Performance and Photosynthetic Ecophysiology of Three Photo-Types of *Dioscorea zingiberensis* under Differing Light Intensities

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Abstract: The performance and photosynthetic ecophysiology of three photo-types of *Dioscorea zingiberensis* were studied. The three types are designated DzTL, DzTM and DzTH, according to their adaptation to low (LL), medium (ML) and high (HL) light intensities, respectively. Under LL (23 – 55 μmol m^{-2} s^{-1}) and simulated natural light (SNL), DzTM grows well with increased longevity, and green leaves which are unspotted; while its leaves became small, light yellow and short-lived under HL (550 – 850 μmol m^{-2} s^{-1}). In contrast, under LL the leaves of DzTH were very large, spotted, light yellow and short-lived; while they were small, green and long-lived under HL. Under HL, DzTH had a much higher chlorophyll content than DzTM. Under LL, DzTM and DzTL had a higher Chl content than DzTH. Among the three types, DzTM had the highest peroxidase activity. DzTL had a higher electron transport rate (ETR), maximal quantum yield (MQY) and effective quantum yield (EQY) than DzTH and DzTL under LL, while DzTH had higher ETR, MQY and EQY than the other two types under ML and HL. Therefore, three different photo-types can be characterized according to their adaptation to LL, ML and HL: DzTL, DzTM and DzTH, respectively.

Key words: *Dioscorea zingiberensis*, photo-type, electron transport rate, light intensity, quantum yield, photosynthesis.

Abbreviations:

Chl:	chlorophyll
DCMU:	3-(3,4-dichlorophenyl)-1,1-dimethylurea
DCPiP:	2,6-dichlorophenolindophenol sodium salt dihydrate
DzTH:	*Dioscorea zingiberensis* high light intensity type
DzTL:	*D. zingiberensis* low light intensity type
DzTM:	*D. zingiberensis* medium light intensity type
EQY:	effective quantum yield
ETR:	electron transport rate
HL:	high light intensity
HPLC:	high performance liquid chromatography
LL:	low light intensity
MDA:	malondialdehyde
ML:	medium light intensity
MQY:	maximal quantum yield
MV:	methyl viologen
SNL:	simulated natural light
PAR:	photosynthetically active radiation
POD:	peroxidase
PSI:	photosystem one
PSII:	photosystem two
Rubisco:	ribulose 1,5-bisphosphate carboxylase/oxygenase
WETC:	whole electron transport chain

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Introduction

Dioscorea zingiberensis C. H. Wright belongs to the Dioscoreaceae family, and is distributed only in the mountainous areas of southern China (Ding et al., 1983[7]). The plant has a very high content of diosgenin (Tang et al., 1979[36]), which is widely used in the pharmaceutical industry to produce corticosteroids, such as cortisone, sexual hormones and anabolic agents. The glycoside of diosgenin, dioscin, has also been found to be effective in improving cardiovascular function (Ding et al., 1983[7]). Since the 1970s, the species has been over-exploited and its natural reserves are now greatly diminished. At present, the production of *D. zingiberensis* as raw material for pharmaceutical uses mainly depends on plantations in the mountainous areas of southern China.

Mountainous areas receive very strong sunlight. High photon flux densities have been shown to cause photoinhibition or photodamage (Farage and Long, 1991[10]; Ögren and Rosenqvist, 1992[30]; Nishio et al., 1994[29]; Andersson and Barber, 1996[2]; Ball et al., 1997[3]; Sonoike, 1998[35]). *D. zingiberensis* plants growing in the wild are often found at the margins of forests and are mainly exposed to scattered light. Therefore, mountainous areas are unsuitable for the optimal growth of *D. zingiberensis* as the yield of rhizomes is not high, thus not meeting the demands of the pharmaceutical industry in China. To date, most studies on cultivation techniques for *D. zingiberensis* have focused mainly on the effects of soil, water and temperature (Huai et al., 1989[16]; Institute of Biology in Sichuan Province, 1974[17]; Zhou et al., 1989[38]), although we have conducted research on the influence of light intensity on the leaf lipids of *D. zingiberensis* (Li et al., 2002[24]) and growth of wild-type plants in the field (Zhu et al., 2001[40]). Choice of an appropriate plant variety is undoubtedly one of the most important factors in successful cultivation in mountainous areas *D. zingiberensis* plants which are adapted to high light intensities are preferable (Li and Wang, 1999[23]). In this study, we

first describe three types of *D. zingiberensis* which have different morphological properties. The performance and some photosynthetic characteristics of these three types were then compared under different light intensities. We conclude that three different photo-types can be distinguished and that a type of *D. zingiberensis* which is adapted to high light intensities would be suitable for cultivation in mountainous areas.

