



Carbon isotope discrimination and water use efficiency of grape varieties, rootstocks and budded grapevines

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Abstract

An investigation was undertaken to study variability in water use efficiency (WUE) of grape varieties, rootstocks and varieties after budding. Significant difference in WUE was observed among varieties, rootstocks and budded varieties. Flame Seedless and Thompson Seedless recorded highest WUE among varieties, while Dog Ridge and Salt Creek rootstocks performed well under limited soil moisture conditions. Budding the least efficient water user (Sharad Seedless) on highly efficient water using rootstock (Dog Ridge) significantly influenced the physiology of scion variety. Significant variability in WUE with respect to carbon isotope discrimination was observed in grape varieties before and after they were budded on drought tolerant rootstocks.

Key words: Grape, rootstocks, water use efficiency, carbon isotope discrimination, budding.

Introduction

Plants have naturally evolved various mechanisms for their survival under limited water conditions. From agronomical point of view, the concept of drought tolerance is linked to the superior crop growth rate under limited water conditions. Hence, the water harvesting and its subsequent utilizations assume greater importance. This concept is also referred to as water use efficiency (WUE), which is the efficiency with which plants use water to produce biomass. Apart from WUE, yet another important trait, which plays a vital role, is transpiration.

The discovery that the plants discriminate against the heavy isotope of carbon ^{13}C during photosynthesis and its subsequent, as a powerful surrogate for WUE has given strong impetus in determining the genetic variability in this physiological trait. The objective is to standardize carbon isotope discrimination (CID or Δ) as a surrogate for WUE to identify genotypes with high WUE based on carbon isotope discrimination technique.

Discrimination against the naturally occurring stable isotope ^{13}C during photosynthetic CO_2 fixation is largely determined by the ratio of the intercellular to atmospheric partial pressure or CO_2 concentration. This ratio is also directly related to the ratio of the instantaneous assimilation and transpiration, a measure of water use efficiency. This relationship has been exploited to establish correlation between CID in leaf tissues and WUE for several crop species. Thus leaf tissue Δ is an integrated plant physiological and external environmental property influencing photosynthetic gas exchange (Farquhar *et al.*, 1982 and Farquhar, 1983)

As grapes is one of the important fruit crops grown in semi arid tropics of Maharashtra and Northern Karnataka, drought is the major problem causing reduced productivity in vineyards. To combat problems associated with drought, use of rootstocks is gaining popularity. Rootstocks are known to influence many physiological activities of the grafted varieties (Sobhana, 1998). Some studies have shown the influence of rootstocks on the

photosynthetic rate of scion leaves as reported by Brown *et al.* (1985), Sharma and Singh (1989) and Iacono *et al.* (1998).

Hence an attempt was made to investigate the occurrence of variability in grape varieties, rootstocks and buddings in respect of physiological behaviour and carbon isotope discrimination.

Materials and methods

Three separate experiments were conducted in the experimental plots of 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 from the bed were transplanted to earthen pots of 14" diameter containing standard potting mixture of FYM, red earth and sand (1:2:1). The plants were maintained under normal culture for six months. After six months plants were irrigated to field capacity before imposing different levels of soil moisture stress. The three 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 4th, 9th and 14th day of the stress cycle.

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

Similarly in second experiment five rootstocks namely Dog Ridge, 1613 C, Salt Creek, St. George and VC clone were tested for water use efficiency and the procedure followed was same as in the first experiment.

Based on the WUE values of the above mentioned four varieties from first experiment and five rootstocks from second experiment at 50 % stress on 14th day, three varieties having highest WUE

namely Flame Seedless, Thompson Seedless and Sharad seedless were selected for studying their performance when budded on the three rootstocks from second experiment namely Dog Ridge, Salt Creek and VC Clone. The nine 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.

