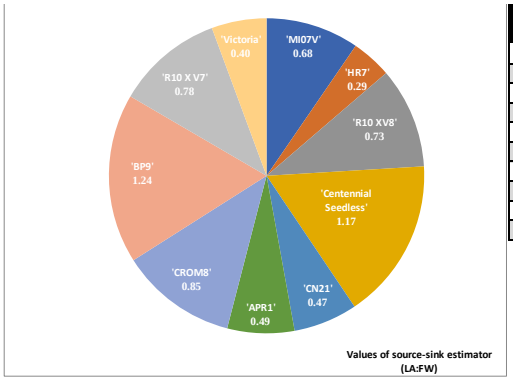


Estimators	LSD 5%
LA:FW	0.00087
intrinsic OA	0.00018
overall OA	0.00022
induced OA	0.00025

Figure 9. Analysis between effects of stressing solutions with and without K^+ in osmotic adjustment estimators and respectively effects of morphological factors in source-sink balance estimator for 10 grapevine genotypes (cultivars and hybrids elite)

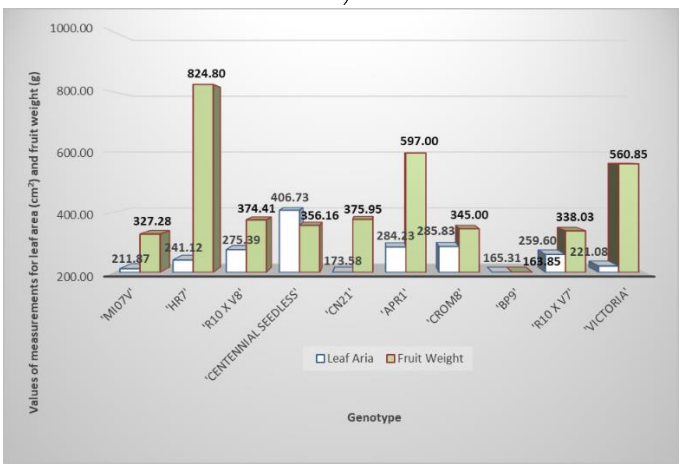
Results of measurements indicate that grapevine genotypes could be separated in two groups:

1. Genotypes which have a higher LA than FW, such as 'Centennial Seedless', leading to the compensatory potassium uptake theory;
2. Genotypes, which have a higher FW than LA, such as 'Victoria', 'HR7', 'APR1' and 'CN21' with good values for source-sink balance (Figure 10b). Standard deviations for LA/FW estimator of several hybrids elite were found higher than cultivar standard deviations. Being hybrids elite in F_1 generations, we recommend a repetition of analysis for this estimator in the next generations. Data from our study are available only for these generations (Figure 10a).



Genotype	Stdev LA:FW
'MI07V'	± 0.26
'HR7	± 0.04
'R10 x V8'	± 0.07
'Centennial Seedless'	± 0.20
'CN21'	± 0.05
'APR1'	± 0.12
'CROM'	± 0.20
'BP9'	± 0.63
'R10 x V7'	± 0.13
'Victoria'	± 0.08

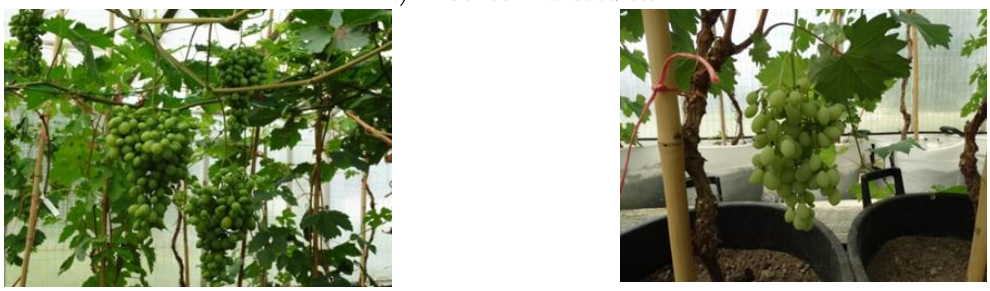
a)



