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Response of Leaf Pigment and Chlorophyll Fluorescence to Light Quality in Soybean (*Glycine max* Merr. var Seoritae)

Sei Joon Park[1], Do Yun Kim[2], Sung Yung Yoo[2,3], Hyun Hee Kim[1], Tae Seok Ko[1], Myong Yong Shim[1], So Hyun Park[2,3], Ji A Yang[2], Ki Cheol Eom[3,4], Sun Hee Hong[5], and Tae Wan Kim[1,2]*

[1]*Institute of Ecological Phytochemistry, Hankyong National University, Ansung 456-749, Korea*
[2]*School of Plant Life and Environmental Science, Department of Phytomedicine, Hankyong National University, Ansung 456-749, Korea*
[3]*Sejong Institute of Data Analysis (SEIDA), Ansung 456-749, Korea*
[4]*Institute of Soil Science, Environment &Underground Water Ltd., Ansung 456-749, Korea*
[5]*School of Ecological and Environmental Science, Korea University, Seoul 136-701, Korea*

Etiolation of plant leaves evoke to be photosynthetically inactive because plant leaves are unable to convert photochlorophyllide to chlorophyllide in the absence of light. In addition, UV-B radiation plays an important role in photomorphogenesis and excessive UV-B radiation decreases photosynthesis and causes to damage to cellular DNA. In the present study, two electrical lights obtained with the ultraviolet lamp and moderate lamp were employed to young plants soybean (*Glycine max* Merr. var Seoritae). After treatment of different lights, young plants were harvested for the determination of pigment contents and chlorophyll fluorescence. The contents of carotenoids and anthocyanins were significantly enhanced with the excessive UV-B radiation. Excessive UV-B light reduced dramatically photosynthetic efficiency causing an irreversible damage on PSII in comparison to the controls treated under normal illumination. As the treatment of normal illumination after dark treatment, the contents of carotenoids and anthocyanains were not changed in the leaves and photosynthetic ability were retained. Therefore, Seoritae soybean leaves might protect themselves from excessive UV-B radiation with up-regulation of antioxidants.

Key words: Soybean, Leaf pigments, Chlorophyll fluorescence.

Introduction

Dark-grown angiosperm seedling contains a chloro-phyllous plastid type known as etioplasts which are transformed into chloroplasts when light is available. They are characterized by a paracristalline prolamellar body which consists of a ternary complex consisting of NADPH, protochlorophyllide oxidoreductase and proto-chlorophyllide (Apel et al., 1980; Oliver et al., 1981). Since etioplast of higher plants are unable to catalyze the conversion of protochlorophyllide to chlorophyllide in the absence of light, they are photosynthetically inactive (Scheumann et al., 1999; Armstrong et al., 2000). A recent report indicates that etioplasts rapidly re-differentiate into photosynnthetically active chloroplasts upon illumination and initial stages of plastid assembly have been studied in the absence of highly-abundant photosynthetic proteins (Baginsky and Gruissem, 2004).

Leaf pigment content can provide valuable insight into the physiological performance of leaves. In accordance with pigment absorptance, this approach may be useful for characterization of the fate of absorbed light in photosynthesis and photo-protective mechanisms. (Sims et al., 1999). Chlorophyll a and b, carotenoid and anthocyanin concentrations correlate to the photosynthetic potential of a plant and give some indication of the physiological status of the plant (Schepers et al., 1996). Chlorophylls tends to decline more rapidly than crotenoids when plants are under

