Changes in Abscissic Acid, Carbohydrates and Nitrogenous Compounds of "Riesling" Grapevines during Induction of Second Shoots by Water-Deficit Stress

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Young "Riesling" grapevines were induced to produce second shoots by water stress before summer pruning. The vines, field-grown and established in isolated soil beds were subjected to water stress by disconnecting irrigation for 15 and 23 days. Water stress enhanced budbreak and cluster formation. In the early stage of water stress, abscissic acid (ABA) content increased in the canes and trunks, while it decreased in the roots. As the stress persisted, ABA content dropped in the canes and trunks, but increased in the roots. The levels of nitrogen, amino acids, and soluble sugars in the canes, trunks, and roots increased during water stress, while the levels of starch decreased. The results were discussed in relation to interactions of ABA with metabolism of amino acids and sugars. A high level of ABA during water stress was proposed to play a role in stimulating nutrient accumulation. A high reserve of nutrients, being available upon resumption of watering, was proposed to stimulate budbreak and subsequently to improve flower formation.

INTRODUCTION

The manipulation of growth cycles by induction of second shoots after summer pruning has been tested for some table grapes, such as "Kyoho" and "Ruby Okuyama" cultivars (Nakagawa, 1972 ; Takei, 1994) in Japan. Other studies have shown the effectiveness of water stress in inducing budbreak and improving fruitfulness in grapevines (Carbonneau and Casteran, 1979 ; Williams *et al.*, 1991).

Hormonal and nutritional status are associated with dormancy and budbreak (Cutting *et al.*, 1991 ; Emmerson and Powell, 1978). Levels of hormones and nutrients change in response to water stress (Andersen *et al.*, 1995 ; Coombe and Monk, 1979). However, it is not clear whether such changes might influence budbreak following rewatering.

The present study was therefore conducted with two objectives: 1) to investigate the effectiveness of water stress before summer pruning in forcing new shoot growth in "Riesling" grapevines; and 2) to investigate the effects of water stress on the levels of hormones, carbohydrates and nitrogenous compounds in different parts of the vines, and their possible correlation with water-stress-induced budbreak.

MATERIALS AND METHODS

Plant materials : Thirty vines of 2-year-old "Riesling" on 5BB virus-free rootstock were used. The vines, field-grown under a polythene sheet covering at the experimental vineyard

at Okayama University, were planted on raised beds which were lined with a polythene sheet under neath. After the commencement of new growth in spring, vines were well watered by drip irrigation to maintain the soil pF at ≤ 1.8 . Fertilizer was given twice a week through irrigation water using a complete liquid-fertilizer of the following composition per 100 L : $\text{KNO}_3 = 27.0$ g, $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O} = 31.7$ g, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O} = 16.7$ g, $\text{NH}_4\text{H}_2\text{PO}_4 = 5.0$ g, $\text{EDTA-Fe} = 1.5$ g, $\text{H}_3\text{BO}_3 = 0.3$ g, $\text{MnSO}_4 \cdot 5\text{H}_2\text{O} = 0.2$ g, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O} = 0.022$ g, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O} = 0.005$ g, $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O} = 0.002$ g. Two shoots per vine were allowed to grow and were all deflowered.

Water stress treatments and sampling : Water stress treatments were started from 16 July 1994, by disconnecting irrigation. The planting beds were covered with aluminium sheets to avoid any water penetration. Ten vines had their water disconnected for 15 days, and another 10 vines for 23 days. The non-stress control vines continued to receive normal irrigation throughout. At the end of the stress treatments, each shoot (cane) was pruned back to 6 nodes (buds). Control vines were pruned on 22 July 1994. All shoots were defoliated. Irrigation was resumed on the day of pruning.

Leaf- and stem-water potentials were measured at mid-day (11.00 to 13.00) by the pressure chamber method, as described by McCutchan and Shackel (1992). Measurements were done on leaves from nodes 5 to 8. Soil samples (two per planting bed) were taken at mid-day with a cork borer, oven-dried to constant weight at 70°C for 72 h, and their water content was determined.

