Bot. Bull. Acad. Sin. (2003) **44:** 31-35

Photosynthetic potentials of in vitro-grown juvenile, adult, and rejuvenated *Sequoia sempervirens* (D. Don) Endl. shoots

Li-Chun Huang, Jui-Hsi Weng, Chiu-Hui Wang, Ching-I Kuo, and Yuh-Jang Shieh*

Institute of Botany, Academia Sinica, Nankang, Taipei, Taiwan 11529

(Received June 7, 2002; Accepted November 7, 2002)

Abstract. In vitro shoot tips of *Sequoia sempervirens* (D. Don.) Endl.—including a juvenile, two adult, and two rejuvenated adult clones—were examined for differences in basic physiological characteristics. Moisture contents were the same, around 85%, for all tissues regardless of origin. Growth rates, determined by fresh weight increase and shoot elongation, were higher for the juvenile and rejuvenated shoots. They correlated with higher total nitrogen contents. Juvenile and rejuvenated shoots also showed higher rates of photosynthesis and respiration, evidenced by faster O_2 evolution and consumption. The photosynthetic rates were associated with more chlorophyll, especially chlorophyll *a*, in the juvenile and the rejuvenated shoots. Nevertheless, identical quantum efficiencies of photosystem II indicated the same photosystems were operating and with equal effectiveness in juvenile, adult, and rejuvenated tissues.

Keywords: Phase change; Photosynthesis; Respiration; *Sequoia sempervirens*; Total nitrogen content.

Introduction

Plant development normally begins with a strictly vegetative juvenile phase and culminates in the sexually reproductive adult phase. Although herbaceous annuals achieve phase change, or maturation, within weeks, in woody perennials (Franclet, 1983), especially trees, this phase change or maturation process can take several years and is associated with changes of different characteristics, e.g., loss of adventitious rooting competence, diminished vigor and growth rate, changes in phyllotaxy, shape and size of leaves, re-orientation of leaves from plagiotropism to orthotropism, and decreased thorniness depending on species. During the past several years we have been employing *Sequoia sempervirens* (D. Don.) Endl., the coastal redwood, as a model to characterize the juvenile and mature phases of plants through their biochemical and physiological differences, as well as to progress in the understanding of the underlying mechanism controlling phase change in trees. This species was chosen because its excised shoots can be easily cultured in vitro on a chemically defined medium without phytohormonal supplements. Also, a phase reversal resulting in emergence of juvenile shoots from adult shoots is readily achieved (Huang et al., 1992). Furthermore, the shoots of the two phases display distinct morphogenetic characteristics. Repeated grafting in vitro of adult shoot tips onto rooted shoot segments of juvenile seedlings eventually results in scion growths with juvenile characteristics, such as a higher capacity for adventitious rooting, vigorous growth, and plagiotropic stems (Huang et al., 1992). Differences in esterase and peroxidase isozymes were observed between juvenile or rejuvenated and adult shoots (Huang et al., 1996). The rate of protein phosphorylation was also higher in the juvenile shoots (Kuo et al., 1995), compared to the adult ones. Although juvenile and rejuvenated *S. sempervirens* produced more ethylene per cultured shoot, the rate of ethylene emission per gram of tissue was found to be the same (Huang et al., 2000). Restriction fragment length polymorphism of mtDNA (mitochondrial DNA) was also pointed out between juvenile and adult phase shoots (Huang et al., 1995). In this report, phase change aspects in tissue-cultured *Sequoia sempervirens* are investigated with respect to the potential for photosynthesis, and this is complemented by information on respiration rates and nitrogen contents.

*Corresponding author. E-mail: yjshieh@gate.sinica.edu.tw

Materials and Methods

Tissues Analyzed

Five different origins of shoots—juvenile (SS), adult from two different trees (AS and AST1), and rejuvenated shoots (RS and RST1) from the two adult *S. sempervirens* were investigated. Stocks of juvenile shoots were initiated from seedlings germinated in vitro. The adult stocks were established by culturing shoot tips excised from trees that were at least 60 years old. Stock cultures of AS were initiated from shoots excised in 1976, and those of AST1 were established from another mature tree in 1994 (Huang et al., 2000). Stocks of rejuvenated shoots, RS and RST1, were derived from AS and AST1, respectively. Rejuvenated shoots were obtained by 5-times grafting of the shoot tips from the two mature trees onto rooted juvenile seedling segments in vitro. Shoots of all five were available in stock cultures, maintained by monthly subculturing on a medium containing MS (Murashige and Skoog, 1962) salts, 3%

