

## Characteristics of Photosynthetic Electron Transport Activity in Isolated Chloroplasts of Korean Ginseng and Radish

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### 人蔘과 무우 葉綠體의 光合成 電子傳達 活性

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#### ABSTRACT

In order to characterize the chloroplasts of Korean ginseng as a semi-shade plant and radish as a sun plant, effects of growth light intensity on photosynthetic electron transport (PS) activity in chloroplasts and superoxide ( $O_2^{\cdot-}$ ) production in thylakoid membrane by irradiation were investigated. High-light chloroplasts of both plants showed higher PS activities than those grown under low growth light intensity. High PS II and low PS I activities in ginseng chloroplasts (ratio of PS II/PS I: 1.1) were observed, but radish chloroplasts showed low PS II and high PS I activities (ratio of PS II/PS I: 0.3). PS II activity of both plants was little affected by temperature in ranges of 15-35 °C. Activities of whole-chain (PS II + I) in ginseng and PS I in radish were increased at high temperature (40 °C). Preincubation of chloroplasts at 40 °C during 30 min, as a mild heat stress, caused rapid decrease in PS II and PS II + I activities of both plants. However PS I activity was not decreased in ginseng and rather increased in radish.  $O_2^{\cdot-}$  production (NBT reduction) in Mehler reaction in the thylakoid membrane was inhibited by DCMU in both plants. DMBIB inhibited  $O_2^{\cdot-}$  production in ginseng, but radish was insensitive to DMBIB. Electron flow system in ginseng thylakoid membrane was more susceptible to damage of photooxidation than that of radish.

**Abbreviations:** PS, photosynthetic electron transport; DCPIP, 2,6-dichlorophenol indophenol; FaCy, ferricyanide; PD, *p*-phenylene diamine; MV, methylviologen; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethyl urea; NBT; *p*-nitro blue tetrazolium chloride; DMBIB, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone.

#### INTRODUCTION

Korean ginseng is one of the most important Oriental herb medicines. Since it has been genotypically adapted to deeply shaded habitats, it is not able to acclimate and grow in full sun conditions. Therefore, it

has long been cultivated under the shade. Because of low growth rate of this plant, it takes about 6 years for good quality even under the most favorable condition for cultivation. In order to improve its low productivity, physiological characteristics of ginseng plant as responses to environmental factors were intensively

investigated during the last decade (Lee, 1988; Park, 1980).

In general, the improvement of ginseng productivity through the increase of photosynthesis is strongly restricted by low light-saturated photosynthetic capacity in ginseng leaves (Lee, 1988) and photodamage in the photosynthetic apparatus induced by exposure to strong light (Yang *et al.*, 1987a,b, 1989). But high temperature as an environmental factor appears to have little influence on photooxidative chlorophyll-bleaching phenomenon (Yang *et al.*, 1987b). Many investigators (Anderson, 1988; Björkman, 1981; Boardman, 1977) have reported, in detail, the characteristics of photosynthesis in many kinds of shade plants. Unfortunately few articles about the characteristics of photosystem in ginseng chloroplasts were published.

In the present study, we tried to get some informations about the characteristics of ginseng chloroplasts in view of the photosynthetic electron transport system (PS), and to compare them with those of radish chloroplasts, as a sun plant.

## MATERIALS AND METHODS

**Plant materials.** One year-old ginseng (*Panax ginseng* C.A. Meyer) roots were grown in shade conditions, where maximum light intensities were 1500, 4500, and 30,000 lux. Radish plants (*Raphanus sativus* L. var. *Altam*) were cultivated in a greenhouse under natural sunlight (ca. 90,000 lux). They were grown in flower beds containing loamy sand mixed with compost. After 4 weeks, plants with 3-4 leaves fully expanded were moved to shade condition (2500 lux) and newly developed leaves were used for chloroplast isolation.

Temperature ranged from 18°C to 30°C during growth period. Nutrient solution was frequently irrigated for radish plants. Chloroplasts were isolated from mature leaves harvested at 9 a.m.

