Drought effects on membrane lipids and photosynthetic activity in different peanut cultivars

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Abstract

The effects of drought on thylakoid acyl lipid composition, photosynthetic capacity (P_{max}), and electrolyte leakage were evaluated in two-months-old peanut cultivars (57-422, 73-30, GC 8-35) growing in a glasshouse. For lipid studies, plants were submitted to three treatments by withholding irrigation: control (C), mild water stress (S1), and severe water stress (S2). Concerning membrane and photosynthetic capacity stability, drought was imposed by polyethylene glycol (PEG 600). In the cv. 73-30 a sharp decrease in the content of thylakoid acyl lipids was observed, already under S1 conditions, whereas cv. 57-422 was strongly affected only under S2. Cv. GC 8-35 had the lowest content of acyl lipids under control conditions, a significant increase under S1 conditions, and only under S2 a decrease occurred. Thus concerning lipid stability, cv. 73-30 was the most sensitive. Among lipid classes, phospholipids and galactolipids were similarly affected, as was MGDG relatively to DGDG. Water deficit imposed by PEG induced a higher increase in electrolyte leakage in cv. 73-30 than in the other cvs. A positive relationship between acyl lipid concentration and membrane integrity was found in all studied cvs. A positive association between acyl lipid concentration, membrane integrity, and P_{max} was found in the cvs. 57-422 and 73-30.

Additional key words: *Arachis hypogaea*; digalactosyldiacylglycerol; galactolipids; membrane stability; monogalactosyl-diacylglycerol; phosphatidylglycerol; phosphatidylinositol; phospholipids; polyethylene glycol; water deficit.

Introduction

According to Kramer (1983), water shortage is the primary constraint to plant growth and productivity over much of land surface. Even in more humid regions, the occurrence of periods of drought which are common in rainfed agriculture often decreases yield substantially (Jacquinot *et al.* 1981, Lawlor and Uprety 1993). Drought limits agricultural production through inhibition of growth and photosynthesis, by decreasing crop leaf area due to fewer, smaller leaves, and by lower rates of photosynthesis per unit leaf area (Kriedemann 1986, Vu *et al.* 1987). Underlying these responses, there are various metabolic events at the subcellular level which should indicate the mechanisms initiating or increasing drought damage (Price and Hendry 1991). Among them, lipid changes are only one example.

Despite differences related to genotype and stress level, changes in the lipid content and composition are a general feature of cell deterioration as a response to most types of stress (Stefanov *et al.* 1992b, Cachorro and Cerda 1993, Gombos *et al.* 1994). Since acyl lipids

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are the major component of plant membranes (Harwood 1980, Webb and Green 1991), contributing to 25-30 % of the mass of plant thylakoid membranes (Anderson 1986), decreases in lipid amount, common in water stressed plants, increase membrane permeability (Zuily-Fodil *et al.* 1990, Navari-Izzo *et al.* 1993a). The ability of plants to maintain, under drought, their membrane integrity would determine their resistance toward drought stress. Thus, this characteristic is widely used as a criterion for drought resistance evaluation (Premachandra and Shimada 1988, Zuily-Fodil *et al.* 1990). On the other hand, membrane disruption leads to a series of events, namely the decrease of photosynthetic activity. The aim of this work was to evaluate differences in the responses to water stress of three peanut cultivars (57-422, 73-30, GC 8-35), concerning changes in acyl lipids content and their relation with membrane permeability and photosynthetic capacity.

Materials and methods

Plants: The effects of drought were evaluated in two-months-old plants (cvs. 57-422, 73-30, and GC 8-35). After germination in Petri dishes, seedlings were placed in 2 500 cm^3 pots (one plant per pot), filled with a mixture of vermiculite and *Trio-hum* substrate (4 : 5, v/v). Plants were grown in a glasshouse, from June to August, under natural irradiation. They were kept well-watered and fed weekly with a Hoagland nutrient solution.

Plant water status: Relative water content (RWC) was determined gravimetrically according to Čatský (1960), in samples of 10 foliar discs of 0.5 cm^2 each, as RWC = [(FM - DM)/(TM - DM)] ×100, where FM is 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.

