

BRIEF COMMUNICATION

Effect of water deficit on photosynthetic and other physiological responses in grapevine (*Vitis vinifera* L. cv. Riesling) plants

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Abstract

The grapevine (*Vitis vinifera* L. cv. Riesling) plants subjected to water deficit were studied for changes in relative water content (RWC), leaf dry mass, contents of chlorophyll (Chl), total leaf proteins, free amino acids, and proline, and activities of ribulose-1,5-bisphosphate carboxylase (RuBPC), nitrate reductase (NR), and protease. In water-stressed plants RWC, leaf dry matter, Chl content, net photosynthetic rate (P_N), and RuBPC and NR activities were significantly decreased. The total leaf protein content also declined with increase in the accumulation of free amino acids. Concurrently, the protease activity in the tissues was also increased. A significant two-fold increase in proline content was recorded.

Additional key words: amino acids; chlorophyll; net photosynthetic rate; nitrate reductase; proline; protease; relative water content; ribulose-1,5-bisphosphate carboxylase.

Water stress has a multifaceted effect on plant growth and metabolism (Hsiao 1973). Under stress, leaves close their stomata, and this is generally believed to be the cause of reduced carbon gain under periods of drought (Kaiser *et al.* 1981). Water stress affects not only the carbon metabolism but also nitrogen metabolism. During water stress nitrate reductase (NR) activity is lost more rapidly than most enzymes (Huffaker *et al.* 1970). The other enzymes of the pathway of nitrate assimilation, nitrite reductase and glutamine synthetase (Taylor *et al.* 1982), are relatively unaffected. Huffaker *et al.* (1970) suggested that a rapid loss of NR activity could be part of a biochemical adaptation to water deficit; shutting off the nitrate assimilation pathway at the first step would reduce energy requirements during periods of stress and prevent accumulation of nitrite and ammonium.

Water stress is the most important factor limiting grapevine growth in the Mediterranean area (Gomez del Campo *et al.* 2000, Flexas *et al.* 2002). Previous studies on the photosynthetic response to drought under field conditions showed that stomatal closure is an early response, which is almost matched by decreases in CO_2 assimilation. This response becomes progressively greater

through summer, as soil water availability decreases and also non-stomatal effects appear (Escalona *et al.* 1997). The purpose of the present study was the investigation of the effects of water deficit on physiological responses in grapevine.

One-year-old grapevine (*Vitis vinifera* L. cv. Riesling) plants were grown in 20 000-cm³ pots containing soil : sand : peat : vermiculite (3 : 1 : 3 : 3) in glasshouse [26/30–16/20 °C day/night temperature, photosynthetic photon flux density (PPFD) of 1 200–1 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$, 14/10 h light/dark cycle, and relative humidity between 65 and 70 %], and then divided into two uniform groups of ten pots each. The first group (control) continued to receive daily irrigation in order to maintain the soil water at the field capacity while in the second group (water deficit) irrigation was stopped. Measurements were made 10 d after the irrigation was stopped. At the time, the plants began to show visual signs of severe water deficiency and values of leaf water potential (Ψ_w) were -0.60 ± 0.05 and -2.00 ± 0.04 MPa for control and water deficit, respectively.

The detached leaves (about 0.3 g fresh mass) were cut and weighed immediately to obtain fresh mass (FM), and

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then they were placed overnight in the dark in a beaker (25 cm³) filled with water. They were reweighed to obtain turgid fresh mass (TM) in the next morning and dry mass after drying at 80 °C for 24 h in drying oven. The relative water content (RWC) of the leaves could be calculated as $RWC = [(FM - DM)/(TM - DM)] \times 100 \%$. Leaf water potential was measured with a WP4 dew-point potential meter (Decagon Device, Pullman, Washington, USA) after gas exchange measurements (Turner 1981). The leaves (about 0.5 g) were homogenized with 10 cm³ of 50 mM sodium phosphate, pH 7.8, containing 2 mM EDTA and 80 mM L-ascorbic acid. After centrifugation at 15 000×g for 20 min, the supernatants obtained were used for determining contents of total leaf protein and free amino acids. These contents in leaves were determined according to Lowry *et al.* (1951) and Moore and Stein (1948). Free proline content was extracted from leaves in 3 % aqueous sulphosalicylic acid and estimated using ninhydrin reagent (Bates *et al.* 1973). The content of chlorophyll (Chl) and protease activity were determi-

ned by the methods of Lichtenthaler (1987) and Davis and Smith (1955), respectively. The assay for ribulose-1,5-bisphosphate carboxylase (RuBPC) and NR activities were carried out as described by Nedunchezian and Kulandaivelu (1991).

Leaf FM and dry mass (DM) markedly decreased in water deficit plants when compared to control (Table 1). These results are in agreement with the earlier report (De Herralde *et al.* 1998, Lu and Zhang 1999). The marked reductions of the leaf DM in water deficit plants was mainly due to reduction in leaf thickness and size. Decreased DM accumulation of leaves as a result of stress may be attributed to the altered carbon and nitrogen metabolisms (Kluge 1976) and due to both senescence and death of leaves, which was considered an avoidance mechanism that allows minimizing water losses (De Herralde *et al.* 1998). A decrease in leaf expansion and thickness of the water deficit leaves indicates that both cell division and enlargement were significantly affected.

