Influence of Different Light Intensities on the Content of Diosgenin, Lipids, Carotenoids and Fatty Acids in Leaves of *Dioscorea zingiberensis*

Huming Li[a], Alfons Radunz[a], Ping He[b] and Georg H. Schmid[a,*]

[a] Lehrstuhl für Zellphysiologie, Fakultät für Biologie, Universität Bielefeld,
Postfach 10 01 31, 33501 Bielefeld, Bundesrepublik Deutschland.
Fax: +49 (521) 1066410. E-mail: G.Schmid@Biologie.Uni-Bielefeld.de
[b] Central South Forestry University, Zhuzhou/Hunan 41206, People's Republic of China

* Author for correspondence and reprint requests

Cultivation of the climbing plant *Dioscorea zingiberensis* at a light intensity of 100 μE · m^{-2} · sec^{-1} yields three different phenotypes. Most of the plants grow as green phenotype (DzW). Two further forms differ in their leaf shape and leaf color. Whereas one type exhibits a more pointed leaf shape in the upper part of the plant with leaves appearing yellow-green with white stripes or hatchings (DzY), the other type shows a more round leaf shape with an intensive yellow-green color (DzT). These three plant types differ in their diosgenin content not only in their rhizomes but also in the chloroplasts. In the rhizomes the diosgenin content in the green form is 0.4%, in the DzY-form 0.6% and in the DzT-form even 1.3% of the dry weight. Furthermore, even in chloroplasts of the green DzW-form and of the DzY-form the presence of diosgenin was demonstrated. It occurs there as the epimeric form yamogenin. The DzT-form contains no yamogenin in its chloroplasts. Besides this, these plant forms differ in their chlorophyll and carotenoid content and in their fatty acid composition. Carotenoids increase from 1.3% of total lipids in the green phenotype to 3.3% in the DzY- and to 4.2% in the DzT-form. This increase refers to β-carotene as well as to lutein and neoxanthin. The chlorophyll content in the green type is 8.1% and lower in the DzY-form with 7%. The highest chlorophyll content is found in the DzT-form with 12%. Fatty acids in the DzY-form and in the DzT-form have a more unsaturated character than in the green phenotype. The content of the monoenoic acid trans-hexadecenoic acid is considerably lower in both phenotypes when compared to the green phenotype. In both phenotypes the quantity of fatty acids with 16 carbon atoms is reduced, whereas fatty acids with 18 carbon atoms occur in higher concentration.

Cultivation of the green phenotype (DzW) at the three light intensities of 10, 100 and 270 μE · m^{-2} · sec^{-1} leads to changes of the diosgenin content in rhizomes, to an increase of leaf dry weight, to a reduction of the grana structure in chloroplasts and therewith to a decrease of the chlorophyll content. The total lipid content is highest under the cultivation at 100 μE · m^{-2} · sec^{-1} and reduced by 30% at 10 and 270 μE · m^{-2} · sec^{-1}. Carotenoids, however, are highest in shaded plants (10 μE · m^{-2} · sec^{-1}) and plants grown under high light conditions of 270 μE · m^{-2} · sec^{-1}. At 100 μE · m^{-2} · sec^{-1} a decrease of saturated fatty acids is observed in comparison to plants grown under shaded conditions.

Introduction

Dioscorea zingiberensis C. H. Wright is a creeping plant, which grows as wild species in the mountain regions of South China (Ding, 1983). It belongs systematically to the *Dioscoreaceae*. When several years old, the plant develops an extended rhizome. In such a rhizome system up to 16.5% of the dry weight are diosgenin (Ding, 1983; Chou et

al., 1965; Tang *et al.*, 1979; Xu and Liu, 1984; Wang and Chou, 1964; Liu and Chen, 1985; Mahato *et al.*, 1982; Tang and Jiang, 1987) which is a steroid sapogenin. Besides this, diosgenin was also detected in chloroplasts. It occurs there in the epimeric form as yamogenin (Tang and Jiang, 1987). Diosgenin serves in the pharmaceutical industry as starting material for the production of corticosteroid sexual hormon preparations as well as for the production of anabolic agents and cardioglycosides. This has made of the plant in the last 20 years in China a valuable raw material source for

Abbreviations: TMSH, Trimethylsulfonium hydroxide.

pharmaceutical industry (Tang and Jiang, 1987). As the quantity of the wild growing plants is not sufficient and as it is known that photosynthesis and growth of this plant depend strongly on the light intensity (He, 1987; Demming-Adams and Adams, 1992; Genty, 1996), the successful cultivation of this plant depends heavily on the knowledge of the necessary light intensities.

