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 ($P_{\text{max}}$), 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 $P_{\text{max}}$ was found in the cvs. 57-422 and 73-30.

Additional key words: Arachis hypogaea; digalactosyldiacylglycerol; galactolipids; membrane stability; monogalactosyldiacylglycerol; phosphatidyglycerol; 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 lowering 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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Abbreviations: DGDG = digalactosyldiacylglycerol; FFA = free fatty acid; GL = galactolipids; MGDG = monogalactosyldiacylglycerol; $P_{\text{max}}$ = photosynthetic capacity; PC = phosphatidylcholine; PG = phosphatidyglycerol; PI = phosphatidylinositol; PL = phospholipids.
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 (Zuiy-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, Zuiy-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 2500 cm³ pots (one plant per pot), filled with a mixture of vermiculite and Triohum 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 Čatsky (1960), in samples of 10 foliar discs of 0.5 cm² 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 cm² 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 (Crisdon 552, Crison Instruments, Spain). The % of relative membrane damage was expressed as an injury index,lace = [I - (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 % and changes in photosynthetic activity, photosynthetic capacity (Pₘₐₓ) 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 CO₂ conditions. For the control plants, the 6-7% CO₂ was provided with 400 mm³ of KHCO₃ (2 M). For the plants submitted to drought, an air flow with 15% CO₂ was provided to completely overcome the stomatal resistance, according to preliminary experiments. Results for Pₘₐₓ 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 scraped 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

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 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).

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 Matttowe 1981, Premachandra and Shimada 1988, Zuiy-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 Zuiy-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 $P_{\text{max}}$ 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 $P_{\text{max}}$ 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 controversial (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, Zufina et al. 1990). Nevertheless, the knowledge of the response under mild water deficit is 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 vulgarius.

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 ± S.E (n = 3).

<table>
<thead>
<tr>
<th>Cv.</th>
<th>Treatment</th>
<th>C</th>
<th>S₁</th>
<th>S₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>57-422</td>
<td>MGDG</td>
<td>748 ± 20 (k,a)</td>
<td>613 ± 19 (b,x)</td>
<td>431 ± 9 (c,x)</td>
</tr>
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<td></td>
<td>DGDG</td>
<td>292 ± 6 (k,a)</td>
<td>253 ± 5 (b,x)</td>
<td>145 ± 3 (c,x)</td>
</tr>
<tr>
<td></td>
<td>GL</td>
<td>1040 ± 14 (k,x)</td>
<td>866 ± 22 (b,x)</td>
<td>676 ± 7 (c,x)</td>
</tr>
<tr>
<td></td>
<td>PC</td>
<td>57 ± 1 (k,x)</td>
<td>50 ± 1 (b,x)</td>
<td>31 ± 2 (c,x)</td>
</tr>
<tr>
<td></td>
<td>PG</td>
<td>74 ± 2 (k,x)</td>
<td>57 ± 1 (b,x)</td>
<td>43 ± 1 (c,x)</td>
</tr>
<tr>
<td></td>
<td>PI</td>
<td>95 ± 0 (k,x)</td>
<td>79 ± 1 (b,x)</td>
<td>48 ± 1 (c,x)</td>
</tr>
<tr>
<td></td>
<td>PL</td>
<td>226 ± 2 (k,x)</td>
<td>186 ± 4 (b,x)</td>
<td>114 ± 5 (c,x)</td>
</tr>
<tr>
<td></td>
<td>total</td>
<td>1267 ± 14 (k,x)</td>
<td>1052 ± 27 (b,x)</td>
<td>689 ± 10 (c,x)</td>
</tr>
<tr>
<td></td>
<td>MGDG/DGDG</td>
<td>3 ± 0 (a,b,x)</td>
<td>2 ± 0 (b,x)</td>
<td>3 ± 0 (c,x)</td>
</tr>
<tr>
<td>73-30</td>
<td>MGDG</td>
<td>712 ± 18 (k,x)</td>
<td>318 ± 3 (c,t)</td>
<td>393 ± 4 (b,x)</td>
</tr>
<tr>
<td></td>
<td>DGDG</td>
<td>345 ± 6 (k,x)</td>
<td>136 ± 4 (c,t)</td>
<td>180 ± 9 (b,x)</td>
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<td>GL</td>
<td>1057 ± 23 (k,x)</td>
<td>453 ± 4 (c,t)</td>
<td>573 ± 6 (b,x)</td>
</tr>
<tr>
<td></td>
<td>PC</td>
<td>73 ± 0 (k,x)</td>
<td>26 ± 1 (c,t)</td>
<td>34 ± 1 (b,x)</td>
</tr>
<tr>
<td></td>
<td>PG</td>
<td>86 ± 1 (k,x)</td>
<td>31 ± 1 (c,t)</td>
<td>43 ± 0 (b,x)</td>
</tr>
<tr>
<td></td>
<td>PI</td>
<td>118 ± 2 (k,x)</td>
<td>46 ± 2 (c,t)</td>
<td>60 ± 0 (b,x)</td>
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<tr>
<td></td>
<td>PL</td>
<td>277 ± 3 (k,x)</td>
<td>103 ± 4 (c,t)</td>
<td>134 ± 7 (b,x)</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>1333 ± 22 (k,x)</td>
<td>566 ± 7 (c,t)</td>
<td>710 ± 12 (b,x)</td>
</tr>
<tr>
<td></td>
<td>MGDG/DGDG</td>
<td>2 ± 0 (k,x)</td>
<td>2 ± 0 (b,x)</td>
<td>2 ± 0 (c,x)</td>
</tr>
<tr>
<td>GC 8-35</td>
<td>MGDG</td>
<td>329 ± 7 (b,a)</td>
<td>513 ± 16 (k,a)</td>
<td>274 ± 9 (c,a)</td>
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<td>DGDG</td>
<td>152 ± 15 (b,a)</td>
<td>197 ± 11 (k,a)</td>
<td>109 ± 9 (c,a)</td>
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<tr>
<td></td>
<td>GL</td>
<td>480 ± 23 (b,a)</td>
<td>709 ± 26 (k,a)</td>
<td>383 ± 17 (c,a)</td>
</tr>
<tr>
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<td>PC</td>
<td>33 ± 2 (b,t)</td>
<td>44 ± 2 (k,a)</td>
<td>24 ± 1 (c,t)</td>
</tr>
<tr>
<td></td>
<td>PG</td>
<td>39 ± 1 (b,t)</td>
<td>49 ± 1 (k,a)</td>
<td>24 ± 0 (c,t)</td>
</tr>
<tr>
<td></td>
<td>PI</td>
<td>51 ± 1 (b,t)</td>
<td>67 ± 2 (k,a)</td>
<td>34 ± 1 (c,t)</td>
</tr>
<tr>
<td></td>
<td>PL</td>
<td>122 ± 5 (b,t)</td>
<td>160 ± 3 (k,a)</td>
<td>82 ± 3 (c,t)</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>602 ± 17 (b,t)</td>
<td>869 ± 28 (k,a)</td>
<td>466 ± 19 (c,a)</td>
</tr>
<tr>
<td></td>
<td>MGDG/DGDG</td>
<td>2 ± 0 (k,a)</td>
<td>3 ± 0 (k,a)</td>
<td>3 ± 0 (a,x)</td>
</tr>
</tbody>
</table>
PEANUT THYLAKOIDS AND DROUGHT

(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 hygroskopica (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 Siegmann-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 Pmax values. Therefore a positive relationship between acyl lipid concentration stability, membrane integrity, and Pmax 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


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