Membrane damage and photosynthetic capacity: For electrolyte leakage test, expanded mature leaves of well hydrated plants were used. Fifteen leaf discs (0.8 cm^2 each) per sample were rinsed three times with deionized water. The discs were floated for 17 h on deionized water (control) or on a 0.5 M solution of polyethylene glycol (PEG) m.m. 600 (*Merck*). After PEG-induced dehydration, discs were carefully washed and floated on deionized water for 24 h, in order to allow rehydration of tissues. Conductivity was monitored along this period, using a conductimeter (*Crison 552, Crison Instruments*, Spain). The % of relative membrane damage was expressed as an injury index, I% = [1 - (T - D/T - W)]×100, calculated according to Vasquez-Tello *et al.* (1990). D and W represent the conductivity of electrolytes released by PEG-treated and control samples, respectively, and T the total electrolyte conductivity measured in the effusate after heating the control sample at 90 °C for 2 h.

To establish a correlation between I% and changes in photosynthetic activity, photosynthetic capacity (P_{max}) was determined in PEG-dehydrated leaf discs, using an oxygen electrode (*LD2/2, Hansatech*, Kings Lynn, UK), at 25 °C under saturating irradiance (1500 μmol m^{-2} s^{-1}) and CO_2 conditions. For the control plants, the 6-7 % CO_2 was provided with 400 mm^3 of $KHCO_3$ (2 M). For the plants submitted to drought, an air flow with 15 % CO_2 was provided to completely overcome the stomatal resistance, according to preliminary experiments. Results for P_{max} were expressed as % of decrease in relation to values obtained in control plants (leaf discs sampled directly from mature leaves of well hydrated plants, presenting RWC values around 95-92 %).

Lipids: For lipid studies, plants were submitted to three treatments by withholding irrigation for two days (control, C), seven days (mild water stress, S1), and twelve days (severe water stress, S2); values of RWC ranged between 95-90, 87-70, and 65-55, respectively. The amount of acyl lipids (MGDG, DGDG, PC, PG, and PI) was determined by thin layer chromatography according to Droppa *et al.* (1987). After chloroplasts isolation, phospholipase D was inactivated by boiling the probes in isopropyl alcohol for 2 min.. The extract, recovered in chloroform containing 0.05 % butylated hydroxytoluene, was quantitatively spotted onto *Silicagel 60* plates to separate the polar lipids in acetone : benzene : water (91 : 30 : 8, v/v/v) as solvent. The plates were sprayed with 1 % 8-anilinonaphthalene sulphonic acid in methanol and viewed under UV radiation. Standards were used for identification. Lipids were scrapped from TLC plates, and concentrations of galactolipids and phospholipids were determined by measuring galactose and phosphorus according to Weischen and Berkotte (1994) and Fiske and Subbarow (1925), respectively. For quantification of losses during the scrape of the applied lipids in the thin-layer chromatography, known amounts of each lipid standard were also applied in the TLC plates. Losses did not exceed 12 % and the obtained results were used for lipid standard curves.

The chlorophyll concentration in lipid extracts was measured spectrophotometrically following the method

of Arnon (1949).

Statistical analysis: For the statistical analysis of values (95 % confidence), a two-way ANOVA was applied to the various studied parameters followed by Tukey test for mean comparison between genotypes (r, s, t) or degrees of water stress (a, b, c).
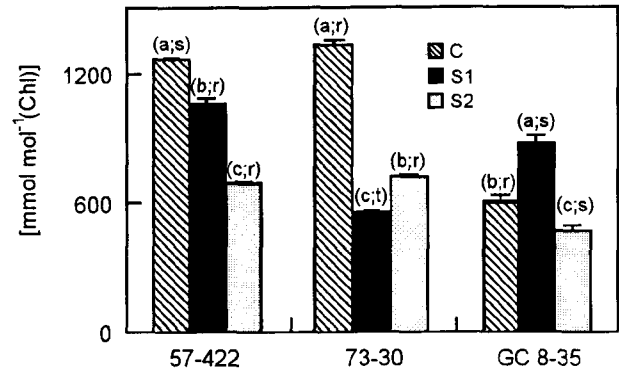
Results

Drought imposed by PEG caused an increase in solute leakage in all the cvs., as observed already at the end of 5 h of rehydration (Fig. 1). However, the cvs. showed differences in the sensitivity to PEG treatment. The solute leakage was higher in cv. 73-30 than in the other two cvs. The photosynthetic capacity also was affected by this treatment, more in cvs. GC 8-35 and 73-30 than in the cv. 57-422 (Fig. 1).

