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Photosynthetic activity and cellular integrity of the Andean legume *Pachyrhizus ahipa* (Wedd.) Parodi under heat and water stress

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Abstract

Pachyrhizus ahipa (Wedd.) Parodi, originally from Latin America, is an agronomy interesting legume crop due to high seed protein content and saccharides-rich tuber root. Its capacity of adaptation to Mediterranean climate, where heat and water stress are frequently associated, is being tested. Two accessions of *P. ahipa* (AC 102 and AC 524) differing in field production were compared as concerns the effects of water stress and high temperature on photosynthetic performance. Membrane integrity was also evaluated through electrolyte leakage (injury index, I%), lipid composition, and ultrastructure observations. Short-term heat stress (40 °C) did not affect net photosynthetic rate (P_N), stomatal conductance (g_s), and most of fluorescence parameters in both accessions, what was consistent with low electrolyte leakage. However, photosynthetic capacity (P_{max}) showed a significant reduction, AC 524 being more affected than AC 102. Relative water content (RWC) below 70 % caused a drastic decrease in P_N and g_s. Fluorescence parameters, P_{max}, and I% were affected in the two accessions, which also presented a strong reduction (42 %) in total fatty acids (TFA). Contents of galactolipids were drastically reduced, and changes in their saturation also occurred, namely a decrease in linolenic acid ($C_{18:3}$) percentage of monogalactosyl-diacylglycerol (MGDG) in both accessions. Thylakoid ultrastructure in AC 524 submitted to drought showed disorganisation of grana stacking. Mitochondria presented signs of injured cristae. When water-stressed plants were subjected to high temperature, photosynthesis and fluorescence parameters did not show significant additional changes in both accessions. The exposure of drought stressed plants to 40 °C further increased electrolyte leakage in AC 524, but not in AC 102. Chloroplasts, mitochondria, and plasmalemma showed an increased disorganisation. Vesicles appeared in the cytoplasm, which became electron-transparent, reflecting a strong reduction in the number of ribosomes. Hence AC 102 was less affected than AC 524 as regards some components of photosynthetic process, namely P_{max} and membrane integrity. This could account for its better yield production previously observed in field grown plants.

Additional key words: chlorophyll fluorescence; drought; high temperature; leakage; membrane lipids; photosystem 2; stomatal conductance; ultrastructure; quantum yield; yam bean.

Introduction

Pachyrhizus (yam bean) is one of the few legume genera with edible tuberous roots. The species *P. ahipa* grows in the subtropical East Andean valleys of Bolivia and Argentina at altitudes between 1 500-3 000 m (Ørting 1996, Sørensen *et al.* 1997). It is an agronomically interesting crop due to its large seed protein content and its

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Abbreviations: DBI = double bond index; DGDG = digalactosyl-diacylglycerol; F_0 = basal fluorescence; F_v/F_m, F_v'/F_m' = photochemical efficiency of photosystem 2 under dark and light, respectively; g_s = stomatal conductance; I% = injury index; MGDG = monogalactosyl-diacylglycerol; P_{max} = photosynthetic capacity; P_N = net photosynthetic rate; PC = phosphatidylcholine; PE = phosphatidylethanolamine; PG = phosphatidylglycerol; PI = phosphatidylinositol; PS2 = photosystem 2; RuBP = ribulose bisphosphate; RH = relative humidity; RWC = relative water content; TFA = total fatty acids; Φ_e = quantum yield of electron transport.
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tuber root rich in saccharides. Its ability to adapt to Southern Europe field conditions was tested, namely in Portugal and Spain (Vaz *et al.* 1998, Leidi and Matos, personal communication), and encouraging root productions (*ca.* 40 t ha^{-1}) were already obtained. In these countries, of Mediterranean climate, the hottest summer months coincide with the driest periods in the year, resulting in a productivity decline mainly due to a decrease in photosynthesis (Lawlor 1995).

Moderate to high temperatures, and water stress inhibit photosynthesis. Depending on the water stress severity, different components of the photosynthetic process are affected. Drought frequently causes rapid stomata closure, resulting in a decrease of water loss through transpiration, internal CO_2 concentration, and leaf photosynthesis. Concomitantly, inhibition or damages in the primary photochemical and biochemical processes may occur (Kaiser 1987, Cornic and Massacci 1996). Optimal growth temperature may vary between 25 and 35 °C, but beyond this threshold range P_N declines sharply, with strong inhibition of photosynthetic electron transport reactions (Berry and Björkman 1980) and/or decreases in ribulose-1,5-bisphosphate carboxylase/oxygenase activity (Law and Crafts-Brandner 1999), limitations of RuBP synthesis, and changes in stromal pH.

Cell membranes are vulnerable to environmental stresses, such as water deficit and high temperature. Structural changes affecting membrane permeability, and therefore cellular compartmentation and functioning, may be reflected in increased membrane leakage under stress conditions (Scherbakova and Kacperska-Palacz 1980, Pham Thi *et al.* 1990, Campos and Pham Thi 1997). Stability of plasmalemma and thylakoid membranes may be a valuable indicator of drought and heat resistance of plants (Ristic *et al.* 1992, Bukhov *et al.* 1999). Thylakoids are predominant components in leaf cells, representing 60-80 % of the total cellular membranes in mesophyll (Webb and Green 1991). They are responsible for radiant energy harvesting and photosynthetic electron transport in plants. Indeed, the saturation level of fatty acids of thylakoid membrane lipids as well as the amount of glycolipids and trans-16:1-PG, are essential in maintaining an efficient photosystem 2 (PS2) electron transport (Horváth *et al.* 1987). Changes in water and temperature regime can modify membrane lipid composition (Harwood 1983, Kuiper 1985, Pham Thi *et al.* 1990), thus affecting plant photosynthetic performance. Damages caused by water shortage and temperature stress are also evidenced by changes in plant ultrastructure, particularly by chloroplast disruption (Lopez-Carbonell *et al.* 1994).

