Effects of Deficit Irrigation Strategies on Vine Water Status, Canopy and Cluster Temperatures, Fruit Total Phenolics, and the Color of Muscat of Alexandria Table Grapes

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(Course of Applied Plant Science)

ブドウ‘マスカット・オブ・アレキサンドリア’に対する灌水制限が樹体の水分，葉温，果実温，果実の全フェノール含量，果皮色に及ぼす影響

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Effects of Deficit Irrigation Strategies on Vine Water Status, Canopy and Cluster Temperatures, Fruit Total Phenolics, and the Color of Muscat of Alexandria Table Grapes

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Effects of different deficit irrigation strategies on vine water status, canopy and cluster temperatures, fruit total phenolics, and the color of white table grapes (*Vitis vinifera* L., cv. Muscat of Alexandria) were compared to a well-irrigated control in 2004 from veraison to harvest at the Okayama University Experimental Vineyard, Japan. The treatments included: (1) well-irrigated control: re-irrigation when the soil moisture tension reached 15 kPa; (2) regulated deficit irrigation (RDI): re-irrigation 4 to 7 days after reaching a soil moisture tension of 15 kPa; (3) fixed partial root-zone wetting (FPRW): one part of the root system was re-irrigated when the soil moisture tension reached 15 kPa; and (4) alternate partial root-zone wetting (APRW): one part of the root system was re-irrigated when the soil moisture tension reached 15 kPa, and every week the irrigated part was switched. As the stress developed in RDI vines, leaf water potential and transpiration rate decreased and canopy and cluster temperatures increased as compared with the control. In contrast, both FPRW and APRW vines had similar leaf water potential and canopy and cluster temperatures, but less leaf transpiration rate as compared with the control. At harvest, fruits from all treatments had higher skin total phenolics and CIELAB *a values than the control. RDI fruit had higher total soluble solids (TSS), a similar acidity, and smaller size compared with the control. FPRW and APRW fruits had slightly higher TSS, lower acidity, and a similar size compared with the control.**

Key words : grape, deficit irrigation, partial root-zone wetting, phenolics, color

Introduction

Appearance is very important in determining the quality of table grapes, especially berry size, color and the conditions of the wax bloom on fruit surface. At veraison the chlorophyll starts to break down and other pigments become apparent. In white or yellow table grape cultivars, the yellowish-green color is due to the presence of minor pigments such as chlorophyll, carotene and xanthophylls[21]. The color development in white table grapes is highly influenced by the cultural and environmental conditions such as temperature and solar radiation[9,13]. The phenolic compounds are present in white table grapes, and especially in skins, where they are responsible for the color and flavor of the fresh fruit[15,16]. The phenolic compounds have several defense functions in plants, and therefore various environmental factors such as temperature, sun light, humidity, and cultural factors, including soil moisture availability and nutrition contribute to their synthesis[12,15,20]. To our knowledge, information on the effects of the new water-efficient irrigation strategies on the yellowish-green color development and total phenolics of white table grapes is still limited. Regulated deficit irrigation (RDI) is a strategy which relies on a precise control of irrigation cut-off in order to apply a mild stress during a critical period of the season to achieve certain beneficial results[7,8]. Partial root-zone drying or what we termed here partial root-zone wetting (PRW) is a new irrigation strategy that requires roots to be simultaneously exposed to wet and dry zones, thus stimulating some of the responses associated with water stress but not resulting in changes in vine water status[5,19]. With both RDI and PRW, water is supplied to vines below the optimum irrigation requirements; however, in RDI water supply is manipulated over time, whereas in PRW water supply is manipulated over space. The decision of whether to use RDI or PRW depends on the objectives of the vineyard and still requires further testing. Recently there is an increasing interest in using the

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infrared thermometry and thermography to measure canopy temperature in grapes and other horticultural crops as a mean for monitoring transpiration and detecting water stress[11,18]. These measurements can be used as a decision support tool to assess growers in irrigation scheduling. In this study, we investigated the effects of several deficit irrigation strategies on vine water status, canopy and cluster temperatures, color and phenolics of white table grapes cv. Muscat of Alexandria. Here we show the results of our investigation where berry skin color and total phenolics were significantly influenced by the deficit irrigation strategies.