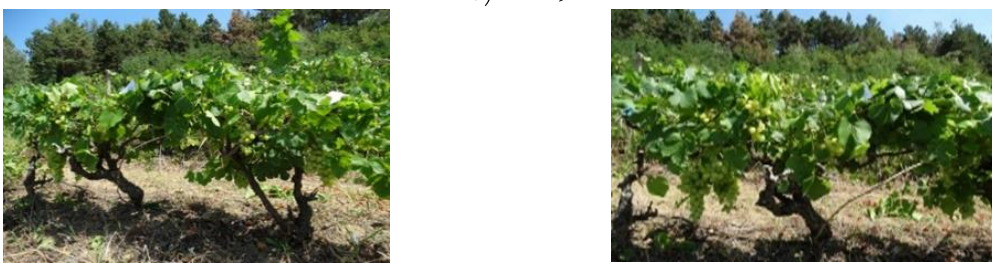
b)

Figure 10. a. Means ± standard deviations of LA:FW estimator for 10 grapevine genotypes (cultivars and hybrids elite); b. Means of leaf area(LA) (cm^2) and fruit weight (FW) (g) for 10 grapevine genotypes (cultivars and hybrids elite)

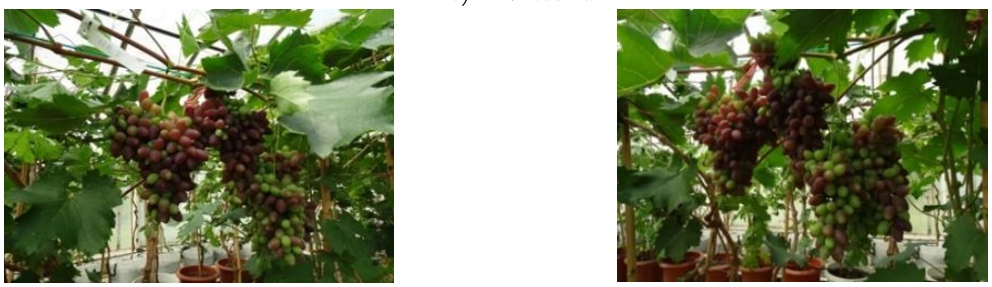
a) 'Centennial seedless'



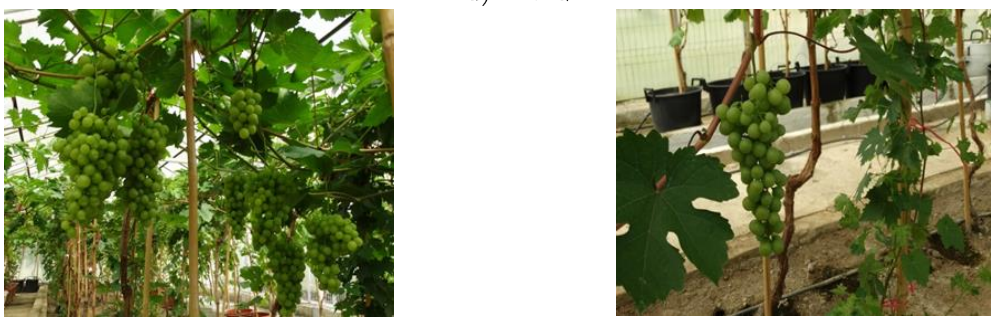
b) 'BP9'



c) 'Victoria'



d) 'HR7'



e) 'MI07V'

Figure 11. Morphological aspects of leaves and bunches for some grapevine genotypes

Relationships between estimators of osmotic adjustment and source-sink balance

Relationship significations were maintained between OA estimators for 10 probes ($R_{C/B;\ C/A}$ = O.82*; $R_{C/A;\ B/A}$ = 0.88**) (Table 3).