sucrose, 0.25% gelrite, and, in mg L^{-1}: *i*-inositol, 100; thiamine•HCl, 1; nicotinic acid and pyridoxine•HCl, 0.5 each; and glycine, 2. Initial growth measurements were made by re-culturing 2-cm long shoot terminals for 14 days. Moisture and nitrogen contents were determined on pooled samples of 1 g of 0.7 cm tall terminals each, consisting of approximately 66 shoot tips per sample. The samples were oven-dried at 70°C overnight, weighed for moisture determinations, and pulverized for Kjeldahl nitrogen analysis using a Kjeltec 2300 Analyser Unit (Foss Tecator, Sweden). For measuring photosynthetic parameters and respiration rates 0.2 g of terminal 0.6-0.7 cm portions of shoots from newly sub-cultured stocks (10 days following transfer to fresh medium) were used. The samples consisted mainly of leaves with a minimum of stem tissue.

Measurements of Photosynthetic Parameters

Photosynthesis rates were based on the measurements of photosynthetic O_2 evolution from 0.3 g of sequoia shoot tips. Photosynthesis and respiration measurements were made using a Hansatech leaf disc oxygen electrode system to trace the O_2 exchange of samples, as described by Delieu and Walker (1981).

Chlorophyll was determined according to Wintermans and De Mots' procedure (1965) after extraction in 96% ethanol.

Ten shoot terminals were employed for each Chlorophyll fluorescence analysis. The shoot terminals were first pre-cultured individually in 25- × 150-mm test tubes for 10 days. Each tube contained 20 ml of the MS medium described above. Chlorophyll fluorescence was measured by means of a PAM 101 chlorophyll fluorometer (H. Walz, Effeltrich, Germany). Just prior to fluorescence measurements, the cultures were placed in darkness, horizontally, for 40 min. The dark fluorescence yield (F_o) was obtained by exciting a 0.7 cm region of each shoot tip with weak red light (1 µmole m^{-2} s^{-1}, emission peak at 650 nm) and fluorescence was detected at wavelength above 700 nm. Each shoot tip was then given one flash (1 s) of saturated light (250 µmole m^{-2} s^{-1}) to obtain the maximal (F_m) fluorescence value. The light signal was recorded and calculated by the software DA-100, proven by the manufacturer to obtain a $(F_m-F_o)/F_m$ ratio. It is a measure of the quantum efficiency, or the potential quantum yield, of photosystem II (Bilger et al., 1995).

Statistical Analysis

Statistical significance was determined by computing standard errors of means or obtaining 95% confidence limits from tables of binomials (Lentner, 1982).

Results

Growth Rates of Experimental Shoots

As expected (Huang et al., 1992), increases of fresh weights and elongation of shoots were significantly greater for the juvenile and rejuvenated shoots than adult shoots (Figures 1A, B).

Moisture and Nitrogen Contents

Juvenile, rejuvenated, and adult phase shoots had the same moisture content, approximately 85%. The total nitrogen content, however, was significantly higher in the juvenile and rejuvenated shoots than in the adult ones (Figure 2). They averaged nearly 5% of the dry weight of juvenile phase tissues. The adult tissues, AS and AST1, contained about 4.5% total nitrogen.

Respiration

Oxygen consumption, or index of respiration rate, was noticeably higher in the juvenile and rejuvenated shoots than in the adult ones. These shoots consumed about 0.3 µmole O_2 per gram tissue per min (Figure 3B). Both adult shoots, AS and AST1, consumed less than 0.25 µmole min^{-1} g^{-1} fwt.