Concerning the drought effects on the total amount of thylakoid acyl lipids (Fig. 2), differences were found among drought severity and cvs. In cv. 57-422, the total amount of thylakoid acyl lipids gradually decreased

Fig. 2. Effects of water stress on concentration of total acyl lipids [mmol mol^{-1}(Chl)] in three peanut cvs. Each value is the mean ± S.E. (n = 3).

along with RWC decline, and in cv. 73-30 a sharp decrease occurred already for the S1 conditions. Cv. GC 8-35 contained the lowest amount of acyl lipids under control conditions, but a significant increase occurred under S1. Moreover, under S2 a decrease occurred (Fig. 2). These changes in the total amount of thylakoid acyl lipids were a result of similar changes observed in PL and GL (Table 1). Although there were no significant differences between them, PL were apparently more affected than GL (Table 1) as was MGDG relatively to DGDG. In general, the MGDG/DGDG ratio was only slightly affected by water stress (Table 1).
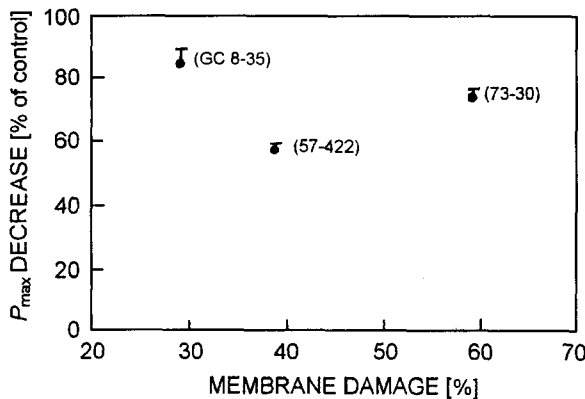
Fig. 1. Correlation of membrane leakage and P_{max} after PEG treatment in three peanut cvs. Each value is the mean ± S.E. (n = 3).

Discussion

At cell level, plant membranes are affected by water deficit, through many processes such as acyl lipid degradation. Therefore, the ability of plants to protect their membranes, under drought conditions, is fundamental for their survival. Solute leakage is widely considered a measure of membrane damage and thus a criterion for evaluation of drought tolerance, since it permits to rank the genotypes in relation to their protoplasmic tolerance to water deficit (Dhindsa and Mattowe 1981, Premachandra and Shimada 1988, Zuily-Fodil et al. 1990, Earnshaw 1993). Our results showed that water deficit leads to membrane injury as previously observed by Premachandra and Shimada (1988) and Zuily-Fodil et al. (1990). However, the studied cvs.

showed differences in their capacity to maintain membrane stability under drought, which determines at least partially the degree of their drought tolerance. Thus, cvs. 57-422 and GC 8-35 displayed a higher drought tolerance.

The P_{max} stability is also a criterion for drought tolerance. We found that under drought this parameter declined in all the cvs. as was observed previously by Kaiser (1987a,b) for a severe stress. However, the photosynthetic apparatus of the cv. 57-422 is probably more able to cope with drought than that of the cvs. GC 8-35 and 73-30, where P_{max} was more affected.

Lipids play an important role in determining the physiological properties of biological membranes

(Raison 1980, Gronewald *et al.* 1982). Although membrane lipid composition influences membrane structure and function, the related implications are still controverse (Navari-Izzo *et al.* 1990, 1995, Pham Thi *et al.* 1990). Despite the reductions in polar acyl lipid content have been considered a common phenomenon, result of drought, this response depends on genotype, water deficit level, growth conditions, *etc.* (Navari-Izzo *et al.* 1990, 1993b, Zúñiga *et al.* 1990). Nevertheless, the knowledge of the response under mild water deficit is

Table 1. Effects of water stress on the thylakoid acyl lipid content [mmol mol^{-1}(Chl)] in the peanut cvs. 57-422, 73-30, and GC 8-35. Each value is the mean $\pm$ S.E (n = 3).

