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Effect of Water Deficit and Propagation Techniques on Solute Accumulation of *Actinidia deliciosa* (cv. Hayward) Vines

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With 3 figures

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Summary

MILONE M. T., LOGGINI B., IZZO R., MURELLI C., MARINONE-ALBINI F. & NAVARI-IZZO F. 1999. Effect of water deficit and propagation techniques on solute accumulation of *Actinidia deliciosa* (cv. Hayward) vines. – Phyton (Horn, Austria) 39 (1): 37–48, with 3 figures. – English with German summary.

Two-year-old kiwifruit vines (*Actinidia deliciosa* cv. Hayward) obtained by micropropagation and from cuttings were used to investigate the effects of three days of water deficit on solute accumulation. The control vines, before (C_0) and after treatment (C_1), were characterized by the same water potential value, irrespective of the propagation technique, while the water potential decreased by 0.9 MPa in micropropagated vines and by 0.6 MPa in vines from cuttings due to water deficit (S_1).

Neither vine showed osmotic adjustment following water deficit. Water deficit caused an increase in total free amino acids, total sugars (glucose + fructose + sucrose + raffinose) and inositol, irrespective of the propagation technique. In micropropagated vines water deficit provoked an increase in proline, valine and leucine, whereas in cutting vines also tyrosine and tryptophan increased.

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Zusammenfassung

MILONE M. T., LOGGINI B., IZZO R., MURELLI C., MARINONE-ALBINI F. & NAVARI-IZZO F. 1999. Einfluß des Wasserdefizites und der Vermehrungsart auf die Anreicherung von gelösten Stoffen in Reben von *Actinidia deliciosa* (cv. Hayward). – Phyton (Horn, Austria) 39 (1): 37–48, 3 Abbildungen. – Englisch mit deutscher Zusammenfassung.

Es wurde an zwei Jahre alten Kiwi-Reben (*Actinidia deliciosa* cv. Hayward), vermehrt als Mikropropagation und Stecklinge, untersucht, wie sich ein dreitägiges Wasserdefizit auf die Anreicherung von gelösten Stoffen auswirkt. Die Kontrollreben, vor (C_0) und nach der Behandlung (C_1), hatten immer ein gleich großes Wasserpotential, unabhängig von der Vermehrungstechnik. Das Wasserpotential hingegen sank um 0,9 MPa in durch Mikropropagation vermehrten Reben und um 0,6 MPa in Reben aus Stecklingen gewonnenen Pflanzen. Keine von beiden Reben zeigte eine osmotische Anpassung als Folge des Wasserdefizites, es bewirkte aber einen Anstieg in den freien Aminosäuren, Zuckern (Glucose + Fructose + Rohrzucker + Raffinose) und Inositol unabhängig von der Vermehrungstechnik. In den durch Mikropropagation gewonnenen Reben rief ein Wasserdefizit einen Anstieg von Prolin, Valin und Leucin hervor, während in den Reben von Stecklingen auch Tyrosin und Tryptophan anstiegen.

Introduction

Water deficit affects many physiological and biochemical processes in plants (HSIAO 1973), resulting in the alteration in some metabolic pathways. Among the major effects are those involving carbohydrate metabolism, with the accumulation of organic solutes (JONES & al. 1980, KAMELI 1990). Carbohydrate changes are of particular importance on account of their direct relationship with physiological processes such as photosynthesis, respiration and nutrient concentration (JONES & al. 1980) and transport (KAMELI & LÖSEL 1993).

Sugars have long been known to increase in a wide range of plants grown at low moisture levels, e.g. in wheat (KAMELI & LÖSEL 1996), and in "resurrection" plants (KAISER & al. 1985). The rate and the extent of the increase in sugar content depend on the environmental conditions, plant species and even on the genotype within the same species (KAMELI & LÖSEL 1993).

In different organisms under conditions of stress, other types of organic solutes increase, including polyols in higher plants, algae and fungi (POPP & SMIRNOFF 1995), amino acids, mainly proline (DELANUEY & VERMA 1993), and glycine-betaine (HANSON 1992) in various plants.