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*Corresponding author : Phone:+82316705081
E-mail: taewkim@hknu.ac.kr

stress or during leaf senescene (Gitelson et al., 1994a, 1994b). Carotenoids (yellow pigments) are the second most abundant pigment in nature, essential components of plant photosynthetic apparatus. Their roles in light harvesting, stabilization of thylakoid membranes, energy distribution in pigment-protein complexes and photoprotection are well-documented (Havaux, 1998). As a result of chlorophyll breakdown, carotenoids were suggested to be involved in photopotection during dismounting of photosynthetic machinery in sensing plants against various harmful environmental factors (Merzlyak et al., 1995, 2002; Strzałka et al., 2003). Carotenoids are known to be sensitive to oxygen, free oxygen, organic radical, undergo organic radical destruction when exposed to light stress in the presence of chlorophyll (Merzlyak et al., 1996; Tregub et al., 1996). While changes in chlorophylls are indicative of stress and phenological status, carotenoid concentration provides complementary information on plant physiological status (Young et al., 1990). Anthocyanins are a group of water-soluble flavonoids that appear pink to purple colours in leaves and other organs. It absorbs light in the UV region of spectrum. Anthocyanins have multiple functions in different plant tissues (Dakora., 1995). In leaves, anthocyanins show to act as a sunscreen, protecting cells from photo-damage, tissues from photoinhibition or light stress (Barker et al., 1997; Steyn et al., 2002; Close et al., 2003). Anthocyanins also act as powerful antioxidants, helping to protect the plant from radicals formed by UV light and during metabolic processes (Burger et al., 1996; Klaper et al., 1996).

The degree of photoinhibition can clearly be determined via measurements of chlorophyll fluorescence relaxation kinetics and depends on the photon flux density of the light stress. Oxygenic photosynthetic for plant requires linear electron transport that is driven by serially operating Photosystem II (PS II) and Photosystem I (PS I) reaction centers (Vacha et al., 2007). Changes in fluorescence yield essentially result from variations in the rates of photochemical energy conversion and nonphotochemical energy dissipation (Mouget et al., 2002). Higher plants develop a variety of photoprotective mechanisms against photoinhibition or the light-dependent loss of photosynthetic efficiency (Chow, 1994; Osmond, 1994). These photoprotective mechanisms are classified as either long-term or short-term responses. The long-term responses include avoidance mechanisms that involve changes in chloroplasts (Björkman et al., 1994; Park et al., 1996) and modulation of the composition of the photosynthetic apparatus by light acclimation (Anderson et al., 1987). The most prominent short-term response is non-photochemical quenching (NPQ), which plays an important role in the photoprotection of PSII in vivo (Zulfugarov et al., 2007).

Several studies have shown that photosystem II (PS II) is often sensitive to ultraviolet-B (UV-B) and it has been assumed to be the most sensitive photosynthetic target for UV-B (Bornman, 1898; Melis et al., 1992). Although UV-B radiation has important regulatory and photomorphogensis roles (Ballare et al., 1995), excessive UV-B radiation reduced photosythesis and growth and injured to DNA (Bray et al, 2005). Treatment with UV-B provoked a decrease in antioxidant enzyme activities and negative impacted on plant cells (Yannarelli et al., 2006). That is, plant exposure to UV-B could impair all major process in photosynthesis including photochemical reactions in thylakoid membranes, enzymatic processes in the Calvin cycle, and stomatal limitations to CO_2 diffusion (Bornman, 1989; Lesser and Neale, 1996; Allen et al., 1998, 1999).

The goal of this study is to examine etioplatid and chloroplastid transformation in relation to different light sources in soybean seedlings. As they are transformed, changes of carotenoid and anthocyanin contents, content and ratios of chlorophylls, and fluorescence parameters are determined in relation to light sources including UV-B stress.

Materials and Methods

The soybean cultivation carried out in grasshouse and chamber at Hanyoung National University, Anseong. Seed of *Glycine max* Merr. var Seoritae, which was collected from Korea. This grown in 400 ㎖ rubber pot. Soybean seedlings were grown for 18days on vermiculite and tap water at 30℃ in grasshouse. Thereafter, it was grown for 3days at chamber in darkness. Then, they were provided two type of the light source (moderate light and Ultraviolet-B) for 3days at same stipulation to cultivate. It used to harvest of first trifoliates for analysis of pigment content and fluorescence kinetic quenching.

