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The Assessment of Ultraviolet Radiation in Vegetable Growth

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植物生長에 미치는 紫外線의 效果

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Abstract

The terrestrial UV flux rapidly increased in late spring, as measured by the chemical actinometry at two elevations (near sea level and 1,100 m above sea level) on Jeju Island. More intense UV fluxes were observed at higher altitudes. Any harmful effects of solar UV-B on the growth of soybean were not detected in UV-B-exclusion experiment. To ascertain the effect of UV radiation on vegetative growth, intact (mol wt 124000) and large (~120000) phytochromes were irradiated with UV-B radiation. In intact phytochrome, the Pfr form accounts for 60% of the total phytochrome under stationary state conditions, whereas it accounts for 50 % in large phytochrome. Calculated quantum yields for the forward and the backward phototransformations of phytochrome by UV were $\phi r=0.016$ and $\phi fr=0.010$ in intact phytochrome, and $\phi r=\phi fr=0.012$ in large phytochrome, respectively.

Introduction

Jeju Island is located in the subtropical region and consists largely of Mt. Halla (1950 m). Therefore, most of vegetations in the island are assumed to be exposed to the ultraviolet-abundant solar radiation, due to low latitude and high altitude.

Ultraviolet radiation has long been known to affect many important photobiochemical reactions (see review by Caldwell[1] and Silberglied[2]). The growth of higher plants has been reported to be accelerated or inhibited by UV, depending upon its amounts. Since germicidal lamps which emit much of UV-C were used as UV source by earlier workers, however, those experimental results gave little information on the effect of the solar UV on the field vegetations[3].

In recent years, the possible depletion of the stratospheric ozone layer due to the impact of te-

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Abbreviations: UV-B, ultraviolet radiation between 280 and 320 nm; UV-C, ultraviolet radiation between 200 and 280 nm; EDTA-Na_4, ethylenediaminetetraacetic acid tetrasodium salt; ME, 2-mercaptoethanol; P, phytochrome; Pr, red light absorbing form of phytochrome; Pfr, far-red light absorbing form of phytochrome.

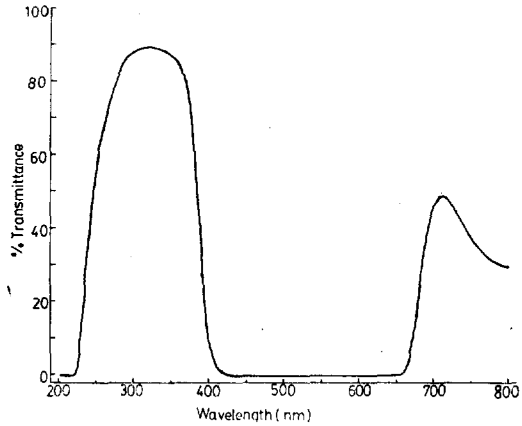

Fig. 1. Optical transmittance of Corning 7-54 UV filter

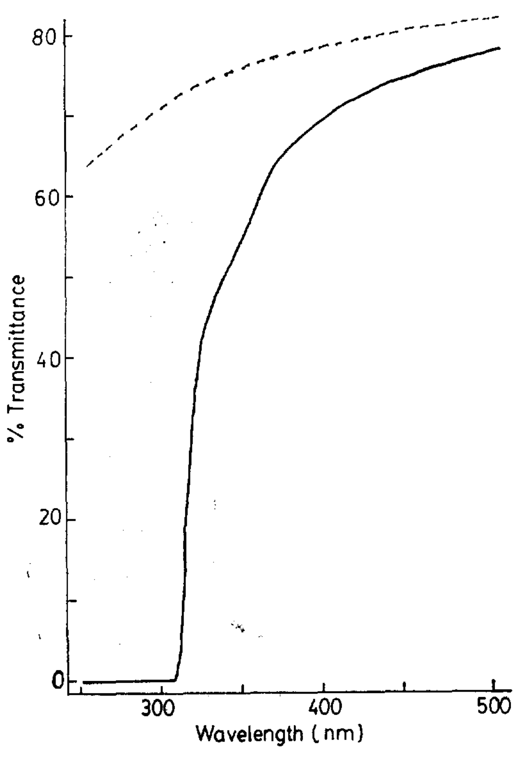

Fig. 2. Optical transmittance of Mylar (Type "D") film and polyethylene film("Polyfilm"; 0.1 mm)

