

## Effects of water deficit stress on leaf and berry ABA and berry ripening in Chardonnay grapevines (*Vitis vinifera*)

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### Summary

Chardonnay vines planted in root zone restricting beds were subjected to water deficit stress by withholding irrigation for a period starting at 15 (early stress; ES) or 25 d (late stress; LS) after veraison and lasting until harvest. Control vines were provided with normal irrigation. Both ES and LS treatments significantly decreased leaf water potential and caused leaf wilting 2 d after the start of each treatment. ABA levels in fruit zone leaves increased significantly 4 and 5 d after the onset of LS and ES treatments, respectively. After 2 d of ES treatment, the berry ABA level greatly increased, while LS and control treatments resulted in a gradual increase in berry ABA. In ES-treated vines, clusters were harvested 10 d after the onset of treatment, 10 d earlier than in LS and control vines, due to the severe berry shrinking and leaf fall. Levels of juice TSS, fructose, glucose, and malate were generally lower than those of LS and control vines. Both ES and LS treatments resulted in significantly higher amino acid levels. The level of proline, however, the amino acid with highest amounts in Chardonnay grape berries, was lowest in ES-treated vines. This study reveals that the effect of water deficit stress on berry ripening of Chardonnay is different depending on the stage of berry ripening, which might be caused by different ABA levels in berries.

**Key words:** water stress, berry ripening, Chardonnay, ABA, amino acid.

### Introduction

High quality wine grapes are mostly produced in dry and cool regions of the world. In Western Japan, a long rainy season from early summer until berry ripening causes excessive shoot growth. This high vigor leads to severe competition for photosynthates between shoots and clusters with negative effects on berry ripening. Thus, wines tend to have a flat taste.

Planting grapevines in isolated soil beds under a plastic-film cover can control vigor by root-zone restriction; this improved berry quality significantly (IMAI *et al.* 1991). Under such conditions, the level of soil moisture can be easily manipulated. A number of reports indicate that water deficit stress during the late stage of berry development improves berry and/or wine quality mainly by decreasing berry size

(MATTHEWS and ANDERSON 1988, PONI *et al.* 1993). On the other hand, early water deficit stress may cause severe inhibition of berry growth and maturation as a result of the decline of photosynthesis (DÜRING 1998).

In this study, we compared the effects of early and late water deficit stress on ripening of Chardonnay berries in relation to changes in the levels of leaf and berry ABA, which is reported to control berry ripening (COOMBE and HALE 1973; DÜRING and ALLEWELDT 1980).

### Material and Methods

Four-year-old Chardonnay vines grafted on SO4 rootstock were used in this study. Thirty-six vines were planted at 0.4 m intervals in 4 raised beds with 2.2 m row spaces in the spring 1997. Each bed was 0.3 m high and 0.5 m wide and isolated from the ground with water-permeable but root-proof plastic sheets (UNICHIKA 200). For three years vines were grown under conventional vineyard management with a spur pruning system. In spring 2000, all the vines were allowed to develop 4 or 5 upright shoots. Three liters of a complete liquid fertilizer (Ohtsuka House Ekihi, No. 1 + No. 2), containing 60 mg l<sup>-1</sup> of N, was applied to each vine twice a week through a trickle irrigation tube. The fertilization level was reduced to one-third at veraison to improve berry ripening. Vines were irrigated when soil water tension reached -10 kPa. After berry set, the number of clusters per shoot was reduced to one or two depending on shoot vigor.

**Treatments and measurements:** Two types of water deficit treatment, early stress (ES) and late stress (LS), were applied to the vines in two beds by withholding irrigation starting on August 1, 14 d after veraison, and 10 d thereafter, respectively. Vines of the third bed were irrigated continuously when soil water reached -10 kPa (control). Changes in soil moisture levels were monitored using tensiometers (Daiki-3100) placed at a depth of 10 cm in each bed. The meters were read daily at 11 a.m. The leaf water status was measured by a pressure chamber (DIK-7002) using 10 leaves that were sampled randomly from the middle part of the shoot. Temperatures of the 5th to the 7th basal leaf were recorded daily at 12 a.m. using an infrared thermometer (Horiba-IT-330).