Chlorophyll and carotenoid were extract in 80%

acetone (v/v) to kept -4℃. Anthocyanin was also extracted in 1% HCl- MeOH (v/v). The clear supernatant obtained after filtered through two layers of cheesecloth and then centrifuged at 480 x g for 3 min (Lichrtenthaler et al., 2007). The content of pigments were measured using an ultraviolet spectrophotometer (Beckman coulter DU 650) under the wavelengths of 663 nm (Chlorophyll a), 647 nm (Chlorophyll b), 470 nm (Carotenoid), 537 nm (Anthocyanin), following Sims and Gamon (1999).

The fluorescence parameters were measured using protocol to quenching analysis by the kinetics image fluorometer (P.S.I., Fluorcam 700MF, CZ). The chlorophyll fluorescence induction kinetics of pre-darkened leaves for 30min before measurement. A dark-adapted leaves are exposed to various induction light sources of actinic and saturant. The continuous actinic light (red LED) amounted to 200μ mol $m^{-2}s^{-1}$ and the source of saturating light (moderate light) pulsed to 1,250μ mol $m^{-2}s^{-1}$. The fluorescence measurements are made using modulated fluorometer (Baker and Rosenqvist, 2004). Other fluorescence parameters (Fv/Fm, Fv'/Fm', R_{FD} and Φ_{PSII}) and values of quenching coefficients (qP, qNP, NPQ) can then be computed by software, using equations of Schreiber et al.(1986).

Results and Discussion

Changes of chlorophylls content Changes of chlorophyll a and b contents, chlorophyll a/b ratio at different light condition were showed at figure 1. Chlorophyll a decreased rapidly during dark treatment, particularly at 72 hr period. As leaves exposed to light, chlorophyll a content increased gradually. However, the chlorophyll a content was not recovered under the UV-B light. The difference of chlorophyll a content between two light conditions were statistically significant when leaves were exposed two lights. Chlorophyll a/b ratio decreased gradually during dark condition and the ratio was significantly lower in UV-B exposed leaves than in natural light exposed seeds.

In general, lowered changes in chlorophyll are indicative of stress and senescence (Richardson, 2002; Young et al., 1990). Chlorophyll b is known to be more sensitive to UV-B radiation than chlorophyll a (Strid and Porra, 1992). On the other hand, chlorophyll a/b has been frequently used as an indicator of plant response to light intensity (Hendry and Price, 1993). Thus, chlorophyll b content detected depletion in young plants soybean under UV-B treatment. In this study, it was clearly observed that a signalling to senescence was progressed with dark and UV-B treatments, following to chlorophyll degradation.

Changes of cartenoids and anthocyanin content Carotenoids and anthocyanins were produced as the young plants exposed to UV-B light. Their contents were significantly higher in UV-B light than in natural light