Materials and Methods

Plant material and growth conditions

The experiment was conducted in 2004 at the Okayama University Experimental Vineyard in Okayama city (long. 133.92°E, lat. 34.66°N), Japan. Nine-year-old grapevines of Muscat of Alexandria (*Vitis vinifera* L.) grafted on SO4 rootstocks were used. Vines were grown individually in raised beds (1.2 m long, 1.1 m wide, and 0.25 m high) under a non-heated poly-house and root-zone restriction condition by installing a water-permeable but root-proof polyester sheet (BDK Lovesheet, Unitica, Japan) below the root-zone. The medium was a mixture of sandy soil, peat moss, and horse manure (4 : 1 : 1 vol/vol). Vines were trained to a unilateral cordon (2.4 m long), and spur-pruned (20 spur per vine); each spur having one shoot carrying one cluster; a sloping shoot-positioned trellis system was used. Weekly fertigation with a complete liquid fertilizer (Ohtsuka House Ekihi, No. 1 + No. 2, Ohtsuka Kagaku, Japan), containing 60 ppm of N was applied, the level of which was reduced to one third at the onset of veraison. A regular pest management program was maintained. Soil moisture tension was monitored by placing tensiometers (DIK-8332, Daiki Rika Kogyo, Japan) at a depth of 15 cm. From bud break to veraison, all vines were irrigated to a soil moisture tension of 3 kPa and re-irrigated when the soil moisture tension approached 15 kPa. Starting at veraison (17 July), four irrigation practices were imposed: (1) well-irrigated control: re-irrigation when the soil moisture tension reached 15 kPa; (2) regulated deficit irrigation (RDI): re-irrigation 4 to 7 days after reaching a soil moisture tension of 15 kPa; (3) fixed partial root-zone wetting (FPRW): one part of the root system was kept un-irrigated while the other part was re-irrigated when the soil moisture tension reached 15 kPa; and (4) alternate partial root-zone wetting (APRW): one part of the root system was allowed to dry while the other part was re-irrigated when the soil moisture tension reached 15 kPa, and every week the irrigated part was switched so that the dry part was irrigated and the wet allowed to dry. For FPRW and APRW, each side of the vine was irrigated independently by dual in-line dripper tubing 30 cm apart from vine trunk, whereas the control and RDI vines were irrigated on both sides at the same time. Each control and RDI vine received 30 L per irrigation, while each vine in PRW (FPRW and APRW) received half of that amount. In conjunction with soil moisture monitoring, we measured the leaf water potential to fine tune the irrigation schedule. Control, RDI, and the wet side of PRW vines were irrigated approximately at 2 to 3, 7, and 2-day intervals, respectively. Treatments were continued for 7 weeks until harvest on 31 August. The total amount of water supplied to the vines from veraison to harvest in the control, RDI, and PRW treatments were 495, 210, and 330 L/vine, respectively.

Sampling and analyses

1. Vine water status, canopy and cluster temperatures, and transpiration rate measurements

The midday leaf water potential (Ψ) measurements were made one day before irrigation was given. The leaves were selected from the middle part of shoots; they were detached and their Ψ was measured immediately in the field by a pressure chamber (DIK-PC40, Daiki Rika Kogyo, Japan). Midday measurements of transpiration rate (E) were performed with a portable infrared gas analyzer (LCA-4, ADC, Shimadzu, Japan). All measurements for leaf Ψ and E were collected using two fully exposed leaves per vine. Midday canopy and cluster temperatures were approximated by the average temperature of 6 mature leaves and 6 representative clusters on both east and west sides of each vine by an infrared thermometer (HORIBA IT-330, Japan).