Several hypotheses have been put forward to explain how solute accumulation might ameliorate the situation of the stressed organisms, however, beneficial effects for stress adaptation is still a subject of controversy. Physical changes in cell size or elasticity can promote stress adaptation. Changes in the elastic properties of cell walls may be im-

portant for concentrating solutes when plants undergo dehydration. However, physical changes alone do not necessarily promote osmotic adjustment, and it seems likely that a combination of solute accumulation and changes in cell volume is involved in adaptation (TURNER 1986).

In contrast to perturbing solutes such as inorganic ions, compatible solutes tend to be excluded from the hydration sphere of proteins and stabilize folded protein structures (LOW 1985). It is probably for this reason that sucrose, proline and glycine-betaine may protect enzyme activities against the increase in salt concentrations following drought (SCHWAB & GAFF 1990). In addition, sucrose (CROWE & al. 1986) and proline (RUDOLPH & al. 1986) may stabilize the membranes. It is likely that proline and inositol also function as hydroxyl radical scavengers (SMIRNOFF & CUMBES 1989).

Actinidia deliciosa needs high relative humidity (75–85%) and an annual rainfall of about 1045–1950 mm (BUWALDA & SMITH 1990). Consequently, when cultivated under Mediterranean climatic conditions, kiwifruit commonly experiences periods of water deficit (SAVÈ & al. 1994).

In preliminary research on *Actinidia* we found an influence of the propagation technique (micropropagated vines resulted less resistant than cutting vines to desiccation during drought) on the response to water deficit (MILONE & al. 1993).

The aim of this work is to evaluate in the leaves of *Actinidia* vines, obtained by micropropagation and from cuttings, the influence of a change in water status on sugar and amino acid contents.

Materials and Methods

Plant material and growing conditions

The experiment was carried out on two-year-old kiwifruit vines (*Actinidia deliciosa* cv Hayward) obtained by micropropagation (A) and from cuttings (B).

Two sets of vines obtained from each propagation technique were grown separately in plastic planter bags. Each bag contained 17 l of a peat/pumice based potting mix. Both vines were grown until two shoots had developed and maintained at field capacity up to the point of stress imposition.

Before stress imposition, 25 vines from each set were chosen as zero time (C_0). Two sets of 25 vines each, obtained by micropropagation and from cuttings respectively, were subjected to water deficit by withholding water for three days (S_1). One set was maintained at field capacity by regular irrigation and considered as a control (C_1). From each set vines in triplicate were chosen and leaves were harvested for analysis.

The fresh weight of leaves was recorded. All residue materials were lyophilized, powdered and stored at –20 °C till analysis. Aliquots of lyophilized material were used for residual humidity determination.

Water status

The water status of the vines was determined through P-V curves using a pressure chamber, as described by QUARTACCI & al. 1990. Leaf water potential (Ψ_w) and its

component potentials, Ψ_π and Ψ_p, were derived according to JOLY & ZAERR 1987. The bulk modulus of elasticity (ε) was derived from P-V curves according to formula: ε = RWC x tan α, where RWC is the relative water content at zero turgor and α is the slope of the regression line of Ψ_p vs RWC.

Sugars and inositol

Before the extraction of inositol and sugars, samples of lyophilized leaves were dipped in chloroform for preliminary separation of epicuticular waxes. After drying on blotting paper, the leaves were homogenized with methanol/water 7/3 (1/100 w/v) under 3 hr magnetic stirring, then left overnight at room temperature, filtered and vacuum concentrated at 35° to give a residue. This residue was partitioned three times between water and ethyl acetate to eliminate lipophilic compounds, then the aqueous phase was evaporated at reduced pressure.

For qualitative and quantitative GC analyses of sugars and inositol, samples of aqueous extracts were converted to trimethylsilylethers (TMSi) with a silylating reagent made up of pyridine, hexamethyldisilazane (HMDS) and trimethylchlorosilane (TMCS) in the ratio of 2/1/1 (v/v/v) and kept in *iso*-octane. Programmed chromatographic separations were run on FID-GC with capillary columns (Mega Carlo Erba, OV-1 or SE-52, 25 m length, 0.32 mm i.d. and 0.1–0.15 µm film thickness). The carrier gas was H_2 at 30 kPa. Typical operating conditions (MARINONE-ALBINI & al. 1994) were: 100° starting temperature, 8° min^{-1} to 250°, 10° min^{-1} to 300°, then held at 300° for 10 min. Peak areas were used for quantification. The concentrations of TMSi derivatives in aqueous extracts were calculated from the calibration curve using linear regression analysis.