Table 3. Correlations between estimators of osmotic adjustment and source-sink balance

Estimators	B/A	C/A	C/B	LA/FW
B/A	1			
C/A	0.88**	1		
C/B	0.46 IS	0.82*	1	
LA/FW	0.28 IS	0.39 IS	0.35 IS	1

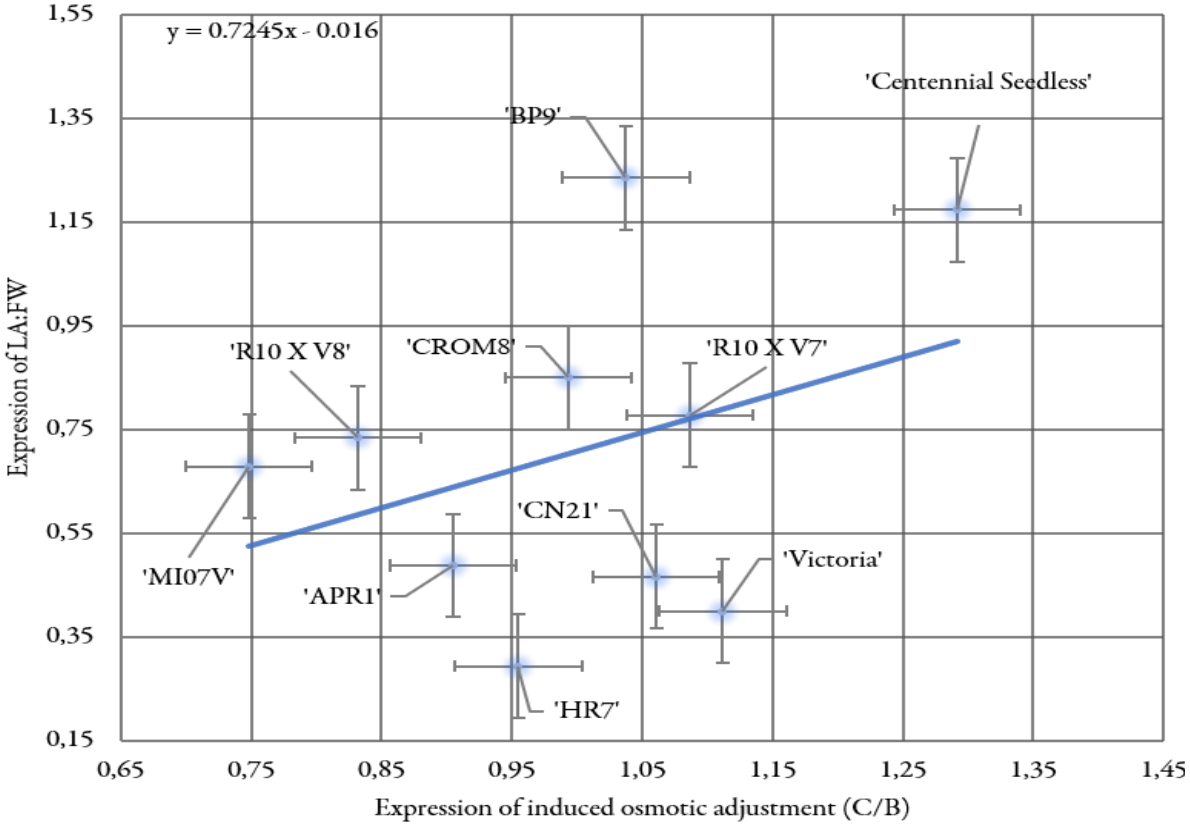


Figure 12. Relationship between induced osmotic adjustment (C/B) and LA:FW
Black line shows the global linear regression for all ten probes. $R^2 = 0.124$ (n.s.); $P < 0.05$; L.S.D. indicate a significance level of 5% 10 probes

Discussion

Variances homogeneity supposed that theoretic distributions for estimators of all variances have the same shape, so, the same variance, being different only as position because of analysed factors effect. Estimation for only general error of all analysis is based on this premise (Săulescu and Săulescu, 1967). In our study, all analyzed estimators had a significant influence on genetic reaction of grapevine genotypes induced by stressing solutions in OA and morphological factors in source-sink balance. Therefore, the estimator analysis had a good response for variances homogeneity.