chnoculture on the steady state composition of atmosphere has been reported[4,5,6]. The reduced ozone layer would increase the influx of the solar UV-B to the earth. Therefore, the possible responses of plants to the elevated UV-B radiation have motivated several recent studies[1,7,8].

Techniques for simulating changes in UV-B under natural ambient conditions are limited at present; however, they might best be achieved in a low-latitude, mininal cloud cover, multiuser facility, which would provide UV-B radiation corresponding to reduced ozone concentrations at more northern latitudes[6].

Most of non-damaging UV-B effects on plant morphogenesis have been shown to be controlled by phytochrome, the ubiquitous photoreceptor for red light-mediated photomorphogenesis in plants (see recent review by Wellman[9]).

In these studies, an attempt was made to measure the terrestrial solar UV flux density on Jeju Island by the method of chemical actinometry. The growth responses of soybean to the exclusion of the solar UV-B were also examined, and the phototransformation of phytochrome induced by UV-B was investigated in order to ascertain the possible correlation between the vegetative growth and the phytochrome involvement under an UV radiation-rich environment.

Materials and Methods

1. Measurement of the terrestrial solar UV flux density

Solar UV flux under the cloudless conditions was repeatedly measured by potassium ferrioxalate actinometry method[10] at two locations of different altitudes; (1) near sea level in Jeju City, and (2) 1,100 m above sea level on Mt. Halla. A 0.006 M $K_3Fe(C_2O_4)_3$ solution in quartz tube was exposed at noon to the sun light filtered by Corning 7-54 UV filter, whose transmittance is shown in Fig. 1. Its slight transmission of long wave region beyond 650 nm was considered not to affect UV measurement because the actinic solution is insensitive to this region of light[11]. The time of exposure was same at the two locations, and the quartz tube containing the actinic solution was aligned to be perpendicular to the solar beam axis.

Number of Fe^{++} ions produced in the exposed solution was analyzed by using Spectronic 20 spectrophotometer, and the number of UV photons was calculated by the use of quantum yield values recommended by Lee and Seliger[12].

2. UV-B exclusion experiment

To examine the effect of the solar UV-B radiation on the vegetations in Jeju Island, the growth of soybean was investigated under the condition of UV-B exclusion from sun light.

Soybean (*Glycine max* L.) plants were grown in the field conditions. When the height of soybean seedling was about 30 cm, or one month after sowing on June 27, different UV transmittance films were used to cover the plants. Polyethylene films (both 0.1 and 0.01 mm) transmitted more than 60% of UV-B band, while Mylar film(.007 Type "D", DuPont) cut all UV-B radiation at 315 nm (Fig. 2). Due to the possible photochemical degradation[13], a Mylar film was replaced every five or seven cloudless days. No substantial change in transmittance during the treatment was observed.

After 100 days of treatment, several vegetative and reproductive characters of soybean were investigated in accordance with the "Standards for the Measurements of Crop Growth" of the Office of Rural Development, Republic of Korea.

3. UV-B-induced phototransformation of phytochrome

Phytochrome was isolated from 5-day-old etiolated oat seedlings (*Avena sativa* L. cv. Garry). Large mol wt phytochrome(~120000) was purified by Affi-Gel Blue chromatography[14], and intact phytochrome (124000) by the method of Vierstra and Quail[15].