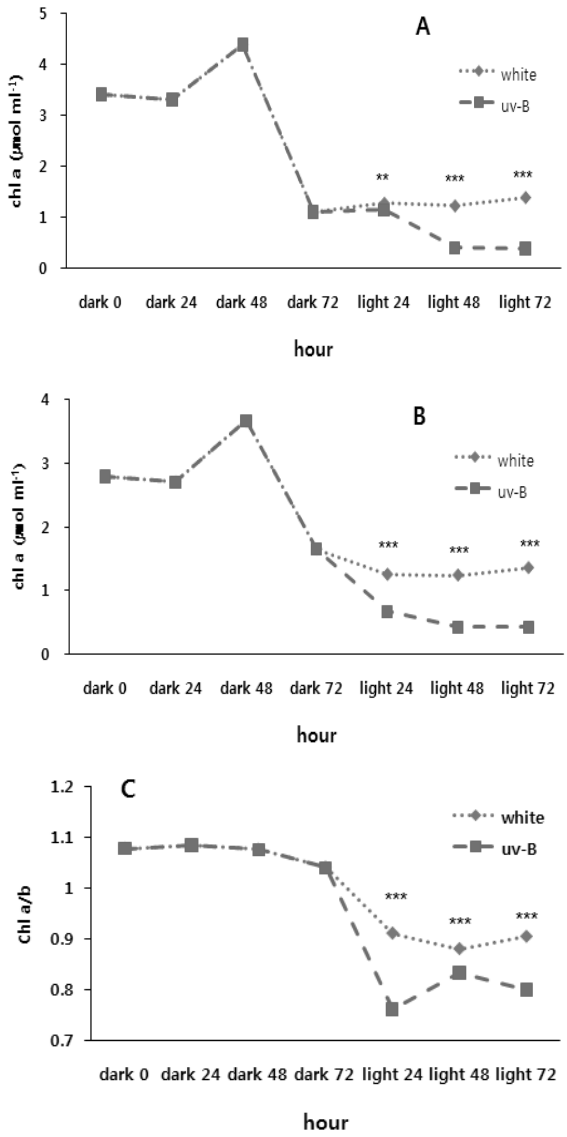

Fig. 1. Effects of treatment with moderate light and UV-B for 3 days after 3 days growth under dark condition on the content of chlorophyll a (A), chlorophyll b (B), and chlorophyll a/b ratio (C) in soybean. *, ** and * represent the statistically significant differences at $p < 0.001$, $p < 0.01$ and $p < 0.05$ level as determined by Student's t-test, respectively.**

(Fig. 2). Carotenoid content was not yielded during dark condition. However, it was occurred as it was exposed to UV-B and increased throughout the cultivation. Although the graph of carotenoid content changes to slowly reduced while absence of light, anthocyanin content were significantly increased when it began to exposure to UV-B. The induction of carotenoid and anthocyanin biosynthesis was triggered by exposure of UV-B radiation rather than natural light condition (moderate light). Therefore, anthocyanins seem to act as protecting from photo-damage to exposed UV-B radiation (Fig. 2) in spite of chlorophylls content were significantly low (Fig. 1).

Increase of carotenoid content is not only related to their essential role in the photosynthetic process, but also to their significant role in the photoprotection of photosynthetic membranes against the large amounts of solar energy absorbed by photosynthetic pigments (Demming-Adams and Adams, 1996; Havaux, 1998; Asada,1999; González et al., 2007). Burger et al. (1996) reported that anthocyanins can helped to protect the plant from radicals formed by UV. Recently, Close er al. (2003) and Steyn et al. (2002) implied that anthocyanins acted as protectants from photo-damage.

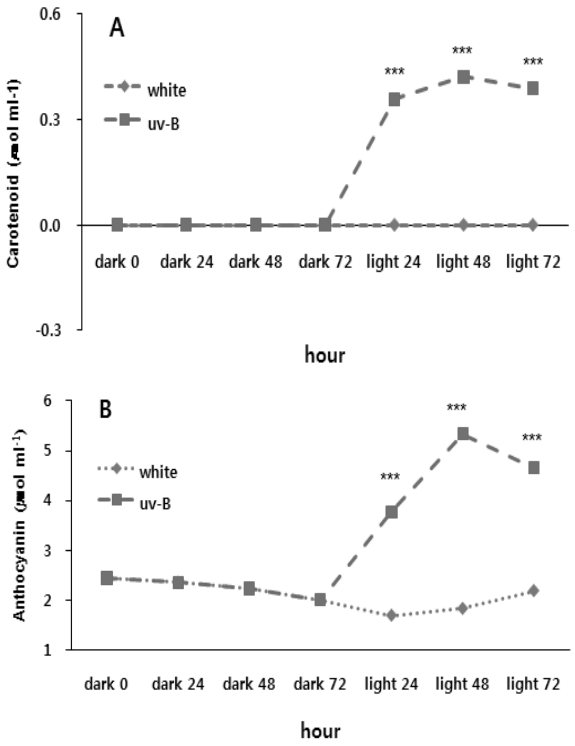

Fig. 2. Effect of different light sources on carotenoid (A) and anthocyanin contents (B). * represent the statistically significant differences at $p < 0.001$ level as determined by Student's t-test, respectively.**