The extent to which water stress and high temperatures affect physical and biochemical aspects of photosynthesis and membrane integrity largely depends on cultivar ability to withstand such stresses. In this work two *P. ahipa* accessions differing in field production were compared as concerns the effects of water stress and high temperature, as well as the interaction of these two factors, on several aspects of photosynthetic process and membrane integrity.

Materials and methods

Plants: Potted plants of two accessions of *Pachyrhizus ahipa* (Wedd.) Parodi (AC 102 and AC 524) were grown in a glasshouse under natural irradiance (noon values usually >1 000 μmol m^{-2} s^{-1}), mean daily temperatures of 30 °C, and relative humidity values between 60 % (early morning) and 40 % (midday). After germination in Petri dishes, plantlets were transferred to 5 000 cm^3 pots filled with a mixture of *vermiculite* : *Triohum* tray substrate (4 : 5). Irrigation was supplied twice a week. A modified (two-fold micronutrients) Hoagland and Snyder (1933) solution was applied when needed.

Water and heat stress treatments: Plants (1.5 months old) were split in two groups: in the first group plants were irrigated twice a week to maintain relative water content (RWC) higher than 85 %. In the second group, plants were subjected to water shortage cycles by withholding irrigation during about 3 weeks. When pot capacity of water stressed plants decreased to 50 %, nutrient solution was applied to both treatments. RWC was calculated according to Čatský (1960) from samples of 10 leaf discs of 0.5 cm^2, as RWC = (FM – DM/TM – DM) × 100, where FM is the fresh mass, TM is the turgid mass after overnight rehydration of the discs in a humid chamber at room temperature, and DM is the dry mass after drying at 80 °C for 24 h.

High temperature treatments were imposed in two ways. For electrolyte leakage test detached leaves of the control and water stressed plants were enclosed in plastic bags and submerged in temperature controlled water bath at 25, 40, and 50 °C for 30 min. For the remaining measurements, whole plants were placed in climatised growth chambers (*Fitoclima 700 EDTU*, *Aralab*, Portugal) at 27/20 °C day/night, 800 μmol m^{-2} s^{-1} irradiance, 14 h photoperiod, and 75 % RH. High temperature treatment (40 °C) was applied under low irradiance (*ca.* 200 μmol m^{-2} s^{-1}) during 4 h. After this treatment, plants were left to adapt to 25 °C in darkness for about 2 h. Before gas exchange measurements plants were left for light adaptation under an irradiance of 800 μmol m^{-2} s^{-1} for at least 1 h.

Gas exchange and fluorescence measurements: Gas exchanges and chlorophyll fluorescence were measured to evaluate water stress and high temperature impact on photosynthetic performance. Photosynthetic capacity (P_{max}) was determined in a leaf disc oxygen electrode *LD2/2* (*Hansatech*, Kings Lynn, U.K.) under irradiance of 450-1 350 μmol m^{-2} s^{-1} (depending on plant status) and

CO_2 (*ca.* 7 %) saturating conditions. P_N and g_s were measured using a portable CO_2/H_2O gas exchange system *LI-6200* (*LI-COR*, Lincoln, USA).

Chlorophyll fluorescence parameters were measured using the *PAM-2000* system (*H. Walz*, Effeltrich, Germany). Leaves were dark adapted for at least 2 h before basal fluorescence (F_0) and photochemical efficiency of PS2 (F_v/F_m) were determined. For the determination of initial F_0 level, a very weak measuring beam switched to 1.6 kHz and at less than 0.1 μmol m^{-2} s^{-1} was used. To obtain F_m a saturating flash of about 6 000 μmol m^{-2} s^{-1} was applied. The PS2 photochemical efficiency in light conditions (F_v'/F_m') and quantum yield of electron transport (Φ_e) were obtained under photosynthetic steady-state conditions according to Genty *et al.* (1989).

Electrolyte leakage measurement: Fifteen leaf discs (0.8 cm^2) were collected from control and treated leaves, rinsed with water, and floated on de-ionised water. Conductivity was monitored with a conductimeter *Crison 522* (*Crison Instruments*, Barcelona, Spain), at room temperature, after 5 h of floating. Membrane leakage was expressed through a relative injury index, I% = [1 – (T – S/T – C)] × 100 (Scherbakova and Kacperska-Palacz 1980), where S and C represent the electrolytes released by stressed and control samples, respectively, and T the total electrolyte content measured after heating the control samples in their effusate at a temperature of 90 °C for 2 h.