Final preparations of the large mol wt phytochrome for UV-induced phototransformation study included 0.1 M phosphate buffer, pH 7.8 at 4°C, containing 50 mM KCl and 0.1 mM EDTA-Na_4, while intact phytochrome 0.1 M potassium phosphate buffer, pH 7.8 at 4°C, including 1.4 mM ME and 1 mM EDTA-Na_4.

A 250 W medium pressure mercury lamp was used as the actinic light source, and a 285 nm band pass interference filter (half band width 19 nm) was used to isolate UV-B band from the light source. The incident UV-B intensity was measured by potassium ferrioxalate method[11,12]. Actinometric solution was irradiated in the same manner as for the phototransformation of phytochrome. The value of the actinic light intensity for the calculation of quantum yield was corrected for the absorption of light by the sample as described by Pratt and Buttler[16].

The phototransformation of phytochrome by UV was followed by the use of a Cary 118C spectrophotometer. Changes in absorbance at 666 nm and 723 (large phytochrome) or 730 nm(intact) were directly read on the spectrophotometer (for large), or on the absorption spectra scanned from 800 to 550 nm (for intact). The total sample absorbance was always less than 0.15 at the actinic wavelength.

To establish the photostationary equilibrium, the Pr and Pfr forms of phytochrome were irradiated until the both initial forms showed similar spectra.

Table 1. Comparision of the solar ultraviolet radiation intensities[a] between two different altitudes in Jeju Island

Altitude	Sea level	1,100 m	Difference
UV intensity ($mol \cdot m^{-2} \cdot s^{-1} \times 10^{-5}$)	7.92	8.63	0.71ns[b]

[a]Figures are average of 11 measurements under cloudless conditions at noon in three seasons.
[b]Result of paired t-test, at 5% level.

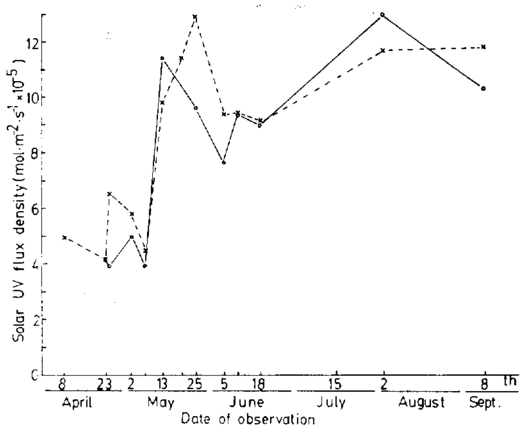

Fig. 3. Seasonal variations in the terrestrial solar UV flux density at two different altitudes in Jeju Island
UV radiation penetrated into quartz tube covered with Corning 7-54 UV filter perpendicular to sun light was measured by potassium ferrioxalate method. ○——○ : at near sea level, ×——× : at 1,100 m above sea level.

Table 2. Vegetative growth of soybean in the open and under UV-B radiation transmitting (0.1 and 0.01 mm polyethylene) and excluding(Mylar) films

Filter treatment	Fresh weight (g/plant)	Plant height (cm)	Stem circumference(mm)	No. of primary branches	No. of secondary branches	No. of nodes per plant
Open (no film)	24.2	74.4	7.2	6.6b[z]	6.8	17.8
Polyethylene (0.1 mm)	32.8	86.2	7.6	8.2a	7.8	19.0
Polyethylene (0.01 mm)	30.6	97.1	6.8	4.4c	7.4	17.6
Mylar	18.4	83.0	7.4	4.6c	7.4	17.0

[z]Mean seperation within columns by DMR, 5% level. Figures without following letters are insignificant within columns.

Table 3. Reproductive growth of soybean in the open and under UV-B radiatlon transmitting(0.1 and 0.01 mm polyethylene) and excluding(Mylar) films

Filter treatment	No. of pods. per plant	Length of pod (cm/pod)	Fresh grain wt (mg/grain)	Dry grain wt (mg/grain)	Dry grain wt (g/plant)
Open (no film)	50.4b[z]	3.3	93	81	4.4b[z]
Polyethylene (0.1 mm)	62.6a	3.5	133	118	7.0a
Polyethylene (0.01 mm)	43.0b	3.6	115	93	3.2c
Mylar	25.2c	3.4	127	112	3.1c

[z]See Table 2.