**ABA analyses:** Two primary leaves near the clusters, as well as 10 berries, were sampled from 10 randomly chosen shoots in each treatment at 2 p.m. at 2-4-day intervals. They were immediately frozen in liquid N<sub>2</sub> and

stored at  $-30\text{ }^{\circ}\text{C}$ . ABA was extracted three times at  $5\text{ }^{\circ}\text{C}$  with 80 % MeOH containing 0.3 % ascorbic acid, acidified to pH 2.5 and partitioned against ethyl acetate. The ethyl acetate fraction was extracted with 5 % sodium hydrogen carbonate. The carbonate extract was washed with petroleum ether, adjusted to pH 2.5, and re-partitioned against ethyl acetate to collect the acidic ethyl acetate fraction. Dried extracts were dissolved in 80 % MeOH and vacuum evaporated. The residue was dissolved in acetone and ABA was methylated with diazomethane for 1 h. ABA determination was performed on a GC (Shimadzu GC-3BE) equipped with an ECD under the following conditions: column type, SE-30 Uniport HP; column temperature,  $220\text{ }^{\circ}\text{C}$ ; injection port temperature,  $200\text{ }^{\circ}\text{C}$ ; carrier gas,  $\text{N}_2$ ; flow rate,  $45\text{ ml min}^{-1}$ . ABA concentration was determined by comparison with S-ABA standard.

**Juice constituents:** For the analyses of berry juice constituents, 30 berries per treatment were randomly sampled from veraison to harvest. They were pressed by hand through doubled gauze to obtain juice. Total soluble solids (TSS) were measured by a refractometer (Atago-30) and titratable acidity (TA) was determined by titrating the juice with 0.1 N NaOH. The concentrations of sugars and acids were determined by GC (Shimadzu GC-14A) equipped with a flame ionization detector after purifying sample juices on an ion-exchange resin and silylating with hexamethyldisilazane and trimethylchlorosilane. For amino acid analysis, 0.5 ml of juice was filtered through a membrane filter (0.45  $\mu\text{m}$ ) and injected into a fully automatic HPLC (JLC-300).

## Results and Discussion

During the experimental period, the weather was either clear or cloudy with day temperatures (1 p.m.) ranging from  $27$  to  $31\text{ }^{\circ}\text{C}$ . Most leaves of the ES-treated vines turned yellow 5 d after the onset of treatment and were completely dried out 3 d later. By contrast, in the LS-treated vines, 6 d after the onset of treatment several basal leaves turned yellow and brown 4 d later.

The water potential of leaves near clusters decreased rapidly after the onset of each treatment. The lowest values ( $-2.35\text{ MPa}$  and  $-2.04\text{ MPa}$ ) were recorded in the ES treatment 5 d after the onset of treatment and in LS 2 d after the onset of treatment, respectively. Berries in ES-treated vines ceased growing 6 d after the onset of withholding irrigation and began to shrink gradually thereafter. They were harvested on August 11, 10 d after the onset of treatment. By contrast, berries in LS-treated vines showed normal growth similar to the control; they were harvested on August 21.

Changes in leaf and berry ABA levels are shown in the Figure. For both treatments, leaf ABA levels increased continuously for 5 d after the onset of each treatment. The leaf ABA level was higher in LS-treated vines than in ES-treated vines when compared 7 d after the onset of treatment. The leaf ABA level in control vines did not change significantly in the same period. On the other hand, ABA levels in berries showed a marked increase in the first two days of ES treatment, the increase being earlier than that of leaf ABA levels.

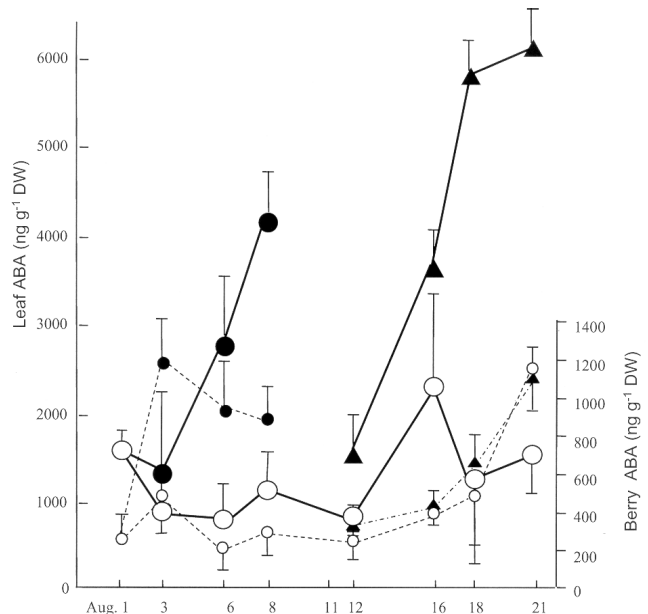


Figure: Effect of water deficit stress on leaf ABA level (solid lines) and berry ABA level (broken lines) in Chardonnay grapevines. Irrigation ceased on August 1 for ES vines (●) and August 11 for LS vines (▲). Control vines (○) were irrigated regularly and kept at  $-10\text{ kPa}$  until harvest time. Vertical bars represent SD,  $n=3$ .