Lipid analysis: One or two leaflets were detached from well-watered and droughted plants, and boiled for 2 min in distilled water to stop lipolytic activities. Lipids were extracted in a mixture of chloroform : methanol : water (1 : 1 : 1, by vol.) according to Allen *et al.* (1966). After evaporation of chloroform under nitrogen, the extracts were re-suspended in a mixture of ethanol : toluene (1 : 4, by vol.) and stored at –20 °C, until further analysis. Fatty acids were saponified and methylated with BF_3 using heptadecanoic acid ($C_{17:0}$) as internal standard, and analysed with a *UNICAM 610* gas chromatograph (*Unicam*, Cambridge, U.K.). Separation was performed on a fused silica capillary column *DB-Wax*, 0.25 mm i.d. × 30 m (*J&W Scientific*, Folsom, CA, USA) coated with polyethylene glycol (*Carbowax*) at a thickness of 0.25 μm. Column temperature was programmed to rise from 80 to 200 °C at a rate of 0.2 °C s^{-1}, after 2 min at the initial temperature. Injector and detector temperatures were 200 and 250 °C, respectively. Carrier gas was hydrogen with a flow rate of 16.6 mm^3 s^{-1}, at a split ratio of 1 : 100 of the sample. Separation of lipid classes was performed by thin layer chromatography as described by Campos and Pham Thi (1997). After visualisation under UV with 0.01 % primuline in 80 % acetone the bands were scraped off, saponified, methylated, and analysed by gas liquid chromatography as described above. The non-saturation degree of total fatty acids and lipid classes was obtained through a double bond index (DBI), calculated according to the formula: DBI = $(\%_{monoenes}) + (2\ \%_{dienes}) + (3\ \%_{trienes})/(\%_{saturated\ fatty\ acids})$ (Mazliak 1983).

Transmission electron microscopy: Small fragments of leaves were fixed overnight at 4 °C in 3 % glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.4. The specimens were washed 3 times for 30 min with buffer, post-fixed in 1 % osmium tetroxide in the same buffer for 2 h, stained in block in 1 % buffered uranyl acetate, dehydrated in an ethanol gradient to absolute ethanol, and embedded in *LR White resin* (Hall and Hawes 1991). Sections (60-80 nm) were cut in an *LKB IV* ultra-microtome (*LKB*, Bromma, Sweden) using glass knives, stained in saturated aqueous solution of uranyl acetate, and post-stained in a 3 % aqueous lead citrate at room temperature. Observations were made with a *Philips 300* transmission electron microscope (*Philips*, Eindhoven, The Netherlands) at 80 kV.

Statistical analysis: Results were statistically analysed by the *Statistix* program version *4.1*, 1985, *94 Analytical Software*, and *Systat for Windows* version *5*.

Results

Effect of high temperature: Well-irrigated plants submitted to short-term exposure to 40 °C did not show significant decreases in P_N, g_s (Fig. 1*A,B*), F_v/F_m, F'_v/F'_m, and F_0 (Fig. 2*A,B,C*) in both accessions, when compared with the control. However, P_{max} values showed significant reductions (Fig. 1*C*) in AC 102 (33 %) and AC 524 (41 %), possibly due to effects on the carboxylation process and/or electron transport (Φ_e) in AC 102 (Fig. 2*D*). At 40 °C, membrane integrity was not affected in both accessions (Table 1). At 50 °C, electrolyte leakage increased significantly in both accessions, but no differences were found between them (Table 1).

Effect of water stress: Severe drought (RWC <70 %) caused a drastic reduction in P_N and g_s (Fig. 1*A,B*). P_{max} and fluorescence parameters were also affected (Figs. 1*C* and 2) in both accessions. Water stress under control temperature (25 °C) caused a similar membrane injury (*ca.* 20 %) in both accessions (Table 1), close to the values observed only at 50 °C. Drought also induced a significant TFA reduction of *ca.* 42 % in the two genotypes (Table 2). Under control conditions no significant differences were observed between the two accessions as regards overall membrane non-saturation (DBI), although AC 524 plants presented slightly higher DBI than AC 102. As a result of drought, DBI showed a tendency to

decrease (15 %) only in AC 524. That could be due to changes in the percentage of the most abundant fatty acid, linolenic acid ($C_{18:3}$), which showed a more pronounced decrease in AC 524 (*ca.* 13 %) than in AC 102 (*ca.* 4 %).

Galactolipid contents were strongly reduced by drought (Fig. 3), MGDG being more affected than DGDG. The content of MGDG decreased 69 % in AC 524 and 60 % in AC 102. DGDG content presented a higher reduction in AC 102 (36 %) than in AC 524 (26 %). Both accessions showed a similar impoverishment (8 % reduction) in the proportion of $C_{18:3}$ in MGDG (Table 3). As concerns DGDG, a significant decrease of $C_{18:3}$ (15 %) was found in AC 102.

Table 1. Changes induced by drought, high temperatures (40 and 50 °C), and the interaction of both factors (drought×40 °C, drought×50 °C) in injury index (I%) of leaf discs of two *P. ahipa* accessions. Means of 3 measurements. *Letters* represent significant differences between treatments ($^{a, b}$) or accessions ($^{r, s}$).

	40 °C	50 °C	Drought	Drought×40 °C	Drought×50 °C
AC 102	0.8^{cr}	15.8^{br}	20.6^{abr}	16.5^{br}	33.6^{ar}
AC 524	2.5^{cr}	23.8^{br}	19.2^{br}	36.1^{as}	40.1^{ar}

Table 2. Total fatty acid (TFA) content [g kg^{-1}(DM)], individual fatty acid [mol %], and double bond index (DBI) of total lipids in well watered (RWC >85 %) and droughted (RWC <70 %) plants of two *P. ahipa* accessions. Means of 3 measurements. *Letters* represent significant differences between treatments ($^{a, b}$) or accessions ($^{r, s}$). RWC, relative water content; $C_{16:0}$, palmitic acid; $C_{16:1}$, palmitoleic acid; $C_{18:0}$, stearic acid; $C_{18:1}$, oleic acid; $C_{18:2}$, linoleic acid; $C_{18:3}$, linolenic acid.