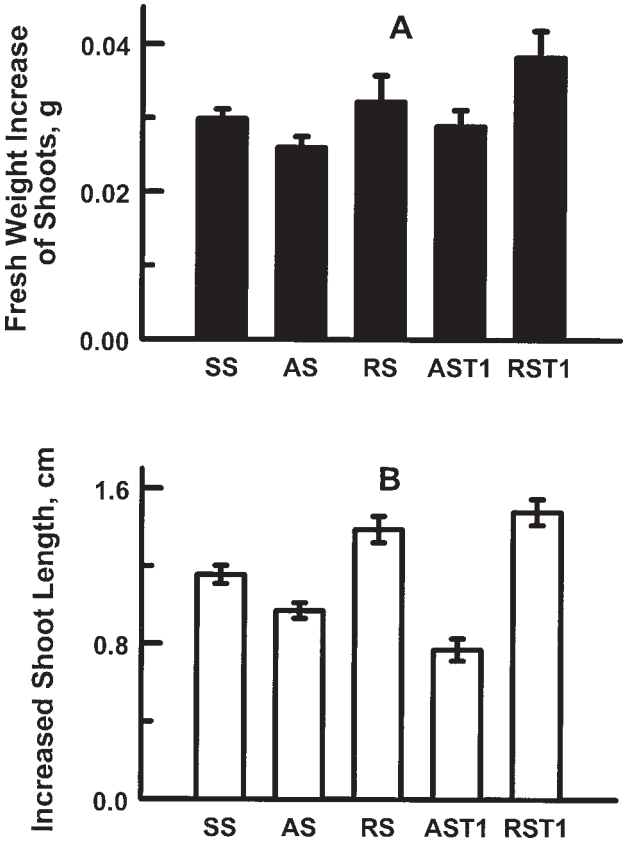

Figure 1. Fresh weight increases (A) and elongation (B) of juvenile (SS), adult (AS and AST1) and rejuvenated (RS and RST1) *S. sempervirens* shoots in vitro. In each case, rejuvenation was achieved by five successive grafts of adult shoot tips onto rooted SS segments in vitro. Values are the mean ± SE (n=3). Bars denote standard errors of means.

Photosynthesis

The rate of photosynthetic oxygen evolution was also noticeably higher in the juvenile (SS) and rejuvenated (RS, RST1) tissues than in the adult (AS, AST1) ones (Figure 3A). The O_2 evolution rates in these tissues ranged from ca. 1.2 to 1.4 μmoles min^{-1} g^{-1} fwt. Those of adult tissues were about 1 μmole min^{-1} g^{-1} fwt.

Chlorophyll Contents

Shown in Figure 4 are the contents of chlorophylls *a* and *b*. Concentrations of both chlorophylls were significantly higher in the juvenile and rejuvenated shoots than in the adult ones although the difference from adult shoots was substantially greater for chlorophyll *a*. The chlorophyll *a*/*b* ratios were significantly lower for adult tissues (2.41 in AS and 2.11 in AST1) compared to the juvenile (2.86 in SS) and rejuvenated ones (2.79 in RS; 3.19 in RST1). Therefore, total chlorophyll was significantly higher in the juvenile and rejuvenated shoots than in the adult ones, which contained less than 600 μg total chlorophyll per gram of tissue. Juvenile and rejuvenated shoots contained nearly 800 μg per gram, in other words, 33% more.

Quantum Efficiency of Photosystem II

Although differing in photosynthetic rates, quantum efficiency was the same for all tissues, juvenile, adult, and rejuvenated (Figure 5).

Discussion

We previously reported on differences between juvenile and adult *S. sempervirens* in protein phosphorylation (Kuo et al., 1995) and esterase and peroxidase isozymes