2. Fruit quality measurements

The fruit diameter was measured in 30 berries per treatment using a digital caliper. To determine juice total soluble solids (TSS) and titratable acidity at harvest, 60 berries per treatment were collected and divided into 3 subgroups including 20 berries each. Berries of each subgroup were peeled, deseeded, and the flesh was homogenized. The homogenate was centrifuged at 6,500 rpm for 10 min. The supernatant was used for juice analysis. TSS of berry juice were measured by a hand refractometer (ATC-1E, Atago, Japan), and titratable acidity (% tartaric acid) by diluting the juice with deionized water and titrating with 0.1 N sodium hydroxide to the phenolphthalein end point. For measuring fruit total phenolics at harvest, 15 berries per treatment were randomly sampled and divided into 3 subgroups comprising 5 berries each. Berries of each subgroup were deseeded and separated into skins and pulps. Samples of 1 g of skins and 2.5 g of pulps were homogenized and extracted in 2% HCl in methanol for 24 h in the dark at room temperature. The extracts were diluted with the same solvent used for extraction to a suitable concentration for analysis. Total phenolics were measured according to the Folin-Ciocalteu reagent method[24,26]. Two hundred microliters of sample extract was introduced in a test tube, 1 mL of Folin-Ciocalteu reagent and 0.8 mL of sodium carbonate (7.5%) were added, and the contents were mixed and allowed to stand for 30 min. Absorption at 765 nm was measured by a spectrophotometer (BECKMAN, DU®530 Life Science UV/VIS, U.S.A.). The total phenolic content was expressed as gallic acid equivalent (GAE) in milligrams per gram of sample, using a standard curve generated with a series of gallic acid concentrations. Fruit skin color was measured in the field using 4 representative clusters on both east and west sides of each vine with a Nippon Denshoku Colormeter (NR–3000, Japan) as CIELAB $L^*a^*b^*$ color system[1-3] (Commission Internationale de l'Eclairage translated as the International Commission on Illumination). The L^* value represents black to white as CIELAB 1986 L^* values increase from 0 to 100; the a^* value represents green to red color as CIELAB 1986 a^* values increase from −60 to +60; and the b^* value represents blue to yellow color as CIELAB 1986 b^* values increase from −60 to +60. Also defined in this system are the hue angle ($h°$); a term used to distinguish the red, yellow, green and blue colors, and the chroma (C^*); a term used to describe the vividness or dullness of color, calculated as $h° = \arctan(b^*/a^*)$ and $C^* = [(a^*)^2 + (b^*)^2]^{1/2}$.

Experimental design and statistical analysis

Three individual vine replicates were assigned for each treatment using a completely randomized block design. Data analysis was done by a one-factor ANOVA. Mean comparisons were performed using the Tukey-Kramer test to examine differences among treatments. Significance was determined at $P < 0.05$ or $P < 0.01$.

Results and Discussion

Midday leaf transpiration rate was significantly lower in all treated vines as compared with the control in the second and fourth measurements (Table 1). However, only RDI vines showed moderate water deficit status as the value of midday leaf Ψ was approximately −0.80 MPa before irrigation, *i.e.*, it was significantly lower than that of control, FPRW and APRW vines at all dates measured (Table 2). The decrease of soil moisture availability under the RDI strategy is acknowledged to reduce leaf Ψ[7]. In contrast, several experiments showed that PRW strategy stimulates some of the responses associated with water stress, such as reduced leaf transpiration rate, but not resulting in changes in leaf Ψ[6,19]. As shown in Tables 3 & 4, we found that canopy and cluster temperatures were highly influenced by the deficit irrigation strategies. In general, differences in canopy temperature were observed among treatments. At harvest (day 45 after veraison) mean canopy temperature in control, FPRW and APRW vines reached approximately 31°C, which was 2°C lower than that in RDI vines (Table 3), whereas mean cluster temperature in control, FPRW and APRW was approxi-

Table 2 Leaf water potential of Muscat of Alexandria grapevines as influenced by the irrigation treatments

Treatment	Day after veraison		
	0	4	45
		(MPa)	
Control	−0.65±0.10a	−0.59±0.10a	−0.67±0.08a
RDI	−0.65±0.10a	−0.83±0.05b	−0.79±0.09b
FPRW	−0.65±0.10a	−0.57±0.07a	−0.65±0.06a
APRW	−0.65±0.10a	−0.62±0.07a	−0.66±0.05a

Abbreviations as in Table 1.