Free amino acids

Free amino acids were extracted for 30 min from aliquots of 500 mg of leaf material with 50 ml of ethanol/water 85/15 (v/v). Each extract was filtered, vacuum dried and dissolved in a known volume of lithium citrate loading buffer (0.2 N, pH 2.2) and analysed. The analysis was carried out with a LKB 4101 Amino Acid Analyzer, equipped with a glass high pressure column (330 × 6 mm), water jacketed and filled with Ultropac 11 ion exchange resin (LKB). The elution was performed with a system of three lithium citrate buffers (LKB): pH 2.6 buffer A; pH 2.94 buffer B; and pH 3.53 buffer C. The amino acids were quantified by comparison with Calbiochem Behring standards.

Statistical analysis

The experimental design was run in triplicate. Data in the tables indicate mean values ± SE. Data reported in the figures were analyzed by ANOVA. The significance of differences was determined according to Tukey's test (P values ≤ 0.01).

Results

Water status of the kiwifruit vines is reported in Table 1. The vines before the treatment (C_0) and those of the control (C_1) showed the same water potentials (Ψ_w) irrespective of the propagation technique. Thereafter Ψ_w decreased by 0.9 MPa in stressed micropropagated vines and

Table 1

Effects of a three day water deficit on water potential (Ψ_w), osmotic potential (Ψ_π), osmotic potential at full turgor (Ψ_π^{100}), pressure potential (Ψ_p) and relative water content (RWC) of leaves of *Actinidia deliciosa* (cv Hayward) vines. A = micropropagated vines; B = vines from cuttings; C_0 = before treatment; C_1 = control; S_1 = after treatment. Results are the means of three replicates ± SE.

	Ψ_w (MPa)		Ψ_π (MPa)		Ψ_π^{100} (MPa)		Ψ_p (MPa)		RWC (%)	
	A	B	A	B	A	B	A	B	A	B
C_0	−1.0±0.12	−1.1±0.06	−1.6±0.05	−1.6±0.02	−1.2±0.04	−1.2±0.03	0.6±0.07	0.5±0.03	77.7±0.76	77.6±0.13
C_1	−1.0±0.12	−1.1±0.06	−1.6±0.05	−1.6±0.02	−1.2±0.01	−1.2±0.03	0.6±0.07	0.5±0.03	77.7±0.76	77.6±0.13
S_1	−1.9±0.03	−1.7±0.18	−2.0±0.03	−1.9±0.11	−1.2±0.03	−1.2±0.02	0.1±0.01	0.2±0.08	75.3±0.66	75.2±0.41

Table 2

Effects of a three day water deficit on fresh weight (FW) and dry weight (DW), bound water and bulk modulus of elasticity (ε) of leaves of *Actinidia deliciosa* (cv Hayward) vines. A = micropropagated vines; B = vines from cuttings. C_0 = before treatment; C_1 = control; S_1 = after treatment. Statistical analysis as in Table 1

	FW (g)		DW (g)		Bound water (%)		ε (MPa)	
	A	B	A	B	A	B	A	B
C_0	8.7±0.26	11.4±1.18	2.1±0.06	2.4±0.54	28.9±2.42	27.2±0.52	11.4±1.93	12.1±0.45
C_1	8.9±0.28	11.0±0.42	2.1±0.05	2.5±0.07	28.9±2.42	27.2±0.52	11.4±1.93	12.1±0.45
S_1	9.1±0.25	8.2±0.14	2.0±0.09	1.9±0.04	24.7±0.94	26.7±0.81	15.0±1.13	12.7±0.77

Table 3

Effects of a three day water deficit on total amino acids and sugars plus inositol of leaves of *Actinidia deliciosa* (cv Hayward) vines. A = micropropagated vines; B = vines from cuttings; C_0 = before treatment; C_1 = control; S_1 = after treatment. Statistical analysis as in Table 1.