Maximum berry ABA levels were approximately 2.4 times higher than in control vines; the level decreased gradually thereafter. By contrast, in LS and control treatments, berry ABA levels increased rather steadily, finally reaching a maximum that was similar to that recorded in the ES treatment.

One of the commonly observed responses of plants is that water deficit stress causes a significant increase in the leaf ABA level. Our results revealed a similar accumulation pattern of leaf ABA for both, ES and LS treatments. However, the increase in berry ABA levels presented an entirely different pattern between ES and LS treatments: it was much faster and larger in the ES treatment than in the LS treatment. It is possible that the rapid accumulation of ABA in ES-treated berries was associated with the rapid degradation of bound ABA in leaves, canes, trunks and roots (KONDO and KAWAI 1998). Furthermore, berry ABA level in control vines showed a final value that was similar to that of LS-treated berries, whereas leaf ABA levels did not change until harvest. These findings indicate that berry ABA levels are not always dependent on leaf ABA levels during ripening. COOMBE and HALE (1973) and DÜRING and ALLEWELDT (1980) reported that the berry ABA level of *V. vinifera* grapes increased significantly after veraison and then decreased at the final stage of berry ripening. In our data for Chardonnay, the final decrease of berry ABA level was not detected in both LS-treated and control vines; this may indicate that the berries harvested on August 21 had not reached full ripeness. In this experiment time of harvest based on juice acidity (not lower than  $4\text{ g l}^{-1}$ ).

Juice TSS increased in ES- and LS- treated vines at almost the same rate as control vines. However, in ES-treated vines, the final average TSS content was 18.5 Brix because of early berry shrinking. DÜRING and ALLEWELDT (1980) noted a strong correlation between berry ABA level and juice TSS content during the early ripening stage of cv. Bacchus. They

Table

Effect of water deficit stress on juice constituents in Chardonnay grape berries at harvest<sup>a</sup>

Treatment and harvest time	TSS (°Brix)	TA <sup>b</sup>	Fructose	Glucose			Tartaric acid	Total amino acid (m mol·l <sup>-1</sup> )
				(g·100 ml <sup>-1</sup> )				
Early stress								
Aug. 11	18.5 b	0.36	7.66 b	9.31 b	0.29 a	0.17 b	19.7 ab	
Late stress								
Aug. 21	20.7 a	0.32	9.77 a	10.56 b	0.14 a	0.34 a	22.7 a	
Control								
Aug. 21	20.0 a	0.35	9.93 a	10.73 a	0.20 ab	0.28 a	18.0 b	

<sup>a</sup> Vines were treated by withholding irrigation starting from August 1 (early stress) and August 11 (late stress). Control vines were irrigated regularly when soil water reached -10 kPa until harvest (August 21). Means were separated by Duncan's multiple range test ( $p < 0.05$ ) for values within each column.

<sup>b</sup> As tartaric acid equivalent.

also demonstrated that ABA infiltration into the fruit cane increased sugar concentration in berries, indicating that berry ABA might be involved in the transport of assimilates and/or their accumulation in the berries. The ineffectiveness of the increased berry ABA levels in the ES treatment to increase TSS accumulation in our experiment may be due to the early and severe dehydration of leaves. Stepwise water deficit treatments should be examined for their ability to induce positively affect berry ripening.

Juice constituents at harvest time are shown in the Table. The levels of fructose and glucose in ES treatment were significantly lower than those in the control and LS treatment, indicating that the ripening process had not been completed. The level of malic acid in ES-treated berries was considerably higher than in LS-treated and control berries. The total amino acid content was significantly higher in LS-treated berries than in control berries. Among the total amino acids, the contents of glutamine, arginine, valine, leucine, isoleucine, and phenylalanine were markedly increased by both water deficit stress treatments. However, the content of proline, the most dominant constituent, was decreased by both treatments.

OKAMOTO *et al.* (2001) reported that water deficit stress treatment of cv. Muscat of Alexandria caused significant increases of several kinds of amino acids in juice at harvest. NDUNG'U *et al.* (1997) reported the remobilization of nitrogenous compounds from dehydrated leaves into other permanent organs, *e.g.* cane, trunk, and root in water-stressed grapevines. During the water deficit stress treatment, they found that due to the decomposition of leaf proteins amino acids were released in large amounts and then translocated into clusters.

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