**Chloroplast and thylakoid membrane isolation.** Chloroplasts were isolated from leaf tissues at 4°C as described by Yu (1987). Leaf tissues were ground in ice-cold grinding medium (0.33 M sorbitol, 10 mM NaCl, 0.1% BSA, 50 mM Tricine-KOH buffer, pH 7.9) using a mortar and pestle. The brei was filtered through 6 layers of gauze and centrifuged at 80 × g for 5 min. The pellet was discarded and the supernatant was recentrifuged at 500 × g for 10 min. The chloroplast

pellet was resuspended in reaction medium containing 0.33 M sorbitol, 2 mM EDTA, 1 mM MgCl<sub>2</sub>, 1 mM MnCl<sub>2</sub>, and 50 mM Hepes-KOH buffer (pH 7.6). For the isolation of thylakoid membrane, the chloroplast pellet was suspended in lysis buffer (10 mM Tricine-KOH, pH 8.0, 150 mM NaCl). After centrifugation at 1500 × g for 10 min, the pellet was resuspended in reaction medium (0.1 M sorbitol, 5 mM NaCl, 5 mM MgCl<sub>2</sub>, 50 mM Hepes-KOH, pH 7.6), generally following the method of Leegood and Malkin (1986).

**Electron transport in chloroplasts.** Rate of O<sub>2</sub> evolution or consumption by chloroplasts was quantified using a Clark-type O<sub>2</sub> electrode (Gilson, 5/6 H Oxygraph, YSI membrane) and O<sub>2</sub> concentration in reaction mixture at a measuring temperature was calibrated by the response of the electrode after addition of solid Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub> in 1 ml H<sub>2</sub>O in reaction vessel (Allen and Holmes, 1986). One ml of reaction mixture containing chloroplasts (50-70 μg chlorophyll/ml) and respective reagents required for photosynthetic electron transport system (PS) was preincubated in the cuvette for 1-2 min and measurement of O<sub>2</sub> concentration was started with illumination by a slide projector (Kodak Carousel 760 H) 15 cm apart from the cuvette. The activity of whole-chain electron transport (PS II + I) was measured as O<sub>2</sub> evolution in the presence of 0.1 mM DCPIP and 2 mM FeCy. The activity of photosystem II (PS II) was determined with 0.5 mM PD and 2 mM FeCy. For PS I activity, O<sub>2</sub> consumption was measured in the mixture supplemented with 0.1 mM MV, 10 μM DCMU, 0.1 mM DCPIP, and 5 mM ascorbate.

**O<sub>2</sub><sup>-</sup> production.** O<sub>2</sub><sup>-</sup> production in thylakoid membrane was followed using a modification of the photochemical assay described by Daub and Hangarter (1983). The reaction mixture consisted of thylakoid membrane (ca. 10 μg chl.), 13 mM methionine, 63 μM NBT, 0.1 M sorbitol, 5 mM MgCl<sub>2</sub>, 5 mM NaCl, and 50 mM Hepes-KOH (pH 7.6) in a final volume of 2 ml. The mixture in a 3 ml cuvette was irradiated by passing the beam of a slide projector through of CuSO<sub>4</sub> solution. O<sub>2</sub><sup>-</sup> production (NBT reduction) was monitored by following the increase in A at 560 nm after every 20 sec irradiation.

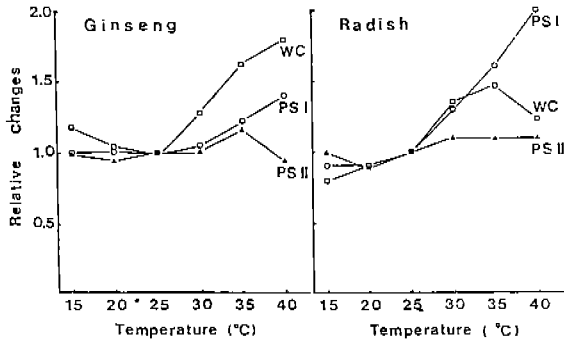
## RESULTS

Effect of growth light intensity on PS activity in in-

**Table 1.** Effect of growth light intensity on activities of photosynthetic electron transport (PS) in intact chloroplasts of Korean ginseng and radish plants