	Amino acids				Sugars plus inositol			
	(μmoles/g DW)		(moles m^{-3} tissue water)		(μmoles/g DW)		(moles m^{-3} tissue water)	
	A	B	A	B	A	B	A	B
C_0	9.6±0.7	15.8±1.8	3.3±0.1	4.6±0.1	179.9± 2.6	308.2±14.2	61.2±0.7	88.9±1.3
C_1	22.4±0.6	25.3±2.8	7.6±0.1	7.9±0.1	221.5±14.9	304.5±16.6	75.7±2.1	94.8±2.2
S_1	35.4±4.5	38.7±1.1	10.3±0.1	12.2±0.1	318.6± 7.1	378.6± 8.4	93.0±1.9	119.6±3.0

0.6 MPa in vines from cuttings in comparison with the controls. The osmotic potentials (Ψ_π) showed the same behaviour, with a decrease in the stressed vines of 0.4 and 0.3 MPa, respectively.

All the vines, independent of propagation technique and treatments, showed constant values of the osmotic potential at full turgor (Ψ_π^{100} = −1.2 MPa). The pressure potential (Ψ_p) also decreased in the stressed vines,

to a greater extent in the micropropagated plants, although it always maintained positive values. The relative water content (RWC) showed a similar reduction in stressed vines obtained both by micropropagation and from cuttings.

After three days of water deficit, neither water from symplast nor ε showed change in the vines from cuttings, in comparison with the control (Table 2). On the contrary, stressed micropropagated vines showed a decrease in water from symplast and an increase in ε (Table 2).

The water status attained by the stressed micropropagated vines (S_1) did not reduce fresh or dry weights of leaves. On the contrary, vines from cuttings suffered a reduction of 25% in fresh as well as in dry weights, in comparison with the final control (C_1) (Table 2).

The content of free amino acids and that of sugars plus inositol were always higher in vines from cuttings in comparison with vines obtained by micropropagation (Table 3).

On dry weight basis total amino acids increased in C_1 by 130% and 60% in micropropagated and cutting vines, respectively. Water deficit caused a further increase in total amino acids in vines from both propagation techniques (Table 3). A similar trend was observed when the previous compounds were expressed as moles m^{-3} tissue water. In the micropropagated vines, the amount of sugars plus inositol increased, on dry weight basis, by 23% in the three day period of growth and by 44% following water deficit, while in the cutting vines they increased by 24% only in the stressed ones. In micropropagated vines, as far as amino acids are concerned, water deficit induced an increase in the percentage of proline, valine and leucine, whereas in cutting vines also tyrosine and tryptophan percentage increased (Figs 1, 2).

Free amino acids, individual sugars and inositol, expressed as the percentage of their total quantities, are reported in Fig. 1, 2 and Fig. 3 respectively. The sequence of sugars and inositol in Fig. 3 follows the gas chromatographic retention times.

The percentage of fructose increased in C_1 and S_1 in both vines but only in cutting vines the percentage of fructose was significantly higher in S_1 than in C_1. Glucose percentage increased only in stressed micropropagated vines, whereas in cutting vines it increased also in C_1. Inositol showed the same trend in both vines, increasing only in the stressed vines. On the contrary, the percentage of sucrose decreased in C_1 and S_1 of micropropagated vines and only in S_1 in vines from cuttings. The percentage of raffinose decreased only in S_1 in both types of vines (Fig. 3).