Effect of stressing solutions with and without K^+ was highly significant in all estimators measured and calculated for osmotic adjustment of grapevine pollen grains. Effect of solutions with K^+, defined by genotype K^+ accumulation capacity, was higher in projected cytoplasm area than the effect of solutions without K^+, indicating that the effect of induced OA plays an important role in maintaining overall OA under osmotic stress conditions than intrinsic OA. Both effects were subordinated to the effect of overall OA. Analysed

grapevine genotype responses in pollen grains for all OA estimators were maintained, independently, on the number of grapevine genotypes (10 or 13), used in the study. An explanation of K^+ accumulation capacity in the sub-cellular location is given by Rogiers (2017). The vacuoles accumulate glucose and fructose at up to 1 M (Conde *et al.*, 2006) and this leads to a doubling of vacuolar K^+ (Keller *et al.*, 2015). Hexose concentrations are important to induce the turgor mediated expansions required for growth. The physiological rationale for this increase in vacuolar K^+ may be related to its potential role in phloem transport. Cytosolic osmolarity is given by a combination of K^+ and others ions as well as organic osmolytes. In our analysis, the correlations of intrinsic and induced OA with overall OA could explain the build-up of cytosolic osmolarity. Stable cytosolic K^+ concentrations are critical for maintaining the activity of enzymes that require K^+ as cofactor (Armengaud *et al.*, 2009). The complexity to determine apoplastic and vacuolar K^+ concentrations is regulated by the presence of gradients in apoplastic and vacuolar K^+ concentrations across the berry. This is driven by the diverse metabolic storage functions of cells according to their location (Rogiers *et al.*, 2017). Our results confirm this explanation by the ratio of mean values calculated for different types of OA mechanisms of pollen cells of elite breeds exposed to PEG solutions with and without K^+ addition. In 'Centennial Seedless' and other hybrid elite pollen cells exposed to PEG solutions with added K^+ had a greater increases of cytoplasm area than pollen cells exposed to stress solutions without K^+, which indicates a good effect and a greater gradient of K^+ in the cells during osmotic adjustment linked to genetic and cellular level. This resulted in induced OA mechanism used by these hybrids and cultivars under severe drought conditions. Under those conditions, the genotype tolerance is characterized by a high K^+ accumulation capacity, comparable to organisms, which use salts to respond quickly to osmotic shock (Galinski, 1995; Rogiers *et al.*, 2017). As in other plant system, greater K^+ concentrations decrease berry's susceptibility to drought, ameliorating oxidative stress, controlling long distance phloem transport and maintaining tissue turgor (Shabala and Pottosin, 2014; Shabala *et al.*, 2016; Rogiers *et al.*, 2017). In contrary, hybrid elites, like 'MI07V' without any pollen cells responses to osmotic stress, are considered as sensitive to drought conditions.

The study found no correlations between estimators of osmotic adjustment and source-sink balance. In some genotypes, the export capacity for photo-assimilates out of leaves is fairly low and induces a high accumulation of soluble carbohydrates in leaves, which triggers an inhibition of photosynthetically activity (Bota *et al.*, 2004a; Lovisolo). The higher LA:FW values of 'Centennial Seedless' and 'BP9' hybrid could be associated with a lower photosynthesis rates (Petrie *et al.*, 2000c; Parker, 2012). Van Bel and Hafke (2005) have been observed that K^+ enters the se/cc when sugars are low and exits se/cc when sugars are high (Smith and Milburn, 1980; van Bel and Hafke, 2005; Rogiers *et al.*, 2017) and this has led to the compensatory potassium uptake theory (van Bel and Hafke, 2005; Rogiers *et al.*, 2017). The results in grapevine with a higher K^+ accumulation capacity than sugar accumulation in the berry (Smart *et. al.*, 1985; Archer and Strauss, 1989; Rojas-Lara and Morrison, 1989; Dokoozlian and Kliewer, 1996; dos Santos *et al.*, 2007; Rogiers *et al.*, 2017) as 'Centennial Seedless', might be explained by the compensatory theory, triggered under sub-optimal light and photosynthesis due to increased canopy shading.