Budding: Chip budding was carried out during September 2003 on one year old rootstocks grown in 14" earthen pots filled with standard potting mixture. When the buddlings attained six months age, two levels of stress namely 100% stress and 50 % and no stress (control) were given for 14 days. The buddlings under 100 % stress could not survive beyond 3-4 days. Hence observations were recorded in only two treatments *i.e.* control and 50 % stress. On 14th day, *i.e.*, at the time of termination of stress treatments gas exchange parameters were measured as explained above.

Carbon isotope discrimination (CID or Δ): The carbon isotope discrimination was determined with an objective to study how far Δ is associated with the observed genetic variability in WUE in crops species. Fully expanded leaves were collected and dried at 70°C for 3-4 days to get constant dry weight. Dried samples were powdered in pestle and mortar. One gram powdered leaf sample was put in glass vials and sent to the laboratory for "National facility for determination of stable isotopes in plants" at Department of Plant Physiology, G.K.V.K., Bangalore for estimation of carbon isotope discrimination (Bindhu Madhava, 2000). As the values are expressed in negative terms lower values means higher discrimination and higher values means lesser discrimination.

Statistical analysis was carried out according to the procedures of Gomez and Gomez (1984).

Results and discussion

Photosynthetic rate, transpiration rate and instantaneous WUE recorded significant difference among the varieties and stress levels on all the days of stress cycle (Table 1, data shown is of 14th day of stress cycle). Both Sharad Seedless and Tas-A-Ganesh did not show any photosynthetic activity on 4th day of the stress cycle at 100 % stress. On 14th day of the stress cycle, maximum reduction in photosynthesis was recorded in Tas-A-Ganesh from non stress to stress conditions. Among the varieties, Flame Seedless recorded maximum photosynthesis of 9.10 $\mu\text{mole m}^{-2} \text{s}^{-1}$ at 50 % moisture stress. Considerable reduction in transpiration rate was recorded on 14th day of stress cycle from non stress to 100 % stress conditions. On 14th day of stress cycle reduction in transpiration rate was more in Flame Seedless and Thompson

Seedless and it was least in Tas-A-Ganesh. Water use efficiency increased with increased soil moisture stress on 9th and 14th day of the stress cycle in all the varieties. But on 4th day of the stress cycle, there was reduction in WUE at 100 % stress compared to 50 % stress. Flame Seedless recorded maximum WUE on 9th and 14th day of the stress cycle at 50 % stress while it was least in Tas-A-Ganesh.

Significant differences in Δ were recorded among varieties at the beginning and at the end of the stress cycle (Table 2). Highest Δ was observed in Thompson Seedless (17.91), while it was least in Sharad Seedless (18.58). With the progress of stress there was decrease in Δ in Flame Seedless, but all the other varieties showed increased Δ . Carbon isotope discrimination values increased from 17.93 to 18.57 in Flame Seedless at 50 % stress while in other varieties there was decrease in Δ values.

Table 2. Influence of soil moisture stress on carbon isotope discrimination (Δ) content in grape varieties

Varieties(V)	0 day	14 th day of stress cycle		
		S1	S2	S3
Flame Seedless	18.57	18.68	17.93	*
Thompson Seedless	17.91	18.64	18.25	*
Sharad Seedless	18.58	19.43	19.77	*
Tas-A-Ganesh	18.05	19.84	20.02	*
	V	S	V x S	
CD (P=0.05)	0.354	0.318	NS	0.450

S1: No stress (100 % irrigation), S2: 50 % stress 50 % irrigation),

S3: 100 % stress (No irrigation)

*: Plants wilted completely and hence observations were not recorded.