Materials and Methods

Cultivation

Three types of *D. zingiberensis* from the Institute of Ecology of Jishou University in P. R. China were named as *D. zingiberensis* type *low light intensity* (DzTL), *D. zingiberensis* type *medium light intensity* (DzTM) and *D. zingiberensis* type *high light intensity* (DzTH), based on observation of morphological characteristics. In the field, DzTL has heart-shaped leaves with white spots along their veins; the leaves of DzTM are also heart-shaped, but they do not have white spots. The leaves of DzTH have white spots, but are triangular instead of heart-shaped. The colours of the leaves of DzTM, DzTL and DzTH in field are dark green, green and light green, respectively. The stem diameters of the three types are in the following descending order: DzTM > DzTL > DzTH.

In order to simulate natural light (SNL) conditions, the plants were grown in a greenhouse beside some other plants, so that they were exposed to scattered light. Only in the afternoons on sunny days could low sunlight illuminate the plants directly for short periods. The light intensity at noon on a cloudy day was about 450 μmol m^{-2} s^{-1} and 1250 μmol m^{-2} s^{-1} on a sunny day. During the experimental period, most days (2/3) were cloudy. The temperature in the greenhouse was 21/25 °C (night/day). For each photo-type, the plants used for the experiment were obtained from a big rhizome that was divided into 5 – 7 small parts (about 6 g fresh weight each). Therefore, each type had 5 – 7 replicates. All plants were fertilized with Wax Super (Firma AGLUKON, Düsseldorf, Germany) once a week. The age of the leaves was checked and marked at least once a week.

The light intensity experiments were conducted in a growth chamber in which the relative humidity was 60% and the photo cycle was 14 h/10 h (light/dark) with a daytime temperature of 27 °C and a nighttime temperature of 22 °C. The light intensities were 39 (23 ~ 55) (LL), 227 (181 ~ 273) (ML) and 700 (550 ~ 850) (HL) μmol m^{-2} s^{-1}. Other conditions were the same as those of the SNL experiment.

The light spectra (Fig. **1**) and intensities in the greenhouse and growth chamber were measured with the QSM-2500 Quantum spectrometer (Techtum Instrument, Sweden).

Measurement of electron transport rate and quantum yield

Because all three photo-types showed better growth under ML than under other light intensities in the growth chamber, leaves from such plants were used to measure the electron transport rate in isolated chloroplasts. Fresh leaves, aged 60 – 65 days, were homogenized in Tris-buffer: 0.05 mol/l Tris, 0.01 mol/l NaCl, 0.4 mol/l sucrose, 0.2% bovine serum albumin, 0.2% pectinase, 0.005 mol/l $MgCl_2$, pH 7.8. The homogenate

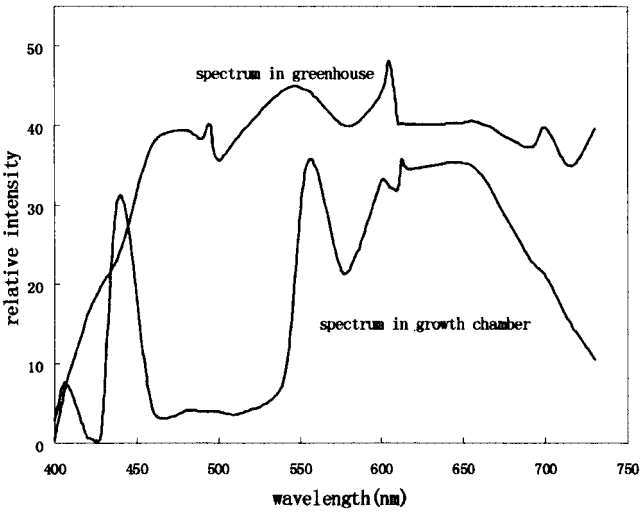

Fig. 1 The spectra in the growth chamber and greenhouse.