At the end of stress treatments, two vines per treatment were sampled and separated into canes (current shoots), trunks and roots. The young second shoots induced following pruning of vines and resumption of irrigation were sampled one week after budbreak. Samples were immediately deep frozen in liquid N_2 , freeze dried and then ground to a fine powder. The samples were stored in darkness with silica gel till analysis.

Chemical analyses : Absciscic acid (ABA) was determined by GLC-ECD, with modifications of the method described by Robinson and Barritt (1990). One gram of sample was extracted 3 times with 80% methanol containing 0.3% ascorbic acid in darkness at 5°C for 24 h. After evaporation of the methanol, the aqueous phase was extrated with ethyl acetate and 5% sodium hydrogen carbonate. The residue remaining after drying in a centrifugal vaporizer (CVE-200D) was dissolved in methanol and spotted on a chromatography paper (Advantec 51B, 2×40 cm). Separation was done by ascending chromatography for 20 cm using isopropanol : 28% ammonia : water (10 : 1 : 1) as a developing solvent system. ABA was recovered from R_f 0.8 to R_f 1.0. The ABA was methylated with diazomethane using acetone-saturated N_2 as a carrier. Two microliter aliquots were injected into a gas chromatograph (Shimadzu GC-3BE) equipped with ^{63}Ni -election capture detector (ECD). The silica column was packed with 1% SE-30 Uniport HP, 80 to 100 mesh. Temperature settings were 220°C and 250°C for the column and injection port, respectively. Carrier gas was N_2 at a flow rate of 45 ml/min. ABA content was calculated using a linear equation generated from chromatographs of (+)-ABA, relating the area of ABA peak to ABA concentration.

Sugars and amino acids were extracted from 500 mg sample with 80% ethanol at 70°C . The supernatant obtained after centrifugation was evaporated and the residue was dissolved in 25 ml of water. Ten milliliters of the aqueous solution was loaded in to a column of Amberlite CG-120 ion exchange resin, followed by 1 ml of 0.1% pentaerythritol as an internal standard. The column was eluted with 50 ml of water to release soluble sugars. Two milliliters of the eluate were freeze dried and the residue resuspended in 0.5 ml pyridine, 0.25 ml hexamethyldisilazane, and 0.25 ml trimethylchlorosilane, and heated at 60°C for 2 h. Two microliter aliquots were injected into a gas chromatograph (Shimadzu GC-14A) equipped with a flame ionization detector (FID).

Amino acids were released from the Amberlite column by eluting with 50 ml of 2 N ammonium hydroxide. The eluate was evaporated and the residue was dissolved in 1 ml of water. A five hundred microliter sample was mixed with an equal volume of lithium citrate buffer (pH 2.2) and filtered through a disposable syringe filter unit (cellulose acetate, 0.45 μ m). Amino acids were analyzed in a fully automated amino acid analyzer (JEOL JLC-300).

The pellet that remained after centrifugation of the ethanolic extracts was oven-dried (60 °C) and used for starch analysis. A fifty milligram sub-sample was incubated with 9.2 N and 4.6 N perchloric acid. Absorbance of derivatized reducing sugars was measured at 660 nm on a Hitachi 101 spectrophotometer. Reducing sugar equivalents were multiplied by 0.9 to obtain starch content.

Total and insoluble nitrogen were determined by a CN-coder (Yanako MT-600). One hundred milligrams of sample were used for total nitrogen, while the residue remaining after extraction of an equal weight of sample with 80% ethanol was used for insoluble nitrogen. Soluble nitrogen was calculated as a difference between total nitrogen and insoluble nitrogen.

Statistics : Data was subjected to analysis of variance as a randomized complete block design.