Material and Methods

Growth of plants

Seeds of the green phenotype of *Dioscocrea zingiberensis* (DzW) were obtained from the Institute of Ecology of the Jishon University in Hunan/China. Seeds were germinated in a greenhouse of the University of Bielefeld. Usually, two seedlings were planted in one flower pot and put for growing into a fully climatized growth chamber. The light-dark cycle was 14 hours light/10 hours dark (Lamp type: Sylvania F96/GRO/VHO/WS). Temperature during the light phase was 27 °C and lowered in the dark period to 22 °C. Humidity was kept at 60% (He *et al.*, 1995). The plants were fertilized twice per week with Wuxal Super (Firma AGLUKON, Düsseldorf, Germany). Different light conditions were used. The light intensities used throughout the growth period were 10, 55, 100 or 270 $\mu E \cdot m^{-2} \cdot sec^{-1}$ respectively. Light intensity was measured with the Quanta-Spectrometer QSM-2500 from Tektrum Instruments, Umeå, Sweden. 45 days after the transplantation into the pots, leaves from the plants were harvested and lipids, carotenoids and fatty acids were determined. Under the light condition of 100 $\mu E \cdot m^{-2} \cdot sec^{-1}$ three phenotypes were observed. Most of the plants exhibited a green phenotype of *Dioscorea zingiberensis* (DzW). However, two further types, different from the phenotype of the green form appeared and are designated as DzT and DzY. The T-form develops rounder leaves and exhibits a green leaf color, sometimes a darkgreen color, whereas the Y-form has a more pointed leaf shape in the upper leaf area and carries white stripes on the leaf surface (Fig. 1). Here the leaf color always appeared yellow-green. The phenotypes emerged from any given seed-lot that is any seed-population yields these three forms.

Lipid extraction and determination of lipids

10 g of fresh leaves were homogenized at room temperature with a solvent mixture of isopropanol, chloroform and methanol (1:1:1, v/v/v) and extracted with this solvent mixture on a glass funnel until the leaf material appeared colorless. The raw lipids were concentrated by destillation of the solvent in a rotation evaporator and subsequently dissolved in a mixture of chloroform/ methanol (2:1, v/v) and centrifuged at 5000 xg. The supernatant was saved and the lipids were washed with a mixture of chloroform water (4:1, v/v). The water phase was shaken twice with chloroform in order to extract all lipids. The chloroform phase was dried and the lipids were dissolved for chromatography in 0.5 ml toluene. Thin layer chromatography was done on silica gel plates in the solvent system chloroform, methanol, acetic acid, water (85:15:10:3.5, v/v/v/v) (He *et al.*, 1995; Radunz, 1969). For the development of glycolipids, the silica gel plates were sprayed with anthrone-sulfuric acid and heated to 95 °C. Galactolipids appear as green-brown and sulfolipids as blue-violet spots. Phospholipids were marked by spraying with phosphatide reagent (He *et al.*, 1996). For the labelling of all lipids the plates were sprayed with phosphomolybdic acid (0.5% in ethanol) and heated for 20 minutes to 100 °C. Lipids appear as dark-blue spots on yellow background.

Carotenoid and fatty acid determination

10 mg lipids were heated with 40 ml of methanolic 0.5 N NaOH for 1.5 h under back flow conditions. 40 ml water were added and the carotenoids were separated by shaking with three portions of 30 ml petrol ether (40–60 °C). The carotenoids were dried and concentrated by vacuum rotation evaporation and dissolved in 100 μl ethanol and analysed by HPLC on a reversed phase RP-18-column (Merck, Darmstadt). The HPLC-facility (Kontron Analytic GmbH, Munich, Germany) was equipped with 2 pumps (LC-pumps 410), a programming module 200, and with the spectrometer Uvikon 720 LC also from Kontron. As a standard authentic carotenoids and chlorophylls from Sigma and Hoffmann-La Roche, Basel, were used. The carotenoid analysis was carried out with solvent mixture A consisting of methanol/water (80:20, v/v) and the solvent mixture B consisting

of methanol/acetone (60:40, v/v). The analysis was started with solvent system A and then switched with a flow rate of 1.5 ml/min to solvent mixture B so that after 20 min towards the end of the analysis, elution was only done with solvent B. For cleaning, the column was washed with solvent mixture B.

For obtaining the fatty acids the solution of the alkaline hydrolysis was acidified with HCl in order to liberate fatty acids. Thereafter fatty acids were taken up by shaking three times with petrol ether (40–60 °C). For drying the fatty acids were concentrated and dissolved in 100 μl hexane. By addition of 100 μl TMSH(trimethylsulfonium hydroxide)-reagent (Macherey and Nagel, Düren, Germany) fatty acids were transformed into their methyl esters and analysed in a gas chromatograph (Hewlett Packard, 58900, Series II plus) via a 10 m ethylene glycolsuccinate capillary column at 190 °C. The carrier gas was nitrogen. The temperature of the injection block and detector was 300 °C (Radunz, 1969; Radunz *et al.*, 1998; He *et al.*, 1996). As fatty acid standards authentic methyl esters from Nu-check Prep. Inc. Elysian, MN, USA were used.

Determination of diosgenin

0.5 g of rhizome or leaves which had been dried at 78 °C were ground and heated with 10 ml 2 N HCl for 3.5 h at 100 °C under backflow in order to split diosgenin from dioscin which is a 3-O-glycoside (Fig. 2). After cooling, the extract was adjusted to pH 7.0 by addition of 2 N NaOH. The tissue brei was dried at 78 °C and for extraction of diosgenin extracted with petrol ether for 4 h at room temperature (Ding, 1983; Radunz and Schmid, 2000). The petrol ether phases were filtered and concentrated to dryness by vacuum rotation evaporation. The remaining diosgenin was taken up in 5 ml chloroform. The analysis and concentration determination was done by HPLC on a reverse phase RP-18-column (Liu and Chen, 1985; Radunz and Schmid, 2000). As diosgenin standard we used diosgenin from Sigma. The solvent phase for the chromatographic analysis was 95% aquous methanol. The flow rate was 0.7 ml/min. Measurements were carried out at 210 nm.