was filtered through eight layers of cheesecloth. Chloroplasts were then obtained by centrifuging the filtrate at 500 g for 5 min. All the above operations were carried out at 0 ~ 4 °C. The chlorophyll content of chloroplasts was measured in extracts of 90% methanol in a spectrophotometer (Schmid, 1971[32]). The oxygen evolution of photosystem two (PSII) and the whole electron transfer chain (WETC), as well as the oxygen uptake of photosystem one (PSI) in isolated chloroplasts of *D. zingiberensis* were measured with a Clark type electrode (Hanstech, Germany) at 25 °C under 2800 μmol m^{-2} s^{-1} of red light which had been passed through a $CuSO_4$ solution. During the process of measurement, the chloroplasts were suspended in Tricine buffer (0.15 mol/l Tricine, 0.03 mol/l KCl, pH 7.5). When the electron transport rates (ETR) of PSII and WETC were being measured, 0.1 ml 0.03 mol/l $K_3[Fe(CN)_6]$, 0.1 ml 0.03 mol/l KCN and 0.1 ml 0.001 mol/l methyl viologen (MV) were added, respectively. For the measurement of the ETR of PSI, 0.1 ml 0.0003 mol/l DCMU (3-[3,4-dichlorophenyl]-1,1-dimethylurea), 0.1 ml 0.003 mol/l MV, 0.1 ml 0.03 mol/l KCN, 0.1 ml 0.005 mol/l DCPiP (2,6-dichlorophenolindophenol sodium salt dihydrate) and 0.1 ml 0.01 mol/l sodium ascorbate were added. All measurements were repeated 3 – 5 times.

The maximum quantum yield (MQY), effective quantum yield (EQY) and ETR of PSII in intact leaves were measured with a portable fluorometer (Mini PAM, Walz) according to Schreiber et al. (1997[34]) and Vest et al. (2000[37]).

Determination of Rubisco, protein, soluble sugar, malondialdehyde content and peroxidase activity

The leaves used in this measurement were 60 – 75 days old. Rubisco (ribulose 1,5-bisphosphate carboxylase/oxygenase) samples were prepared according to He et al. (1996[14]). Rubisco content was determined by rocket-immune electrophoresis according to Schmid et al. (1993[33]).

Protein content was measured according to Bradford (1976[4]). Soluble sugar was measured according to Roughan and Batt (1968[31]) Chlorophyll content was measured according to Schmid (1971[32]).

Table 1 Performance of the three photo-types of *D. zingiberensis* under different light conditions

	Light intensity	DzTH	DzTM	DzTL
Leaf area (cm^2)	HL	33.25 ± 5.03	7.30 ± 2.12	–
n > 25	ML	38.10 ± 6.35	25.74 ± 2.10	18.40 ± 6.22
	LL	41.88 ± 4.47 (gr)[1] 70.4 ± 13.3 (ye)[2]	27.41 ± 5.01	24.82 ± 18.67
	SNL	37.52 ± 13.32 (gr)	24.19 ± 15.83	24.2 ± 11.32
Leaf longevity[3] (days)	HL	125 ± 10	85 ± 18	–
n = 5	ML	133 ± 13	131 ± 15	78 ± 16
	LL	123 ± 12 (gr) 75 ± 5 (ye)	155 ± 21	158 ± 24
	SNL	145 ± 18 (gr)	141 ± 23	131 ± 15
Total biomass (g; dry weight)	HL	33.0 ± 5.4	12.5 ± 2.6	–
n = 5 ~ 7	ML	37.5 ± 2.8	37.4 ± 7.9	21.3 ± 5.1
	LL	21.5 ± 2.0	17.3 ± 1.4	24.1 ± 0.9
	SNL	39.4 ± 3.3	38.0 ± 5.3	24.6 ± 7.3

[1] green leaves; [2] yellow leaves; [3] only the first leaf of each plant was observed.

For determination of malondialdehyde (MDA) and peroxidase (POD), leaves were first homogenized with 62.5 mM phosphate buffer (pH 7.8) at 0 °C, and then centrifuged for 20 min at 15 000 g at 4 °C. The supernatant was used to determine the content of MDA according to Chen (1991[5]) and the activity of POD according to Zhu et al. (1990[39]). One unit of POD activity was defined as 0.01 of the change of absorbance at 470 nm.