Discussion

Kiwifruit, irrespective of propagation technique, after three days of water depletion showed a mild water deficit stress (Table 1). The smal-

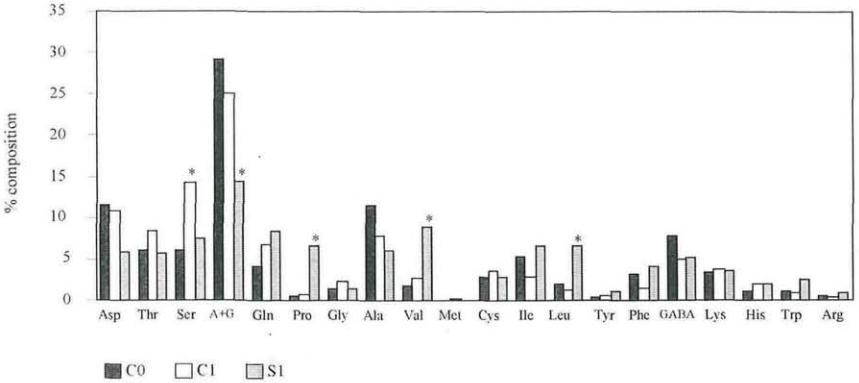

Fig. 1. Effects of a three day water deficit on free amino acids in leaves of *Actinidia deliciosa* cv. Hayward micropropagated vines. *Significant differences ($P \leq 0.01$) as assessed by ANOVA.

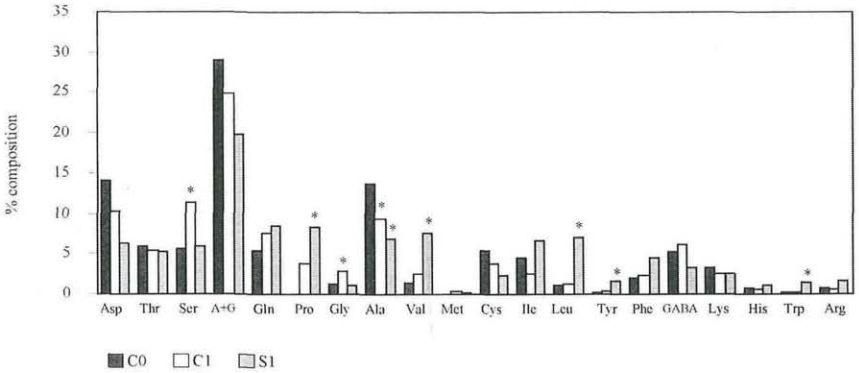

Fig. 2. Effects of a three day water deficit on free amino acids in leaves of *Actinidia deliciosa* cv. Hayward cutting vines. *Significant differences ($P \leq 0.01$) as assessed by ANOVA.

ler Ψ_p reduction in vines from cuttings compared with micropropagated vines (Table 1) may be related to the fact that only vines from cuttings maintained the same value of ε following water deficit (Table 2). A tissue with a lower value of ε is elastic and results in a smaller decrease in turgor, for the loss of a given volume of water, than a more rigid tissue. In fact, in sunflower, following moderate and severe water deficit, the decrease in ε contributed to an increase in Ψ_p (SGHERRI & NAVARI-IZZO 1995).

Although in the vines under water stress the increasing content of organic solutes measured did not result in an osmotic adjustment, the plant dry mass reduction and water loss were prevented only in micropropagated vines (Table 2). The contribution of the sum of amino acids and sugars plus

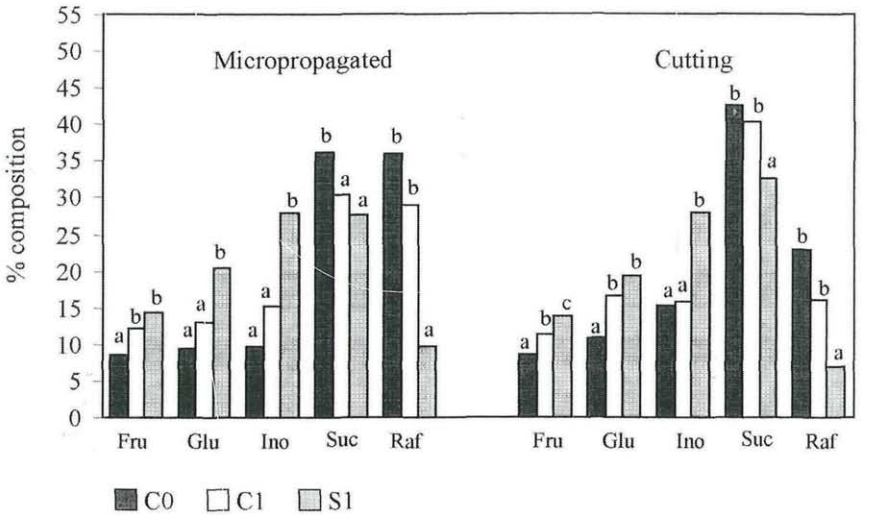

Fig. 3. Effects of a three day water deficit on fructose, glucose, inositol, sucrose and raffinose contents in leaves of *Actinidia deliciosa* cv. Hayward vines. Different letters indicate significant differences ($P \leq 0.01$) as assessed by ANOVA.