RESULTS

Soil and vine water status

The water content of well-watered soil ranged from 20 to 30% (w/w). After disconnecting irrigation, soil water content decreased rapidly, reaching 5% by the 5th day of water cut-off. Leaf and stem water potentials ranged from -0.8 to -1.6 MPa for well-watered vines. In water-stressed vines, the values dropped to -2.0 MPa by the 5th day of water cut-off. Water potential measurements could not be continued beyond the 5th day because the leaves had turned yellow.

Budbreak and fruitfulness of second shoots

Water stress enhanced budbreak and cluster formation, and the longer the duration of stress, the greater the effects (Table 1). Budbreak showed apical dominance in well-watered vines, being restricted to the uppermost 2 buds. Water stress was effective in overcoming this apical dominance tendency.

Hormonal changes in the vines

The 15-day water stress resulted in an increase of ABA contents of canes and trunks, but the levels in roots dropped. As the water stress persisted up to 23 days, the trend was reversed such that the ABA contents of canes and trunks dropped, while those of roots increased (Fig. 1).

Nutritional changes in the vines

Nitrogen contents of canes, trunks and roots increased in water-stressed vines (Table 2). Generally, the longer the duration of stress, the higher the nitrogen levels. Upon induction of second shoots, the young shoots of vines that had been water-stressed contained more nitrogen than those of well-watered vines.

Fig. 1 Changes in abscisic acid (ABA) contents of parts of "Riesling" grapevines subjected to water stress before summer pruning. Vertical bars represent standard error at 5% level.

Table 1 Effect of water stress before summer pruning on budbreak and fruitfulness of the second shoots in “Riesling” grapevines.

Treatment	Budbreak (%) per nodal position ^z						Clusters/shoot ^y
	1	2	3	4	5	6	
Control	0	0	0	0	58	96	1.5±0.5
15-day water stress	0	0	14	64	93	100	1.7±0.2
23-day water stress	25	44	35	69	75	75	1.8±0.3

^z Buds are numbered from the base of the primary shoots.

^y Values are mean±S.D.

Table 2 Total and soluble nitrogen contents (mg/100 mg dry wt) in parts of “Riesling” grapevines during induction of second shoots by use of water stress before summer pruning.

Treatment	Vine parts ^z			
	Cane	Trunk	Root	Young shoots
Control				
Soluble N	0.13	0.19	0.20	0.55
Total N	0.54	0.53	0.93	3.95
15-day water stress				
Soluble N	0.23	0.25	0.29	0.73
Total N	0.78	0.68	1.42	4.57
23-day water stress				
Soluble N	0.39	0.33	0.56	0.74
Total N	0.80	0.69	1.57	4.27

	Significance ^y	
	Treatments	Vine parts
Soluble N	**	**
Total N	*	**

^z Canes, trunks and roots were sampled at the end of water stress, when vines were pruned and watering resumed. Young shoots were sampled one week after budbreak.

^y *, ** Significant at $p \leq 0.05$ or 0.01, respectively.

Similarly, water stress caused an increase of amino acids contents (Table 3). The increase was significant for arginine, aspartic acid and threonine. Glycine, threonine, arginine and glutamic acid were the predominant amino acids in canes. In trunks, glycine and threonine were predominant, while in roots, arginine and glutamic acid were predominant. In the newly induced second shoots, amino acids contents were higher in vines that had been water stressed than in well-watered vines. Glutamic acid and threonine were the predominant amino acids in the young shoots.

Starch contents of canes, trunks and roots decreased during water stress, but soluble sugars increased (Table 4). Roots contained more starch compared to trunks and canes. Moreover, after 23 d of water stress, canes and trunks contained more sugars but less starch, while roots contained more starch but less sugars. Newly induced second shoots contained more sugars but less starch, and the 23-day water stress elevated the sugar content of young shoots.

DISCUSSION

While soil water content decreased to a minimum after 5 days of irrigation cut-off, by which time most of the leaves had turned yellow, vines were able to withstand irrigation cut-off

Table 3 Contents of amino acids ($\mu\text{mol/g}$ dry wt) in parts of “Riesling” grapevines during induction of second shoots by use of water stress before summer pruning.