Other genotypes, such as 'Victoria' and 'HR7' hybrid elite were found to increase K^+ during the application of osmotic stress in the cell and lower source-sink ratios which could be associated with an increase of photosynthesis rates. This could be explained by an increase of photoassimilate export capacity and a decrease sugar accumulation as a consequence of severe water stress (Quick *et. al.*, 1992; Lovisolo *et al.*, 2010).

Conclusions

The pollen grain test for OA can be used for all grapevine genotypes and different age. The cytometry analysis used for pollen grains test shows flexibility and stability for all types of locations (pot, soil vs. greenhouse, field). A good screening of grapevine genotypes depends on reaction homogeneity, stressing

solutions, temperature and phenological stage. Genotype responses in pollen grains for all estimators were obtained, independent of the number of varieties used in the study. The analyzed estimators had a good response for variances homogeneity. In the analysis, significant differences were found between genotypes, for estimators of OA and source-sink balance. The induced OA mechanism following higher K^+ supply during the application of osmotic stress is a characteristic of drought tolerant genotypes under the influence of water stress. 'Centennial Seedless' and 'BP9' hybrid showed the best responses of induced osmotic adjustment and lower photosynthesis rates given by the higher average ratio values of LA:FW. Other genotypes, such as 'Victoria' and 'HR7' hybrid reacted with an increase of osmotic stress after K^+ application and lower source-sink ratios, which could be associated with an increase of photosynthesis rates. No correlations were found between mechanisms expressed by analyzed estimators indicating that they are activated and functional separately one by another; sometimes only in a compensatory way.

Authors' Contributions

Conceptualization: MD; Data curation: C-MC, MFB, GN, AT, IDS, RS; Formal analysis: MD, C-MC, MFB; Funding acquisition: Ministry of Agriculture and Rural Development; Investigation: MD, C-MC, MFB, GN, RS; Methodology: MD, C-MC, MFB, GN, RS; Project administration: C-MC, MFB; Resources Software: National Research and Development Institute for Biotechnology in Horticulture Ștefănești-Argeș, Romania; Writing-original draft: MD; Writing-review and editing: MD, RS, AT. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

Acknowledgements

This work was supported by "Breeding of table grape cultivars assortment for obtaining tolerant genotypes on stress environment conditions with the keeping of high qualitative and profitable standardizations-ADER 7.1.3" and "Using of national viticole germplasm for obtaining the new grapevine cultivars with the highest quantitative and qualitative potential regarding abiotic and biotic tolerance-ADER 7.2.3". We acknowledge project managers: Dr. Aurora Ranca from Research and Development Station for Viticulture and Enology, Murfatlar, Romania and Dr. Mărioara Pușcaliu from Research and Development Station for Viticulture and Enology, Odobești, Romania for their research assistance. Also, we acknowledge The Molecular Biology, Plant Protection, Virology and Breeding Laboratories from National Research and Development Institute for Biotechnology in Horticulture, Ștefănești-Argeș for their technical assistance.