Beside the determination by HPLC, diosgenin was also analysed by thin-layer chromatography on silica gel G-layers in the solvent system chloroform/petrol ether (40–60 °C bp) 4:1 (v/v). The Rf-value was identical (0.65) to that obtained with the diosgenin standard from Sigma.

Chlorophyll was determined according to Schmid (1971) in 10% aqueous methanol.

Results and Discussion

Dioscorea zingiberensis plants which are grown from seeds in a fully automatic growth chamber under identical growth conditions with respect to temperature, humidity, nutrition and a light intensity of $100 \, \mu E \cdot m^{-2} \cdot sec^{-1}$ always represent a plant population consisting of 3 phenotypes. A majority of the *Dioscorea z.* (DzW) plants has a green phenotpye but additionally two other phenotypes are observed (Fig. 1). They differ from the green type with respect to leaf shape and form but also with respect to their physiological properties. The two plants are denoted as DzT- and DzY-form. Leaves of the DzT-form have round shaped leaves and appear green or light-green (Fig. 1), whereas leaves of the DzY-form have a more pointed leaf shape and are intensively yellow-green with a peculiar white hatching (Fig. 1). However, the descisive difference is the diosgenin content in the rhizomes which makes these plants to be a valuable object for the pharmaceutical industry. The diosgenin content in two phenotypes (DzY and DzT) is in comparison to the green type, where it amounts to 0.4% of dry weight, strongly increased. Thus, the content in the rhizome of the DzY-form is 0.65% and in the DzT-form 1.3% of the dry weight. Besides the occurrence of diosgenin in the rhizomes, diosgenin was also detected in low quantities in chloroplasts of the green phenotype (DzW) and of the DzY-form. In chloroplasts diosgenin occurs in the epimeric form as yamogenin (Fig. 2). In chloroplasts of the DzT-phenotype diosgenin is apparently lacking. For the evaluation of chloroplast structure and function this observation is of great interest (Luckner, 1990), above all as it is known that sapogenins cause permeability changes in cell membranes. The interrelationship between localisation of diosgenin in chloroplasts and its function will be published elsewhere.

In view of these findings it is of interest to analyse leaf lipids, carotenoids and fatty acids of these morphologically differnt plant types. In an analysis
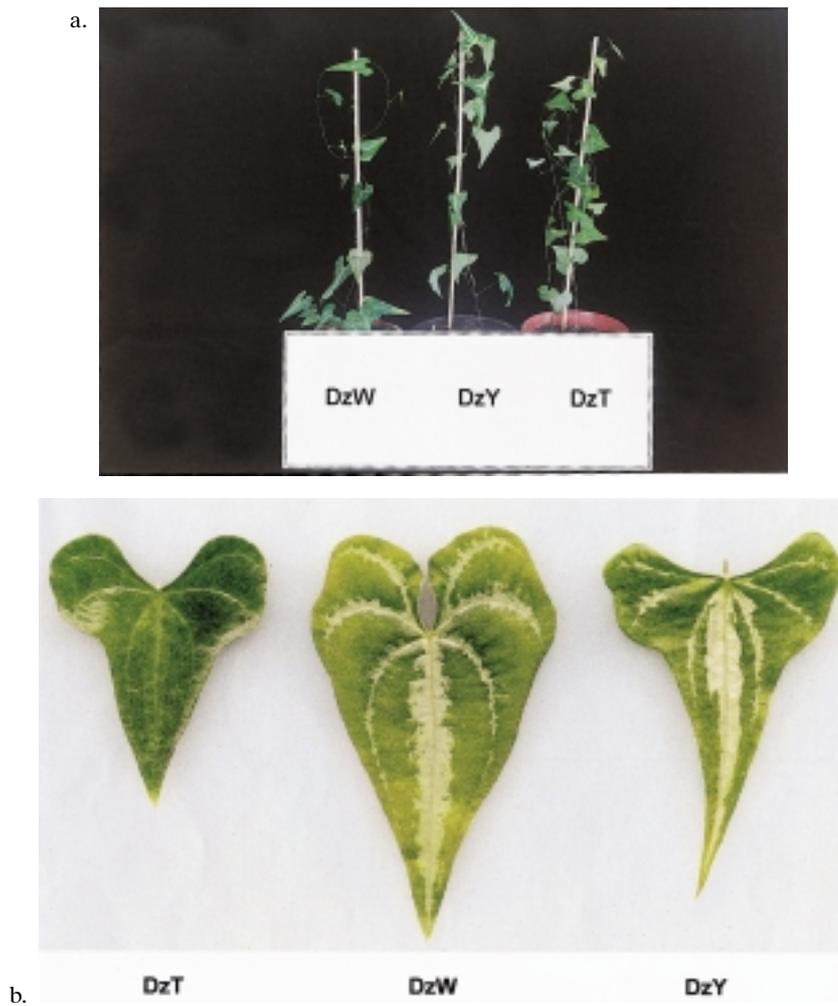
Fig. 1. *Dioscorea zingiberensis*: a. Plant phenotypes DzW, DzY, DzT, b. Leaf shape of the three phenotypes observed when grown under the light intensity of 100 μE · m^{-2} · s^{-1} and otherwise identical conditions. DzW, normal green type; DzY, yellow-green phenotype; DzT, green or light-green phenotype.