Determination of diosgenin

Diosgenin in the rhizomes of the three photo-types grown under medium light intensity was extracted according to Tang et al. (1979[36]) and diogenin content was determined by high performance liquid chromatography (HPLC). HPLC analysis was carried out with a device manufactured by Hewlett Packard (Böblingen, Germany), on a reversed phase column (LiChropher® 100 RP-18 (5 μm), No. 419272, Merk, Darmstadt, Germany). The mobile phase was 95% methanol. Samples dissolved in 100% ethanol were injected through an auto-sampler AS-200 (Merck and Hitachi). The flow rate of the solvent was 0.7 ml/min. The detector used was a diode detector. Measurements were carried out at 210 nm. Evaluation of signals utilized an integrator (3390A, Hewlett Packard).

A control sample of diosgenin (Sigma) was analysed under identical conditions and the following standard curve was obtained:

$$Y = 7.32 \cdot 10^{-8} X \quad (R = 0.999)$$

where Y is the diosgenin concentration in extracted solution (mg ml^{-1}), and X the area of the diosgenin peak.

Growth and biomass

In our experiment, it took 4 – 5 months for *D. zingiberensis* to complete development and growth from germination to the end of fluorescence measurement in the growth chamber and the greenhouse, and 5 – 7 months in the field (Ding et al., 1983[7]). During different developmental stages of the plants, leaf size, weight per unit leaf area, chlorophyll content and composition were measured. Growth and leaf colour were checked every week. Dead leaves from different developmental stages were collected and dried at 78 °C. Plants were harvested after the end of fluorescence measurements, and leaves, stem and rhizomes were then dried at 78 °C.

Results and Discussion

Performance of the three photo-types

The three types of *D. zingiberensis,* DzTL, DzTM and DzTH, cultivated in a growth chamber and greenhouse, showed significantly different performance. In particular, their leaf characteristics changed with the light intensity of their environment (Table **1**). However, their flowers were morphologically identical regardless of light conditions. Under HL, the leaves of DzTM became almost colourless, while those of DzTH remained green although some DzTH leaves did become lighter in colour. DzTL produced hardly any leaves under HL. Under LL, DzTH had yellow leaves and the leaf area of the first 15 – 20 leaves increased significantly. Under ML, DzTH and DzTM grew well, but DzTL did not grow as well as the other two types. Compared to the plants in the growth chamber, all the plants in the greenhouse which were illuminated by natural light grew better; this may have been caused by differences in the light quality (Fig. **1**). The growth chamber was deficient in wavelengths between 460 – 540 nm. The leaf longevities of DzTL under ML and of DzTM under HL were much shorter than those under LL. The longevity of the yellow leaves of DzTH under LL was much shorter than that of its green leaves under HL.

Even though there are no white spots on the leaves of DzTM in the wild and under LL, under ML light colour spots of the same shape as those on the leaves of DzTL and DzTH appeared. The spots were visible especially on the first several leaves of a plant in the seedling stage; becoming gradually lighter and lighter until they were invisible. DzTL plants grown in ultra-

Table 2 Ecophysiological characteristics of the three photo-types of *Dioscorea zingiberensis* under different light conditions

