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Statistical Modeling of the Effect of Physio-Biochemical Parameters on Water Use Efficiency of Grape Varieties, Rootstocks and Their Stionic Combinations under Moisture Stress Conditions

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Abstract: The effect of various physio-biochemical parameters on the water use efficiency (WUE) of grape varieties, rootstocks and budded vines was studied in 3 separate experiments at 3 levels of irrigation (100%, 50% and no irrigation) for 14 days of stress cycle. None of the varieties or rootstocks could survive beyond 4 days under the no irrigation treatment. Considerable genetic variability was observed for WUE with respect to photosynthetic rate, transpiration rate, stomatal conductance, root to shoot length ratio, abscisic acid, zeatin riboside, carbon isotope discrimination etc. among grape rootstocks, viz., Dogridge, 1613 C, Salt Creek, St. George and *Vitis champinii* (VC) clone, and varieties, viz., Flame Seedless, Thompson Seedless, Sharad Seedless and Tas-A-Ganesh under 50% moisture stress. Among the rootstocks Dogridge was the most efficient water user, followed by Salt Creek and VC clone, while among the varieties Flame Seedless was the most efficient water user, followed by Thompson Seedless and Sharad Seedless at 50% moisture stress. Sharad Seedless on its own root, which was the least efficient water user under 50% soil moisture stress, increased its WUE when budded on efficient water using rootstocks like Dogridge and Salt Creek. A strong positive correlation was observed between WUE and photosynthetic rate, stomatal conductance, root to shoot length ratio and abscisic acid, while a negative correlation was observed with cytokinin and transpiration rate. The regression model revealed photosynthetic rate and transpiration rate as the major contributing factors for variation in WUE of rootstocks, varieties and budded grape vines under moisture stress conditions.

Key Words: Grape, varieties, rootstocks, budded vines, water use efficiency, correlation, regression model, abscisic acid

Introduction

Grape is an important fruit crop in India. Although it originated in a temperate region, it became well acclimatized to the subtropics and tropics of India. Water stress is not a problem in the subtropics, but it is one of the major abiotic constraints in Central and Southern India. Among the several adaptive strategies, improving the efficiency of water use for biomass production is perhaps the most relevant mechanism in drought tolerance (Lincoln and Eduardo, 2002). The physiological mechanisms related to drought tolerance vary from genotype to genotype. It is necessary to screen genotypes for drought tolerance taking into consideration all aspects like photosynthesis rate, transpiration rate, WUE, stomatal conductance, and relative water content at different levels of water stress. Even though plants experience water stress, they have developed several adaptive mechanisms to overcome drought-related harmful effects. There are several mechanisms operating in plants for drought tolerance. Increasing water use efficiency (WUE) is the common adaptation by perennial crops under drought conditions, and when plants are provided with copious water the question of minimizing the water use does not arise at all. Therefore, WUE is understood under water-limited conditions.

Rootstocks have a profound effect on the vigor of the scion and the size and shape of its canopy in various species. The rooting behavior of the rootstocks has an obvious effect on the water relation of the scion leaves. Basic research revealed that photosynthesis and stomatal

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conductance, which are the main physiological parameters in drought tolerance, are affected by the rootstocks used and its effect is scion specific. Another important aspect in rootstock usage is that when water availability is limited root growth is generally less inhibited than shoot growth. This characteristic is thought to be adaptive, whereby the root continues to explore the soil for water while inhibition of shoot growth together with stomatal closure restricts transpiration. An important feature of the root system response is the ability of some roots to continue elongating at the water potential lower than those that inhibit shoot growth (Westgate and Boyer, 1985).

Since grafting/budding provides a new root system to the scions grafted on them, it is not understood whether the leaf physiology of the own rooted rootstock has the same effect on the scion leaves after grafting. Some studies in *Hevea* spp. have shown that the greater the photosynthetic rate of the rootstock seedlings (before grafting), the greater is the photosynthetic rate of the scion (after grafting), but the stomatal conductance of the rootstocks and the scions was not correlated (Sobhana, 1988).

Various physiological, biological and morphological parameters involved in overcoming water stress are interrelated and some are not dependent. Hence, to determine the relationship among various physiological and biochemical parameters involved in improving the WUE of grape varieties, rootstocks and their stionic combinations, an experiment was conducted and the observations were subjected to correlation and regression analysis.