Cv.	Treatment	C	S$_1$	S$_2$
57-422	MGDG	748 $\pm$ 20 [a;r]	613 $\pm$ 19 [b;r]	431 $\pm$ 9 [c;r]
	DGDG	292 $\pm$ 6 [a;s]	253 $\pm$ 5 [b;r]	145 $\pm$ 3 [c;s]
	GL	1040 $\pm$ 14 [a;r]	866 $\pm$ 22 [b;r]	676 $\pm$ 7 [c;r]
	PC	57 $\pm$ 1 [a;s]	50 $\pm$ 1 [b;r]	31 $\pm$ 2 [c;r]
	PG	74 $\pm$ 2 [a;s]	57 $\pm$ 1 [b;r]	43 $\pm$ 1 [c;r]
	PI	95 $\pm$ 0 [a;s]	79 $\pm$ 1 [b;r]	48 $\pm$ 1 [c;s]
	PL	226 $\pm$ 2 [a;s]	186 $\pm$ 4 [b;r]	114 $\pm$ 5 [c;r]
	total	1267 $\pm$ 14 [a;s]	1052 $\pm$ 27 [b;r]	689 $\pm$ 10 [c;r]
	MGDG/DGDG	3 $\pm$ 0 [ab;r]	2 $\pm$ 0 [b;r]	3 $\pm$ 0 [a;r]
73-30	MGDG	712 $\pm$ 18 [a;r]	318 $\pm$ 3 [c;t]	393 $\pm$ 4 [b;r]
	DGDG	345 $\pm$ 6 [a;r]	136 $\pm$ 4 [c;t]	180 $\pm$ 9 [b;r]
	GL	1057 $\pm$ 23 [a;r]	453 $\pm$ 4 [c;t]	573 $\pm$ 6 [b;r]
	PC	73 $\pm$ 0 [a;r]	26 $\pm$ 1 [c;t]	34 $\pm$ 1 [b;r]
	PG	86 $\pm$ 1 [a;r]	31 $\pm$ 1 [c;t]	43 $\pm$ 0 [b;r]
	PI	118 $\pm$ 2 [a;r]	46 $\pm$ 2 [c;t]	60 $\pm$ 0 [b;r]
	PL	277 $\pm$ 3 [a;r]	103 $\pm$ 4 [c;t]	134 $\pm$ 7 [b;r]
	Total	1333 $\pm$ 22 [a;r]	566 $\pm$ 7 [c;t]	710 $\pm$ 12 [b;r]
	MGDG/DGDG	2 $\pm$ 0 [a;rs]	2 $\pm$ 0 [b;r]	2 $\pm$ 0 [a;s]
GC 8-35	MGDG	329 $\pm$ 7 [b;s]	513 $\pm$ 16 [a;s]	274 $\pm$ 9 [c;s]
	DGDG	152 $\pm$ 15 [b;t]	197 $\pm$ 11 [a;s]	109 $\pm$ 9 [c;t]
	GL	480 $\pm$ 23 [b;s]	709 $\pm$ 26 [a;s]	383 $\pm$ 17 [c;s]
	PC	33 $\pm$ 2 [b;t]	44 $\pm$ 2 [a;s]	24 $\pm$ 1 [c;s]
	PG	39 $\pm$ 1 [b;t]	49 $\pm$ 1 [a;s]	24 $\pm$ 0 [c;s]
	PI	51 $\pm$ 1 [b;t]	67 $\pm$ 2 [a;s]	34 $\pm$ 1 [c;t]
	PL	122 $\pm$ 5 [b;t]	160 $\pm$ 3 [a;s]	82 $\pm$ 3 [c;t]
	Total	602 $\pm$ 17 [b;t]	869 $\pm$ 28 [a;s]	466 $\pm$ 19 [c;s]
	MGDG/DGDG	2 $\pm$ 0 [a;s]	3 $\pm$ 0 [a;r]	3 $\pm$ 0 [a;s]

fundamental to study plant survival capacity under drought, and influences its drought tolerance degree, since under severe water deficit even the drought tolerant genotypes are more or less affected.