Treatment and vine part ^z	ARG ^y	ASP ^y	GABA ^y	GLU ^y	GLY ^y	PHE ^y	THR ^y	Others	Total
Control									
Cane	0.13	0.33	1.47	0.22	0.89	0.02	0.33	0.29	3.68
Trunk	0.08	0.25	1.25	0.07	0.32	0.02	0.17	0.16	2.32
Root	0.37	0.06	0.68	0.10	0.17	0.01	0.03	0.21	1.63
Young shoots	0.07	0.81	0.04	0.25	2.40	0.16	1.88	3.89	9.50
15-day water stress									
Cane	0.86	1.04	0.51	2.05	2.79	0.17	1.79	1.07	10.28
Trunk	0.37	0.65	0.45	1.45	3.35	0.70	1.92	2.11	11.00
Root	0.71	0.18	0.08	0.63	0.14	0.02	0.14	0.32	2.22
Young shoots	0.40	1.67	0.17	1.67	8.85	0.21	1.08	3.05	17.10
23-day water stress									
Cane	4.52	1.73	1.42	4.09	6.14	0.47	4.99	1.61	24.97
Trunk	0.80	1.08	0.85	0.69	3.72	0.27	1.90	3.06	12.37
Root	5.16	0.50	1.33	5.02	1.73	0.13	1.23	0.78	15.88
Young shoots	0.72	1.34	0.17	0.28	3.54	0.22	3.92	1.17	11.36
Significance ^x									
Treatments	*	*	NS	NS	NS	NS	*		
Vine parts	NS	*	NS	NS	NS	NS	NS		

^z Canes, trunks and roots were sampled at the end of water stress, when vines were pruned and watering resumed. Young shoots were sampled one week after budbreak.

^y ARG, arginine; ASP, aspartic acid; GABA, γ -aminobutyric acid; GLU, glutamic acid; GLY, glycine; PHE, phenylalanine; THR, threonine.

^x NS, * Nonsignificant or significant at $p \leq 0.05$, respectively.

Table 4 Starch and soluble sugars contents (mg/100 mg dry wt) in parts of “Riesling” grapevines during induction of second shoots by use of water stress before summer pruning.

Treatment	Vine parts ^z			
	Cane	Trunk	Root	Young shoots
Control				
Sugars	1.3	1.4	1.0	3.4
Starch	3.2	2.2	5.9	1.6
15-day water stress				
Sugars	1.4	1.9	1.8	3.2
Starch	1.4	1.4	6.8	2.6
23-day water stress				
Sugar	2.5	2.3	2.0	4.4
Starch	1.3	1.2	3.6	2.1
Significance ^y				
	Treatments		Vine parts	
Sugars	**		**	
Starch	NS		**	

^z Canes, trunks and roots were sampled at the end of water stress, when vines were pruned and watering resumed. Young shoots were sampled one week after budbreak.

^y NS, ** Nonsignificant or significant at $p \leq 0.01$, respectively.

for up to 23 days with positive results to budbreak and cluster formation. This shows that soil water status and vine foliar symptoms are unreliable indicators of water stress level in relation to budbreak induction. Enhancement of budbreak and inflorescence formation in “Riesling”

grapevines by water stress agrees with the findings of Williams *et al.* (1991) on "Perlette" grapevines.

Planting vines on isolated soil beds made cutting off underground water from reaching the roots possible. Under this planting system, referred to as "restricted rooting volume culture" (Imai *et al.*, 1991), disconnecting irrigation was effective in inducing water stress within a short time. Use of water stress for budbreak induction in vines under ordinary field planting would depend on a reliable dry period.