Materials and Methods

Three separate experiments were conducted in the experimental plots of the Indian Institute of Horticultural Research, Bangalore, during 2002-2003 and 2003-2004. In the first experiment rooted cuttings of the varieties Flame Seedless, Thompson Seedless, Sharad Seedless and Tas-A-Ganesh (Source: Germplasm collection of National Research Centre for Grapes, Pune, India) from the bed were transplanted to pots 36 cm in diameter containing a standard potting mixture of farm yard manure (FYM), red earth and sand (1:2:1). The potting mixture was porous with a water holding capacity of 30%. The plants were maintained under uniform cultural practices like irrigation, fertilizer application, weeding and plant protection measures for 6 months. After 6 months plants were irrigated to field capacity before imposing different levels of soil moisture stress. To calculate the field capacity, a pot filled with a known volume of potting mixture was placed in a large plastic bucket and irrigated with a known quantity of water and kept for 6 h to attain the field capacity. The drained water was collected in the bucket. After 6 h the amount of drained water in the plastic bucket was measured and was subtracted from the total amount of water applied. The obtained value was treated as the volume of the irrigation water that has to be applied to attain 100% field capacity (100% irrigation). Half the amount of this was considered as 50% irrigation.

The 3 treatments were S1: No stress (100% irrigation), S2: 50% stress (50% irrigation) and S3: 100% stress (no irrigation). The above treatments were given for 14 days and periodic observations were recorded for various physiological parameters on the 4th, 9th and 14th day of the stress cycle. The irrigation was done manually.

Gas exchange parameters: The gas exchange parameters, namely photosynthetic rate (A), stomatal conductance (gs) and transpiration rate (E), were measured using a portable open photosynthetic system (Model LCA-3, ADC, UK). WUE at the single leaf level (A/E) was calculated using the photosynthesis and transpiration rate values.

Water relation parameters: Relative water content was determined as per the procedures of Barrs and Weatherly (1962), leaf water potential was measured using water potential system CR-7 (Campbell Scientific Inc, USA) and leaf osmotic potential was measured using a vapor pressure osmometer 5100 C (Wescor). For calculating specific leaf weight, the leaf samples were collected from a definite position on the plants and the leaf area was measured with a leaf area meter. Later leaves were oven dried at 70 °C for 72 h. The specific leaf weight was calculated by the following formula: SLW = leaf dry weight / leaf area. The values were expressed as mg cm^{-2}.

Estimation of ABA and Cytokinins: The fully developed immature leaves were collected in an icebox and were brought to the laboratory. Leaves were washed in distilled water and 10 g leaf tissue was ground in 80%

methanol and filtered using Whatman filter paper. The residue was left overnight in 80% methanol. It was filtered and filtrates were pooled. The filtrate was dried using a rotary flash evaporator at 35 °C under vacuum and the residue was dissolved in distilled water. The water extract was partitioned 3 times against di-ethyl ether after adjusting pH to 3.0 with 0.1 N HCl.

The ether fraction was dried in the flash evaporator at 35 °C and the residue was dissolved in 5 ml of Tris buffer (20 mM, pH: 7.4) for ABA estimation.

The aqueous phase was partitioned with water-saturated n-butanol at pH 8.0. The butanol fraction was dried under reduced pressure at 35 °C in the flash evaporator and the residue was dissolved in 5 ml of Tris buffer (20 mM, pH: 7.4) for estimation of cytokinins like zeatin riboside and dihydro zeatin riboside (ZR and DHZR).

ELISA was used to quantify ABA (Weiler, 1980) and cytokinins (Barthe and Stewart, 1985) in plant tissue employing laboratory raised polyclonal antibodies. The quantity of hormones was expressed as ng g^{-1} fresh weight of the tissue.

Carbon isotope discrimination (CID or Δ): One gram powdered leaf sample was put in glass vials and sent to the laboratory for the National Facility for Determination of Stable Isotopes in Plants at the Department of Plant Physiology, G.K.V.K., Bangalore, for estimation of CID using an isotope ratio mass spectrometer (Bindhu Madhava, 2000).

Estimation of potassium, calcium and magnesium: At the beginning of the experiment leaf samples were collected from all the varieties, rootstocks and budded vines, and the potassium was estimated by flame photometer, while calcium and magnesium were estimated using an atomic absorption spectrophotometer.

Similarly, in the second experiment 5 rootstocks, namely Dog ridge, 1613 C, Salt Creek, St. George and VC clone (Source: Germplasm of National Research Centre for Grapes, Pune, India), were tested for WUE and the procedure followed was the same as that in the first experiment.