		$<C_{16:0}$	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	TFA	DBI
AC 102	Watered	10.2^{as}	27.9^{ar}	5.3^{ar}	1.4^{ar}	0.9^{br}	7.9^{ar}	46.5^{ar}	35.1^{ar}	4.1^{ar}
	Droughted	10.4^{ar}	27.8^{ar}	3.9^{br}	1.8^{ar}	2.8^{ar}	8.9^{ar}	44.4^{ar}	20.3^{br}	4.0^{ar}
AC 524	Watered	11.6^{ar}	24.3^{bs}	4.7^{ar}	1.4^{ar}	1.1^{br}	7.2^{ar}	49.8^{ar}	40.0^{ar}	4.6^{ar}
	Droughted	10.4^{ar}	28.4^{ar}	3.6^{br}	2.0^{ar}	2.4^{ar}	10.0^{as}	43.2^{ar}	23.3^{br}	3.9^{ar}

Table 3. Effects of drought on fatty acid composition [mol %] of galactolipids in well-watered (RWC >85 %) and droughted (RWC <70 %) plants of two *P. ahipa* accessions. Means of 3 measurements. SE is indicated for the main fatty acids. *Letters* represent significant differences between treatments ($^{a, b}$) or accessions ($^{r, s}$). RWC, relative water content; MGDG, monogalactosyl-diacylglycerol; DGDG, digalactosyl-diacylglycerol. Symbols for fatty acids as in Table 2.

			$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
AC 102	Watered	MGDG	4.27^{ar}	1.54^{ar}	0.66^{ar}	0.65^{ar}	2.97^{br}	89.90^{ar}
		DGDG	32.53^{ar}	1.35^{br}	2.29^{br}	0.68^{ar}	4.15^{br}	58.99^{ar}
	Droughted	MGDG	5.51^{ar}	0.54^{br}	1.84^{br}	1.95^{br}	8.02^{ar}	82.14^{br}
		DGDG	35.88^{ar}	1.12^{ar}	2.77^{ar}	0.94^{ar}	8.09^{ar}	51.20^{br}
AC 524	Watered	MGDG	4.59^{ar}	1.33^{ar}	0.49^{ar}	0.76^{br}	3.25^{br}	89.58^{ar}
		DGDG	38.94^{ar}	1.19^{ar}	2.08^{br}	1.15^{ar}	4.21^{br}	52.43^{as}
	Droughted	MGDG	4.34^{ar}	0.98^{ar}	0.65^{ar}	1.56^{ar}	10.26^{ar}	82.21^{br}
		DGDG	35.56^{ar}	1.07^{ar}	2.79^{ar}	2.53^{ar}	9.80^{ar}	48.25^{ar}

As regards drought impact in phospholipid amounts, in AC 102 all the phospholipids showed some degree of reduction, diphosphatidylglycerol (DPG) and phosphatidylglycerol (PG) being more affected (Fig. 3). In AC 524 the content of PG and phosphatidylethanolamine (PE) increased in relation to irrigated plants, while phosphatidylinositol (PI) was maintained and contents of phosphatidylcholine (PC), neutral lipids (NL), and particularly DPG, decreased (Fig. 3*B*).

Since under drought AC 524 seemed to be more affected than AC 102 at least as regards P_{max} and membrane integrity, ultrastructure observations were conducted only in the genotype AC 524. Severe water deficit induced alterations in ultrastructure of the mesophyll cells, particularly as regards chloroplasts and mitochondria, as observed in Fig. 4*A* compared with the control (Fig. 4*B*). Different thylakoid organisation, from well-structured grana to partial membrane degradation, was observed in different chloroplasts of the same cell (Fig. 4*A,C,D*) or even in close areas of the same chloroplast (Fig. 4*C*). The most evident effect was thylakoid non-stacking in some areas of the chloroplasts (Fig. 4*A,C,D*). Swelling and undulation of the thylakoids also occurred, as well as changes in their orientation and their

partial degradation (Fig. 4*C*). The mitochondria presented signs of injured cristae. The cytoplasm did not reveal obvious changes at this observation level (Fig. 4*E*).

High temperature and water stress interaction: When water-stressed plants were submitted to short-term high temperature (40 °C), most of the parameters regarding photosynthesis and fluorescence did not show significant additional changes, in both accessions (Figs. 1 and 2). However, these conditions significantly increased membrane damage (Table 1) and ultrastructure disorganisation (Fig. 5*A*) when compared with plants submitted only to short-term high temperature (40 °C). AC 102 showed similar leakage when compared to droughted plants, but AC 524 presented significantly higher values resulting in a significant difference between the two accessions in interaction (drought×40 °C) treatment (Table 1). The chloroplasts rounded up and presented disarranged thylakoid membranes and, in some cells, the chloroplast envelopes and the plasmalemma lost their integrity. Mitochondria showed some structural disorganisation, as inferred from internal and external membrane integrity (Fig. 5*B*). Accordingly, net respiration rate decreased to about half when comparing water stressed and drought×40 °C treated plants (not shown). The cytoplasm became electron-transparent, reflecting a strong reduction in the number of ribosomes. Plasmalemma invaginations and exocytic extrusion vesicles occurred as well (Fig. 5*A,B*).