Table 1 Leaf transpiration rate of Muscat of Alexandria grapevines subjected to different irrigation strategies

Treatment[a]	Day after veraison				
	0	4	12	25	30
			$(\mathrm{mmol} \cdot \mathrm{m}^{-2} \cdot \mathrm{s}^{-1})$		
Control	4.96±0.68a[b, c]	3.95±0.59a	4.30±0.53a	4.77±0.63A	3.85±0.37A
RDI	4.96±0.68a	3.12±0.56b	3.22±0.87b	3.41±0.80B	3.40±0.41AB
FPRW	4.96±0.68a	3.22±0.49b	3.57±0.68b	3.65±0.45B	3.25±0.52B
APRW	4.96±0.68a	3.22±0.47b	3.82±0.56ab	3.63±0.92B	3.28±0.54B

[a] Control = Full irrigation; RDI = Regulated deficit irrigation; FPRW = Fixed partial root-zone wetting; APRW = Alternate partial root-zone wetting.

[b] Values in columns followed by the same letter are not significantly different. Uppercase letters indicate significant difference at $P < 0.01$; lowercase letters indicate significant difference at $P < 0.05$.

[c] Values are means ± standard deviation.

mately 33°C and it was 1°C lower than that in RDI vines (Table 4). Canopy and cluster temperatures were inversely correlated with vine water status in RDI vines. With the decrease in midday leaf Ψ of RDI vines, transpiration was decreased; meanwhile, canopy and cluster temperatures rose. This was probably because of the marked decrease in the transpiration cooling capacity of RDI vines compared with the control vines. A feature of water-stressed vines is the increased leaf temperature[25]. As leaf water potential decreases leaf temperature increases[22]. We did not fully understand why the decreased transpiration rates of PRW leaves were not reflected in increased leaf temperature. However, it is reported that changes in leaf angle (away from the sun) in stressed canopies prevents higher leaf temperature irrespective of transpiration rate[11]. Berry juice TSS and acidity contents as well as berry diameter values during berry development from veraison through harvest are illustrated in Fig. 1. TSS increased during ripening and reached values of approximately 16, 17, 17, and 18% for control, FPRW, APRW, and RDI fruits, respectively, when we took the last sample on day 45 after veraison (Fig. 1A). Juice titratable acidity decreased during the course of the experiment, but more in PRW and RDI than the control (Fig. 1B). At harvest, PRW treatments had a lower acidity level than the control, whereas the acidity level of RDI juice was similar to the control. There was a steady increase in berry diameter throughout ripening, but markedly higher in control and PRW than in the RDI (Fig. 1C). In a previous work[7], we reported and discussed the effects of deficit irrigation on quality of Muscat of Alexandria table grapes. Table 5 shows the mean values of L^*, a^*, b^*, h°, and C^* for the fruit as affected by the irrigation treatments. From the first to the second measurement, the fruit starts to lose the dark green color, and there were no significant differences in fruit color among treatments. At harvest (day 45 after veraison), fruits from all treatments had significantly higher a^* values than the control, indicating that control fruit had more intense green color than that of RDI, FPRW and APRW fruits. Although the control fruit had slightly lower values for L^* and b^*, and slightly higher values for h° and C^* than that of the other treatments, the differences were only significant between the control and APRW fruit in h° value, *i.e*., control fruits had more green color and less yellowish color compared

Table 3 Canopy temperature of Muscat of Alexandria grapevines subjected to different irrigation strategies

Treatment	Day after veraison		
	12	30 (°C)	45
Control	36.06±2.11b	31.39±1.51b	30.53±0.68B
RDI	38.73±2.31a	33.50±2.11a	32.52±0.91A
FPRW	36.58±2.18ab	31.81±1.06ab	30.93±0.54B
APRW	35.74±1.18b	31.48±1.57b	30.66±0.72B

Abbreviations as in Table 1.

Table 4 Cluster temperature of Muscat of Alexandria grapevines subjected to different irrigation strategies

Treatment	Day after veraison		
	26	35 (°C)	45
Control	34.62±0.52b	33.34±0.80B	32.64±0.41B
RDI	36.06±0.74a	34.84±0.99A	33.63±0.75A
FPRW	34.98±1.10b	34.03±1.10AB	33.01±0.68AB
APRW	34.93±0.70b	34.02±0.76AB	32.76±0.35B

Abbreviations as in Table 1.