Based on the WUE values of the above-mentioned 4 varieties from the first experiment and the 5 rootstocks from the second experiment at 50% stress on the 14th day, 3 varieties having the highest WUE, namely Flame Seedless, Thompson Seedless and Sharad Seedless, were selected for studying their performance when budded on the 3 rootstocks from the second experiment, namely Dog ridge, Salt Creek and V.C clone. The 9 stionic combinations were Flame Seedless on Dog ridge, Salt Creek and VC clone; Thompson Seedless on Dog ridge, Salt Creek and VC clone; and Sharad Seedless on Dog ridge, Salt Creek and VC clone. When the budded plants were 6 months old, 2 levels of stress, namely 100% stress and 50%, and no stress (control) were given for 14 days. The budded plants under 100% stress could not survive beyond 3-4 days. Hence, observations were recorded in only 2 treatments of the control and 50% stress. On the 14th day, i.e. at the time of termination of stress treatments, gas exchange parameters were measured as explained above.

Statistical Modeling

As a first step, linear correlation coefficients (r) among various physiological parameters with WUE under soil moisture stress were computed individually for all the data sets. Then a multiple regression model for WUE versus various physiological parameters was constructed individually for all the data sets. However, these models are not robust against the basic assumption of regression approach, viz. independent variables should not be related among themselves, commonly known as the problem of multi-co-linearity, as indicated by their respective values of Variance Inflation Factor (VIF), being above 10. To this end, a refined step-wise regression model was developed to identify the best indicators of WUE (Ryan, 1997). As a measure of goodness of fit, the values of the coefficient of determination (R^2) (Kvalseth, 1985) were calculated as below:

Coefficient of Determination (R^2)

$$R^2 = 1 - [\Sigma (Y_t - \hat{Y})^2 \ / \ [\Sigma (Y_t - \bar{Y})^2],$$

where Y_t represents the WUE of the t^{th} sample.

However, inclusion of an additional independent variable in the selected candidate model will always boost the computed R^2 value (Kvalseth, 1985). Hence, to ensure the statistical significance of the computed regression coefficients, they were subjected to detailed t-test statistic analysis (Ryan, 1997).

Results and Discussion

Correlation studies in grape varieties, rootstocks and budded vines

As a first step, linear correlation coefficients (r) among various physiological parameters with WUE under soil moisture stress were worked out individually for all the 3 experiments and are presented in Tables 1-3. Perusal of these tables indicated that in grape varieties WUE is highly significant ($P < 0.01$) and positively correlated with A ($r = 0.95$), CID ($r = 0.84$), and SLW ($r = 0.72$). However, in the case of grape rootstocks WUE is highly significant and positively correlated with A ($r = 0.96$), OP ($r = 0.77$), ABA ($r = 0.72$), WP ($r = 0.70$), and RSLR ($r = 0.69$), and negatively correlated with E ($r = -0.73$). However, in the case of budded vines instantaneous WUE is highly significant and positively correlated with A ($r = 0.77$), ABA ($r = 0.74$), and DM ($r = 0.57$), and negatively correlated with E ($r = -0.79$). The Statistical Package for the Social Sciences (SPSS v11.0) was utilized extensively for statistical analysis.

Statistical models of grape varieties, rootstocks and budded vines

The statistical model developed by regressing instantaneous WUE on physiological parameters of grape varieties (Table 4) indicated that about 99.8% of the variability in instantaneous WUE could be collectively accounted for by all the physiological parameters. Further, to identify the most significant contributing factor, again the model was optimized. Results of the optimized model presented in Table 4 showed that about 94.9% of the variability in instantaneous WUE could be attributed to A and RSLR only.

The statistical model developed by regressing instantaneous WUE on physiological parameters of grape rootstocks (Table 5) indicated that about 99.8% of the variability in instantaneous WUE could be collectively accounted for by all the physiological parameters. Further, to arrive at the most significant physiological parameters, which could attribute the maximum contribution to instantaneous WUE, the model was optimized and the results of the optimized model presented in Table 5 showed that about 90.6% of the variability in instantaneous WUE could be attributed to A and E only.

The statistical model developed by regressing instantaneous WUE on physiological parameters of budded vines (Table 6) indicated that about 97.9% of the variability in instantaneous WUE could be collectively

Table 1. Correlation matrix among various physiological parameters in grape varieties under soil moisture stress.