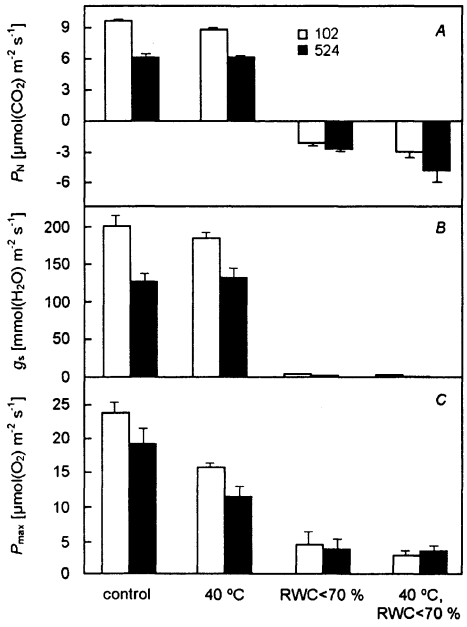

Fig. 1. Effect of high temperature (40 °C), water stress (measured by means of relative water content, RWC), and interaction of these two factors in (*A*) net photosynthetic rate (P_N), (*B*) stomatal conductance (g_s), and (*C*) photosynthetic capacity (P_{max}) of two *P. ahipa* accessions. Means of 4 measurements ± SE.

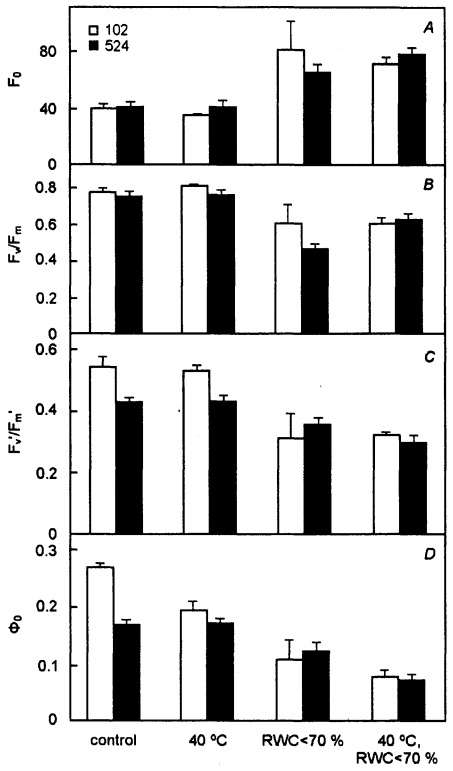

Fig. 2. Effect of high temperature (40 °C), water stress (measured by means of relative water content, RWC), and interaction of these two factors in (*A*) basal fluorescence (F_0), (*B* and *C*) the photochemical efficiency of PS2 under dark (F_v/F_m) and photosynthetic steady-state conditions (F_v'/F_m'), and (*D*) quantum yield of PS2 electron transport (Φ_e) of two *P. ahipa* accessions. Means of 4 measurements ± SE.

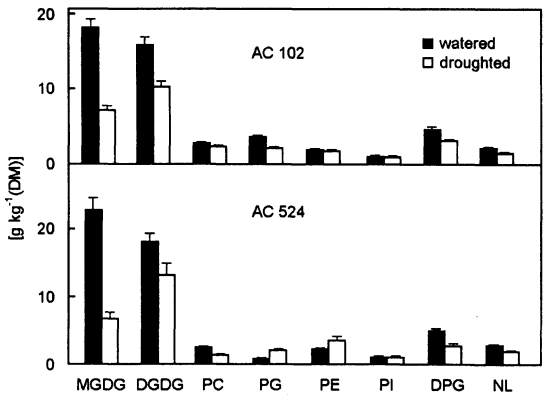

Fig. 3. Changes induced by drought in the lipid classes of two *P. ahipa* accessions. MGDG, monogalactosyl-diacylglycerol; DGDG, digalactosyl-diacylglycerol; PC, phosphatidylcholine; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; PI, phosphatidylinositol; DPG, diphosphatidylglycerol; NL, neutral lipids. Means of 3 measurements ± SE.