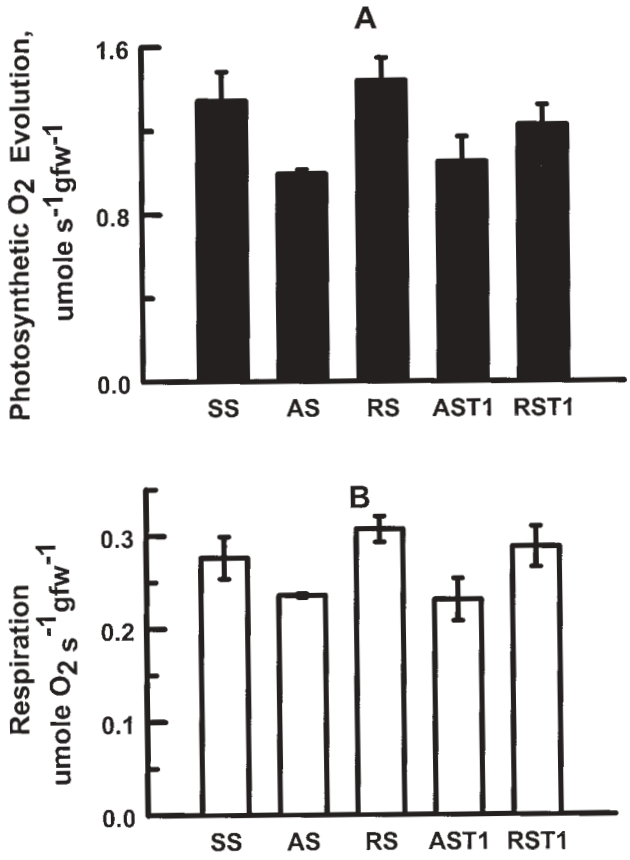

Figure 3. Photosynthetic (O_2 evolution) (A) and respiration (O_2 consumption) (B) rates of juvenile (SS), adult (AS and AST1), and rejuvenated (RS and RST1) *S. sempervirens* shoots. Values are the mean ± SE (n=3). Bars denote standard errors of means.

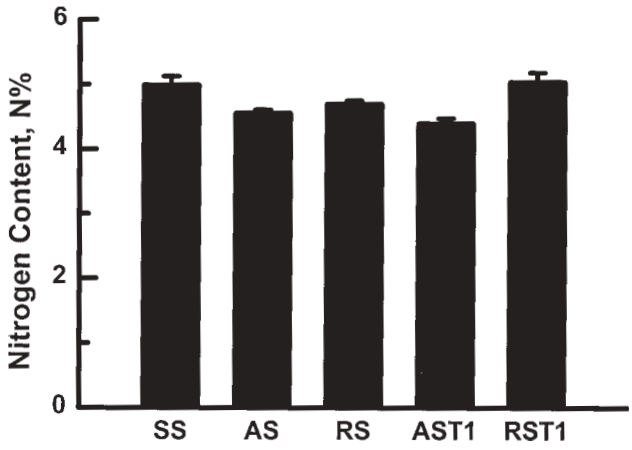

Figure 2. Total nitrogen contents in shoots of juvenile (SS), adult (AS and AST1), and rejuvenated (RS and RST1) *S. sempervirens*. Values are the mean ± SE (n=3). Bars denote standard errors of means.

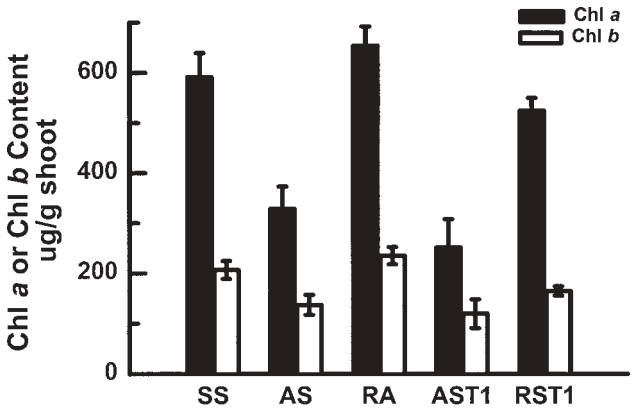

Figure 4. The chlorophyll *a* and chlorophyll *b* contents of juvenile (SS), adult (AS and AST1), and rejuvenated (RS and RST1) *S. sempervirens* shoots. Values are the mean ± SE (n=3). Bars denote standard errors of means.

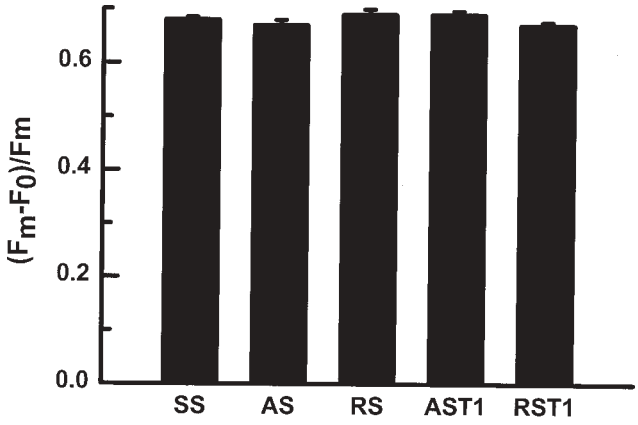

Figure 5. Quantum efficiency of photosystem II in vitro juvenile (SS), adult (AS and AST1) and rejuvenated (RS and RST1) *S. sempervirens* shoots. Values are the mean ± SE (n=10). Bars denote standard errors of means.