	A/E (Y1)	gs	ABA	RSLR	RSDWR	CID	A	ZR	OP	WP	RWC	SLW
A/E	1.00											
gs	-0.19	1.00										
ABA	0.68	0.01	1.00									
RSLR	0.70	-0.07	0.46	1.00								
RSDWR	0.50	0.12	0.28	0.13	1.00							
CID	**0.84**	-0.12	0.72	0.78	0.43	1.00						
A	**0.95**	-0.17	0.55	0.54	0.54	0.77	1.00					
ZR	**-0.71**	-0.17	-0.57	0.83	0.35	0.93	0.64	1.00				
OP	-0.63	0.00	-0.39	-0.71	-0.43	-0.83	-0.62	-0.84	1.00			
WP	0.37	-0.07	0.56	0.03	0.29	0.51	0.45	0.37	-0.22	1.00		
RWC	0.63	-0.31	0.35	0.46	0.51	0.51	0.65	0.58	-0.60	0.13	1.00	
SLW	**0.72**	-0.32	0.61	0.57	0.36	0.77	0.60	0.75	-0.40	0.40	0.31	1.00

(Note: Bold values are significant at $P < 0.01$)

WUE: Water use efficiency, gs: Stomatal conductance (μ CO_2 m^{-2} s^{-1}), ABA: Abscissic acid (ng g^{-1}), RSLR: Root to shoot length ratio, RSDWR: root to shoot dry weight ratio, CID: carbon isotope discrimination (‰), A: Photosynthetic rate (μ CO_2 m^{-2} s^{-1}), ZR: zeatin riboside (ng g^{-1}), OP: Osmotic potential (-Mpa), WP: water potential (-Mpa), RWC: relative water content (%), SLW: specific leaf weight (mg cm^{-2}).

Table 2. Correlation matrix among various physiological parameters in grape rootstocks under soil moisture stress.

	A/E	gs	E	A	ABA	ZR	RSLR	RSDWR	RWC	SLW	WP	OP	CID
A/E	1.00												
gs	0.14	1.00											
E	**-0.73**	0.02	1.00										
A	**0.96**	0.20	-0.51	1.00									
ABA	**0.72**	-0.20	-0.71	0.59	1.00								
ZR	-0.61	-0.03	0.22	-0.65	-0.71	1.00							
RSLR	**0.69**	-0.25	-0.80	0.53	0.92	-0.55	1.00						
RSDWR	0.25	-0.41	-0.49	0.10	0.69	-0.39	0.74	1.00					
RWC	0.15	0.62	-0.05	0.20	-0.32	0.10	-0.23	-0.40	1.00				
SLW	0.50	-0.29	-0.69	0.35	0.43	-0.21	0.48	0.37	-0.08	1.00			
WP	**0.70**	0.17	-0.67	0.63	0.52	-0.37	0.64	0.22	0.35	0.38	1.00		
OP	**0.77**	-0.08	-0.88	0.61	0.77	-0.38	0.81	0.65	-0.02	0.67	0.64	1.00	
CID	-0.13	0.44	0.52	0.07	-0.52	-0.10	-0.56	-0.38	0.38	-0.26	-0.16	-0.37	1.00

(Note: Bold values are significant at $P < 0.01$)

A/E: Water use efficiency, gs: Stomatal conductance (μ CO_2 m^{-2} s^{-1}), E: transpiration rate (m mole H_2O m^{-2} s^{-1}), A: Photosynthetic rate (μ CO_2 m^{-2} s^{-1}), ABA: Abscisic acid (ng g^{-1}), ZR: zeatin riboside (ng g^{-1}), RSLR: Root to shoot length ratio, RSDWR: root to shoot dry weight ratio, RWC: relative water content (%), SLW: specific leaf weight (mg cm^{-2}), WP: water potential (-Mpa), OP: Osmotic potential (-Mpa), CID: carbon isotope discrimination (‰).

Table 3. Correlation matrix among various physiological parameters in budded vines under soil moisture stress.