	Light intensity	DzTH	DzTM	DzTL
Chl (μg cm^{-2})	HL	32.6 ± 13.25	3.3 ± 1.10	–
	ML	35.6 ± 3.85	63.33 ± 12.11	28.94 ± 6.45
	LL	41.4 ± 4.62 (gr) [1] 13.87 ± 1.56 (ye) [2]	75.45 ± 8.33	65.78 ± 15.32
	NL	38.06 ± 3.23 (gr)	57.76 ± 6.43	28.89 ± 4.66
Chl/protein (%) [3]	ML	8.35 ± 0.32	7.19 ± 0.45	4.39 ± 0.33
Chl a/Chl b	HL	4.3 ± 0.5	6.4 ± 0.7	–
	ML	3.1 ± 0.3	3.0 ± 0.6	4.1 ± 0.4
	LL	2.3 ± 0.1 (gr) 4.5 ± 0.4 (ye)	2.5 ± 0.2	2.8 ± 0.1
	NL	2.9 ± 0.2 (gr) 4.8 ± 0.3 (ye)	2.9 ± 0.5	3.0 ± 0.2
Soluble sugar (%; dry weight)	HL	7.49 ± 0.35	7.83 ± 1.45	–
	ML	8.09 ± 1.23	15.36 ± 4.45	11.08 ± 2.34
	LL	5.50 ± 0.77	11.5 ± 3.21	24.8 ± 8.75
	NL	13.56 ± 3.2	18.34 ± 4.32	18 ± 2.33
MDA (nM g^{-1}; dry weight)	HL	179.34 ± 26.80	102.34 ± 13.07	–
	ML	166.32 ± 20.46	48.9 ± 8.34	84.75 ± 10.47
	LL	112.49 ± 10.04	43.77 ± 6.60	71.27 ± 4.95
	NL	98.38 ± 7.43	35.43 ± 3.46	55.20 ± 5.38
Peroxidase (u/mg protein) [4]	HL	16.45 ± 1.43	21.73 ± 2.13	–
	ML	12.01 ± 0.94	17.33 ± 1.88	6.53 ± 1.13
	LL	10.05 ± 1.12	15.45 ± 1.50	5.52 ± 1.65
	NL	9.86 ± 0.82	13.59 ± 2.11	4.13 ± 1.86
Rubisco (μg cm^{-2})	LL	101 ± 12 (gr) 55 ± 6 (ye)	105 ± 10.6	111 ± 15

[1] green leaves; [2]yellow leaves; [3] chlorophyll content/protein ratios in isolated chloroplasts; [4] one unit of peroxidase activity was defined as 0.01 of the change of absorbance at 470 nm.

low light (13 μmol m^{-2} s^{-1}) were entirely green with no white spots. DzTH always had spotted leaves under both HL and LL. This suggests that the appearance of white spots may be controlled by the light intensity of the environment. The spots on the leaves of DzTL and DzTM can be induced by certain light intensities and different photo-types have different thresholds for the appearance of such spots. DzTM had a much higher threshold for this than DzTL.

With regard to leaf area, all three types of *D. zingiberensis* responded in the same way to light intensity in that their leaf areas increased under LL and decreased under HL. Under LL, the big yellow leaves of DzTH, with a low Rubisco content (Table **2**), only appeared in the early seedling stage. The leaves then gradually became green and their Rubisco content increased. This may be related to gene expression. The leaves of DzTM became smaller and smaller with increasing exposure to HL. After two months, leaves near the flower became colourless and very small, with a mean leaf area of only 7.3 cm^2; the leaves at same position under ML had a mean area of about 24 cm^2. This conforms to the conclusion established by many authors (Lichtenthaler et al., 1981[26]; Lichtenthaler, 1981[25]; Heuvelink and Marcelis, 1996[15]) that plants grow small leaves under HL and big leaves under LL.

Biomass is a very important indicator of the degree of adaptation of a plant to its environment. Cho et al. (1998[6]) and Erick (1994[9]) showed that the biomass of plants decreased with decreasing light intensity. However, Egerton et al. (2000[8]) demonstrated that *Eucalyptus pauciflora* plants could benefit from a reduction in irradiance. Our opinion is that the plants used by Cho et al. and Erick et al. may show adaptation to high light intensities, while *E. pauciflora* may show adaptation to lower light intensities. The results in our experiment indicated that plants could attain their maximal biomass under light intensities which are most favourable for them. Our studies also showed that high light intensities could cause a reduction in biomass due to photoinhibition of photosynthesis, which accords with the results of Laing et al. (1995[20]).

Of the three photo-types in the growth chamber, DzTL and DzTH had the highest biomass under LL and HL, respectively. Under ML, DzTM and DzTH had almost the same biomass. For each photo-type, DzTL had higher total biomass under LL than under ML; DzTM had much higher biomass under ML than under HL and LL; and DzTH had almost the same amount of biomass under both HL and ML, much more than under LL. In SNL in the greenhouse, the biomass of all three types of *D. zingiberensis* exceeded that of plants in the growth chamber. This

Table 3 Electron transport rates of photosystems in isolated chloroplasts of three types of *Dioscorea zingiberensis* grown under medium light intensities*