Table 1. Changes of relative water content (RWC), leaf biomass, contents of chlorophyll (Chl), total leaf proteins, free amino acids, free proline, net photosynthetic rate (P_N), and protease, ribulose-1,5-bisphosphate carboxylase (RuBPC), and nitrate reductase (NR) activities in control and water deficit grapevine plants. Means $\pm$ SE of five replications.

Parameter		Control	Water deficit
RWC	[%]	93.4 $\pm$ 4.3	44.6 $\pm$ 2.2
Leaf area	[cm ² plant ⁻¹]	164.0 $\pm$ 8.1	72.0 $\pm$ 3.5
Leaf fresh mass	[g plant ⁻¹]	2.43 $\pm$ 0.13	0.63 $\pm$ 0.03
Leaf dry mass	[g plant ⁻¹]	0.60 $\pm$ 0.03	0.27 $\pm$ 0.01
Chl <i>a</i>	[g kg ⁻¹ (FM)]	1.90 $\pm$ 0.10	1.14 $\pm$ 0.07
Chl <i>b</i>	[g kg ⁻¹ (FM)]	0.57 $\pm$ 0.02	0.45 $\pm$ 0.03
Chl (<i>a+b</i>)	[g kg ⁻¹ (FM)]	2.47 $\pm$ 0.13	1.59 $\pm$ 0.08
Total leaf protein	[g kg ⁻¹ (FM)]	46.84 $\pm$ 2.40	30.44 $\pm$ 1.60
Free amino acids	[g kg ⁻¹ (FM)]	146.4 $\pm$ 7.1	401.5 $\pm$ 21.1
Free proline	[g kg ⁻¹ (FM)]	30.65 $\pm$ 1.80	78.69 $\pm$ 3.80
Protease activity	[mg ⁻¹ (BSA hydro.) kg ⁻¹ (prot.) s ⁻¹]	2.05 $\pm$ 0.15	4.04 $\pm$ 2.30
P_N	[μ mol m ⁻² s ⁻¹]	11.32 $\pm$ 0.56	0.90 $\pm$ 0.05
RuBPC	[nmol(CO ₂) kg ⁻¹ (protein) s ⁻¹]	42.32 $\pm$ 2.40	25.39 $\pm$ 1.40
NR activity	[nmol(NO ₂) kg ⁻¹ (FM) s ⁻¹]	48.12 $\pm$ 2.30	21.65 $\pm$ 1.60

RWC is considered a measure of plant water status, reflecting the metabolic activity in tissues and used as a most meaningful index for identifying legumes with contrasting differences in dehydration tolerance (Sinclair and Ludlow 1986). In the present study, water deficit treatment significantly decreased the RWC (Table 1). RWC of several stressed crop plants were evident from the studies of Nagy *et al.* (1995) and El Hafid *et al.* (1998).

The marked reduction of total Chl in water deficit plants was due to the decrease of both Chl *a* and Chl *b* contents (Table 1). Chl *a* was degraded more than Chl *b* under deficit. These results are in agreement with earlier reports (Alberte *et al.* 1977, Jeyaramraja *et al.* 2005). Such water deficit induced reduction in Chl content has been ascribed to loss of chloroplast membranes, excessive swelling, distortion of the lamellae vesiculation, and

the appearance of lipid droplets (Kaiser *et al.* 1981).

Environmental stress could evoke compensatory metabolic changes through modification and modulation of the quantity and quality of proteins (Ramagopal 1987). The total leaf protein content decrease due to water deficit consequently resulted in a significant accumulation of amino acids (Table 1). Similar reports were observed in other plant species (Good and Zaplachinski 1994). The decrease in protein content and simultaneous elevation in amino acid pool under stress in the present study could be explained by enhanced proteolysis and decreased protein synthesis (Thakur and Thakur 1987). The elevated protease activity in the present study points to enhanced proteolysis in grapevine plants (Table 1). Thakur and Thakur (1987) reported that water-stressed maize plants showed a high protease activity over the control plants. The

elevated protease activity in plants under stressful conditions appears to be a part of an adaptive potential, since it also led to the accumulation of pool of free amino acids (Rai *et al.* 1983). Amino acid accumulation associated with water stress may actually be a part of an adaptive process contributing to osmotic adjustment (Ali Ahmad and Basha 1998).

Water deficit triggers the accumulation of proline in many different plant and crop species (Csonka and Hanson 1991, Delauney and Verma 1993). A significant accumulation of proline was recorded in water deficit treatment compared to control (Table 1). Further, the magnitude of accumulation of proline was dependent on severity of stress. The accumulation of proline may contribute to maintaining proper balance between extra-cellular and intracellular osmolarity under water stress. However, the significance of proline accumulation is controversial (Hare and Cress 1997). Other functions have been proposed for this response, such as free radical scavenging or nitrogen storage of pH regulation (Delauney and Verma 1993).

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