by thin layer chromatography on silica gel G layers the lipids of the 3 phenotypes were compared (data not shown). As in leaf lipids of all higher plants (He *et al.,* 1996; Radunz, 1966a; Douce and Joyard, 1980) glycolipids appear in the highest concentration. Here, monogalactosyldiglyceride is the main component followed by digalactosyldiglyceride. Sulfoquinovosyldiglyceride occurs in lesser quantitites. Among phospholipids phosphatidylcholine is strongest followed by phosphatidylethanolamine, phosphatidylglycerol and phosphatidylinositol. Diphosphatidylglycerol also occurs in traces. The striking observation is that in the DzY-form the two anionic lipids phosphatidylglycerol and sulfoquinovosyldiglyceride but also phosphatidylinositole occur in lesser amounts than in the green phenotype (DzW) and in the DzT-form (data not shown). The fatty acid pattern of *Dioscorea z.* leaves corresponds to the fatty acid composition of leaves of other higher plants (Table I) (Douce and Joyard, 1980; Radunz, 1966b; Bednarz *et al.*, 1988). The main amount is formed by fatty acids with 18 carbon atoms and these make up for nearly 3/4 of all fatty acids, with linolenic and linoleic acid being the major components. Relatively high appears the portion of oleic acid, which in
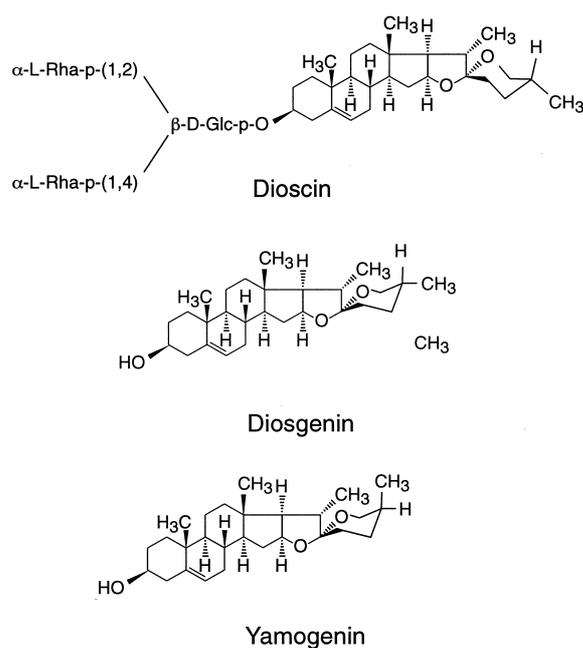
Fig. 2. Structure of dioscin, diosgenin and yamogenin.

other higher plants occurs usually only with half the amount. Fatty acids with 16 carbon atoms make up for 1/5 to 1/4 of total fatty acids, with palmitic acid being the main component. It is noted that, despite the fact that these plants have been cultivated in a growth chamber (and not outdoor), in leaves of *Dioscorea* plants, the portion of saturated fatty acids is high and makes up for 20–30% of total fatty acids. Short-chain carbonic acids with 12, 14 and 15 carbon atoms as well as long-chain saturated fatty acids with 20 carbon atoms occur only in low concentration as in other higher plants. Fatty acids with 22 and more carbon atoms occur only in traces.

The two above described phenotypes DzT and DzY differ from the green type DzW in their fatty acid pattern and also in their carotenoid composition (Table II). In both phenotypes fatty acids with 16 carbon atoms appear diminished and carbonic acids with 18 carbon atoms are correspondingly increased in their concentration. The saturation degree appears lesser in both phenotypes with the unsaturated fatty acids being stronger developed, despite the fact that the ratio of linolenic and linoleic acid is in both phenotypes inversed in comparison to the green type DzW. Thus, in the leaves

Table I. Fatty acid composition of leaf lipids of *Dioscorea zingiberensis* plants which differ under cultivation at identical environmental conditions as temperature, humidity, nutrients and a light intensity of 100 μE m^{-2} s^{-1} in their phenotype. DzW corresponds to the normal green type, DzT to a light-green phenotye with more round leaves and DzY to a yellow-green phenotpye with white-hatched leaves. Fatty acids are given in % of total fatty acids.

Fatty acids	*Dioscorea zingiberensis* Phenotype		
	DzW	DzT	DzY
$C_{12:0}$	0.3	0.5	0.3
$C_{12:x}$	3.5	0.1	2.0
$C_{14:0}$	0.8	1.3	0.8
$C_{15:0}$	0.3	0.3	0.3
$C_{16:0}$	19.2	16.4	17.0
$C_{16:1 \ cis}$	0.8	0.7	0.9
$C_{16:1 \ trans}$	1.2	0.	0.7
$C_{18:0}$	2.7	3.6	2.5
$C_{18:1}$	4.4	7.0	4.2
$C_{18:2}$	7.1	9.9	6.8
$C_{18:3}$	59.6	59.4	64.5
C_0	24.7	22.1	20.9
C_x	75.3	77.9	79.1
C_{16}	21.2	17.9	18.6
C_{18}	73.8	79.9	78.0

C_{12-18} gives the carbon atom chain length of the acid; the second number gives the number of double bounds. $C_{12:x}$ means undefined number and position of double bounds; C_0 means total quantity of saturated fatty acids; C_x means total quantity of unsaturated fatty acids. Values are averages of 3 to 5 determinations; maximal deviations 5%. With reference to dry weight 10 g of leaf dry weight (DzW) contain 1.3 g of total fatty acids ($C_0 + C_x$).