Our results suggest that the cv. 57-422 is a drought tolerant genotype, since it showed, relative to the cv. 73-30, a high stability in lipid content under mild stress, as previously observed in the drought tolerant *Haberlea*

rhodopensis and *Ramonda serbica* (Stefanov *et al.* 1992a) and in drought resistant genotypes of *Vigna unguiculata* (Pham Thi *et al.* 1990).

The drought tolerant behaviour of cv. GC 8-35 was clear, since under moderate drought stress it presented a significative increase in acyl lipids, and therefore a higher ability to preserve membrane integrity similarly as in drought resistant genotypes of *Hordeum vulgaris*

(Zúñiga *et al.* 1990) and *Vigna unguiculata* (Pham Thi *et al.* 1990). According to our results, the response of cv. 73-30 is typical of drought sensitive plants. The sharp decrease in acyl lipids under moderate water deficit was also found in *Zea mays* (Navari-Izzo *et al.* 1989), *Helianthus annuus* (Navari-Izzo *et al.* 1990), and in drought sensitive genotypes of *Vigna unguiculata* (Pham Thi *et al.* 1990).

Although opposite results have been reported by some authors (Pham Thi *et al.* 1990, Stefanov *et al.* 1992a), the obtained results suggest that PL are more sensitive to water deficit as was observed by Navari-Izzo *et al.* (1989, 1990, 1995). However, decrease in both PL and GL affects plants that grow under drought, since the hydrolytic enzymes that are responsible for their breakdown may cause formation of FFA (Navari-Izzo *et al.* 1990, Pham Thi *et al.* 1990). This process inhibits photoactivity of the chloroplasts giving further explanation for the diminished photosynthetic activity observed under drought stress. The resulting increase of FFA and FFA/PL molar ratio may destabilise membranes (Senaratna *et* al. 1984, Navari-Izzo *et al.* 1990, 1993b).

In the cv. 73-30, a pronounced decline in MGDG content was observed under moderate water stress. This has been considered as a feature of the more sensible cvs. (Pham Thi *et al.* 1990, Hubac *et al.* 1992). Besides the analyses of the individual changes of MGDG and DGDG, the MGDG/DGDG ratio is also an indication of the environmental stress effects on membranes structure and function. Contrarily to what was observed in *Boea hygroscopica* (Navari-Izzo *et al.* 1995), our results showed that the MGDG/DGDG ratio was slightly affected by drought stress as was previously observed for

temperature stress in *Pisum sativum* (Chapman *et al.* 1983). According to Quinn and Williams (1983) and Siefermann-Harms *et al.* (1987), this means that the relationship between the electron transfer among the antennae, the core of photosystems, and the bilayer arrangement might remain unchanged under our water stress conditions.

In conclusion, we found that drought stress induced a strong decrease in the content of acyl lipids of cv. 73-30, giving further explanation for the increase in permeability observed in this genotype. The obtained results showed a positive relationship between acyl lipid stability and membrane integrity in all the cvs. The lowest membrane damage was observed in cv. GC 8-35, which showed increases in lipid content under moderate water stress, and therefore a high ability to preserve membrane integrity. On the contrary, cv. 73-30, which suffered the highest lipid decreases, also showed significant levels of membrane damage, expressed through electrolyte leakage. Although cv. GC 8-35 presented the highest membrane stability, as referred above, it showed the sharpest decreases in P_{max} values. Therefore a positive relationship between acyl lipid concentration stability, membrane integrity, and P_{max} was found only for cvs. 57-422 and 73-30. This was not the case of cv. GC 8-35, as was also observed in *Lupinus albus* (Meyer *et al.* 1992). According to these authors, this might be due to the fact that the lowering of photosynthetic activity was not a consequence of membrane damage caused by changes in polar lipid content and composition, but rather a result of destruction or inactivation of ATPases.

References

Anderson, J.M.: Photoregulation of the composition, function, and structure of thylakoid membranes. - Annu. Rev. Plant Physiol. **37**: 93-136, 1986.

Amon, D.I.: Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris.* - Plant Physiol. **24**: 1-15, 1949.