Changes of PS II maximum efficiency The phase of histograms exhibited a similarity between Fv/Fm and F'v/F'm (Fig. 3). Damage to PS Ⅱ as indicated potential Fv/Fm and F'v/F'm decreased with light stress, but it is have been no sooner exposure to moderate light than their duty of PS II photochemical function recovered. The result of Fv/Fm correlated with UV-B radiation and moderate light condition except lightning for 4 hour. Although results of F'v/F'm have smilar significance, just only two point have statistically significant by treatment lights. Upon exposure to excess light, the D1 protein of PS II reaction center inactivated by phosphorylation and then degraded, leading to an inactive PS II center. Fv/Fm can be used as an indicator of this process since there is a good correlation between degradation of D1 protein measured by radioactive labelling and Fv/Fm (Rintamaki et. al., 1995). In healthy leaves, Fv/Fm value is always close to 0.8, a lower value indicates that a propotion of PS Ⅱ reaction centers are damaged, a phenomenon called photoinhibition, often observed in plants conditions. Thus, Values of their parameter were reduced slightly under dark condition and UV-B radiation.

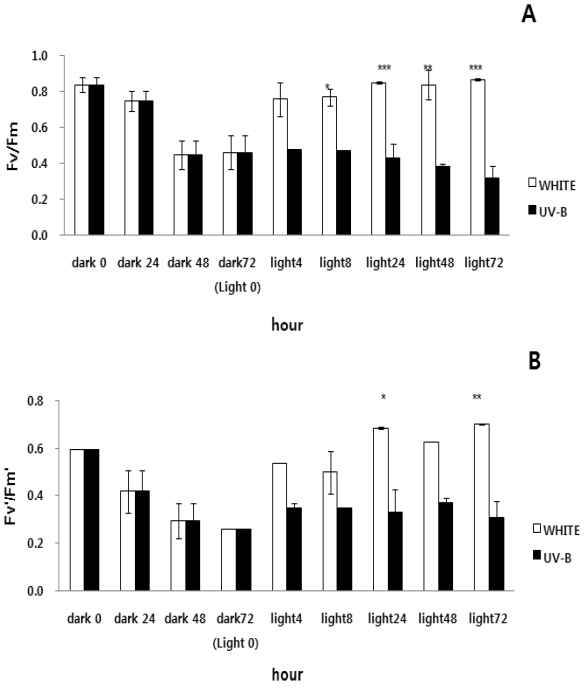

Fig. 3. Changes of Fv/Fm (A) and F'v/F'm (B) yield by light sources. *, ** and * represent the statistically significant differences at $p < 0.001$, $p < 0.01$ and $p < 0.05$ level as determined by Student's t-test, respectively.**

Changes in PS II operating efficiency The PS II operating efficiency has been reduced rapidly during dark

condition (Fig. 4). Φ_{PSII} was changed to increase when it exposed to normal lights. However, it was significantly differed by illumination of UV-B radiation after dark conditions. It is assumed that Φ_{PSII} have been lost of photosynthetic function in darkness and then recovered their function during moderate light. The PS II was damaged by UV-B radiation contrastively. They were significant only when light was exposed for 24 and 72 hours. The others were not statistically significant. Also, on the basis of histograms of the PS II operating efficiency, Φ_{PSII} was similar to F'v/F'm (Fig. 3B). Ralph (2005) had implied that Φ_{PSII} provides a more realistic impression of the leaves overall photosynthetic condition as it is under ambient light condition, whereas Fv/Fm estimates indicate the maximum potential photosynthetic once light stress is removed.

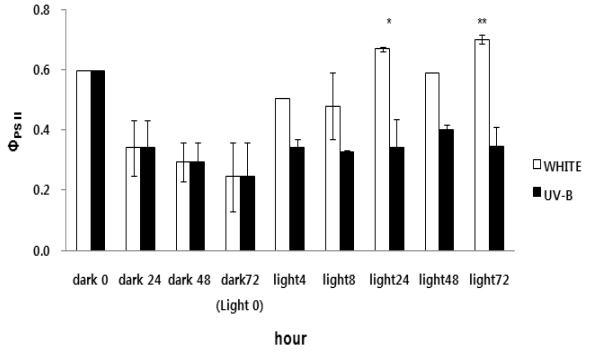

Fig. 4. Changes of PS Ⅱ operating efficiency (Φ_{PSII}) by different light sources. ** and * represent the statistically significant differences at $p < 0.01$ and $p < 0.05$ level as determined by Student's t-test, respectively.