Table II. Carotenoid content of leaves of *Dioscorea zingiberensis* plants which differ under cultivation of identical environmental conditions as temperature, humidity, nutrients and a light intensity of 100 μE m^{-2} s^{-1} in their phenotypes (DzW, DzY, DzT).

Carotenoids	*Dioscorea zingiberensis* Phenotype		
	DzW	DzY	DzT
Neoxanthin	0.11	0.12	0.20
Violaxanthin	0.05	0.23	0.14
Lutein	1.00	2.22	3.11
β-Carotene	0.14	0.69	0.74
Carotenoids	1.3	3,3	4.2
Chlorophyll	8.1	6.9	12.0

Carotenoids are given in % of lipids. Values are averages of at least 3 separate determinations and deviate by maximally 5%.

of the DzT-form the content of linolenic acid is unchanged in comparison to the green type and only linoleic acid occurs in higher concentration. Inversely, in the white-hatched leaves of the DzY-form linoleic acid is reduced and linolenic acid occurs by 10% more. A decisive difference between the green type and the two other phenotypes refers to the occurence of hexadecenoic acid with trans-configuration. This trans-fatty acid which occurs in *Dioscorea zingiberensis*, as well as in lipids of other higher plants and which as an ester component is coupled to phosphatidylglycerol occurs in the phenotypes DzY and DzT to a lesser degree which permits the conclusion that the grana structure of the thylakoid membrane is not too much developped. According to Tremolières *et al.* (1978, 1982) this acid functions as a structure-forming element in grana formation. In particular, it appears that the fatty acids with 12 carbon atoms are strongly present in the normal green type DzW. However, in the other phenotypes, at least in the light-green form DzT, these acids are strongly reduced. The location of the number of double-bounds for these 12-C-carbonic acids was not yet determined but this will be done in a further publication.

Carotenoids of *Dioscorea zingiberensis* correspond with respect to their composition to carotenoid patterns of green leaves of other higher plants (Isler, 1971; Siefermann, 1987; Krinsky, 1979) (Table II). Carotenoids as β-carotene and the xanthophylls lutein, violaxanthin and neoxanthin occur. As shown by thin layer chromatographic analyses zeaxanthin occcurs also. However, a quantitative analysis of zeaxanthin via HPLC on a RP-18 column with the described HPLC-device is not possible, as the retention times of zeaxanthin are identical to those of lutein. In leaf lipids of the normal green form DzW total carotenoids make up for 1.3% of total lipids. This corresponds to a carotenoid/chlorophyll ratio of 1:6. The order of magnitude of this carotenoid to chlorophyll ratio compares to relationships seen in lipids of leaves of other higher plants. In the two other phenotypes, due to an increase in carotenoids, the carotenoid/chlorophyll ratio changes bringing the carotenoid/chlorophyll ratio in the DzT-forms with light-green leaves to 1:3 and in the DzY-forms with yellow-green leaves even to 1:2. This increase of carotenoids in the two phenotypes

DzY and DzT refers to β-carotene as well as to the xanthophylls lutein and violaxanthin. Concerning the neoxanthin content, only in the DzT-form a doubling occurs, but not in the DzY-form.

In further investigations we have looked at the influence of different light intensities on the diosgenin synthesis in the normal green type of *Dioscorea zingiberensis*. For this purpose plants of the DzW-form were cultivated in a growth chamber at different light intensities, namely at 10, 30, 50, 100 and 270 $\mu mol \cdot m^{-2} \cdot sec^{-1}$. The outside conditions with respect to humidity, nutrient salt supply and temperature were identical.

As seen from Table III, the diosgenin content of one year and two years old plants is very different. In the rhizomes of the one year old plants the content is low, but increases in two years old plants strongly. Moreover, it is clearly seen that a dependence of diosgenin synthesis on certain high light conditions exists. Light intensities around 55 $\mu E \cdot m^{-2} \cdot sec^{-1}$ are best (Table III). The diosgenin content in the rhizomes is highest at the light intensities of 55 and 100 $\mu E \cdot m^{-2} \cdot sec^{-1}$ and makes up for 0.45 to 0.55% of the dry weight. Under the strongly shaded conditions of 10 $\mu E \cdot m^{-2} \cdot sec^{-1}$ and at extremely strong light intensities, e.g. 270 $\mu E \cdot m^{-2} \cdot sec^{-1}$ the diosgenin content decreases by one half.

In context with the diosgenin synthesis in these plants we show that also the lipid content of leaves as well as their carotenoid and chlorophyll content

Table III. Content of diosgenin in rhizomes of green type plants (DzW) of *Dioscorea zingiberensis* grown under different light intensities.