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

Archer E, Strauss HC (1989). Effect of shading on the performance of *Vitis vinifera* L. cv. Cabernet Sauvignon. South African Journal of Enology and Viticulture 10:74-77. *https://doi.org/10.21548/10-2-2290*

Armengaud P, Sulpice R, Miller AJ, Stitt M, Amtmann A, Gibon Y (2009). Multilevel analysis of primary metabolism provides new insights into the role of potassium nutrition for glucolysis and nitrogen assimilation in *Arabidopsis* roots. Plant Physiology 150:772-785. *https://doi.org/10.1104/pp.108.133629*

Bota J, Stasyk O, Flexas J, Medrano H (2004b). Effect of water stress on partitioning of ^{14}C-labelled photosynthates in *Vitis vinifera*. Functional Plant Biology 31:697-708. *https://doi.org/10.1071/FP03262*

Chouzouri A, Schultz HR (2005). Hydraulic anatomy, cavitation susceptibility and gas-exchange of several grapevine cultivars of different geographic origin. Acta Horticulturae 689:325-331. *https://doi.org/10.17660/ActaHortic.2005.689.38*

Clarkson DT, Hanson JB (1980). The mineral nutrition of higher plants. Annual Review in Plant Physiology 31:239-298. *https://doi.org/10.1146/annurev.pp.31.060180.001323*

Cohen (1992b). Statistical power analysis. Current Directions in Psychological Science 1(3):98-101. *https://doi.org/10.1111/1467-8721. ep 10768783*

Conde BC, Agasse A, Glissant D, Tavares RM, Geros H, Delrot S (2006). Pathways of glucose regulation of monosaccharide transport in grape cells. Plant Physiology 141:1563-1577. *https://doi.org/10.1104/pp.106.080804*

Daudet FA, Lacointe A, Gaudillère JP, Cruiziat P (2002). Generalized Münch coupling between sugar and water fluxes for modeling carbon allocation as affected by water status. Journal of Theoretical Biology 214:481-498. *https://doi.org/10.1006/jtbi.2001.2473*

David M, Tița A, Toma DI, Ciobotea CM, Bănuță FM (2020). Pollen grain expression of osmotic adjustment as a screening method on drought tolerance in several wine and table grape genotypes. Notulae Scientia Biologicae 12(4):869-883. *https://doi.org/10.15835/12/4108/43*

Delas J, Molot C, Soyer JP (1989). Qualité et constitution des raisins de cuve: fertilization minérale de la vigne et teneurs en potassium des baies, des moûts et des vins. In: 4Ème Symposium International d'Oenologie-Actualités Oenologiques, Bordeaux, France, Dunod, Paris, pp 1-6.

Dokoozlian N, Kliewer MW (1996). Influence on light on grape berry growth and composition varies during fruit development. Journal of the American Society for Horticultural Science 121:869-874. *https://doi.org/10.21273/JASHS.121.5.869*

Dobrei A, Rotaru L, Morelli S (2008). Ampelografie [Ampelography]. In: Dobrei A, Rotaru L, Morelli S (Eds). Ampelografie [Ampelography]. Solness, Timișoara.

Dry PR, Loveys BR, Mccarthy MG, Stoll M (2001). Strategic irrigation management in Australian vineyards. Journal International des Sciences de la Vigne et du Vin 35:129-139. *https://doi.org/10.20870/oeno-one.2001.35.3.1699*

Düring H, Lang A, Oggionni F (1987). Patterns of water flow in Riesling berries in relation to developmental changes in their xylem morphology. Vitis 26:123-131. *https://doi.org/10.5073/vitis.1987.26.123-131*

Findlay N, Oliver KJ, Nii N, Coombe BG (1987). Solute accumulation by grape pericarp cells: IV. Perfusion of pericarp apoplast via the pedicel and evidence for xylem malfunction in ripening berries. Journal of Experimental Botany 38:668-679. *https://doi.org/10.1093/jxb/38.4.668*

Fouquet R, Leon C, Ollat N, Barrieu F (2008). Identification of grapevine aquaporins and expression analysis in developing berries. Plant Cell Reports 27:1541-1550. *https://doi.org/10.1007/s00299-008-0566-1*