Gas exchange parameters like photosynthesis and transpiration rate recorded significant differences among rootstocks and stress levels on all the days of the stress cycle (Table 3). Similarly instantaneous WUE significantly differed among rootstocks and stress levels. Rate of photosynthesis decreased with increased soil moisture stress. At 100 % moisture stress 1613 C recorded least photosynthesis while Dog Ridge recorded highest photosynthetic rate. On 14th day of the stress cycle at 50 % stress Dog Ridge recorded highest photosynthesis followed by Salt Creek while least was in 1613 C. Similarly transpiration rate reduced drastically with increased soil moisture stress in all the rootstocks. The rate of reduction in transpiration rate was maximum in Dog Ridge and Salt Creek while it was least in St. George and 1613 C. On 14th day of stress cycle, Dog Ridge had least transpiration rate while St. George had highest transpiration rate at 50 % stress. Instantaneous WUE increased with increased soil moisture stress on 14th of the stress cycle in all the rootstocks. But on the 4th day of the stress cycle WUE was lesser under 100% stress than under

Table 1. Influence of moisture stress on gas exchange parameters in grape varieties on 14th day of stress cycle

Varieties (V)	Photosynthetic rate ($\mu\text{mole m}^{-2} \text{s}^{-1}$)			Transpiration rate ($\text{m mole m}^{-2} \text{s}^{-1}$)			Water use efficiency ($\mu\text{mole /m mole}$)		
	S1	S2	S3	S1	S2	S3	S1	S2	S3
Flame Seedless	10.0	9.10	*	10.50	6.90	*	0.96	1.33	*
Thompson Seedless	9.63	8.00	*	10.40	7.80	*	0.91	1.02	*
Sharad Seedless	7.50	7.06	*	10.33	8.90	*	0.72	0.78	*
Tas-A-Ganesh	7.83	5.73	*	10.76	9.50	*	0.72	0.60	*
	V	S	V x S	V	S	V x S	V	S	V x S
CD (P=0.05)	1.080	1.380	NS	0.371	0.321	0.643	0.134	0.116	0.232

S1: control (100% irrigation); S2: 50% stress (50% irrigation); S3: 100% stress (no irrigation)

*: Plants were wilted completely and hence observations were not recorded.

Table 3. Influence of moisture stress on gas exchange parameters in grape rootstocks on 14th day of stress cycle

Rootstocks (R)	Photosynthetic rate ($\mu\text{ mole m}^{-2}\text{s}^{-1}$)			Transpiration rate ($\text{m mole m}^{-2}\text{s}^{-1}$)			Water use efficiency ($\mu\text{ mole /m mole}$)		
	S1	S2	S3	S1	S2	S3	S1	S2	S3
Dog Ridge	8.30	9.36	*	9.76	7.06	*	0.84	1.31	*
1613 C	5.70	4.83	*	9.46	8.33	*	0.60	0.58	*
Salt Creek	6.80	9.23	*	9.76	7.63	*	0.69	1.21	*
St. George	10.60	7.20	*	10.33	9.40	*	1.02	0.76	*
VC clone	7.03	8.40	*	10.06	7.93	*	0.69	1.06	*
	R	S	R x S	R	S	R x S	R	S	R x S
SEm±	0.324	0.251	0.262	0.113	0.087	0.196	0.040	0.031	0.070
CD ($P=0.05$)	0.937	0.726	1.624	0.327	0.253	0.567	0.118	0.091	0.204

S1: control (100% irrigation); S2: 50% stress (50% irrigation); S3: 100% stress (no irrigation)

*: Plants were wilted completely and hence observations were not recorded.

50 % stress among all the rootstocks. Dog Ridge recorded highest WUE on 14th day at 50 % stress followed by Salt Creek, while 1613 C had the least WUE.

Table 4. Influence of soil moisture stress on carbon isotope discrimination (Δ) level in grape rootstocks

Rootstocks	0 day	14 th day of stress cycle		
		S1	S2	S3
Dog Ridge	20.49	20.52	19.00	*
1613 C	19.66	19.48	19.01	*
Salt Creek	21.09	21.32	19.95	*
St. George	21.40	21.45	20.63	*
VC clone	21.33	21.38	20.18	*

Note: Values were not analyzed statistically and are average of two replications only.