Response of ABA levels to water stress was dependent on duration of stress and the part of vine considered. During the early stages of water stress, ABA contents increased in canes and trunks but decreased in roots. As the stress continued, the trend was reversed, with ABA contents dropping in canes and trunks, and increasing in roots. This might be a suitable hormonal condition for budbreak after rewatering. ABA has been shown to play a role in bud regeneration and flower development in *Citrus* (Goldschmidt, 1980 ; Maggon and Singh, 1995).

Increase in ABA contents during water stress and correlations of ABA with dormancy have been reported (Emmerson and Powell, 1978 ; Robinson and Barritt, 1990). Water stress and ABA induce the synthesis of certain proteins in plants (Blackman *et al.*, 1992 ; Bradford and Chandler, 1992). The role of these proteins (variously referred to as "stress proteins," "maturation proteins," "dehydrins," "responsive to ABA proteins") has been suggested to be mainly a protective one, enabling plant tissues to withstand desiccation and to reduce desiccation-induced cellular damage following rehydration.

Water stress caused increases in the contents of ABA, nitrogen and amino acids in "Riesling" grapevines. The results show that during water stress, nitrogen was remobilized from the senescing leaves to canes, trunks and roots. Glycine, threonine, arginine, and glutamic acid were the predominant amino acids during water stress. Arginine level is a good indicator of the nitrogen status of grapevines (Kliewer and Cook, 1971). Amino acids contents increased markedly in the xylem fluid of plum trees subjected to water stress (Andersen *et al.*, 1995). Our findings support the idea of water stress- and ABA-induced amino acid accumulation and protein synthesis. The increased amino acids might serve as nitrogen storage compounds or as precursors of protein synthesis. This would subsequently promote budbreak and flower formation following rewatering.

Starch contents decreased, while soluble sugars increased during water stress. Increase in soluble sugars during water stress is important for osmoregulation (Andersen *et al.*, 1995 ; Wang and Stutte, 1992). However, attention may also have to be focused on the high levels of sugars as a readily utilizable energy source, thus enhancing budbreak upon watering resumption. Several reports have shown ABA-mediated changes in sugars (Archbold, 1988 ; Ofosu-anim and Yamaki, 1994). The existence of an interaction between ABA and sugars in water stressed grapevines may not be ruled out.

The present study was conducted in early-summer, and bud dormancy at this stage can be classified as the para-type. It is well known that paradormancy is overcome by shoot tip removal, and even more readily if the leaves and lateral shoots are also removed (Mullins *et al.*, 1992). Dormancy and budbreak are controlled by a complex interaction of water, hormones, and assimilates. It seems like water-stress-induced budbreak is a process under interactive control by hormones and nutrients.

In this research, the yield and quality of fruit borne on the second shoots were not determined. Further work should focus on these parameters and compare with the crop normally produced on primary (first) shoots, with emphasis on quality of the berries for wine production.

CONCLUSION

The promotive effect of water stress on budbreak during paradormancy could be explained on basis of a hormonal and nutritional interaction. The role of increased ABA levels during water stress might be related to enhancement of nutrients accumulation. The high reserve of nutrients, being available upon resumption of watering, stimulated budbreak and subsequently improved flower formation. A second role of nutrient accumulation during water stress (especially amino acids and probably proteins, and sugars) might be related to the osmoregulation mechanism, thus protecting buds against desiccation damage.

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〈和文抄録〉

水分ストレスが“リースリング”ブドウ樹のABA、窒素および
炭水化物栄養の変化と2番枝の発育に及ぼす影響

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地表と隔離したベッドに植えた“リースリング”の幼樹に、7月中旬から15日または23日間、かん水を中断して水分ストレスを与えると、夏季剪定後の2番枝の発芽と花穂の着生が促進された。ストレス15日目には母枝のABA含量は低下し、根では増加した。また、ストレスが進むにつれて、樹体各部の全窒素とアミノ酸、糖含量が著しく増加し、デンプン含量が減少した。これらの結果から、水分ストレスによって増加したABAは養分の蓄積を促進したと考えられ、この養分蓄積が剪定後の2番枝の発芽と花穂の発育を良好にしたと推察される。