Light intensity [$\mu E \cdot m^{-2} \cdot s^{-1}$]	Content of diosgenin	
	1 year old plant	2 years old plant
270	0.05	0.21
100	0.06	0.45
55	0.2	0.55
30	0.07	0.28
10	0.03	0.18

Diosgenin is given in percent of dry weight. Values are differences of 3 to 5 individual determinations and differ by maximally 5%. In 2 years old plants grown at a light intensity of 55 $\mu E \cdot m^{-2} \cdot s^{-1}$, 1 g of rhizome dry matter contains 55 mg diosgenin. In leaf material the content is 10 to 12 times lesser. 1 g leaf dry matter contains nevertheless 1–4 mg diosgenin depending on the light conditions.

Table IV. Lipid, carotenoid and chlorophyll content of leaves of DzW-plants grown under different light intensities.

Light intensity [$\mu E \cdot m^{-2} \cdot s^{-1}$]	Lipids in % of dry weight	Carotinoid/ Lipid	Chlorophyll *a/b*	Chlorophyll/ Lipid
10	13.4	0.363	1.90	0.11
100	18.8	0.246	2.64	0.077
270	13.7	0.280	2.88	0.068

Values are averages of 3 to 5 individual determinations and deviate by maximally 5%.

and the fatty acid composition exhibit differences. Cultivation under increasing light intensities reduces grana stacks considerably and increases the intergrana regions (data not shown). This is the morphological differentiation seen in chloroplasts of most higher plants (Schmid *et al.*, 1966; Homann and Schmid, 1967). Corresponding to this change in chloroplast structure it was to be expected that the lipid content of the leaves would change. According to earlier lipid analyses of leaves and chloroplasts with *Antirrhinum majus*, it is known (Radunz, 1966) that half of the leaf lipids are localized in chloroplasts whereas the other half is distributed over cell membranes like the plasmalemma, tonoplast, cytoplasmic reticulum, in the cytoplasma and waxes of the leaf surface. Plants of DzW cultivated under a light intensity of 100 $\mu E \cdot m^{-2} \cdot sec^{-1}$ have a very high lipid content in comparison to other plants (Table IV). It represents 19% of dry weight. In plants cultivated under shaded conditions as well as in plants grown under very high light intensities the lipid content is reduced by 30% (Table IV). The chlorophyll a/b ratio increases with increasing light intensity from 1.9 at 10 $\mu E \cdot m^{-2} \cdot sec^{-1}$ to 2.6 at 100 $\mu E \cdot m^{-2} \cdot sec^{-1}$ and to 2.88 at 270 $\mu E \cdot m^{-2} \cdot sec^{-1}$. This increase of the chlorophyll a/b ratio is due to the reduction of grana stacks in the lamellar system and therewith connected to the decrease of the chlorophyll b content.

The fatty acids pattern of DzW-plants cultivated under different light intensites shows strong differences (Table V). These differences refer to the chain lengths and the degree of saturation. Plants that have been grown at 10 $\mu E \cdot m^{-2} \cdot sec^{-1}$ which corresponds to strongly shaded conditions contain up to 67% of their total fatty acids in form of unsaturated acids. The rest are saturated acids. The main components are with 53% linolenic acid and with 25% palmitic acid. A ten-fold increase of the light intensity to 100 $\mu E \cdot m^{-2} \cdot sec^{-1}$ leads to an

Table V. Fatty acid composition of leaf lipids of DzW-plants grown under different light intensities.

	Light intensity [$\mu E \cdot m^{-2} \cdot s^{-1}$]		
Fatty acids	10	100	270
$C_{12:0}$	0.3	0.3	0.4
$C_{12:x}$	0.1	3.5	0.4
$C_{14:0}$	1.0	0.8	1.0
$C_{15:0}$	0.6	0.3	0.5
$C_{16:0}$	25.0	19.2	24.0
$C_{16:1}$ cis	1.5	0.8	1.4
$C_{16:1}$ trans	0.3	1.2	1.6
$C_{16:2}$	0.1	–	0.4
$C_{18:0}$	4.5	2.7	3.0
$C_{18:1}$	5.5	4.4	4.7
$C_{18:2}$	7.9	7.1	8.5
$C_{18:3}$	53.4	59.6	53.5
$C_{20:0}$	1.8	–	0.6
C_0	33.2	23.3	29.5
C_x	66.8	76.7	70.5
C_{16}	26.9	21.2	27.4
C_{18}	71.3	73.8	69.7

Fatty acids are given in% of total fatty acids. $C_{12:x}$ means undefined number and position of double bounds; C_0 means total quantity of saturated fatty acids; C_x means total quantity of unsaturated fatty acids. Values are averages of 3 to 5 determinations and deviate by maximally 5%. With reference to dry weight the amount of total fatty acids is considerable. 10 g leaf dry matter contain 1.3 g fatty acids ($C_0 + C_x$).