Effect on nonphotochemical quenching Nonphotochemical quenching (NPQ) indicate an increase in environment stresses. In this study, NPQ was significant reduced by dark condition or excessive light (Fig. 5). It may mean that NPQ was used to dissipate excess energy from photosystem. It was presumed that function of PS Ⅱ to be lost by light stress. It was expectedly assumed that chloroplasts became prolamellar body which was photosynthetically inactive on account of etioplasts changed from chloroplasts at dark condition. Etioplasts can rapidly re-differentiate into photosynthetically active chloroplasts upon illumination, and they were often uesd to study initial events in plastid in the absence of highly-abundant photosynthetic proteins (Baginsky and Gruissem, 2004). NPQ could play a role in restoration to PS II function as soon as exposure normal light (Fig. 5). However, photosynthetical function has been irreparable damaged when exposed to UV-B radiation.

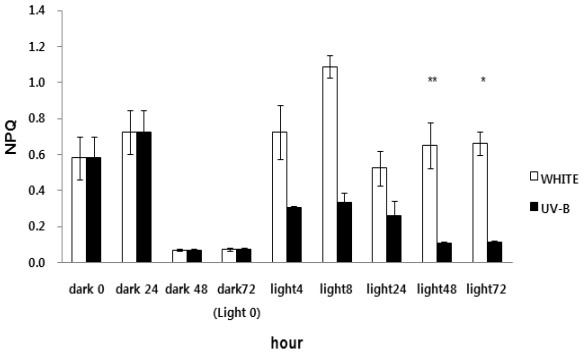

Fig. 5. Changes of nonphotochemical quenching (NPQ) by different type of light source. ** and * represent the statistically significant differences at $p < 0.01$ and $p < 0.05$ level as determined by Student's t-test, respectively.

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콩의 광질에 대한 엽 색소 및 엽록소 형광반응 연구

박세준[1] · 김도연[2] · 유성녕[2,3] · 김현희[1] · 고태석[1] · 심명룡[1] · 박소현[2,3] · 양지아[2] · 엄기철[3,4] · 홍선희[5] · 김태완[1,2*]

[1]한경대학교 식물생태화학연구소, [2]한경대학교 식물생의약전공, [3]세종데이터해석연구원,
[4]환경수맥 (주)토양연구소, [5]고려대학교 환경생태공학부

서리태콩 (*Glycine max* Merr. var Seoritae)에서 광원에 따른 엽의 광합성 변화를 구명하기 위하여 콩의 제 1복엽이 완전 전개되었을 때 3일 동안 빛을 차단한 후 UV-B 와 일반광에 노출시켜 색소 함량과 엽록소 형광반응의 변화를 측정하였다. 암처리에서 엽록소 함량은 감소하고, 일반 광에서 회복하였다. 카로티노이드와 안토시아닌 함량은 UV-B 조사한 처리구에서 증가하였다. 엽록소 형광분석을 이용한 광합성 능률을 분석한 결과, 암처리가 진행 됨에 따라 Fv/Fm, F'v/F'm, Φ_{PSII} 및 NPQ는 감소하였다. 모든 변수들은 일반광에 노출되면서 회복하였으나 UV 처리한 것은 암처리 72시간의 수치와 큰 변화가 없었다. 이를 통하여 암처리 48시간 경과함으로 엽록체가 에티오플라스트로 전환되며, 일반광을 조사하였을 시 광합성 관련 광계가 복구되지만, UV-B의 강한 광이 조사되었을 때 광계가 회복되지 못하는 것으로 사료되었다.

중심어 : 콩, 엽 색소, 엽록소 형광