A significant difference in Δ was observed among rootstocks. The least Δ was observed in 1613 C at the beginning of the experiment followed by Dog Ridge and Salt Creek. However, it was interesting to note that, there was reduction in Δ at 50 % stress on 14th day among all the rootstocks. Dog Ridge recorded least Δ (19.00 %) followed by 1613 C (19.01 %). The magnitude of reduction in Δ from non stress to stress was more in Dog Ridge and Salt Creek and was least in 1613 C and St. George (Table 4).

Flame Seedless and Thompson Seedless on their own roots recorded higher photosynthetic rate and decreased transpiration rate at 50 % stress on 14th day stress cycle. Sharad Seedless and Tas-A-Ganesh recorded least photosynthetic rate and high transpiration rate at 50 % stress on 14th day of stress cycle (data not shown). It is evident from Table 5 that rate of transpiration was not influenced by rootstocks under control conditions. There was decrease in the rate of transpiration at 50 % stress in all the stionic combinations except Sharad Seedless on VC clone. Flame Seedless on Dog Ridge exhibited least transpiration rate followed by Sharad Seedless on Dog Ridge. All the three varieties on VC clone rootstock transpired more than either on Dog Ridge or Salt Creek. Similarly all the varieties budded on Dog Ridge and Salt Creek recorded more photosynthetic rate and the least photosynthetic rate was recorded in varieties budded on VC clone (Table 4).

Budding the varieties on Dog Ridge rootstock increased instantaneous water use efficiency of all the three varieties. Least WUE was recorded in Sharad Seedless budded on VC clone (Table 5). Sharad Seedless and Tas-A-Ganesh recorded least WUE on 14th day of stress cycle at 50 % stress while highest was recorded in Flame Seedless followed by Thompson Seedless.

Water use efficiency is more important than the higher rate of

Table 5. Influence of soil moisture stress on gas exchange parameters in budded vines on 14th day of stress cycle

Rootstock / Scion	Control (100 % irrigation)			50 % stress (50% irrigation)		
	Dog Ridge	Salt Creek	V.C.Clone	Dog Ridge	Salt Creek	V.C.Clone
Photosynthetic rate ($\mu\text{ mole m}^{-2}\text{s}^{-1}$)						
Flame Seedless	6.80	7.86	7.63	6.80	7.46	6.46
Thompson Seedless	5.80	7.76	7.46	6.73	7.20	6.36
Sharad Seedless	6.86	8.00	6.46	7.16	7.36	5.93
CD ($P=0.05$)	NS	NS	NS	NS	NS	NS
Transpiration rate ($\text{m mole m}^{-2}\text{s}^{-1}$)						
Flame Seedless	7.86	8.46	9.23	6.30	6.86	9.00
Thompson Seedless	8.23	8.80	8.46	7.33	8.43	7.93
Sharad Seedless	8.83	9.09	8.90	6.53	7.63	8.96
CD ($P=0.05$)	NS	NS	NS	0.554	NS	0.960
Water use efficiency ($\mu\text{ mole /m mole}$)						
Flame Seedless	0.85	0.92	0.82	1.09	1.09	0.71
Thompson Seedless	0.70	0.87	0.87	0.89	0.85	0.80
Sharad Seedless	0.76	0.87	0.73	1.09	0.96	0.66
CD ($P=0.05$)	NS	NS	Interaction	Rootstock	Scion	Interaction

Table 6. Influence of soil moisture stress on carbon isotope discrimination (Δ) level in budded vines

Rootstock / Scion variety	Control (100 % irrigation)			50 % stress (50% irrigation)		
	Dog Ridge	Salt Creek	V.C.Clone	Dog Ridge	Salt Creek	V.C.Clone
Flame Seedless	19.21	19.51	19.63	16.21	17.47	17.90
Thompson Seedless	19.37	19.67	19.79	16.61	17.20	17.56
Sharad Seedless	20.13	20.43	20.55	16.19	18.33	18.49

Note: Data not analyzed statistically and are average of two replications only.

photosynthesis or decreased rate of transpiration under drought conditions. Riesling variety of grape grafted on SO-4 and 5BB rootstocks had highest water use efficiency though there was higher stomatal conductance and photosynthesis in un-grafted Riesling variety (Williams and Smith, 1991). In the present investigation the higher water use efficiency in few scion rootstock combinations may be due to 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 reduced transpiration rate. Because in few scion / rootstock combinations (Sharad Seedless on VC clone) at 50 % stress, there was reduction in photosynthesis and also increase in transpiration which resulted in lower water use efficiency.