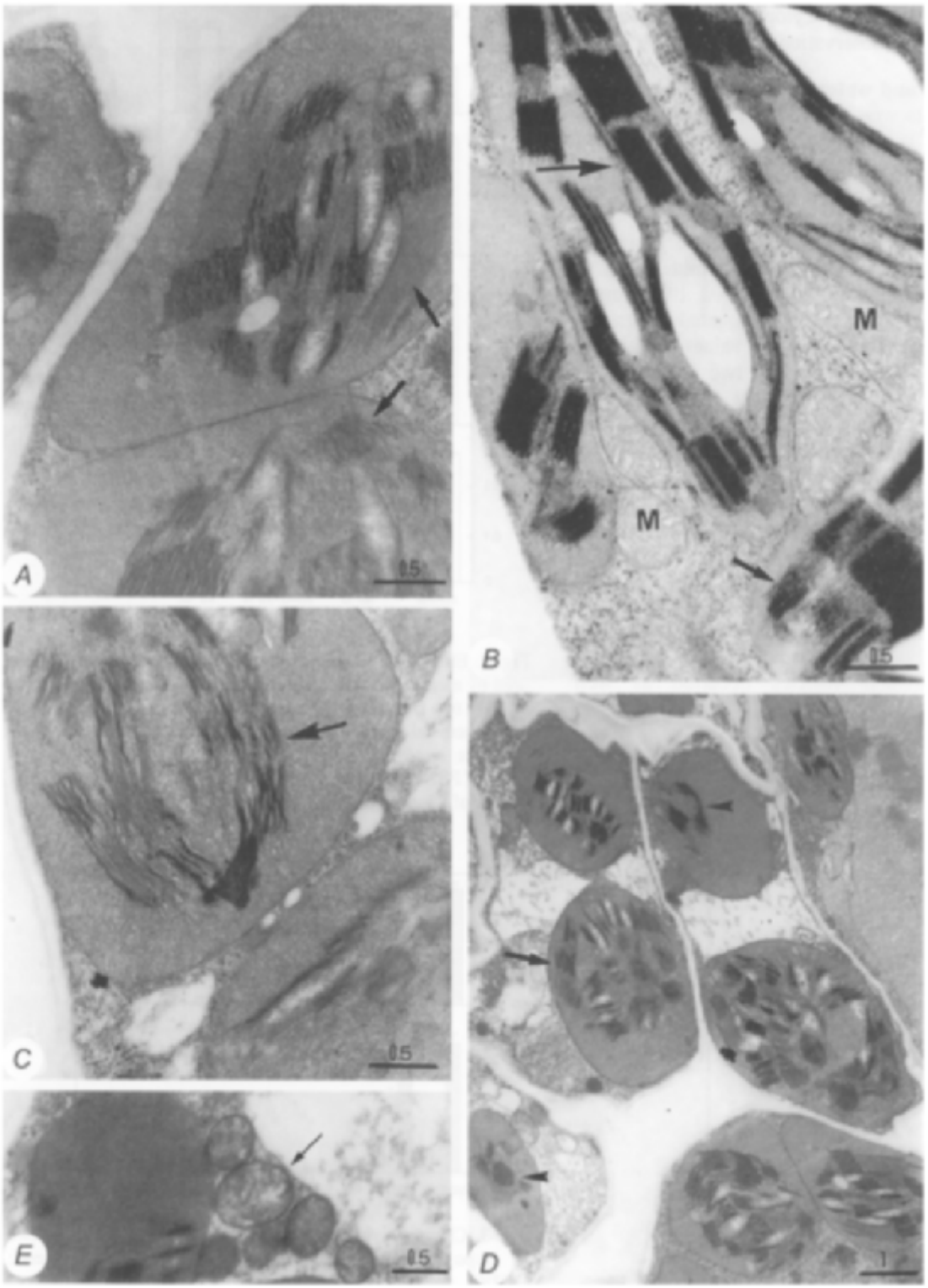

Fig. 4. Ultrastructural aspects of mesophyll cells of *P. ahipa* plants (AC 524) submitted to water stress conditions (*A, C, D, E*) compared with control (*B*). *A* – Changes in chloroplast grana being visible non-stacking in some areas (*arrows*). *B* – Chloroplasts with normal stacking grana (*arrows*) and mitochondria (M) with well developed cristae. *C* – Swelling and undulation of thylakoids, changes in their orientation, and partial degradation (*arrows*). *D* – Several mesophyll cells with different levels of thylakoid disorganisation. Well-structured grana stacks (*arrow*), partial membrane degradation of thylakoids (*arrow head*). *E* – Mitochondria with injured cristae (*arrow*). Scale bars in μm.