	A/E	RWC	E	A	gs	ABA	DM	K	Mg	CID
A/E	1.00									
RWC	0.48	1.00								
E	**-0.79**	-0.48	1.00							
A	**0.77**	0.26	-0.25	1.00						
gs	-0.13	-0.38	0.47	0.37	1.00					
ABA	**0.74**	0.58	-0.76	0.35	-0.61	1.00				
DM	**0.57**	0.60	-0.61	0.28	-0.31	0.65	1.00			
K	-0.20	-0.19	0.10	-0.20	0.05	-0.11	-0.14	1.00		
Mg	-0.27	-0.26	0.36	-0.05	0.16	-0.31	-0.18	0.21	1.00	
cid	-0.32	-0.38	0.22	-0.23	0.19	-0.57	-0.42	-0.31	0.22	1.00

(Note: Bold values are significant at $P < 0.01$)

A/E: Water use efficiency, RWC: relative water content (%), E: transpiration rate (m mole H_2O $m^{-2}s^{-1}$), A: Photosynthetic rate (μ CO_2 m^{-2} s^{-1}), gs: Stomatal conductance (μ CO_2 m^{-2} s^{-1}), ABA: Abscisic acid (ng g^{-1}), DM: dry matter (%), K: potassium (%), Mg: magnesium (%), CID: carbon isotope discrimination (‰).

Table 4. Statistical model expressing relationship between WUE and physio-biochemical parameters in grape varieties.

	Dependent variable as A/E: Instantaneous WUE						
Variable	Equation						R^2 statistics
All	Y = -3.86 + 0.001 ABA + 0.103 RSLR + 0.018 RSDWR – 0.107 CID + 0.13A						0.998
	Se (b)	(0.001)	(0.03)	(0.01)	(0.16)	(0.02)	
	t-stat	1.9	4.14	0.24	-0.67	5.52	
	-0.01 ZR – 0.45B OP + 0.04 WP + 0.003 RWC + 0.289 SLW						
	Se (b)	(0.005)	(0.36)	(0.05)	(0.005)	(0.103)	
	t-stat	-2.35	-1.22	0.76	0.61	2.8	
Optimized model	Y = -0.429 + 0.157 A + 0.061 RSLR						0.949
	Se (b)	(0.017)	(0.20)				
	t-stat	8.981	3.033				

Se (b): Standard Error of regression coefficients.

A/E: Water Use Efficiency, ABA: Abscisic acid, RSLR: Root to shoot length ratio, RSDWR: root to shoot dry weight ratio, CID: Carbon isotope discrimination, A: Photosynthetic rate, ZR: Zeatin riboside, OP: Osmotic potential, WP: water potential, RWC: Relative water content, SLW: Specific leaf weight.

Table 5. Statistical model expressing relationship between WUE and physio-biochemical parameters in grape rootstocks.

	Dependent variable as A/E: WUE						
Variable	Equation						R^2 statistics
All	Y = 2.616 - 0.066 gs - 0.156 E + 0.123 A – 0.01 ABA - 0.007 ZR						0.998
	Se (b)	(0.412)	(0.05)	(0.013)	(0.001)	(0.007)	
	t-stat	0.162	-3.09	9.52	-8.41	-1.125	
	+ 0.0009 RSLR – 0.007 RSDWR - 0.0037 RWC - 0.021 SLW						
	Se (b)	(0.055)	(0.065)	(0.005)	(0.034)		
	t-stat	0.017	-0.112	-0.73	-0.613		
	- 0.03 WP + 0.07 OP - 0.03 CID						
	Se (b)	(0.048)	(0.15)	(0.41)			
	t-stat	-0.68	0.475	-0.78			
Optimized model	Y = 1.0 + 0.125 A - 0.123 E						0.906
	Se (b)	(0.003)	(0.008)				
	t-stat	36.31	-15.2				

Se (b): Standard Error of regression coefficients.

A/E: Water Use Efficiency, gs: Stomatal conductance, E: transpiration rate, A: Photosynthetic rate, ABA: Abscisic acid, ZR: Zeatin riboside, RSLR: Root to shoot length ratio, RSDWR: root to shoot dry weight ratio, RWC: Relative water content, SLW: Specific leaf weight WP: water potential, OP: Osmotic potential, CID: Carbon isotope discrimination.

Table 6. Statistical model expressing relationship between WUE and physio-biochemical parameters in budded grape vines.

	Dependent variable as A/E: WUE						
Variable	Equation						R^2 statistics
All	Y = 1.157 - 0.0005 RWC - 0.108 E + 0.134 A – 0.207 gs + 0.0002 ABA						0.979
	Se (b)	(0.001)	(0.013)	(0.014)	(0.195)	(0.02)	
	t-stat	- 0.427	-8.5	9.72	-1.06	0.196	
	- 0.001 DM – 0.02 K + 0.017 Mg - 0.009 CID						
	Se (b)	(0.005)	(0.041)	(0.43)	(0.015)		
	t-stat	-0.278	-0.48	0.04	-0.632		
Optimized model	Y = 0.937 - 0.117 E + 0.127 A						0.986
	Se(b)	(0.006)	(0.007)				
	t-stat	-18.12	17.16				

Se (b): Standard Error of regression coefficients.