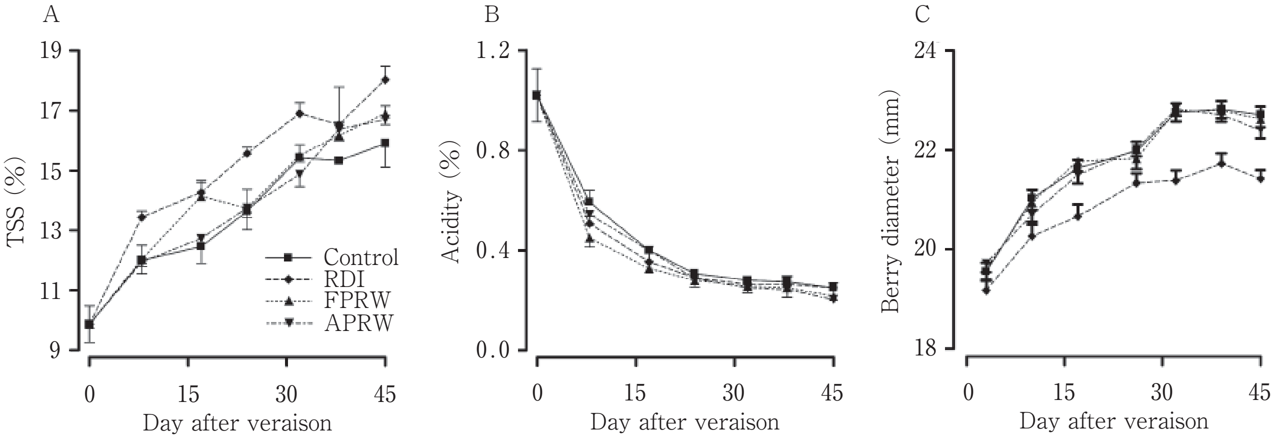

Fig. 1 (A) Total soluble solids (TSS), (B) titratable acidity (tartaric acid), and (C) berry diameter of Muscat of Alexandria table grapes as influenced by the deficit irrigation strategies imposed from veraison through harvest.
T bars=SD. Abbreviations as in Table 1.

to the other treatments. Starting at veraison, we did not severely control the lateral shoot growth in all treatments, and we observed that control vines had more vigorous lateral shoot growth than the other treatments. There is the possibility of a direct relationship between the late-season lateral shoot growth and fruit composition[4,10]. As regards fruit total phenolics (Fig. 2), we found that the total phenolic contents in RDI and PRW fruit skins were significantly higher than that in the control (Fig. 2A). However, we did not find any significant differences in flesh total phenolics among treatments (Fig. 2B). Previous studies showed that the contents of phenolic compounds in fruit of Muscat of Alexandria grapes and other white grape cultivars fall steadily during ripening, and that there were significant seasonal and cultivar variations[13,17]. Several studies reported that water deficit stress during grape berry maturation results in small berries but they contain more total phenolics, tannins, and anthocyanins in their skins[14,20,23]. There are two likely causes for the potential effect of deficit irrigation on the accumulation of phenolic compounds in grape skins: an indirect and positive response due to the concentration effect as a result of reduced berry size, and a direct response on phenolic biosynthesis which can be positive or negative depending on the type of phenolic compound and the details of irrigation deficit, *i.e.*, timing, duration, degree and rate[23].

Conclusion

Our measurements for the TSS, acidity, fruit size, color and total phenolic contents of Muscat of

Table 5 L^*, a^*, b^*, hue angle ($h°$), and chroma (C^*) values of Muscat of Alexandria table grapes subjected to different irrigation treatments

Treatment	L^*	a^*	b^*	$h°$	C^*
Day 0 after veraison					
Control	40.29±1.84	−7.36±1.94	7.50±1.00	133.80±9.72	10.65±1.15
Day 18 after veraison					
Control	41.76±1.32a	−6.14±1.48a	7.52±1.40a	129.40±9.47a	9.83±1.20a
RDI	42.52±1.14a	−6.48±1.46a	7.85±1.66a	129.17±3.15a	10.11±2.00a
FPRW	41.37±0.60a	−5.77±1.84a	6.87±0.94a	129.61±9.61a	9.14±1.46a
APRW	42.38±1.23a	−5.44±0.62a	7.14±1.58a	127.95±6.34a	9.03±1.34a
Day 45 after veraison					
Control	41.36±1.37a	−5.63±0.94b	8.28±1.34a	124.56±7.49a	10.09±1.06a
RDI	42.91±1.36a	−4.42±0.57a	8.76±1.22a	117.75±4.70ab	9.90±1.08a
FPRW	42.10±1.73a	−4.50±1.15a	8.59±1.51a	119.00±5.97ab	9.87±1.63a
APRW	42.37±1.47a	−4.53±1.03a	8.88±1.41a	116.11±6.23b	9.93±1.44a

Abbreviations as in Table 1.

