State of the lipid and fatty acid composition in chloroplasts of *Nicotiana tabacum* under the influence of an increased CO$_2$ partial pressure of 700 p.p.m.

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**Abstract**
We analysed chloroplast lipids of *Nicotiana tabacum* var. John William’s Broadleaf, cultivated under an increased P$_{CO_2}$ of 700 p.p.m. Glycolipids and phospholipids remain constant under these conditions, whereas the carotenoid content undergoes a quantitative change. The saturation degree of fatty acids increases due to an increase in palmitic acid and decreases in hexadecatrienoic acid and linolenic acid.

**Introduction**
Cultivation of *Nicotiana tabacum* plants under an increased P$_{CO_2}$ of 700 p.p.m. led to changes in the structural protein subunits of photosystem II and photosystem I. This refers above all to the core peptides, light-harvesting complex (LHCP) subunits and proteins of the proximal antenna [1,2]. In the context of these observed changes it was of interest to also analyse the lipids of the chloroplast membrane and the lipids which by intermolecular interactions are very tightly adsorbed on to protein subunits. These analyses were performed because in total leaf lipids of *N. tabacum* and the Chinese tung oil tree *Aleurites montana*, which were cultivated under increased-CO$_2$ conditions, quantitative changes of the membrane lipid composition were found [3-5]. Despite the fact that the total amount of glycolipids remained constant, individual glycolipids exhibited variations. Whereas MGDG (monogalactosyldiacylglycerol) was decomposed by 10% more than in the control, DGDG (digalactosyldiacylglycerol) and SQDG (sulphoquinovosyldiacylglycerol) were more intensively synthesized. Among phospholipids, which represent the main lipids of the cell membrane, the thylakoid-membrane-forming phospholipids PG (phosphatidylglycerol), PC (phosphatidylcholine) and PI (phosphatidylinositol) were increased under these conditions. Plastoquinone, which functions as an electron and proton carrier, was increased 2-fold in chloroplasts [6].

**Methods**
Chloroplasts were isolated by centrifugation in a sucrose-density gradient [7]; sucrose was subsequently removed by washing with water. In this procedure the envelope membranes are disrupted and the stroma is washed out, yielding in these preparations only thylakoid membranes. For the extraction of lipids the chloroplasts were boiled for 1 h in ethanol under back-flow with the remaining lipids being extracted with acetone and diethyl ether. The lipids were taken up with diethyl ether. Glycolipids and phospholipids were analysed by TLC on silica gel plates and photo-metrically determined by means of anthrone...
reagent, or in the case of phospholipids with Fiske-Subbarow reagent [4]. Pigments were also photometrically determined and fatty acids were analysed by GC [4].

Results and discussion

As seen from Table 1, as the main lipid of the thylakoid membrane MGDG remained unchanged under these conditions and, despite the fact that a slight increase in SQDG and a decrease in DGDG was observed, the total amount of glycolipids remained constant. Also the phospholipids, which represent the cell-membrane lipids, showed a small variability in PG, but no quantitative changes, as with the glycolipids.

As expected, the strongest changes were found in terms of pigment composition. Thus, the chlorophyll ratio decreased due to the reduction of the chlorophyll content. This in turn is due to the slight reduction of the LHCP under these increased-CO$_2$ conditions. The strongest changes were found with carotenoids. This concerns, in the first place, the two carotenoids violaxanthin and zeaxanthin, involved in the xanthophyll cycle. It appears that violaxanthin under increased Pco$_2$, which causes the same changes in the plant as high illumination, as shown by us earlier [1,2], was transformed to zeaxanthin. The two main carotenoids β-carotene and lutein, which exert a protective function in redox reactions, were more strongly synthesized, whereas neoxanthin was decomposed.

The results of the GC fatty acid analyses are summarized in Table 2. It is very clear that the saturation degree of fatty acids increased. This is due to an increase in palmitic acid and to the decrease of the trienoic fatty acids hexadecatrienoic and linolenic acid. The chain length of the fatty acids remained unchanged.

Taking together earlier analyses of total leaf lipids showing that leaf lipids are subject to larger quantitative changes under increased-CO$_2$ conditions [3–5], studies showing that half of leaf lipids occur inside chloroplasts and the other half outside [8] and, as described here, the observed constancy of chloroplast lipids, it is concluded that under the increased Pco$_2$ of 700 p.p.m. the lipids of other cell organelles such as mitochondria and the Golgi apparatus, as well as lipids of cell membranes, such as the endoplasmic reticulum, the tonoplast and the plasmalemma, have undergone changes.

| Table 1 |
| Composition of chloroplast lipids of N. tabacum var. John William’s Broadleaf, cultivated under an increased Pco$_2$ of 700 p.p.m. in air |

Lipid compositions are given as a percentage of total lipids. Chl a+b, chlorophylls a+b; Vio, violaxanthin; Zea, zeaxanthin; β-Co, β-carotene; Lu, lutein; Neo, neoxanthin; STGL, sterylglucoside.