A/E: Water Use Efficiency, RWC: Relative water content, E: transpiration rate, A: Photosynthetic rate, gs: Stomatal conductance, ABA: Abscisic acid, DM: dry matter, Mg: magnesium, K: Potassium, CID: Carbon isotope discrimination.

accounted for by all the physiological parameters. Further, the model was again optimized to identify the most significant factors responsible for explaining 97.9% of the variability in instantaneous WUE. Results of the optimized model presented in Table 6 showed that about 98.6% of the variability in instantaneous WUE could be attributed to A and E only.

Further, under all the optimized models, corresponding regression coefficients were statistically significant (being greater than 2.0 in absolute value). This further strengthens the statistical validity of the selected models with an optimum number of physiological parameters to explain maximum variability in intrinsic/instantaneous WUE in all 3 data sets.

Relationship between WUE, water relations and gas exchange parameters

A significant positive correlation was observed between WUE and photosynthetic rate. The reduction in photosynthesis may be associated with the decreased stomatal conductance in all the varieties. The reduction in photosynthesis in grape vine leaves was higher under drought conditions than in moderately and fully irrigated plants. The present study supports the earlier findings reported by Delgado et al. (1995) on stomatal conductance and photosynthesis in grape vines.

As the transpiration rate fell under drought conditions in response to decreased stomatal conductance, there was an increase in WUE in Flame Seedless and Thompson Seedless. In Sharad Seedless and Tas-A-Ganesh, there was a decrease in WUE, which may be due to the higher transpiration rate under moisture stress conditions (Table 7). Such an increase in WUE at decreasing water potential was observed by Behboudian et al. (1986) in many pistachio varieties. They observed reduced WUE at leaf water potential beyond –3.0 Mpa. As the water potential decreases beyond –3.0 Mpa perhaps the mere survival of plants will be more important than economy of water use.

Many photosynthetic parameters like electron transport reaction, carboxylation efficiency, intrinsic WUE and respiration were more strongly correlated with stomatal conductance than with water status itself (Medrano et al., 2002). WUE is more important than the higher rate of photosynthesis or decreased rate of transpiration under drought conditions. The Riesling grape variety grafted on SO-4 and Kober 5 BB rootstocks had the highest WUE although there were higher stomatal conductance and photosynthesis in the

Table 7. Physiological parameters of grape varieties and rootstocks at 100% irrigation and 50% irrigation at the end of the stress cycle.

	A		E		gs		WUE		RWC		WP		ABA		ZR		CID		RSLR		K
	100%	50%	100%	50%	100%	50%	100%	50%	100%	50%	100%	50%	100%	50%	100%	50%	100%	50%	100%	50%	100%
Flame Seedless	10.0	9.10	10.50	6.90	0.57	0.41	0.96	1.33	87.36	71.94	1.15	1.31	14.62	133.8	64.26	47.35	18.68	17.93	1.55	4.02	1.35
Thompson Seedless	9.63	8.00	10.40	7.80	0.52	0.40	0.91	1.02	87.39	71.17	1.21	1.48	24.62	49.9	80.42	52.91	18.64	18.25	2.79	4.25	1.21
Sharad Seedless	7.50	7.06	10.33	8.90	0.42	0.39	0.72	0.78	81.98	67.04	1.28	1.51	20.32	31.76	38.31	27.09	19.43	19.77	3.31	1.39	1.23
Tas-A-Ganesh	7.83	5.73	10.76	9.50	0.43	0.36	0.72	0.60	80.25	61.04	1.23	1.64	15.43	32.31	35.19	24.85	19.84	20.02	4.41	2.33	1.33
P < 0.05	1.38		0.37		0.04		0.13		5.04		NS		12.72		3.43		0.31		0.73		NS
Dog ridge	8.30	9.36	7.06	7.06	0.53	0.35	0.84	1.31	84.93	71.37	1.12	1.31	66.37	163.8	43.41	32.85	20.52	19.00	1.09	3.50	1.37
1613 C	5.70	4.83	8.33	8.33	0.44	0.36	0.60	0.58	82.54	73.40	1.47	1.66	19.35	25.92	63.49	47.12	19.48	19.01	1.17	1.52	0.90
Salt Creek	6.80	9.23	7.63	7.63	0.58	0.39	0.69	1.21	85.20	78.06	1.11	1.26	32.46	78.05	52.18	34.32	21.32	19.95	0.91	2.19	0.97
St. George	10.60	7.20	9.40	9.40	0.52	0.38	1.02	0.76	82.34	74.08	1.34	1.70	12.17	31.38	71.16	30.92	21.45	20.63	0.85	1.37	1.22
V.C. Clone	7.03	8.40	7.93	7.93	0.44	0.39	0.69	1.06	86.50	77.82	1.39	1.43	22.46	34.42	53.43	35.16	21.38	20.18	0.75	1.99	1.01
P < 0.05	0.32		NS		0.11		2.57		0.39		0.10		5.91		3.23		*		0.384		NS