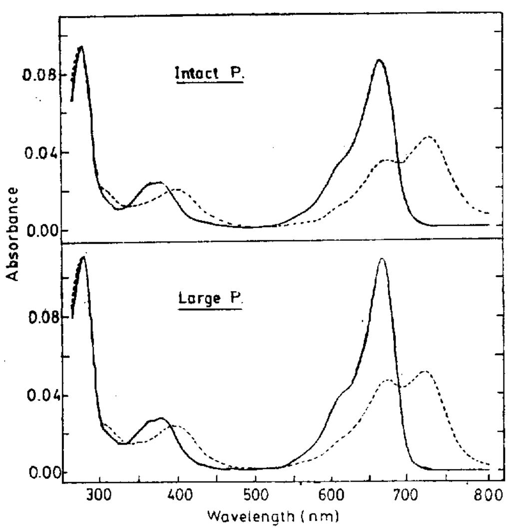

Fig. 4. Absorption spectra of intact and large phytochrome

Intact phytochrome was dissolved in 0.1 M potassium phosphate buffer, pH 7.8 at 4°C containing 1.4 mM EDTA-Na_4, while large phytochrome in 0.1 M sodium phosphate buffer, pH 7.8 at 4°C containing 50 mM KCl and 0.1 mM EDTA-Na_4. Solid lines for Pr and broken lines for Pfr(red-light saturated).

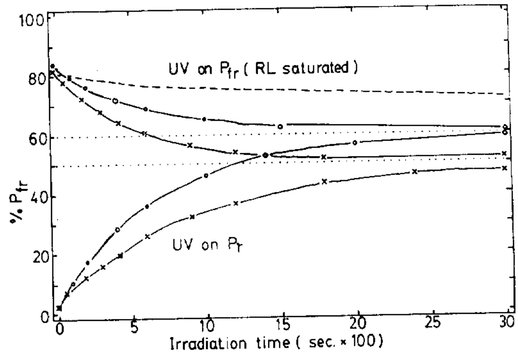

Fig. 5. Time courses of UV-B-induced phototransformation of phytochrome

○—○ for intact phytochrome, ×—× for large phytochrome, and upper broken line for dark reversion of large Pfr. The incident intensity of actinic light was $2.13 \times 10^{-6}\ mol \cdot m^{-2} \cdot s^{-1}$.

Calculation of %Pfr was based on 81% Pfr under red light saturated conditions for large phytochrome (see review by Song[17]) and 86% for intact phytochrome[15]. Loss in absorbance of phytochrome during UV irradiation was compensated for as follows: If there is no loss in absorbance,

$$\text{Absorbance} = A_{666,t} + A_{730,t} \times \frac{\Delta A_{666}}{\Delta A_{730}}$$

would be constant, where $A_{666,t}$ and $A_{730,t}$ are absorbances at 666 and 730 nm after irradiation of t seconds, respectively, and ΔA_{666} and ΔA_{730} are changes in absorbance at two wavelengths during phytotransformation by red and far-red light. $\varepsilon_{668,r} = 1.21 \times 10^{5} l mol^{-1} cm^{-1}$[18] for intact phytochrome, and $\varepsilon_{667,r} = 1.2 \times 10^{5}$[19] were used to estimate the extinction coefficient of phytochrome at 285 nm. Rate constants and quantum yields were calculated by the use of the equations derived by Buttler *et al.*[20], using the initial transformation rates.