	Growth light intensity (K Lux)	PS* activity			Ratio		
		II+I	II ( $\mu\text{mole O}_2/\text{mg chl. hr}$ )	I	I/II+I	II/II+I	II/I
Korean ginseng	1.5(LL)	13.8 (84)	35.6 (88)	30.0 (82)	2.2	2.6	1.2
	4.5(LL)	12.4 (76)	29.0 (72)	31.1 (85)	2.5	2.3	0.9
	30.0(HL)	16.4 (100)	40.4 (100)	36.7 (100)	2.2	2.5	1.1
1.1	$\bar{X}$				2.3	2.5	
Radish	2.5(LL)	9.8 (70)	16.7 (81)	40.3 (58)	4.7	1.7	0.4
	90.0(HL)	14.0 (100)	20.6 (100)	69.0 (100)	4.9	1.5	0.3
	$\bar{X}$				4.8	1.6	0.4

\*II+I, II, and I: Activities of whole-chain, PS II, and PS I, respectively. PS activity was measured at 25 °C in the presence of required reagents (PS II+I, 0.1 mM DCPIP + 2 mM FeCy; PS II, 0.5 mM PD + 2 mM FeCy; PS I, 0.1 mM MV + 10  $\mu\text{M}$  DCMU + 0.1 mM DCPIP + 5 mM ascorbate). The figures in parentheses show per cent.



**Fig. 1.** Temperature-dependent change in activity of photosynthetic electron transport system (PS) in chloroplasts isolated from Korean ginseng and radish leaves grown under light conditions of 4.5 klux and 90 klux, respectively. The results were average values from 3 separate experiments. Activity at 25 °C represents 1.0. WC, Whole-chain (PS II + I); PS II, Photosystem II; PS I, Photosystem I.

tact chloroplasts was shown in Table 1.

The chloroplasts of both ginseng (30 klux) and radish (90 klux) grown under high light intensity (HL) showed higher PS activities compared to those adapted to low growth light intensity (LL). The PS activities of the LL chloroplasts decreased to around 80% of those of the HL chloroplasts in ginseng plants. The

**Table 2.** Temperature-dependent change in activity ratio between each photosynthetic electron transport system in intact chloroplasts of Korean ginseng and radish

Temp. (°C)	Korean ginseng			Radish		
	I/II+I	II/II+I	II/I	I/II+I	II/II+I	II/I
15	1.4	2.6	1.9	3.5	2.1	0.6
20	1.6	2.8	1.8	3.4	1.9	0.6
25	1.6	3.1	2.0	3.5	1.9	0.6
30	1.5	2.4	1.6	3.4	1.6	0.5
35	1.3	2.3	1.8	3.7	1.4	0.4
40	1.6	1.9	1.2	5.8	1.9	0.4
$\bar{X}$	1.5	2.5	1.7	3.9	1.8	0.5

reduction in PS activities of the LL chloroplasts was more prominent in radish, as a sun plant, especially, the LL chloroplasts showed only 60% of PS I activity of the HL chloroplasts. In spite of decrease in PS activities under LL, the ratios between respective PS activities were little affected by growth light intensity. Radish chloroplasts showed higher PS I and lower PS II activities (PS II/PS I: 0.4) compared to ginseng chloroplasts (PS II/PS I: 1.1).

The PS activities were measured at various measuring temperature in range of 15-40 °C (Fig. 1).