NS: Nonsignificant, *; values not analyzed statistically

A: Photosynthetic rate (µ CO_2 m^{-2} s^{-1}), E: Transpiration rate (m mole H_2O m^{-2} s^{-1}), gs: Stomatal conductance (µ CO_2 m^{-2} s^{-1}), WUE: Water Use Efficiency, RWC: Relative water content (%), WP: water potential (-Mpa), OP: Osmotic potential (-Mpa), ABA: Abscisic acid (ng g^{-1}), ZR: Zeatin riboside (ng g^{-1}), CID: carbon isotope discrimination (‰), RSLR: Root to shoot length ratio, K: Potassium (%).

ungrafted Riesling variety. In the present investigation the higher WUE in a few scion rootstock combinations may be due to an interaction between rootstocks and scions. It is important to have increased WUE (Flame Seedless and Sharad Seedless on Dog ridge) under drought conditions rather than increased photosynthesis or a reduced transpiration rate, because in some scion/rootstock combinations (Sharad Seedless on VC clone) at 50% stress, there was a reduction in photosynthesis and an increase in transpiration, which resulted in lower WUE (Table 8). A strong negative correlation was observed between WUE and both transpiration rate and stomatal conductance (Tables 1-3).

Relationship between hormones and WUE

A strong positive correlation was observed between ABA content and WUE & root to shoot length ratio. The increase in ABA in the drying roots, combined with the availability of water drawn from the wetted roots, may have an impact on root growth as it has been shown that grape vines subjected to partial root drying (PRD) irrigation show increased root development in the deeper soil layer when compared with fully irrigated controls (Dry et al., 2000). It is also known that ABA can maintain root growth under conditions of low soil moisture, which results in inhibition of shoot growth (Sharp, 1996).

The effect of root growth may be augmented by the reduction in cytokinin concentration observed in roots of grape vines and in the very large difference in ABA to cytokinin ratio that occurs in roots during PRD cycles (Auer, 1996). The reduction in the shoot development in severely water-stressed vines could be due to a reduction in cytokinin supply from the roots (Dry and Loveys, 1999). Studies by Zhang and Davies (1987) also demonstrated that ABA inhibits leaf area growth with a concentration dependence similar to the relationship observed from ABA accumulation in plants subjected to drying soils.

Relationship between nutrient elements and WUE

Potassium also plays a major role in the opening and closing of stomata as well as in activating enzymes in the photosynthetic process. In the present study, a strong negative relation was observed between WUE and transpiration rate. Specific leaf weight was negatively correlated with K (data not shown) in budded plants, suggesting that increased K reduces specific leaf weight in Flame Seedless. Under water stress, plants well supplied with K quickly close the stomata, thus preventing excessive water loss. In other words, when the plants obtain sufficient water, the stomata open wide, leading to higher CO_2 assimilation, thus leading to the conclusion that K increases WUE.

In the present study also, high K content in Flame Seedless and Dog ridge must have influenced their increased WUE, as explained above. Among the budded plants, Flame Seedless and Sharad Seedless on Dog ridge had higher K content and the same combination had higher WUE and lower transpiration rate (Table 8). Specific leaf weight was negatively correlated with K in budded plants, suggesting that increased K reduces specific leaf weight in Flame Seedless. Similarly, a negative correlation between K and SLW was obtained by Brown and Byrd (1997) in 4 cereal crops. The explanation is that low SLW with high mineral content was due to genetic variation in mineral uptake and was associated with some process other than transpiration. It is possible that mineral uptake is more closely associated with leaf area than leaf weight and that secondary leaf thickening or other cause of high SLW result in dilution of leaf minerals.