Cachorro, A.D., Cerda, A.: Effects of saline stress and calcium on lipid composition in bean roots. - Phytochemistry **32**: 1131-1136, 1993.

Čatský, J.: Determination of water deficit in discs cut out from leaf blades. - Biol. Plant. **2**: 76-77, 1960.

Chapman, D.J., De-Felice, J., Barber, J.: Influence of winter and summer growth conditions on leaf membrane lipids of *Pisum sativum* L. - Planta **157**: 218-223, 1983.

Dhindsa, R.S., Mattowe, W.: Drought tolerance in two mosses: correlated with enzymatic defence against lipid peroxidation. - J. exp. Bot. **32**: 79-91, 1981.

Droppa, M., Masojidek, J., Rózsa, Z., Wolak, A., Horváth, L., Farkas, T., Horváth, G.: Characteristics of Cu deficiency-

induced inhibition of photosynthetic electron transport in spinach chloroplasts. - Biochim. biophys. Acta **891**: 75-84, 1987.

Earnshaw, W.J.: Stress indicators: electrolyte leakage. - In: Hendry, G.A.F., Grime, J.P. (ed.): Methods in Comparative Plant Ecology. A Laboratory Manual. Pp. 152-154. Chapman & Hall, London - Glasgow - New York - Tokyo - Melbourne - Madras 1993.

Fiske, C.H., Subbarow, Y.: The colorimetric determination of phosphorus. - J. biol. Chem. **66**: 375-400, 1925.

Gombos, Z., Wada, H., Hideg, E., Murata, N.: The unsaturation of membrane lipids stabilises photosynthesis against heat stress. - Plant Physiol. **104**: 563-567, 1994.

Gronewald, J.W., Abou-Khalil, W., Weber, E.J., Hanson, J.: Lipid composition of plasma membrane enriched fraction of maize roots. - Phytochemistry **21**: 859-862, 1982.

Harwood, J.L.: Plant acyl lipids: structure, distribution, and analysis. - In: Stumpf, P.K., Conn, E.E. (ed.): The Biochemistry of Plants. A Comprehensive Treatise. Vol. 4.

Pp. 2-48. Academic Press, New York - London - Toronto - Sydney 1980.

Hubac, C., Guerrier, D., Ferran, J., Trémolières, A.: Change of lipid composition during water stress on two genotypes resistant or susceptible to drought. - Plant Physiol. Biochem. 27: 737-744, 1989.

Jacquinot, L., Forget, M., Edah, K.A.: Résistance à la transpiration chez le riz pluvial (*Oryza sativa*). Ètude d'un test de criblage variétal. - Agron. trop. 36: 247-252, 1981.

Kaiser, W.M.: Effects of water deficit on photosynthetic capacity. - Physiol. Plant. 71: 142-149, 1987a.

Kaiser, W.M.: Non-stomatal, primary dehydration effects on photosynthesis: possible mechanisms for reversible and irreversible damage. - Curr. Top. Plant Biochem. Physiol. 6: 119-133, 1987b.

Kramer, P.J.: Water Relations of Plants. - Pp. 342-389. Academic Press, New York 1983.

Kriedemann, P.E.: Stomatal and photosynthetic limitations to leaf growth. - Aust. J. Plant Physiol. 13: 15-31, 1986.

Lawlor, D.W., Uprety, D.C.: Effects of water stress on photosynthesis of crops and the biochemical mechanism. - In: Abrol, Y.P., Mohanty, P., Govinjee (ed.): Photosynthesis: Photoreaction to Plant Productivity. Pp. 419-449. Kluwer Academic Publ., Dordrecht - Boston - London 1993.

Meyer, S., Hung, S.P.N., Trémolières, A., de Kouchkovsky, Y.: Energy coupling, membrane lipids and structure of thylakoids of Lupin plants submitted to water stress. - Photosynth. Res. 33: 95-107, 1992.

Navari-Izzo, F., Milone, M.T.A., Quartacci, M.F., Pinzino, C.: Metabolic changes in wheat plants subjected to water-deficit stress programme. - Plant Sci. 92: 151-157, 1993a.