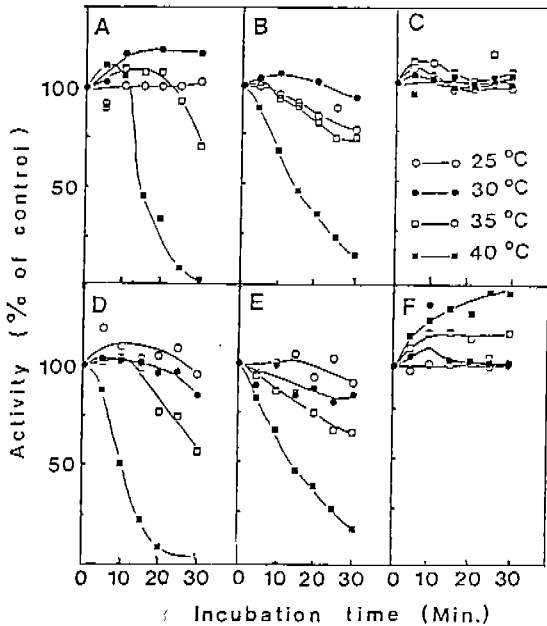


Fig. 2. Effects of thermal stress on electron transport (PS) activity in chloroplasts isolated from Korean ginseng (A, B, C) and radish (D, E, F) leaves. Photochemical activities were measured at 25°C. The activity at the beginning of incubation was represented as 100%. A, D: PS II + I; B, E: PS II; C, F: PS I.

Activities of PS II + I, PS II, and PS I at 25°C were 21.9, 69.1, and 35.0  $\mu\text{M O}_2/\text{mg chl./hr}$  for ginseng and 14.2, 27.4, and 50.1  $\mu\text{M O}_2/\text{mg chl./hr}$  for radish chloroplasts, respectively. PS II activity in both ginseng and radish chloroplasts was little changed by the temperature in range of 15-35°C. Activities of PS II + I (whole-chain) in ginseng and PS I in radish were sensitively increased with the temperature increase from 25°C to 40°C. But PS II + I activity in radish and PS II activity in ginseng were inhibited at 40°C. PS I activity in ginseng was slightly increased at high temperature.

The change in ratio between each PS activity at measuring temperature ranged from 15°C to 40°C was not so great than the ratio difference between two species (Table 2), which was very prominent, i.e. high PS II/PS I ratio (1.6) in ginseng and low ratio (0.6) in radish, as shown in Table 1.

In order to investigate the effects of mild heat stress on PS activity in intact chloroplasts, PS activities were measured at 25°C after the preincubation at various temperature during 30 min (Fig. 2).

Preincubation during 30 min in temperature range

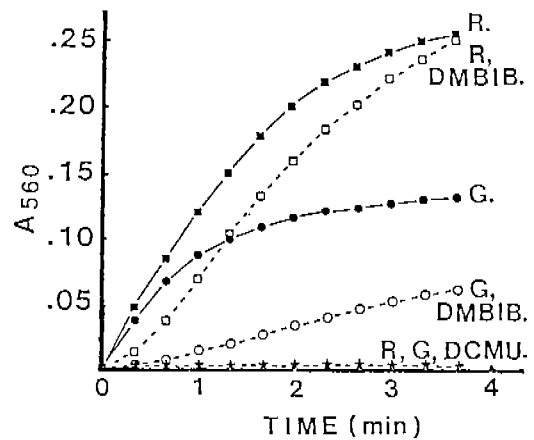


Fig. 3. Effects of DCMU and DMBIB on the production of  $\text{O}_2^-$  in thylakoid membrane of ginseng (G) and radish (R). The reaction mixture contained thylakoid membrane (ca. 10  $\mu\text{g}$  chlorophyll), 13 mM methionine, 63  $\mu\text{M}$  NBT, 0.1 M sorbitol, 5 mM  $\text{MgCl}_2$ , 5 mM NaCl and 50 mM Hepes-KOH (pH 7.6) in a final volume 2 ml. 0.5 mM DCMU or 50  $\mu\text{M}$  DMBIB was added.  $\text{O}_2^-$  production (NBT reduction) was monitored by following the increase in absorbance (A) at 560 nm.

of 25-40°C caused little changes in PS I activity in ginseng chloroplasts (Fig. 2C). Radish chloroplasts showed rather slight increase in PS I activity by heat treatment (Fig. 2F). Activities of PS II were markedly inhibited in both plants by heat stress of 40°C (Fig. 2B and E). Concomitant reduction of PS II + I activity could be observed during the preincubation at 40°C (Fig. 2A and D).

$\text{O}_2^-$  production (NBT reduction) in the thylakoid membrane of ginseng and radish by irradiation was shown in Fig. 3.