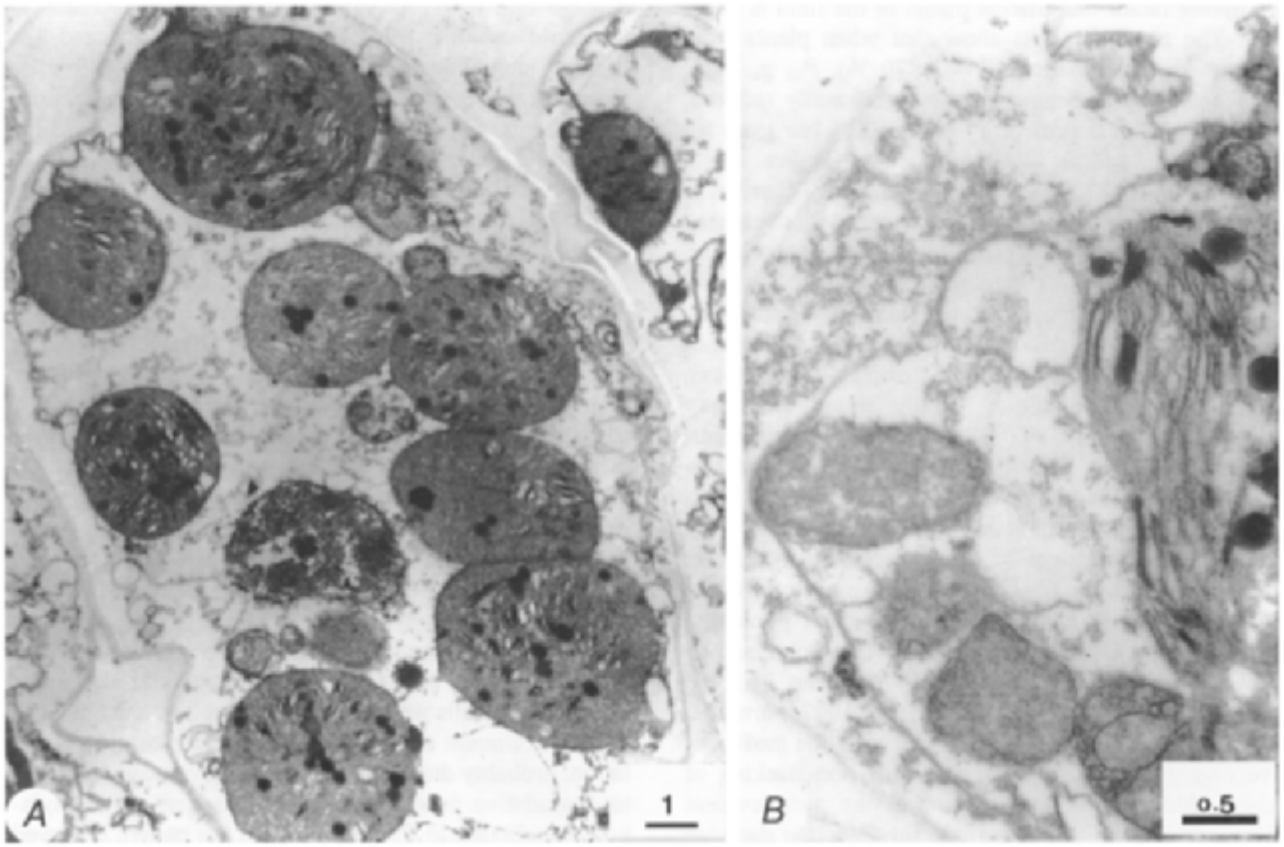

Fig. 5. Ultrastructure aspects of mesophyll cells of *P. ahipa* plants (AC 524) submitted to water stress and high temperature. Electron-transparent cytoplasm with numerous vesicles and plasmalemma invaginations. *A* – Chloroplast shape changed becoming spherical with the disarrangement of thylakoids. *B* – Destruction of mitochondrial cristae. Scale bars in μm.

Discussion

The two studied accessions (AC 102 and AC 524) seem to endure short-term high temperatures, since P_N, g_s, and fluorescence parameters did not decrease after a 40 °C treatment applied 2-3 h before measurements. This assumption was further supported by electrolyte leakage results. They reflected the absence of a significant membrane damage for 40 °C. According to Yordanov (1995) pea leaves exposed to heat stress (40 °C) in light did not show inhibition of oxygen evolution and fluorescence, what was already referred by Havaux (1992). In our case unexpected P_{max} lowering occurred and may be undetectable within the obtained range of P_N values since P_{max} values were considerably higher than P_N values.

Heat stress induces hyper-fluidisation of thylakoid membranes affecting lipid protein interactions and causing various perturbations, such as phase transitions of lipids and conformational changes, therefore altering their functions, namely PS2 reactions. However, such thylakoid structural changes are completely reversible when temperature returns to control values (Yordanov 1995), what could explain the stability observed as regards fluorescence measurements in *P. ahipa*. These short-term adaptive processes take place within minutes or hours and rapidly modify the thermal sensitivity of the photochemical apparatus of photosynthesis. Decreases in biosynthesis and activation of RuBPCO can occur (Yordanov 1995) and, although reversible (Feller *et al.* 1998), may probably take a longer time to be re-established to control values. That may have contributed to the P_{max} decrease observed in both accessions after the short-term 40 °C treatment. Thus other changes in carbon reduction cycle may have occurred.

When temperatures rose to 50 °C, membrane injury occurred in both accessions (Table 1) indicating that this temperature overcame the heat threshold range and photosynthetic parameters were most probably affected. Thylakoid membranes are particularly heat-sensitive (Gounaris *et al.* 1984), PS2 functions being readily inactivated under heat stress (Havaux 1996). Temperatures in the range of 40.0-47.5 °C induce dissociation of LHC2 from the PS2 core, and at temperatures above 52 °C denaturation of protein components of LHCP occurs, resulting in almost complete fluorescence inhibition (Yordanov 1995).

Another factor that affects plants in the field is water stress. The present results show that when plants were submitted to water deficit (RWC <70 %), P_N, g_s, P_{max}, and fluorescence parameters were significantly reduced. Similar results were previously obtained by our team for other legume species (Ramalho and Chaves 1992, Lauriano *et al.* 1997, 2000, Matos *et al.* 1998, Campos *et al.* 1999). These results are consistent with increased electrolyte leakage and membrane lipid degradation, which expressed loss of membrane integrity in AC 102 and AC 524. Lipid amounts were reduced by water deficit, probably due to decreased biosynthesis and stimulation of degradation processes. Effectively under stress, membrane lipids containing highly unsaturated fatty acids, as is the case of MGDG and DGDG in thylakoids, are main targets for hydrolytic and peroxidative processes, which cause membrane damage and loss of cell compartmentation (Pham Thi *et al.* 1990, Sahsah *et al.* 1998). Severe structural changes of chloroplast ultrastructure in water stressed leaves of AC 524 confirmed the loss of membrane integrity and thus impairments of photosynthetic processes. Our results agree with those obtained by several authors (Vieira da Silva 1976, Pham Thi and Vieira da Silva 1980, Utrillas and Alegre 1997, Farrant *et al.* 1999), which considered chloroplasts as the most sensitive organelles to water stress. Grana non-stacking of stressed chloroplasts of *P. ahipa* was the most evident change detected, as also described for *Cynodon dactylon* (Utrillas and Alegre 1997). The formation of grana stacks is controlled by surface electrical charges (Barber 1986), thus being affected by the ionic content of cells. In contrast to well-watered plants the sharp decrease in sodium concentration observed in stressed *C. dactylon* induced decreases in grana stacking (Utrillas and Alegre 1997). In a similar way it is possible that in *P. ahipa* the electrolyte leakage due to water stress can lead to a decrease in thylakoid stacking. Mitochondria were also affected, the damage including swelling and injury of cristae. Similar aspects were observed in short-term laboratory experiments on cotton (Pham Thi and Vieira da Silva 1980) and in long-term stressed field-grown plants of *C. dactylon* (Utrillas and Alegre 1997).

When water stress and high temperatures (40 °C) interacted, no significant additional decreases occurred in P_N, P_{max}, and fluorescence, possibly due to the severe physiological constraints already imposed by strong dehydration. Decreases in the $C_{18:3}$ of DGDG observed in droughted leaves of AC 102 may contribute to the maintenance of membrane leakage when 40 °C and water stress were superimposed. In contrast to results obtained in AC 102, membrane leakage further increased in AC 524. Effectively decreased non-saturation of thylakoid membrane lipids may confer thermal stability to membranes (Thomas *et al.* 1986), improving photosynthesis and growth at temperatures of 35 °C or more (Murakami *et al.* 2000).