inositol to osmotic potential, calculated from Table 3 according to Vant't Hoff relation, passed from 0.16 (C_0) to 0.19 (C_1) and to 0.25 MPa (S_1) in micropropagated vines and from 0.23 (C_0) to 0.25 (C_1) and to 0.32 MPa (S_1) in vines from cuttings, being less than 20%. Thus not all of the osmotically important solutes have been accounted for (JONES & al. 1980).

Since there are numerous solutes which appear to accumulate significantly during adaptation to water deficit, it is important to know if all the solute changes observed arise independently from each other or if some primary biochemical response induces changes in the level of several solutes. It is possible that such a response is transduced through changes in hormonal levels. Such changes in turn must affect N assimilation since the majority of the solutes accumulated are NO_3^-, proline and/or other amino acids like γ-amino butyric acid and quaternary NH_4^+ compounds (HANDA & al. 1983).

Although present in low amount total amino acids increased remarkably following water deficit (Table 3) in vines from both propagation techniques. The quantitative and qualitative changes in free amino acids (Figs. 1, 2) suggest that also *Actinidia deliciosa* may primarily respond to water deficit by altering the rates of amino acids metabolism. However, the increase in total free amino acids can also derive from protein hydrolysis and/or from an inhibition of protein synthesis, such as in maize subjected to drought (NAVARI-IZZO & al. 1990).

Among amino acids an important role in drought resistance has been attributed to proline accumulation (SIVARAMAKRISHNAN & al. 1988, VENEKAMP 1989), although it is still not clear whether accumulation of proline can provide any biochemical adaptation for plants during water deficit (NAVARI-IZZO & al. 1990, SUNDARESAN & SUDHAKARAN 1995). In addition, proline accumulation is not always linked to osmotic adjustment and can exert a possible toxic role in the inhibition of malate dehydrogenase and nitrate reductase activities (NIKOLOUPOULOS & MANETAS 1991).

In *Actinidia deliciosa* vines an increase in proline as well as in other amino acids (Fig. 1) has been monitored following water deficit, but their contribution to osmotic potential was very low. Furthermore, amino acid accumulation (Table 3) did not cause osmotic adjustment (Table 1) as in maize (NAVARI-IZZO & al. 1990), so that quantitative and qualitative changes in their amounts, monitored in the present experiment, may be responsible for other defence mechanisms (RUDOLPH & al. 1986, MILONE & al. 1993) or may result from alterations of metabolism (PEREZ-ALFOSEA & al. 1993).

In the vines from both propagation techniques sucrose and raffinose were the major sugars, and following water deficit they significantly decreased, different from the "resurrection" *Sporobolus stapfianus*, where sucrose showed the highest increase (MARINONE-ALBINI & al. 1994). However, these resurrection plants, differently from our experiment, were almost completely dehydrated. Consequently, in *Sporobolus stapfianus* the increase in sucrose and raffinose, observed by MARINONE-ALBINI & al. 1994, is probably necessary to preserve the integrity of membranes during desiccation and rehydration.

Under the growth conditions used in the present experiment, glucose, fructose and especially inositol, which all increased in the stressed vines (Fig. 1), might play a more important role than sucrose and raffinose during water deficit. The differences between propagation techniques observed here in terms of glucose accumulation accompanying the decrease in water potential may be physiologically important in helping the plants to withstand the effects of reduced water potential and eventually in the utilization of glucose for growth after stress is relieved (KAMELI & LÖSEL 1993). The increase in fructose and glucose was probably caused mainly by hydrolysis of oligosaccharides which decreased under water deficit conditions (Fig. 3).