increase of the degree of unsaturated fatty acids, by 16% to 77% of total fatty acids, whereas saturated ones decrease to a value of 23% of total fatty acids. This change is caused by the increase in linolenic acid. It is noteworthy that linoleic acid does not follow linolenic acid. To the contrary, a decrease is observed and at very high light intensities at best a small increase in linoleic acid can be seen. Also monoenoic acids as oleic and $C_{16:1}$ acids decrease with an increase of the light intensity to 100 $\mu E \cdot m^{-2} \cdot sec^{-1}$ and remain at high light intensities more or less constant. Corresponding to the increase of unsaturated fatty acids a decrease of saturated fatty acids occurs if light intensities

are increased to 100 µE · m^{-2} · sec^{-1}. This change refers essentially to the main component of saturated fatty acids which is palmitic acid. Stearic acid behaves in parallel to palmitic acid. Noteworthy is the behaviour of the C$_{16:1}$ trans fatty acid which increases from 1.2% of total fatty acids in shaded plants to 1.6% if plants are cultivated under extremely high light intensities. This is valid despite the fact that under these light conditions the grana structure is strongly reduced as an electron microscopy study of the chloroplast structure has shown. This fatty acid is the only fatty acid with trans-configuration and is coupled as ester component to the anionic lipid phosphatidylglycerol. It is this fatty acid which is thought to be decisive for grana structure (Trémolières *et al.*, 1978, 1982). Here, however, together with the diminuation of the grana structure an increase in trans-hexadecenoic acid is observed.

Furthermore unsaturated fatty acids with 12 carbon atoms undergoe an enormous change in dependence on light intensity. The content of these acids increases many-fold when the cultivation light intensity is lifted to 100 µE · m^{-2} · sec^{-1}. However, under extreme light intensities a strong decomposition seems to occur.

Also the carotenoids do not remain constant when the cultivation light intensities are increased from 10 to 270 µE · m^{-2} · sec^{-1} (Table VI). In plants with a strong grana structure which are those grown at low light intensity as well as in plants with a low grana structure which are those grown at the highest light intensity, carotenoids appear increased in comparison to plants grown at a medium light intensity of 100 µE · m^{-2} · sec^{-1}. Also the ratio of carotenoids to chlorophyll does not remain constant. Whereas this ratio is 1:5 in plants grown at 100 µE · m^{-2} · sec^{-1} and in the strongly shaded plants this ratio is reduced to half under very high light intensities which is apparently due to the reduction of the grana structure (Table VI).

Table VI. Carotenoid content of leaf lipids of DzW-plants grown under different light intensities.

Carotenoids	Light intensity [µE · m^{-2} · s^{-1}]		
	10	100	270
Neoxanthin	0.03	0.11	0.26
Violaxanthin	0.09	0.05	0.05
Lutein	1.88	1.19	2.03
β-Carotene	0.16	0.18	0.35
Total carotenoids	2.16	1.53	2.69
Chlorophyll	10.9	7.7	6.8

Carotenoids are given in % of lipids. Values are averages of 4 separate determinations. Maximal deviations 5%.

This differing behaviour of the carotenoid concentration is obviously due to the function of the carotenoids (Isler, 1971; Siefermann, 1987; Krinsky, 1979; Koyama, 1991; Bassi *et al.*, 1993) which on the one hand participate in light absorption and on the other hand have protective functions. With a strong increase of the grana strucutre (i.e. grana stacking) under shaded light conditions lutein and violaxanthin appear in considerably higher concentrations. In contrast to this under very high light intensities which goes in parallel with a reduced grana strucutre, the protective function of carotenoids gains more importance which manifests itself in the increase of carotenoids like lutein, neoxanthin and β-carotene. However, violaxanthin decreases with the increase of light intensity. This decrease of violaxanthin under high light conditions corresponds to the behaviour of the xanthophyll cycle (Siefermann, 1987; Hager, 1966, 1969; Yamamoto, 1967) in which violaxanthin is transformed to zeaxanthin under high light conditions, and is retransformed to violaxanthin under normal light conditions.

Bassi R., Pineau B., Dainese P. and Marquardt J. (1993), Carotenoid-binding proteins of photosystem II. Eur. J. Biochem. **212**, 297–303.

Bednarz J., Radunz A. and Schmid G. H. (1988), Lipid composition of photosystem I and II in the tobacco mutant *Nicotiana tabacum* NC 95. Z. Naturforsch. **43c**, 423–430.

Chou J., Wu D. and Huang W. (1965), Studies on the saponin compounds of plants in Yunnan. Acta Pharmaceutica Sinica **12(6)**, 392–398.

Demmig-Adams B. and Adams W. W. (1992), Photoprotection and other responses of plants to high light stress. Annu. Rev. Plant Physiol. Plant Mol. Biol. 43, 599–626.

Ding Z. (1983), Resource plant of steroid hormone. Publishing House of Science (Chinese), Beijing, pp. 15–45.

Douce R. and Joyard J. (1980), Plant galactolipids. In: The Biochemistry of Plants. A Comprehensive Treatise (P. K. Stumpf and E. E. Conn, eds.), Vol. 4, Lipids: Structure and Function. Academic Press, New York, London, pp. 321–357.

Genty B. (1996), Regulation of light utilization for photosynthetic electron transport. In: Photosynthesis and the environment (Neil R. Baker, Ed.), Kluwer Academic Publ., pp. 67–99.

Hager A. (1966), Die Zusammenhänge zwischen lichtinduzierten, reversiblen Xanthophyllumwandlungen und Hill-Reaktion. Ber. Deutsch. Bot. Ges. **79**, 94–107.