Relationship between carbon isotope discrimination and WUE

A significant negative correlation was found between intrinsic WUE and CID. Even though WUE was higher in Flame seedless, the photosynthetic rate was lower than that in other varieties where WUE was less. This can be explained as per Hubick et al. (1988), who stated that if environmental factors influence the conductance, leading to changes in WUE, it is evident that internal and ambient partial pressure influences WUE, which, in turn, influences CID. WUE is not only altered by vapor pressure deficits through gs, but moisture stress also alters stomatal and non-stomatal factors.

Meinzer et al. (1998) also obtained an inverse relationship between WUE and CID in 5 coffee genotypes. Under adequate irrigation, higher WUE in coffee genotypes resulted from reduced stomatal apertures rather than increased photosynthesis capacity at a given gs. Apparently contrasting behavior has been reported for well-irrigated peanut genotypes, where productivity and CID were negatively correlated, suggesting that variations in CID were the result of variation in photosynthesis capacity and therefore pi/pa at similar levels of gs.

Table 8. Physiological parameters of budded vines at 100% irrigation and 50% irrigation at the end of the stress cycle.

	A		E		gs		WUE		RWC		WP		ABA		CID		K
	100%	50%	100%	50%	100%	50%	100%	50%	100%	50%	100%	50%	100%	50%	100%	50%	100 %
Flame on Dog ridge	6.80	6.80	7.86	6.30	0.27	0.27	0.85	1.09	88.47	81.94	0.95	1.17	26.96	62.79	19.21	16.21	1.80
Flame on Salt reek	7.86	7.46	8.46	6.86	0.36	0.36	0.92	1.09	86.20	76.20	1.15	1.39	23.79	58.76	19.51	17.47	1.66
Flame on VC clone	7.63	6.46	9.23	9.00	0.43	0.43	0.82	0.71	79.13	65.17	1.19	1.51	20.02	32.16	19.63	17.90	1.91
Thompson on Dog ridge	5.80	6.73	8.23	7.33	0.38	0.38	0.70	0.89	88.40	77.86	1.17	1.53	20.21	39.06	19.37	16.61	1.85
Thompson on Salt Creek	7.76	7.20	8.80	8.43	0.47	0.47	0.87	0.85	88.65	78.53	1.05	1.40	17.75	28.07	19.67	17.20	1.70
Thompson on VC clone	7.46	6.36	8.46	7.93	0.45	0.45	0.87	0.80	83.78	64.64	1.23	1.64	15.59	24.38	19.79	17.56	1.91
Sharad on Dog ridge	6.86	7.16	8.83	6.53	0.32	0.32	0.76	1.09	88.37	76.30	1.03	1.44	24.85	50.97	20.13	16.19	1.48
Sharad on Salt Creek	8.00	7.36	9.09	7.93	0.42	0.42	0.87	0.96	86.90	79.62	1.00	1.55	22.26	40.39	20.43	18.33	1.25
Sharad on VC clone	6.46	5.93	8.90	8.96	0.37	0.37	0.73	0.66	83.91	66.49	1.04	1.51	18.11	28.32	20.55	18.49	1.45
$P < 0.05$	NS		0.96		0.06		0.13		NS		NS		5.23		*		NS

NS: Nonsignificant, *; values not analyzed statistically

A: Photosynthetic rate (μ CO_2 m^{-2} s^{-1}), E: Transpiration rate (m mole H_2O m^{-2} s^{-1}), gs: Stomatal conductance (μ CO_2 m^{-2} s^{-1}), WUE: Water use efficiency, RWC: Relative water content (%), WP: water potential (-Mpa), ABA: Abscisic acid (ng g^{-1}), CID: Carbon isotope discrimination (‰), K: Potassium (%).

Conclusion

In the present study, there was considerable genetic variability in WUE among various genotypes, rootstocks and budded grape vines. The genotypes differed significantly in various physiological parameters like WUE, photosynthetic rate and transpiration rate before and after budding on rootstocks. A strong positive correlation was observed between WUE and photosynthetic rate, stomatal conductance, root to shoot length ratio and ABA among grape rootstocks and varieties. A negative relationship was observed between WUE and transpiration rate, zeatin riboside etc., but the statistical model developed by regressing WUE on physiological parameters indicated more than 97% of variability in WUE collectively was accounted for by all physiological parameters. However, the stepwise regression equation suggests more than 90% of the variability in WUE was accounted for by photosynthetic rate, transpiration rate and root to shoot length ratio to some extent.

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