In wheat, inhibition of growth, observed at the time when sugar content increased markedly, supports the suggestion that reduction in growth is the main cause of sugar accumulation (KAMELI & LÖSEL 1996). From the continuous measurement of leaf elongation in maize plants under water stress, VOLKENBURGH & BOYER 1985 concluded similarly that solute accumulation in the growing cells occurred after the elongation rate was re-

duced. It is possible that the slight growth reduction in stressed vines from cuttings (Table 2), was responsible for the lack of sugar synthesis induction. There is strong evidence which implies that photosynthesis is the main source of accumulation of organic solutes under water stress, as indicated by the cessation of osmotic adjustment in *Centrosema* resulting from stomatal closure (LUDLOW & al. 1983).

When polyols are present at high concentrations, such as in *Fabaceae* and mangroves, they must make a significant contribution to osmotic adjustment (POPP & SMIRNOFF 1995); however, as shown by the Ψ_{π}^{100} osmotic adjustment did not occur in kiwifruit (Table 1). Therefore the inositol accumulation observed here is probably related to other factors. It is possible that inositol, which acts as a hydroxyl radical scavenger (SMIRNOFF & CUMBES 1989), may counteract a water deficit induced increase of oxygen radical production (PRICE & HENDRY 1991, SGHERRI & al. 1996)

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References

BUWALDA J. G. & SMITH G. S. 1990. Acquisition and utilization of carbon mineral nutrients, and water by the kiwifruit vine. – Hort. Rev. 12: 307–347.

CROWE L. M., WOMERSLEY C., CROWE J. H., APPEL L. & RUDOLPH A. 1986. Prevention of fusion and leakage in freeze-dried liposomes by carbohydrates. – Biochim. Biophys. Acta. 861: 131–140.

DELANUEY A. J. & VERMA D. P. S. 1993. Proline biosynthesis and osmoregulation in plants. – Plant J. 4: 215–223.

HANDA S., BRESSAN R. A., HANDA A. K., CARPITA N. C. & HASEGAWA P. M. 1983. Solutes contributing to osmotic adjustment in cultured plant cells adapted to water stress. – Plant Physiol. 73: 834–843.

HANSON A. D. 1992. Compatible solute synthesis and compartimentation in higher plants. – In: SOMERO G. N., OSMOND C. B. & BOLIS C. L. (Eds.), Water and life, pp. 52–60. – Springer-Verlag, Berlin.

HSIAO T. C. 1973. Plant responses to water stress. – Ann. Rev. Plant Physiol. 24: 519–570.

JOLY R. J. & ZAERR J. B. 1987. Alteration of cell-wall water content and elasticity in Douglas-fir during periods of water deficit. – Plant Physiol., 83: 418–422.

JONES M. M., OSMOND C. B. & TURNER N. C. 1980. Accumulation of solutes in leaves of sorghum and sunflower in response to water deficits. – Aust. J. Plant Physiol. 7: 193–205.

KAISER K., GAFF D. F. & OUTLAW W. H. Jr. 1985. Sugar contents of leaves of desiccation sensitive and desiccation tolerant plants. – Naturwiss. 85: 608–609.

KAMELI A. 1990. Metabolic responses of wheat plants to water stress and their role in drought resistance. – Ph. D. thesis, University of Sheffield, UK.

— & LÖSEL D. M. 1993. Carbohydrates and water status in wheat plants under water stress. – New Phytol. 125: 609–614.

— & — 1996. Growth and sugar accumulation in durum wheat plants under water stress. – New. Phytol. 132: 57–62.

Low P. S. 1985. Molecular basis of the biological compatibility of nature's osmolytes. – In: Gilles R. and Gilles-Baillien M. (Eds.), Transport processes, iono- and osmoregulation. pp. 469–477. – Springer Verlag, Berlin.

Ludlow M. M., Chu A. C. P., Clements R. J. &. Kerslake R. G. 1983. Adaptation of species of *Centrosema* to water stress. – Austr. J. Plant Physiol. 10: 119–130.