Results and Discussion

Fig. 3 shows the seasonal variations in the solar UV flux density measured at two different elevations on Jeju Island. The UV flux increased rapidly in late spring at both altitudes and the intensity in summer was more than double that in spring. Even though the difference was not statistically significant at 5% level (Table 1), more intense UV flux was observed at higher elevation (9% higher at 1,100 m elevation than near sea level).

Since these values (Fig. 3 and Table 1) are the results measured by the actinic solution in quartz tube covered by UV filter, the actual incident UV flux density should be corrected for the reciprocal factor of the transmittance of quartz and the filter.

These results obtained here by the chemical actinometry can not be compared with those of others (for example, Bird *et al.*[21] and Green *et al.*[22]), who used sophisticated radiometric methods in order to determine the precise spectral intensities. These preliminary results, however, could be considered to show the possibility of using the simple method of chemical actinometry as an economical tool for monitoring the seasonal or annual variations in the terrestrial solar UV flux density.

Vegetative and reproductive growth data for soybean under several different UV transmittance films are presented in Tables 2 and 3. No statistically significant differences were recognized among treatments in various vegetative characters, except for fewer primary branches under UV-B exclusion(Table 2). There was no treatment effect on the development of pod and bean seed, while the number of

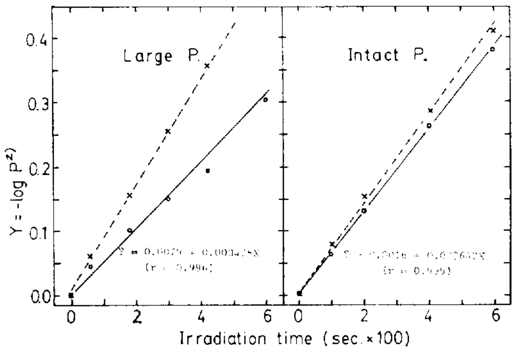

Fig. 6. Semilog plot of the proportion of phytochrome transformed vs. time of irradiation with UV-B light

○——○ : Pr to Pfr, and ×——× : Pfr to Pr.

$^{z)}\ P = \dfrac{\%Pfr,\ t - \%Pfr,\ \infty}{\%Pfr,\ 0 - \%Pfr, \infty}$

pods and the resulting dry weight of bean seed per plant were decreased by UV-B excluding Mylar film treatment (Table 3).

Enhanced UV-B radiation has been used in various plants, resulting in damages to photosynthetic system[23], depressing net photosynthesis and growth[13] and affecting leaf development[24,25], especially under low visible light conditions. Broad-leaved species with C_3 type of carbon assimilation were reported to be more susceptible to UV-B alterations of morphological and biochemical characteristics than the narrow-leaved species with C_4 type photosynthesis[26]. Teramura *et al.*[3] observed adverse effect of UV-B on net photosynthesis of soybean at low photosynthetically active radiation levels but it had little consequence at light levels that normally saturate photosynthesis in the field. No significant differences in the growth of several plants among the treatments of different UV-transmitting films were observed at a 3,000 m elevation, only exception being longer wheat stems under UV-B excluding film than under UV-B radiation[27].

During the present experiments, the frame and test plants had to be rehabilitated after the devastation by a typhoon (Sept. 25~28, 1983) which, in addition to the excessive rain during the flowering season, compromised the precision of UV-B exclusion effect. No evidence for a signfiicant harmful effect of the solar UV-B on soybean growth was detected in this experiment. In order to draw conclusion on the solar UV-B effects on the vegetations in Jeju Island, however, more plants should be tested.

The absorption spectra of purified phytochromes scanned by Cary 118C spectrophotometer are shown in Fig. 4. Difference between the spectra of two phytochromes was noticed in the absorption band by the chromophore of Pfr forms (650~800 nm).

Fig. 5. indicates the time courses of the phototransformation of phytochrome during irradiation with UV-B (flux density 2.13×10^{-6} mol·m^{-2}·s^{-1}).