Type	PSI+II	PSII	PSI	PSII/PSI	WETC/PSII	WETC/PSI
DzTL	15.33 ± 3.52	16.69 ± 2.33	32.72 ± 4.25	0.60	1.28	0.47
DzTM	27.29 ± 4.11	43.10 ± 7.45	37.70 ± 4.11	1.14	0.63	0.72
DzTH	39.02 ± 2.32	57.23 ± 9.04	52.02 ± 11.23	1.10	0.68	0.75

* The measurement was carried out at the late seedling stage; unit: μmol O_2 h^{-1} mg^{-1} Chl.

meant that either the fluctuation of light intensity or short-wave light favoured the accumulation of biomass. It should be noted that the total biomass of DzTL in one generation was related to the form of propagation. The plants of DzTL grown from seeds attained much less biomass than those grown from rhizomes. Therefore, plants grown from rhizomes are preferred in commercial cultivation. The above results show that DzTL, DzTM and DzTH show adaptation to LL, ML and HL, respectively.

Malondialdehyde (MDA) content, peroxidase (POD) activity and chlorophyll content and composition

The three photo-types also showed different photosynthetic and ecophysiological characteristics under different light intensities (Table **2**). The content of MDA reflects the degree of membrane peroxidization and is often used as an index of the adaptation of a plant to its environment. DzTH had the highest content of MDA among the three photo-types. In the growth chamber, DzTH had nearly twice as much MDA as DzTL and four times as much MDA as DzTM under ML. No correlation was found between the MDA content of the three photo-types and their adaptation potential to light. In the greenhouse, all three photo-types had a lower content of MDA than in the growth chamber, suggesting that either fluctuation of light intensity or short-wave light is beneficial to the growth of *D. zingiberensis*.

Peroxidase (POD) is a protective enzyme that can remove active oxygen and therefore protects the membrane system from oxidation. The activity of POD in the three photo-types showed a positive correlation to MDA content, with correlation coefficients in DzTH, DzTM and DzTL being 0.87, 0.96 and 0.99, respectively. The MDA content/POD activity ratios (MDA/POD) of DzTH under HL, ML, LL and SNL were 10.9, 13.8, 11.2 and 9.97, respectively; those of DzTM under HL, ML, LL and SNL were 4.71, 2.82, 2.83 and 2.6, respectively; and those of DzTL under ML, LL and SNL were 12.95, 12.91 and 13.37, respectively. These results show that DzTH plants have the potential to adapt to HL as their MDA/POD values under HL did not increase but decreased instead. DzTM had low and stable MDA/POD ratios under ML and LL, but had much higher MDA/POD ratios under HL; therefore DzTM could not grow normally under HL. The stable MDA/POD ratios of DzTL in ML, LL and SNL meant that DzTL can maintain normal function under these conditions.

The chlorophyll content and composition of *D. zingiberensis* were influenced by light conditions. Our results support the work of other authors that plants grown in shade or under LL have a much higher chlorophyll content and a lower ratio of Chl a/Chl b compared to plants grown in sunlight or under HL (Anderson, 1986[1]; Larcher, 1994[21]; Melis and Harvey, 1981[27]). In natural light, the Chl a/Chl b ratios in the green leaves of the three types were almost identical (2.9 – 3.0). However, the ratio in the yellow leaves of DzTH was 4.8. Under HL, the Chl a/Chl b ratios of DzTH were much lower than those of DzTM and DzTL. DzTH had big yellow and smaller green leaves under LL, with much less chlorophyll in the yellow leaves compared to the green ones. The leaves of DzTM under HL were yellow or almost colourless and had little chlorophyll with a very high Chl a/Chl b ratio. Therefore, DzTH shows better adaptation to higher light intensities than DzTM and DzTL. In order to observe the recovery of plants from photodamage caused by HL, two DzTM plants with very small colourless leaves were removed to the greenhouse and illuminated naturally. A month later, the colourless leaves gradually became green, with the chlorophyll content increasing to 6.1 μg cm^{-2}; and the malondialdehyde content and the POD activity decreasing to 55 nM g^{-1} and 13.2 u mg^{-1} protein, respectively (see Table **2** for the basal values).

Rubisco content

A quantitative immunological determination of Rubisco showed that there was not much difference in the Rubisco content (weight per unit leaf area) of the green leaves in all three types under LL. However, the big yellow leaves of DzTH contained much less Rubisco than its green leaves. In terms of unit leaf area, these yellow leaves photosynthesized poorly, but the total photosynthesis might not be low owing to their large areas. The relationship between the content of Rubisco and leaf size needs further investigation and elucidation.