Marinone-Albini F., Murelli C., Patritti G., Rovati M., Zienna P. & Vita Finzi P. 1994. Low-molecular weight substances from the resurrection plant *Sporobolus stapfianus*. – Phytochemistry 37: 137–142.

Milone M. T. A., Murelli C., Navari-Izzo. F. & Izzo R. 1993. Water relations and epicuticular waxes in *Actinidia* vines subjected to water stress. – Giorn. Bot. Ital. 127: 961–962.

Navari-Izzo F., Quartacci M. F. &. Izzo R. 1990. Water-stress induced changes in protein and free aminoacids in field-grown maize and sunflower. – Plant Physiol. Biochem. 28: 531–537.

Nikoloupoulos D. & Manetas Y. 1991. Compatible solutes and in vitro stability of *Salsola soda* enzymes: proline incompatibility. – Phytochemistry 30: 411–413.

Pèrez-Alfocea F., Estan M. T., Caro M. & Guerrier G. 1993. Osmotic adjustment in *Lycopersicon esculentum* and *L. pennellii* under NaCl and polyethylene glycol 6000 iso-osmotic stresses. – Physiol. Plant. 87: 493–498.

Popp M. &. Smirnoff N. 1995. Polyol accumulation and metabolism during water deficit. – In: Smirnoff N. (Ed.), Environment and plant metabolism flexibility and acclimation, environmental plant biology, pp. 199–216. Bios Scientific Publishers, Oxford.

Price A. H. & Hendry G. A. F. 1991. Iron-catalysed oxygen radical formation and its possible contribution to drought damage in nine native grasses and three cereals. – Plant Cell Env. 14: 477–484.

Quartacci M. F., Navari-Izzo F. & Izzo R. 1990. Water status and associated changes in phospholipids and glycolipids of maize seedlings submitted to field water deficits. – Agr. Med. 120: 211–219.

Rudolph A. S., Crowe J. H. & Crowe L. M. 1986. Effects of three stabilizing agents -proline, betaine, and trehalose – on membrane phospholipids. – Arch. Biochem. Biophys. 245: 134–143.

Savè R., Olivella C., Biel C., Adillon J. & Rabella R. 1994. Seasonal patterns of water relationships, photosynthetic pigments and morphology of *Actinidia deliciosa* plants of the Hayward and Tomiri cultivars. – Agron. 2: 121–126.

Schwab K. B. & Gaff D. F. 1990. Influence of compatible solutes on soluble enzymes from desiccation-tolerant *Sporobolus stapfianus* and desiccation sensitive *Sporobolus pyramidalis*. – J. Plant Physiol. 137: 208–215.

Sgherri C. L. M. &. Navari-Izzo F. 1995. Sunflower seedlings subjected to increasing water deficit stress: oxidative stress and defence mechanisms. – Physiol. Plant. 93: 25–30.

— Pinzino C. & Navari-Izzo F. 1996. Sunflower seedlings subjected to increasing stress by water deficit: Changes in $O_2^{\cdot-}$ production related to the composition of thylakoid membranes. – Physiol. Plant. 96: 446–452.

SIVARAMAKRISHNAN S., PATEL V. Z., FLOWER D. J. & PEACOCK J. M. 1988. Proline accumulation and nitrate reductase activity in contrasting sorghum lines during mid-season drought stress. – Physiol. Plant. 74: 418–426.

SMIRNOFF N. & CUMBES Q. J. 1989. Hydroxyl radical scavenging activity of compatible solutes. – Phytochemistry 28: 1057–1060.

SUNDARESAN S. &. SUDHAKARAN P. R. 1995. Water stress-induced alterations in the proline metabolism of drought-susceptible and -tolerant cassava (*Manihot esculenta*) cultivars. – Physiol. Plant. 94: 635–642.

TURNER N. C. 1986. Adaptation to water deficits: A changing perspective. – Austr. J. Plant Physiol. 13: 175–190.

VENEKAMP J. H. 1989. Regulation of cytosol acidity in plants under conditions of drought. – Physiol. Plant. 76: 112–117.

VOLKENBURGH E. V. & BOYER J. S. 1985. Inhibitory effects of water deficit on maize leaf elongation. – Plant Physiol. 77: 190–194.