(Huang et al., 1996). Other investigators found differences in esterase and peroxidase isozymes between juvenile and adult *Betula* (Brand and Lineberger, 1992) and *Persea americana* (Sanchez-Romero et al., 1993). The differences were proposed as markers for distinguishing the juvenile and mature phases rather than clues to the mechanism underlying phase change. Perhaps more closely related to the mechanism might be the different protein phosphorylation patterns observed in juvenile and adult *S. sempervirens* by Kuo et al. (1995). In *Castanea sativa*, Amo-Marco et al. (1993) identified two polypeptides of 38 and 44 kDa uniquely associated with the adult phase. Similarly, Bon and Monteuuis (1991) observed a membrane-associated protein of 16 kDa in juvenile and rejuvenated *Sequoiadendron giganteum* which was lacking in the mature forms. In still another instance, Hand et al. (1996) reported of a 28 kDa protein in juvenile *Prunus avium*. Unfortunately, no progress reports have been made by these investigators. Our investigation of *S. sempervirens* led us to discover an association between developmental phases and mtDNA (Huang et al., 1995). The focus of our continuing investigation is on establishing a causal relationship. Applications of exogenous gibberellin and cytokinin can sometimes induce temporary phase reversal, consistently with the higher contents of gibberellins found in juvenile *Hedera helix* (Frydman and Wareing, 1973) and of cytokinins in juvenile *Hevea brasiliensis* (Perrin et al., 1997) compared to the mature phase.

Phase change investigations were extended to photosynthesis with a view to obtaining additional information for reliably characterizing the two phases. The higher photosynthetic rates, nitrogen content, and respiration rates observed in the juvenile and the rejuvenated *S. sempervirens* shoots are consistent with the more vigorous and rapid growth that characterize the juvenile stage. The increased nitrogen enables enhanced protein synthesis, and the required energy is derived from respiration. Nevertheless, the higher photosynthetic oxygen evolution and higher chlorophyll content of juvenile or rejuvenated shoots were not suggestive of a more efficient photosynthetic system. The virtual identity among all tissues, i.e., juvenile, adult and rejuvenated, of the $(F_m - F_o)/F_m$ ratios signified no differences in the physiological state of photosynthetic apparatus in intact tissues. Under optimal physiological conditions this parameter was found to have the value of 0.83 (Demmig and Björkman, 1987). The value for sequoia was about 0.76. Differences in the observed rates of photosynthesis are not reflective of differences in basic mechanisms.

Acknowledgements. The investigation was supported by grants from the National Science Council (NSC 89-2313-B-001-017) and the Institute of Botany, Academia Sinica.

Literature Cited

Amo-Marco, J.B., N. Vidal, A.M. Vieitez, and A. Ballester. 1993. Polypeptide markers differentiating juvenile and adult tissues in chestnut. J. Plant Physiol. **142:** 117-119.

Bilger, W., U. Schreiber, and M. Bock. 1995. Determination of the quantum efficiency of photosystem II and of non-chemical quenching of chlorophyll fluorescence in the field. Oecologia **102:** 425-432.

Bon, M.C. and O. Monteuuis. 1991. Rejuvenation of a 100-year-old *Sequoiadendron giganteum* through in vitro meristem culture. II. Biochemical arguments. Physiol. Plant. **81:** 116-120.

Brand, M.H. and R.D. Lineberger. 1992. In vitro rejuvenation of *Betula* (Betulaceae): biochemical evaluation. Am. J. Bot. **79:** 626-635.

Delieu, T. and D.A. Walker. 1981. Polarographic measurement of photosynthetic oxygen evolution by leaf discs. New Phytol. **89:** 165-178.

Demmig, B. and O. Björkman. 1987. Comparison of the effect of excessive light on chlorophyll fluorescence (77 K) and photon yield of O_2 evolution in leaves of higher plants. Planta **171:** 171-184.

Franclet, A. 1983. Rejuvenation: theory and practical experiences in clonal silviculture. *In* L. Zsuffa, R.M. Raturer, and C.W. Yeatman (eds.), Clonal Forestry: Its Impact on Tree Improvement and Our Future Forests. Part 2. Canadian Tree Improvement Association, Toronto, pp. 96-134.