Ganbale F, Uozumi N (2006). Properties of shaker-type potassium channels in higher plants. The Journal of Membrane Biology 210:1-19. *https://doi.org/10.1007/s00232-006-0856-x*

Galinski EA (1995). Osmoadaptation in bacteria. Advances in Microbial Physiology 37:272-328. *https://doi.org/10.1016/S0065-2911(08)60148-4*

Glăman G, Dejeu L, Brânduşe E, Şerdinescu A, Ion M (2018). Soiuri noi de viță-de-vie și portaltoi create în România [New grapevine cultivars and rootstock genotypes obtained in Romania]. In: Glăman G, Dejeu L, Brânduşe E, Şerdinescu A, Ion M (Eds). Ampelografia României [Romanian Ampelography] IX. Ceres Bucureşti [Bucharest] pp 63-64, 81-83, 185-186, 215-217, 395, 396, 397, 407, 408.

Hale CR (1977). Relation between potassium and the malate and tartrate contents of grape berries. Vitis 16:9-19. *https://doi.org/10.5073/vitis.1977.16.9-19*

Hays WL (1981). Statistics. In: Hays WL (Ed). Holt Rinehart and Winston, New York.

Keller M, Zhang Y, Shrestha PM, Biondi M, Bondada BR (2015). Sugar demand of ripening grape berries leads to recycling of surplus phloem water via the xylem. Plant Cell and Environment 38:1048-1059. *https://doi.org/10.1111/pce.12465*

Lang A (1983). Turgor-related translocation. Plant Cell and Environment 6:683-689. *https://doi.org/10.1111/1365-3040.ep11589312*

Lovisolo C, Perrone I, Carra A, Ferrandino A, Flexas J, Medrano H, Schubert A (2010). Drought-induced changes in development and function of grapevine (*Vitis* spp) organs in their hydraulic and non-hydraulic interactions at whole-plant level: a physiological and molecular update. Functional Plant Biology 37:98-116. *https://doi.org/10.1071/FP09191*

Morgan JM (1999). Pollen grain expression of a gene controlling differences in osmoregulation in wheat leaves: a simple breeding method. Australian Journal of Agricultural Research 50:953-962. *https://doi.org/10.1071/AR98143*

Parker AK (2012). Modelling phenology and maturation of the grapevine *Vitis vinifera* L.: varietal differences and the role of leaf area to fruit weight ratio manipulations. PhD Thesis, Lincoln University, New Zealand.

Patil BS, Ravikumar RL (2011). Osmotic adjustment in pollen grains: a measure of drought adaptation in sorghum? Current Science 100(3):377-382. *https://www.researchgate.net/publication/257299541*

Petrie PR, Trought MCT, Howell GS (2000c). Influence of leaf ageing, leaf area and crop load on photosynthesis, stomatal conductance and senescence of grapevine (*Vitis vinifera* L. cv. Pinot Noir) leaves. Vitis 39:31-36. *https://doi.org/10.5073/vitis.2000.39.31-36*

Pratelli R, Lacombe B, Torregrosa L, Gaymard F, Romieu C, Thibaud J, Sentenac H (2002). A grapevine gene enconding a guard cell K^+ channel displays developmental regulation in the grapevine berry. Plant Physiology 128:564-577. *https://doi.org/10.1104/pp.010529*

Quick WP, Chaves MM, Wendler R, David M, Rodrigues ML, Passaharinho JA, ... Stitt M (1992). The effect of water stress on photosynthetic carbon metabolism in four species grown under field conditions. Plant Cell and Environment 15:25-35. *https://doi.org/10.1111/j.1365-3040.1992.tb01455.x*

Roby G, Matthews MA (2004). Relative proportions of seed, skin and flesh, in ripe berries from Cabernet Sauvignon grapevines grown in a vineyard either well irrigated or under water deficit. Australian Journal of Grape and Wine Research 10:74-82. *https://doi.org/10.1111/j.1755-0238.2004.tb00009.x*