<table>
<thead>
<tr>
<th>Lipid composition</th>
<th>Chl a+b</th>
<th>a/b ratio</th>
<th>MGDG</th>
<th>DGDG</th>
<th>SQDG</th>
<th>STGL</th>
<th>PG</th>
<th>PI</th>
<th>β-Co</th>
<th>Lu</th>
<th>Zea</th>
<th>Vio</th>
<th>Neo</th>
</tr>
</thead>
<tbody>
<tr>
<td>N. tabacum control</td>
<td>36.5</td>
<td>1/2.7</td>
<td>28.3</td>
<td>20.4</td>
<td>4.9</td>
<td>0.4</td>
<td>0.4</td>
<td>3.6</td>
<td>1.1</td>
<td>0.8</td>
<td>0.84</td>
<td>2.00</td>
<td>0.15</td>
</tr>
<tr>
<td>N. tabacum CO$_2$</td>
<td>35.2</td>
<td>1/2.4</td>
<td>28.4</td>
<td>18.6</td>
<td>6.5</td>
<td>0.4</td>
<td>3.1</td>
<td>1.1</td>
<td>0.9</td>
<td>1.42</td>
<td>3.12</td>
<td>0.51</td>
<td>0.28</td>
</tr>
</tbody>
</table>

| Table 2 |
| Fatty acid composition of lipids of chloroplasts of N. tabacum var. John William’s Broadleaf cultivated under an increased Pco$_2$ of 700 p.p.m. in air |

Fatty acids are given as percentage of total fatty acids.

<table>
<thead>
<tr>
<th>Fatty acid composition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C$_{12:0}$</td>
</tr>
<tr>
<td>---------------------------</td>
</tr>
<tr>
<td>N. tabacum control</td>
</tr>
<tr>
<td>N. tabacum CO$_2$</td>
</tr>
</tbody>
</table>

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References

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Role of lipids in molecular thermoadaptation mechanisms of seagrass Zostera marina

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Abstract
By using the combined approaches of micro-calorimetric analysis and polarizing microscopy, the crystal–liquid crystal–isotropic melt phase transition of major phospholipids (phosphatidylycerine and phosphatidylethanolamine) and glycolipids (monogalactosyldiacylglycerol and digalactosyldiacylglycerol) isolated from marine seagrass Zostera marina were investigated. Here we discuss the results in view of the fatty acid structures of the lipids studied.

Introduction
As is known, primary thermoadaptation changes occur on the molecular level. These changes can be reached first of all at the expense of reorganization of the composition and intramolecular structure of membrane lipids. The liquid-crystalline state of lipid matrix is necessary for physiological and biochemical functions of membranes and depends on different environmental factors, with the most important effects being those of temperature [1,2]. There has been relatively little research investigating the physico-chemical changes of plant lipids during temperature adaptation [3]. In contrast to animals, plant membrane lipids are predominantly represented by glycolipids [4–6], which are not only structural components of chloroplasts, but also participate in photosynthetic processes [4,5]. Zostera marina belongs to the marine macrophytes and carries out an important role in marine ecosystems [7,8]. Also it is used in medicine, agriculture etc. [8]. With this view, the present work was a phase-transition study of major glycolipids [monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG)] as well as phospholipids [phosphatidylcholine (PC) and phosphatidylethanolamine (PE)] of Z. marina. In addition, fatty acid composition, which defines the thermotropic behaviour of lipids [3,9–12], was also analysed.

Materials and methods
Seagrass Z. marina was harvested in Possiet Bay (Sea of Japan) in August at 20 °C in seawater. Freshly collected seagrass was cleaned and heated to inactivate enzymes as described in [13]. Total lipid extracts from about 10 kg of seagrass were obtained according to the method of Folch et al. [14]. Crude glyco- and phosho-lipids were isolated by column chromatography on silica gel by elution with acetone, acetone/benzene/acid/water (200:30:3:10, by vol.) and a gradient of chloroform and methanol. Then, lipids were purified by preparative silica TLC using chloroform/methanol/water (65:25:4, by vol.). Chromatographically pure lipids were solubilized in chloroform and introduced into standard