Large variation was observed for Δ on budded vines compared to varieties on their own roots. On all the rootstocks there was reduced Δ in the varieties after budding. This suggests the genetic variability of scion varieties in Δ before and after budding. Flame Seedless and Sharad Seedless budded on Dog Ridge have less Δ (16.19) than on Salt Creek (18.33) and VC clone (18.49). But, it was least in Thompson Seedless when budded on Dog Ridge. Also there was reduction in Δ with increased soil moisture stress. The reduction was more in scion varieties grafted on Dog Ridge rootstock than on Salt Creek and V.C.clone (Table 6).

Carbon isotope discrimination v/s water use efficiency: The selection of genotypes which uses available water in an effective way and thus produce high biomass is an important criterion for increasing productivity in drought conditions. Apart from improved methods in determining WUE such as gas exchange measures and gravimetric methods, attempts have been made to measure WUE in field conditions using crop growth models. For large scale breeding program, the pioneering work of Dr. Farquhar who found ^{13}C isotope discrimination during photosynthesis is related to WUE is worth mentioning. Since then, ^{13}C discrimination is being used as a tool to identify genotypes with high WUE. The environmental factors which alters the photosynthetic rate and stomatal conductance would also alter WUE and may have significant influence on carbon isotope discrimination.

Significant differences in carbon isotope discrimination were recorded among genotypes at the beginning of the experiment suggesting the genotypic response in fixing carbon dioxide. But at the end of the stress cycle, there was decrease in carbon isotope discrimination (Δ), and was least in Flame Seedless followed by Thompson Seedless. The reduction in Δ was in accordance with earlier results of White *et al.* (1990), Osario *et al.* (1998) and Read *et al.* (1991). The results indicate that soil water deficit exacerbates the rate of change in Δ and water use efficiency, because Δ negatively correlates with stomatal conductance and transpiration rate which was notably reduced in plants subjected to water stress.

Significant negative correlation was found between intrinsic WUE and Δ ($r = -0.66$). Even-though WUE was more in Flame seedless,

photosynthetic rate was less than in other varieties where WUE was less. It can be explained as per Hubick *et al.* (1988) which 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 inturn influences Δ . The WUE is not only altered by vapour pressure deficits through g_s , but moisture stress also alters stomatal and non-stomatal factors.

Meinzer *et al.* (1998) also got inverse relationship between WUE and Δ in five coffee genotypes. Under adequate irrigation, higher WUE in coffee genotypes resulted from reduced stomatal aperture rather than increased photosynthesis capacity at a given stomatal conductance. Apparently contrasting behaviour has been reported for well irrigated peanut genotypes, where productivity and Δ were negatively correlated suggesting that variation in Δ were the results of variation in photosynthesis capacity and therefore pi/pa at similar levels of stomatal conductance.

White *et al.* (1990) could observe greater biomass associated with higher Δ values under rainfed conditions suggesting that bean genotypes achieving a greater biomass also had higher internal carbon concentration values than in irrigated conditions. Under less irrigated conditions, the bean lines with higher Δ values should have consumed available soil moisture to yield high biomass. Significant correlation was observed between Δ and biomass, root length and leaf area index.

In the present study, there was genetic variability in WUE with respect to carbon isotope discrimination. The behaviour of genotypes differed significantly in Δ before and after budding on different rootstocks. Dog Ridge rootstock was known to increase WUE of Flame Seedless and Sharad Seedless when Δ and other physiological parameters were compared. But, Thompson Seedless increased its water use efficiency when budded on Dog Ridge, which is confirmed by the least Δ in this combination at 50 % stress.

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