Rogiers YS, Zelmari AC., Walker RR, Deloire A, Tyerman SD (2017). Potassium in the grape (*Vitis vinifera* L.) berry: transport and function. Plant Science 8:1629. *https://doi.org/10.3389/fpls.2017.01629*

Rojas-Lara BA, Morrison JC (1989). Differential effects of shading fruit or foliage on the development and composition of berries. Vitis 28:199-208. *https://doi.org/10.5073/vitis.1989.28.199-208*

Săulescu NA, Săulescu NN (1967). Câmpul de experiență [Trial field]. Editura Agro-Silvică, Bucharest.

Simonneau T, Lebon E, Ledru-Coupel A, Marguerit E, Rossdeutsch L, Ollat N (2017). Adapting plant material to face water stress in vineyards: Which physiological targets for an optimal control of plant water status? OENO One 51(2):167-179. *https://doi.org/10.20870/oeno-one.2016.0.0.1870*

Shabala S, Pottosin I (2014). Regulation of potassium transport in plants under hostile conditions: implications for abiotic and biotic stress tolerance. Physiologia Plantarum 151:257-279. *https://doi.org/10.1111/ppl.12165*

Shabala S, Bose J, Fuglsang AT, Pottosin I (2016). On a quest for stress tolerance genes: membrane transporters in sensing and adapting to hostile soils. Journal of Experimental Botany 67:1015-1031. *https://doi.org/10.1093/jxb/erv465*

Smart RE, Robinson JB, Due GR, Brien CJ (1985). Canopy microclimate modification for the cultivar Shiraz. II. Effects on must and wine composition. Vitis 24:119-128. *https://doi.org/10.5073/vitis.1985.24.119-128*

Smith JAC, Milburn JA (1980a). Osmoregulation and the control of phloem-sap composition in *Ricinus communis* L. Planta 148:28-34. *https://doi.org/10.1007/BF00385438*

Smith JAC, Milburn JA (1980b). Phloem turgor and the regulation of sucrose loading in *Ricinus communis* L. Planta 148:42-48. *https://doi.org/10.1007/BF00385440*

Steel RG, Torrie JH, Dickey DA (1977). Principles and procedures of statistics: a biometrical approach, 3erd eds. WCB MC Graw-Hill, Boston.

Steen HB (1990a). Characteristics of flow cytometers. In: Melamed MR, Lindmo T, Mendelsohn ML (Eds). Flow Cytometry and Sorting. John Wiley and Sons, New York, USA.

Thompson M, Holbrook N (2003). Scaling phloem transport: water potential equilibrium and osmoregulatory flow. Plant Cell and Environment 26:1561-1577. *https://doi.org/10.1046/j.1365-3040.2003.01080.x*

Tyree MT, Fensom DS (1970). Some experimental and theoretical observations concerning mass flow in the vascular bundles of *Heracleum*. Journal of Experimental Botany 21:304-324. *https://doi.org/10.1093/jxb/21.2.304*

Țârdea C, Rotaru L (1996). Ampelografie [Ampelography. Universitatea Agronomică și de Medicină Veterinară 'Ion Ionescu de la Brad', Iași, pp 222.

van Bel AJE, Hafke JB (2005). Physiochemical determinants of phloem transport. In: Holbrook NM, Zwieniecki MA (Eds). Vascular transport in plants. Burlington, CA, Academic Press, pp 19-44. *https://doi.org/10.1016/B978-012088457-5/50004-6*

VIVC (2019). Vitis International Variety Catalogue. JKI Federal Research Centre for Cultivated Plants. *https://www.vivc.de*

Williams LE, Matthews MA (1990). Grapevine. In: Stewart BA, Nielsen DR (Eds). Irrigation of Agricultural Crops. Agronomy Monograph No. 30 (Madison: ASA-CSSA-SSSA), pp 1019-1055.