Table 4. Photostationary state and quantum yields for UV-B-induced phototransformation[a] of phytochrome

Items	Intact phytochrome	Large phytochrome
%Pfr, ∞	60	50
ϕr	0.016	0.012
ϕfr	0.010	0.012
$\phi r/\phi fr$	1.5	1.0

[a]Medium pressure mercury lamp of 250W was used as actinic light source, and UV-B band was isolated through the interference filter (CW 285 nm, and HBW 19 nm). The incident intensity was 2.13×10^{-6} mol·m^{-2}·s^{-1}.

In intact phytochrome, the Pfr form accounted for 60% of the total phytochrome under stationary state conditions, while it accounted for 50% in large phytochrome.

Pratt and Buttler[16] observed 60% Pfr under stationary state at 280 nm UV light in small (60,000 mol wt) phytochrome.

Intact phytochrome showed almost identical rate constants for both forward and reverse phototransformations, while large phytochrome showed a marked difference (Fig. 6). The difference observed might be due to dark reversion of large Pfr during UV irradiation (see upper broken line in Fig. 5). Thus, the Pr→Pfr rate was used for the calculation of quantum yields.

Table 4 summarizes the results of UV-B-induced phototransformation of phytochrome. Lower %Pfr under stationary state in large phytochrome than intact phytochrome could be explained with increased ϕfr and decreased ϕr. It is yet to be further explored whether the observed difference in quantum yields between the two phytochrome preparations are due to the difference in the efficiency of energy transfer from the UV-B absorbing amino acids (tryptophane in particular) to the phytochrome chromophore, or due to dark reversion of large Pfr. Our results, however, suggest that intact phytochrome favors a higher Pfr level possibly due to a preferential energy transfer from aromatic amino acid residue to the Pr chromophore.

The fact that UV-B itself transforms Pr to Pfr up to 60%, should be considered in explaining the UV-B-induced morphogenesis (for example, flavonoid synthesis in parsley[28]), which is controlled by the red or far-red light after UV-B irradiation.

Conclusion

Using the chemical actinometry method, it was found that the UV-B radiation increases with the altitude of Mt. Halla, Jeju. However, no significant UV damages were observed with soybean plants. UV-B radiation is effective in establishing photostationary equilibrium between the Pr and Pfr forms of phytochrome, suggesting that UV-B radiation activates/maintains phytochrome-mediated morphogenic and developmental processes to underscore the lack of significant UV-B damages to soybean plants on Mt. Halla, Jeju.

要 約

化學的 光量測定方法을 利用하여 濟州島의 海邊과 海拔 1,100 m 高地에서 봄부터 가을사이의 太陽光線中 紫外線 含量을 測定한 結果, 여름과 초가을의 紫外線 含量은 봄에 비해서 倍 以上으로 나타났으며 高地帶에서는 더 많은 紫外線 含量이 測定되었다. 圃場條件에서 UV-B部分(280~320 nm) 紫外線의 透過率이 서로 다른 필름을 被覆하여 大豆의 生長發育을 調査했는데, 光線中 UV-B의 害作用은 認定되지 않았다. 紫外線이 植物의 光形態形成에 미치는 效果를 究明하기 위하여 光形態形成에 관여하는 植物의 光受容體 色素인 파이토크롬에 UV-B를 쪼이면서 파이토크롬의 變化를 追跡하였던바, 分子量 124,000인 파이토크롬은 60% Pfr, 分子量~120,000은 50% Pfr의 光平衡 狀態를 보였다.

光變換中 quantum yield는 分子量 124,000의 파이토크롬에서는 $\phi r=0.016$, $\phi fr=0.010$이었으며 分子量 ~120,000은 $\phi r=\phi fr=0.012$였다. 圃場狀態에서 供試

된 大豆에 UV-B의 害作用이 觀察되지 않은 것은, UV-B 가 파이토크롬을 變換시켜 파이토크롬이 관여하는 防禦的 形態形成 및 發育을 促進시키기 때문이라고 推論되었다.

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