Soluble sugar

The soluble sugar content varied with light intensity in all three types. DzTH and DzTM had a higher soluble sugar content under greenhouse conditions than in the growth chamber. However, DzTL had the highest level of soluble sugar under LL in the growth chamber. In the growth chamber, DzTH had the higher level of soluble sugar under ML than in HL and LL, as did DzTM. This differs from the findings of Lichtenthaler et al. (1981[26]) who showed that the level of soluble carbohydrate was significantly higher in leaves grown in sunlight and under HL than leaves grown in shade and LL. This difference may have resulted from the heavy photodamage in our plants.

Electron transport rate

Table **3** shows that the absolute values of electron transport rate (ETR) of the whole electron transport chain (WETC) were less than those of PSI or PSII. However, the values of the ETR/WETC and PSI, PSII increased or decreased together. In DzTM

and DzTH, the ETRs of PSII were greater than those of PSI, while in DzTL, PSII ETR was less than PSI ETR. Under our experimental conditions, DzTH, DzTM and DzTL had the highest, intermediate and lowest values of ETR of WETC, PSII and PSI, respectively. These results indicate that there is a positive correlation between the ETR and the adaptation potential of the plants to light, i.e., the greater the ETR, the higher the adaptation potential of the plants to HL. This finding is similar to that of Lichtenthaler et al. (1981[26]) who showed that leaves grown in sunlight had very high CO_2 fixation rates.

The light-dependent electron transport rates in intact leaves of the three photo-types of *D. zingiberensis* grown under simulated natural light conditions were measured with a mini-PAM quantum yield analyser (Fig. **2**). The curves for the three types had the same shapes: under low light intensities, the ETR increases rapidly in a straight line, while with incremental increases in light intensity, the ETR increases more and more slowly. The difference between the three curves was the absolute value of ETRs under the same light intensity. When the light intensity was lower than 160 μmol m^{-2} s^{-1}, DzTL had the highest ETR, with the next higher being DzTM. When the light intensity was higher than 160 μmol m^{-2} s^{-1}, DzTH had higher ETRs than both DzTM and DzTL. Thus, when the photosynthetically active radiation was 1000 μmol m^{-2} s^{-1}, the electron transport rate of DzTH exceeded 120 μmol electrons m^{-2} s^{-1}, but those of DzTM and DzTL were about 80 and 85 μmol electrons m^{-2} s^{-1}, respectively. These results suggest that DzTH should be capable of adapting to HL.

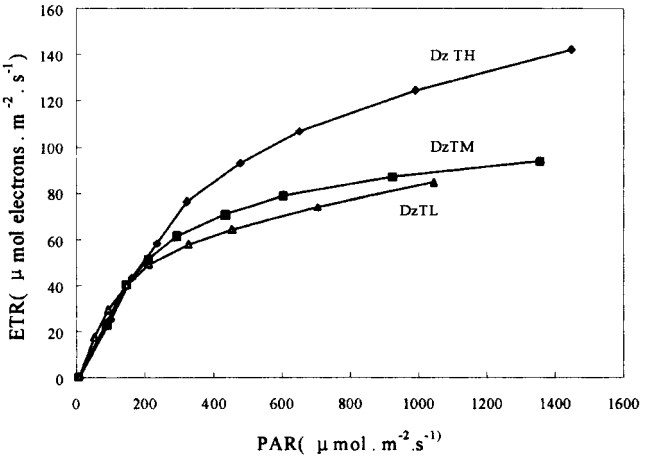

Fig. 2 The light dependence in leaves of three ecotypes of *D. zingiberensis* in natural light.

Degree of adaptation of the three photo-types to their environments

MQY is the maximal quantum yield (the ratio of variable fluorescence to maximum fluorescence) of a dark-adapted sample (Genty et al., 1989[11]). Table **4** shows that, in the growth chamber, most plants had healthy, functional leaves as the MQYs were not low except for those of DzTM under HL. Comparing the three photo-types, DzTL had the greatest MQY under LL, while DzTH had the greatest MQYs under both ML and HL. In addition, the MQYs of the three photo-types changed with light intensities. The MQY of DzTL in ML was less than in LL. However, both DzTM and DzTH had their highest values of MQY in ML.