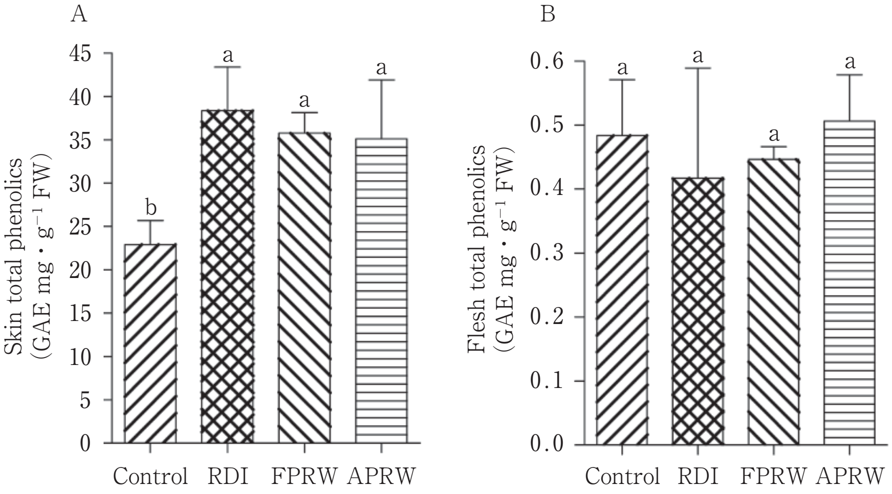

Fig. 2 Total phenolic contents in skins (A) and flesh (B) of Muscat of Alexandria table grapes as affected by the deficit irrigation strategies at harvest.

T bars=SD. Lowercase letters above SD bars indicate significant difference at $P<0.05$. Abbreviations as in Table 1.

Alexandria table grapes subjected to different deficit irrigation strategies revealed that these quality characteristics were highly influenced by the treatments. This could possibly be due to the differences in vine water status and the stress-associated responses that developed in RDI and PRW vines. We used infrared thermometry as a tool for assessing the degree of water-deficit stress, and it appears to be of practical value. Our results indicate that deficit irrigation strategies must be regarded as one of the factors determining the ripening processes of table grapes in terms of appearance and edible qualities. Further work is needed to identify the specific individual phenolic compounds and pigment compositions in fruit that are particularly sensitive to the water stress-associated responses.

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ベレゾーン期から収穫期までの灌水制限処理が‘マスカット・オブ・アレキサンドリア’ブドウ（*Vitis vinifera* L.）の水分条件，葉温，果実温，果実の全フェノール，果皮色に及ぼす影響を，十分に灌水した樹と比較した．実験は2004年に岡山大学農学部内の実験圃場で行った．処理区は，１）土壌水分張力が15kPa に達したときに灌水する対照区，２）土壌水分張力が15kPa に達してから４～７日後に灌水する制限灌水区，３）土壌水分張力が15kPa に達したときに根域の半分に灌水する片側灌水区，４）片側灌水する根域部分を１週間ごとに変更する交互灌水区とした．制限灌水区では水分ストレスが強まるにつれて葉の水ポテンシャルと蒸散速度が対照区よりも低下し，果実温が高くなった．しかし，片側灌水区と交互灌水区では，葉の水ポテンシャルと葉温，果実温は対照区と同程度で，蒸散速度が低下した．収穫期の果皮の全フェノールと CIELAB *a** 値は，灌水を制限した各区では標準区より高かった．制限灌水区の果実は，標準区より糖度が高く，酸度は低く，果粒は小さかった．片側灌水区，交互灌水区では糖度がやや高く，酸度は低く，果粒の大きさは同程度であった．