As a result of the interaction between water stress and high temperature (40 °C), some ultrastructural alterations in mesophyll cells were noteworthy, such as a decrease in ribosome amount and an increase in cytoplasmic vesiculation, probably due to lowering of biosynthesis rates and to degradative processes. The disarrangement of thylakoid membranes in *P. ahipa* follows the pattern observed in bean plants submitted to water stress and high temperature (42 °C) treatment (Stoyanova and Yordanov 1999).

Taken altogether the present results suggest that AC 102 was less affected as regards some components of photosynthetic process, namely P_{max} and membrane integrity. This could account for its better yield production previously observed in field grown plants.

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Fabian P.: **Leben im Treibhaus. Unser Klimasystem – und was wir daraus machen.** – Springer, Berlin – Heidelberg – New York – Hongkong – London – Mailand – Paris – Tokio. 2002. ISBN 3-540-43361-6. 258 pp., GBP 16.50, sFr 40.00, € 24.95.

Weather has always been of prime interest for people. Many activities (*e.g.* agriculture, forestry) depended on it directly. Practically everybody is influenced by the actual weather conditions every day and in every place. No doubt, weather forecasts on TV are one of the most carefully followed news items. At present, many researchers as well as the public are interested in the climate and weather because of the increasing number of extreme weather events (floods, droughts, very high—or lack of—precipitation) and the awareness of global climate change. For this reason, it is desirable for the scientific community to offer new publications, both of high scientific standard and those addressing the broad public, that aid understanding of the complexity of long-term climatic events, as well as the rapid changes in the weather at various locations. The reviewed book has appeared just at the right time and contributes to better understanding of both climate development (change) on the Earth and more local weather.

The purpose of this book "Life in a greenhouse" is precisely characterised by its subtitle "Our climatic system – and what do we make of it". It is systematically focused on human intervention into natural climatic phenomena. It starts with a Preface by the author, explaining the principle philosophy of the book. In my opinion, the intention of the author, that the book should be of use not only for researchers in different fields, but also for laymen and policy makers interested in the problems of our environment, is fully justified.

Chapter 1 (17 pp.) is entitled "How things began: the evolution of the Earth's atmosphere". The text briefly describes the very beginning of our planet and its subsequent evolution, emphasising the origin, increase in concentration, and decisive role of oxygen in the atmosphere. Chapter 2 (55 pp.) "The Earth's greenhouse" is one of the most extensive and explains the composition of the atmosphere, principles of the greenhouse effect, global cycles of water and other components, El Niño phenomenon, and climatic zones of the Earth. Chapter 3 (38 pp.) describes "The role of the biosphere in the climatic system" and describes the biological components of the geosphere and hydrosphere, and global carbon, nitrogen, and sulphur cycles. The role of forests in climate is also described in reasonable detail. Chapter 4 (19 pp.) is entitled "Natural climatic variations – the variable climatic history of the Earth"; its content is obvious from the title. Chapter 5 (57 pp.) is quite extensive and deals with "Changes in the environment as consequences of human interventions". The individual subchapters are devoted to photo-smog, burning of biomass, acid rain, consequences of air traffic, and global climate changes. Here, even without reading the previous chapters, any interested reader could gain qualified information on the global temperature increase, sea temperature and sea level increases, iceberg melting and glacier retreat, and changes in distribution of precipitation. Again, special attention has been paid to the effects of climate change on forests. A relatively unexpected content has been included into Chapter 6 (10 pp.) "International treaties on the protection of the environment", in which both the Montreal (ozone hole) and Kyoto (CO_2 concentration reduction) protocols are depicted. The final Chapter 7 (9 pp.) is devoted to "Future development", describing various scenarios of future climate changes and possibilities for preventing the realisation of the worst one.

The text is accompanied by 33 pp. of instructive colour figures, maps, and graphs. The list of references contains 426 items ranged according to their first appearance in the text. I am not very happy with such an arrangement of the literature list. It could be fine for somebody who studies the text systematically from the beginning to its end. However, it is extremely difficult to locate an appropriate author, whom the reader would like to find. At the end of the book, an Index (6 pp.) is included, which helps considerably in finding the appropriate topic.

It is possible that specialists in climatology would not find this book exhausting and extensive enough. But such an objection is not relevant for the majority of readers of Photosynthetica. Photosynthesis is irreplaceable in the carbon cycle, determining the content of the greenhouse gases in the atmosphere and global climate change. Hence, the majority of our readers are more or less interested in what happens with current weather and climate. This book provides better understanding of the relationships between plants and the environment at the level of the canopies and biomes. Furthermore, the author successfully gives an account not only on the scientific basis of climate on the Earth, but also on its many links to the biosphere and human activities. This broad aspects make the book a valuable source of information for the majority of readers of Photosynthetica, as well for many others who would like to get more precise insight into what happens and what is going to happen to our climate. The text is very well arranged and intelligibly written.

I am afraid that outside German speaking countries, understanding of the German language considerably decreased during the last decades. The book deserves to be available to all who are interested, in the "international scientific language", *i.e.* English. And I believe that among English speaking scientists and the public, there are many potential readers who would acknowledge an English version of this book, because it clearly describes a serious problem for both many scientists and a considerable part, if not all, of our population.

L. NÁTR (*Praha*)