When plants of the three photo-types of *D. zingiberensis* were photosynthesizing under stable conditions in the growth chamber, their effective quantum yields (EQY) (Genty et al., 1989[11]) were measured *in situ*. The data in Table **4** show that under LL, DzTL and DzTM had their highest EQYs, while DzTH had its highest EQY under ML. DzTM exhibited poor adaptation to HL, with its EQY under HL being 35.5% of that under LL. The EQY of DzTH under HL was 89.4% of that under ML, reflecting the high adaptive potential of DzTH to HL.

Although most fluorescence is emitted by Chl a of PS II at room temperature, the fluorescence yield is also influenced by many factors affecting photochemical (qP) and non-photochemical quenching (NPQ) (Krause and Weis, 1991[19]; Kramer, 1996[18]; Gilmore, 1997[13]; Müller et al., 2001[28]). These include PSII cooperativity and heterogeneity, size of the plastoquinone pool and rate of its reoxidation, rate of electron transport beyond PSI, such as carbon metabolism, and rate of electron donation to P_{680}, and many other factors including the structure and functional states of each component related to electron transport. Under different light conditions, the key factor influencing fluorescence yield is different. qP may play an important role in conditions with limited light, while in conditions with excess light, NPQ may quench a lot of Chl a fluorescence. Therefore, EQY can function as a comprehensive indicator of photosynthesis and reflect the degree of adaptation of a plant to its environment. Our results show that the degree of adaptation of DzTL was much greater under weak light than under strong light, while that of DzTH under strong light was greater than under weak light.

Table 4 Maximum quantum yield (MQY) and effective quantum yield (EQY) of the three photo-types of *Dioscorea zingiberensis* under different light conditions

Type	LL		ML		HL	
	MQY	EQY	MQY	EQY	MQY	EQY
DzTL	0.816 ± 0.016	0.743 ± 0.017	0.795 ± 0.015	0.650 ± 0.021	–	–
DzTM	0.810 ± 0.025	0.736 ± 0.009	0.813 ± 0.017	0.695 ± 0.023	0.361 ± 0.135	0.261 ± 0.110
DzTH (gr)[1]	0.790 ± 0.033	0.720 ± 0.011	0.833 ± 0.023	0.764 ± 0.013	0.786 ± 0.018	0.683 ± 0.015
DzTH (ye)[2]	0.481 ± 0.043	0.382 ± 0.025				

[1] green leaves; [2] yellow leaves.

The content of diosgenin

The diosgenin content in the rhizomes of DzTL, DzTM and DzTH grown under ML were 0.43%, 0.65% and 1.31%, respectively (Table **2**). Theoretically, the degree of adaptation of a plant to its environment can influence its photosynthesis which plays a key role in primary metabolism. Secondary metabolism is regulated by primary metabolism. Therefore, there is some relationship between the secondary metabolite, diosgenin, and the light adaptation potential (EQY) or the electron transport rate (ETR) of each type. The correlation coefficient between WETC ETR and diosgenin content in the three phototypes under ML is 0.97; that between EQY and the diosgenin content is 0.99. This correlation requires further investigation and confirmation. DzTH, a photo-type which is adapted to high light intensity, is the preferred variety for cultivation in the mountains. DzTL and DzTM can be cultivated in a shady location or under other plants.

Conclusion

This paper shows that each type of *Dioscorea zingiberensis* responds in its own way to different light conditions. The performance and physiological characteristics of the three types show that DzTL, DzTM and DzTH are adapted to low, medium, and high light intensities, respectively. Under HL, the LL phototype DzTL and ML photo-type DzTM would suffer serious photodamage and would not grow normally. Therefore, when cultivated commercially, they should be planted under appropriate light intensities.

This study suggests that the effective quantum yield (EQY) of PSII at room temperature can be used as a comprehensive indicator of photosynthesis and the degree of adaptation of a plant to its environment. Our results also suggest that there may be a relationship between the adaptation of *D. zingiberensis* plants to differing light intensities and their diosgenin content. In addition, our work provides good experimental material (three photo-types of *Dioscorea zingiberensis*) for studying the evolutionary mechanism of photo-adaptation.

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