Hager A. (1969), Lichtbedingte pH-Erniedrigung in einem Chloroplasten-Kompartiment als Ursache der enzymatischen Violaxanthin-Zeaxanthin-Umwandlung; Beziehungen zur Photophosphorylierung. Planta **89**, 224–243.

He P. (1987), The effects of light regime on growth characteristics of chloroplasts and photosynthesis of the Chinese pine tree (*Pinus taboliaformis*) seedlings. Doctoral thesis, Beijing Forestry University.

He P., Bader K. P., Radunz A. and Schmid G. H. (1995), Consequence of high CO_2-concentrations in air on growth and gas-exchange rates in tobacco mutants. Z. Naturforsch. **50c**, 781–788.

He P., Radunz A., Bader K. P. and Schmid G. H. (1996), Quantitative changes of the lipid and fatty acid composition of leaves of *Aleurites montana* as a consequence of growth under 700 ppm CO_2 in the atmosphere. Z. Natuforsch. **51c**, 833–840.

Homann P. H. and Schmid G. H. (1967), Photosynthetic reactions of chloroplasts with unusual structures. Plant Physiol. 42, 1619–1632, **1967**.

Isler G. O. (1971), Carotenoids, Birkhäuser Verlag, Basel, Stuttgart.

Koyama Y. (1991), Structures and functions of carotenoids in photosynthetic systems. J. Photochem. Photobiol. B, Biol., **9**, 265–280.

Krinsky N. I. (1979), Carotenoid protection against oxidation. Pure Appl. Chem. Vol. **51**, 649–660.

Liu C. and Chen Y. (1985), Isolation and identification of protosaponins from fresh rhizomes of *Dioscorea zingiberensis* Wright. Acta Botanica Sinica **27(1)**, 68–74.

Luckner M. (1990), Secondary Metabolism in Microorganism, Plants and Animals. Springer Publ., pp. 214–215.

Mahato S. B., Ganguly A. N. and Sahu N. P. (1982), Steroid saponins. Phytochemistry **21(5)**, 959–978.

Radunz A. (1966a), Chlorophyll- und Lipidgehalt der Blätter und Chloroplasten von *Antirrhinum majus* in Abhängigkeit von der Entwicklung. Z. Pflanzenphysiol. **5**, 395–406.

Radunz A. (1966b), Über die Fettsäuren in Blättern und Chloroplasten von *Antirrhinum majus* in Abhängigkeit von der Entwicklung. Flora, Abt. A, **157**, 131–160.

Radunz A. (1969), Über das Sulfoquinovosyl-diacylglycerin aus höheren Pflanzen, Algen und Purpurbakterien. Hoppe-Seyler's Z. Physiol. Chem. **350**, 411–417.

Radunz A. (1972), Lokalisierung des Monogalaktosyl-diglycerids in Thylakoidmembranen mit serologischen Methoden. Z. Naturforsch. **27b**, 822–826.

Radunz A. (1976), Localization of the tri- and digalactosyldiglyceride in the thylakoid membrane with serological methods. Z. Naturforsch. **31c**, 589–593.

Radunz A., He P. and Schmid G. H. (1998), Analysis of the seed lipids of *Aleurites montana*. Z. Naturforsch. **53c**, 305–310.

Radunz A. and Schmid G. H. (2000), Wax esters and triglycerides as storage substances in seeds of *Buxus sempervirens*. Eur. J. Lipid Sci. Technol. **102**, 734–738.

Schmid G. H., Price J. M. and Gaffron H. (1966), Lamellar structure in chlorophyll deficient but normally active chloroplasts. J. Microscopie 5, 205–212.

Schmid G. H. (1971), Origin and properties of mutant plants: Yellow tobacco. In: Methods in Enzymology, Vol. 23 (San Pietro, A., ed.). Academic Press, New York, London 171–194.

Siefermann H. D. (1987), The light-harvesting and protective function of carotenoids in photosynthetic membranes. Physiol. Plant. **69**, 561–568.

Tang S. and Jiang Z. (1987), Three new steroidal saponins from the aerial part of *Dioscorea zingiberensis*. Acta Botanica Yunnanica **9(2)**, 233–238.

Tang S., Zhang H., Dong Y., Li H. and Ding Z. (1979), Identification and content of steroidal sapogenins in *Dioscoreaceae* plants. Acta Botanica Sinica **21(2)**, 171–176.

Trémolières A., Dubacq J. P. and Drapier D. (1982), Unsaturated fatty acids in maturing seeds of sunflower and rape: Regulation by temperature and light intensity. Phytochemistry **21**, 41–45.

Trémolières H., Trémolières A. and Mazliak P. (1978), Effects of light and temperatures on fatty acid desaturation during the maturation of rape seed. Phytochemistry **17**, 685–687.

Wang M. and Chou T. (1964), Determination of diosgenin in plants. Acta Pharmaceutica Sinica **11(4)**, 235–241.

Xu L. and Liu A. (1984), Determination of diosgenin in *Dioscorea*. Acta Pharmaceutica Sinica **19(2)**, 141–145.

Yamamoto H. Y. (1967), Light-induced interconversation of violaxanthin and zeaxanthin in New Zealand spinach-leaf segments. Biochim. Biophys. Acta **141**, 342–347.