The amount of  $\text{O}_2^-$  produced in radish thylakoid membrane through Mehler reaction was much higher than that in ginseng. The  $\text{O}_2^-$  generation per time unit in ginseng decreased more rapidly under irradiation, compared to that in radish (Fig. 3G and R). DCMU inhibited completely the  $\text{O}_2^-$  production in thylakoid membrane of both plants. The strong inhibition of  $\text{O}_2^-$  production by DMBIB could be observed in ginseng. But such inhibitive effect of DMBIB in radish was transient in the first 40 sec irradiation period, and the  $\text{O}_2^-$  generation was soon recovered.

## DISCUSSION

Photosynthetic acclimation of the plants in natural environments is quite limited, because the potential for

photosynthetic acclimation is different among species and even populations of the same species (Anderson, 1986; Berry and Björkman, 1980). The light intensity under the shade in ginseng field is 4-7 klux, and the light saturation point is about 10 klux at 20-25 °C (Lee, 1988). However, the highest photosynthesis occurs in ginseng leaves grown under the shade of 15% transmittance of natural sunlight, about 20 klux (Lee, 1988). Under the high-light condition, severe photooxidative damage occurs in the photosynthetic apparatus of shade plants (Krause, 1988; Yang *et al.*, 1987a, b). But the radish requires high light intensity for the growth and does not suffer the photodamage even under the natural sun light.

In the present study, the high-light chloroplasts of ginseng and radish showed higher PS activities than the low-light chloroplasts (Table 1), as reported in other shade plants by Boardman (1977) and Björkman (1981). Under 2.5 klux, the growth inhibition of radish leaves could be discernible, but that of ginseng could not even under 1.5 klux. From the result that the PS I activity of radish chloroplasts was more decreased under low-light growth condition than that of ginseng chloroplasts (Table 1), it was disclosed that radish suffered severely from light deficiency.

Acclimation of ginseng to the shade condition may result in high PS II activity (high ratio of PS II/PS I: 1.1-1.6, Tables 1 and 2) for efficient light energy harvest (Anderson, 1986), as shown in a model of possible organization of the photosynthetic system in sun and shade leaves by Björkman (1981). That may cause the photoinhibition and photooxidative damage under HL condition by excessive transfer of excitation energy from the light-harvesting pigment molecules (Kyle *et al.*, 1985; Krause, 1988). But in radish, low activity of PS II compared with that of high PS I (PS II/PS I: 0.4-0.6) may restrict excessive electron flow from PS II even under the full sun light.

Changes in PS activities of the intact chloroplasts in range of 15 °C to 40 °C were different between ginseng and radish (Fig. 1). Although PS II activity in ginseng was reduced at 40 °C, PS II + I activity in ginseng increased with the increment of the measuring temperature from 25 °C to 40 °C. In view of the high ratio of PS II/PS I (1.2 at 40 °C), that might be attributed to the slight increase of PS I activity. In radish chloroplasts, inhibition of PS II + I activity at 40 °C could be observed, in spite of the steady increase of PS I activity. This suggests that the whole-chain activity of radish may be

limited at high temperature through some other mechanisms.

In summer, when the early leaf fall often occurs in ginseng fields, sun light accompanies high temperature over 38 °C, which may cause leaf damage. From the study about the effect of mild heat stress on PS activities (Fig. 2), PS II activity of the intact chloroplasts was sensitively reduced in both plant species by preincubation at 40 °C. That accompanied the inhibition of whole-chain activity. But PS I activity was not inhibited by mild heat treatment in ginseng, and rather stimulated in radish, as reported by other investigators (Berry and Björkman, 1980; Gounaries *et al.*, 1983; Sabat and Mohanty, 1989; Thomas *et al.*, 1986). They concluded that the increase in PS I rate was associated not only with uncoupling of chloroplasts by heat treatment but also with some alteration of thylakoid membrane structure, imposing change in the DCPIP H<sub>2</sub> electron donate site and therefore ensuing an increase of PS I catalyzed electron transport activity. From the data, it can be suggested that high temperature may also accelerate the photodamage in summer through inhibition of PS II activity.