Frydman, V.M. and P.F. Wareing. 1973. Phase change in *Hedera helix* L. I. Gibberellin-like substances in the two growth phases. J. Exp. Bot. **24:** 1131-1138.

Hand, P., R.T. Besford, C.M. Richardson, and S.D. Peppitt. 1996. Antibodies to phase related proteins in juvenile and mature *Prunus avium*. J. Plant Growth Reg. **20:** 25-29.

Huang, H.-J., Y. Chen, J.-L. Kuo, T.-T. Kuo, C.-C. Tzeng, B.-L. Huang, C.-M. Chen, and L.-C. Huang. 1996. Rejuvenation of *Sequoia sempervirens* in vitro: changes in isoesterases and isoperoxidases. Plant Cell Physiol. **37:** 77-80.

Huang, L.-C., C.-I. Kuo, C.-H. Wang, T. Murashige, and T.-C. Huang. 2000. Ethylene evolution by juvenile and adult developmental phases of *Sequoia sempervirens* shoots cultured in vitro. Bot. Bull. Acad. Sin. **41:** 263-266.

Huang, L.-C., L.-Y. Lin, C.-M. Chen, L.-J. Chen, B.-L. Huang,

and T. Murashige. 1995. Phase reversal in *Sequoia sempervirens* in relation to mtDNA. Physiol. Plant. **94:** 379-383.

Huang, L.-C., S. Lius, B.-L. Huang, T. Murashige, E.F.M. Mahdi, and R. Van Gundy. 1992. Rejuvenation of *Sequoia sempervirens* by repeating grafting of shoot tips onto juvenile rootstocks in vitro. Plant Physiol. **98:** 166-173.

Kuo, J.-L., H.-J. Huang, C.-M. Cheng, L.-J. Chen, B.-L. Huang, L.-C. Huang, and T.-T. Kuo. 1995. Rejuvenation in vitro: modulation of protein phosphorylation in *Sequoia sempervirens*. J. Plant Physiol. **146:** 333-336.

Lentner, C. (ed.). 1982. Geigy Scientific Tables, ED 8, Vol. 2. Ciba-Geigy, Basle, Switzerland, pp. 89-102.

Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol. Plant. **15:** 473-497.

Perrin, Y., P. Doumas, L. Lardet, and M.-P. Carron. 1997. Endogenous cytokinins as biochemical markers of rubber-tree (*Hevea brasiliensis*) clone rejuvenation. Plant Cell Tissue Organ Cult. **47:** 239-245.

Sanchez-Romero, C., M.L. Garcia-Gomez, F. Pliego-Alfaro, and A. Heredia. 1993. Peroxidase activities and isoenzyme profiles associated with development of avocado (*Persea americana* M.) leaves at different ontogenetic stages. J. Plant Growth Regul. **12:** 95-100.

Wintermans, J.F.G.H. and A. De Mots. 1965. Spectrophotometric characteristics of chlorophyll and their pheophytins in ethanol. Biochim. Biophys. Acta **109:** 448-453.

紅杉試管幼年、老齡、及其復幼植莖光合作用之潛力

黃麗春　翁瑞禧　王秋惠　郭靜儀　謝昱暐

中央研究院植物研究所

紅杉不同生長相 (Developmental Phases) 即幼年期與老齡期植株建立之試管培養，比較其試管內光合作用之生理特性為本研究之主要探討。以一種幼年苗，二株成齡老樹及該二成齡老株返老還輕復幼後，培養之試管無性系取其植莖為試驗材料，發現二種幼年性即幼年莖與復幼莖與成齡老株植莖，細胞水份含量相同，約為 85%。分析生長速率（植莖生長長度及鮮重增加）及含氮量，發現二幼年性皆高於成齡老株。幼年莖與復幼莖其光合作用及呼吸作用速率亦優於成齡老株。總葉綠素含量，尤其葉綠素 *a*含量，幼年莖與復幼莖顯著高於成齡老株，但二生長相，無論幼年期或老齡期之 $(F_m - F_o)/F_m$ 相同，顯示二生長相葉之光合系統的電子傳遞效率並無差異。

關鍵詞：紅杉；生長相；光合作用；呼吸作用；含氮量。