Navari-Izzo, F., Quartacci, M.F., Izzo, R.: Lipid changes in maize seedlings in response to field water deficit. - J. exp. Bot. 40: 675-680, 1989.

Navari-Izzo, F., Quartacci, M.F., Melfi, D., Izzo, R.: Lipid composition of plasma membranes isolated from sunflower seedlings grown under water stress. - Physiol. Plant. 87: 508-514, 1993b.

Navari-Izzo, F., Ricci, F., Vazzana, C., Quartacci, M.F.: Unusual composition of thylakoid membranes of the resurrection plant *Boea hygroscopica*: changes in lipids upon dehydration and rehydration. - Physiol. Plant. 94: 135-142, 1995.

Navari-Izzo, F., Vangioni, N., Quartacci, M.F.: Lipids of soybean and sunflower seedlings grown under drought conditions. - Phytochemistry 29: 2119-2123, 1990.

Pham Thi, A.T., Vieira da Silva, J., Mazliak, P.: The role of membrane lipids in drought resistance of plants. - Bull. Soc. bot. Fr. 137: 99-114, 1990.

Premachandra, G.S., Shimada, T.: Evaluation of polyethylene glycol test of measuring cell membrane stability as a drought tolerance test in wheat. - J. Agr. (Cambridge) 110: 2000-2005, 1988.

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

Quinn, P.J., Williams, W.P.: The structural role of lipids in photosynthetic membranes. - Biochim. biophys. Acta 737: 223-266, 1983.

Raison, J.K.: Membrane lipids: structure and function. - In: Stumpf, P.K., Conn, E.E. (ed.): The Biochemistry of Plants. A Comprehensive Treatise. Vol. 4. Pp. 57-81. Academic Press, New York - London - Toronto - Sydney 1980.

Senaratna, T., McKersie, B., Stinson, R.H.: Association between membrane phase properties and dehydration in soybean axes. - Plant Physiol. 76: 759-762, 1984.

Siefermann-Harms, D., Ninneman, H., Yamamoto, H.Y.: Reassemble of solubilized chlorophyll-protein complexes in proteolipid particles - comparison of monogalactosyl-diacylglycerol and two phospholipids. - Biochim. biophys. Acta 892: 303-313, 1987.

Stefanov, K., Markovska, Y., Kimenov, G., Popov, S.: Lipid and sterol changes in leaves of *Haberlea rhodopensis* and *Ramonda serbica* at transition from biosis into anabiosis and vice versa caused by water stress. - Phytochemistry 31: 2309-2314, 1992a.

Stefanov, K., Popova, I., Nikolova-Damyanova, B., Kimenov, G., Popov, S.: Lipid and sterol changes in *Phaseolus vulgaris* caused by lead ions. – Phytochemistry 31: 3745-3748, 1992b.

Vasquez-Tello, A., Zuily-Fodil, Y., Pham Thi, A.T., Vieira da Silva, J.B.: Electrolyte and Pi leakage and soluble sugar content as physiological tests for screening resistance to water stress in *Phaseolus* and *Vigna* species. - J. exp. Bot. 41: 827-832, 1990.

Vu, J.C., Allen, L.H., Jr., Bowes, G.: Drought stress and elevated CO_2 effects on soybean ribulose *bis*phosphate carboxylase activity and canopy photosynthetic rates. - Plant Physiol. 83: 573-578, 1987.

Webb, M.S., Green, B.R.: Biochemical and biophysical properties of thylakoid acyl lipids. – Biochim. biophys. Acta 1060: 133-158, 1991.

Weischen, R., Berkotte, M.: Soluble and insoluble sugars. - In: Weischen, R., Berkotte, M. (ed.): Ecophysiology and Book of Methods. Pp. C37-C38. Ultrecht 1994.

Zuily-Fodil, Y., Vazquez-Tello, A., Vieira da Silva, J.: Effect of water deficit on cell permeability and on chloroplast integrity. - Bull. Soc. bot. Fr., Actual. bot. 137: 115-123, 1990.

Zúñiga, G.E., Fernandez, J., Cristi, R., Alberdi, M., Corcuera, L.J.: Lipid changes in barley seedlings subjected to water and cold stress. - Phytochemistry 29: 3087-3090, 1990.