Under high-light intensity, ginseng leaves are damaged such as photooxidative-destruction of chlorophyll pigments and membrane, and leaf fall (Yang *et al.*, 1987a, b). Yang *et al.* (1989) reported that chlorophyll-bleaching in ginseng chloroplasts was induced by the treatment of <sup>1</sup>O<sub>2</sub> in reaction medium and other active oxygens (H<sub>2</sub>O<sub>2</sub>, O<sub>2</sub><sup>-</sup>) induced this phenomenon under irradiation. They concluded that these active oxygens might be the major agents causing the photooxidative damage.

Although the amount of O<sub>2</sub><sup>-</sup> generated through Mehler reaction in thylakoid membrane under irradiation was higher in radish than in ginseng, the inhibition of O<sub>2</sub><sup>-</sup> production per time unit by irradiation was more severe in ginseng thylakoid membrane than in radish, when compared on a chlorophyll basis (Fig. 3 G and R). This suggests that the electron transfer system from PS II to PS I of ginseng thylakoid is more susceptible to photodamage than that of radish. Considering that the NBT reduction in the reaction mixture was completely inhibited by DCMU in both plants without any chlorophyll-bleaching (Fig. 3, DCMU), the photodamage in this experiment might be caused by O<sub>2</sub><sup>-</sup> formed through electron flow to PS I. Moreover, ginseng showed a sensitive inhibition of O<sub>2</sub><sup>-</sup> formation by DMBIB treatment, which was known as a inhibitor

of electron flow through the cytochrome  $b_6/f$  complex (Sandmann and Böger, 1986; Allen and Holmes, 1986). But in radish this inhibitive effect of DMBIB was completely recovered by successive irradiation (Fig. 3, DMBIB).

From the data of the present study, it can be suggested that the ginseng chloroplasts also have the general characteristics as those of other shade plants, such as high PS II and low PS I activities, and high susceptibility to photodamage. Difference in DMBIB sensitivity between both plants suggests that the biochemical characters of electron flow system through cytochrome  $b_6/f$  complex in ginseng is different from those in radish. Further study will be focused on the repair system in photoinhibition and the mechanism of the nondestructive dissipation of excessive excitation energy in the intact chloroplasts.

## 적 요

반음지성 식물인 인삼의 엽록체내의 광합성 전자전달계(PS)에 관한 기초 지식을 얻고자 광의 강도와 온도처리별 전자전달계 활성을 조사하여 양지성 식물인 무우와 비교하였다. 고광도하에서 자란 식물체의 엽록체가 저광도의 엽록체보다 모든 PS 활성이 높았다. 인삼의 엽록체는 PS II 활성이 PS I 활성보다 높았으며(PS II/PS I : 1.1-1.6), 무우 엽록체는 PS I 활성이 PS II 활성보다 월등히 높았다(PS II/PS I : 0.4-0.6). 측정온도에 따른 PS 활성변화를 측정할 때, PS II 활성은 15-30°C 범위에서 거의 일정하였으며, 40°C까지는 온도상승에 따라 인삼에서는 PS II + I 활성이, 무우에서는 PS I 활성이 계속 증가하였다. 온도 stress(온탕 25-40°C, 5-30분)에 따른 PS 활성변화를 보면, 인삼과 무우 공히 PS II 및 PS II + I 활성이 고온에서 급격히 감소하였고, PS I 활성은 감소하지 않았고 무우의 경우 오히려 증가하였다. 엽록체 thylakoid membrane을 분리하여 광을 조사하였을 때 Mehler 반응에 의해 생성되는 superoxide( $O_2^-$ )의 양(NBT reduction)을 측정하였던 바, 인삼보다 무우에서 많았으며, 광조사 시간이 경과함에 따라 인삼의  $O_2^-$  생성능이 무우에서보다 급격히 감소하였다. DCMU에 의해서 두 식물 공히  $O_2^-$  생성이 완전 억제되었고, DMBIB에 의해서는 인삼에서 크게 억제된 반면, 무우에서는 억제효과가 아주 적어서, 두 식물간에 반응차이가 뚜렷하였다.

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