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Effect of UV-B radiation on seedlings of two *Solidago virgaurea* populations from the Mt. Hakusan area of Japan

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We collected seeds of *Solidago virgaurea* plants growing at different altitudes on the Mt. Hakusan area in Japan and cultivated them in a naturally-lit green house. Three-week-old seedlings were irradiated with supplemental UV-B for 12 h each day for 1 and 2 weeks. After a week of irradiation the seedlings of the population collected from the higher altitude at Oh-nanjiho (ON) had accumulated more anthocyanins than those from the lower altitude at Bettoh-deai (BD). Levels of anthocyanins in the ON seedlings were highly correlated with the dose of UV-B radiation and the correlation was also observed after 2 weeks. The growth of the third leaves was retarded by UV-B radiation in both populations. The extent of growth retardation in the third leaves was correlated with the dose of UV-B radiation in both populations. However, no significant difference in the extent of leaf area growth was observed between the ON and BD populations. The increase in plant fresh weight was extensively inhibited in the ON seedlings after 1 week of UV-B radiation. The inhibition was recovered to those in the BD population by 2 weeks irradiation. These results indicate that these populations respond differentially to supplementary UV-B radiation during the first week. Because flavonoids such as anthocyanins play an important role in protection against UV-B radiation in many plants, populations growing at higher altitude may be better able to adapt to increased global levels of UV-B radiation.

Key words: *Solidago virgaurea*, UV-B, anthocyanins, plants, growth

INTRODUCTION

The UV-B part of sunlight (290-320 nm wavelength) has received much attention in recent years, because it is predicted that thinning of the stratospheric ozone layer from contamination of the atmosphere by chlorofluorocarbons will lead to an increase in the amount of UV-B radiation reaching the Earth's surface. Supplemental UV-B radiation has been found to inhibit leaf expansion and to increase production of flavonoids that absorb UV light and act as UV protectors. As the intensity of UV-B increases with altitude, plants that populate the higher altitudes may adapt to greater levels of UV-B radiation. However, it has not been shown whether they respond differentially to it. *Solidago virgaurea* L. is a wild species from the family *Asteraceae* that is distributed in the Mt. Hakusan area of Japan. Several populations of it are growing at 1260 m to 2670 m. Therefore, this species is suitable for research about the effects of UV-B radiation on

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the wild plants. In the present study, we examined the effect of UV-B radiation on *Solidago virgaurea* seedlings whose seeds had been collected from different two altitudes in the Mt. Hakusan area.

MATERIALS AND METHODS

Plant material. Seeds of *Solidago virgaurea* plants growing at Bettoh-deai (BD, 1260 m in altitude) and at Oh-nanjiho (ON, 2670 m in altitude) in the Mt. Hakusan area were collected *in situ*. They were sown on vermiculite and germinated in temperature-controlled greenhouse. The plants were grown for 3 weeks at 25 ℃ during the day and 20 ℃ at night with a constant relative humidity of 70 %. The pots were fertilized with 4000-fold dilution of Hyponex daily.

UV-B irradiation and plant measurement. Three-week-old plants were transferred to an artificially illuminated growth chamber (Conviron CMP3244), and preconditioned with 12 h of light at 18 ℃ and 12 h of darkness at 15 ℃ for a day before the experiment began. UV-B was irradiated with white light from fluorescent lamps (250μmol $m^{-2}s^{-1}$ of photosynthetic photon flux density) during light periods. UV-B was supplemented from fluorescent sun lamps (FL20SS·BRN/18; Toshiba) with a glass filter (UV-29, Hoya) to cut off UV light with a wavelength shorter than 280 nm. For the analyses, 20 seedlings were cut above the soil at 0, 7 and 14 d after the start of UV-B irradiation. The fresh weight of each was measured, and then anthocyanins were extracted as described below. The third leaves from another 20 seedlings were determined by using a Macintosh computer equipped with a scanner and imaging software (NIH Image).

Measurement of anthocyanins. Each plant cutting was transferred to a 2-mL microtube and immersed in 1 mL of 90% methanol containing 1 % (w/v) hydrochloric acid. The tubes were then tightly sealed with o-ring screw caps and incubated at 40 ℃ for several hours. The absorbance of each extract at a wavelength of 518 nm was measured with a spectrophotometer (UV-2200; Shimazu).

RESULTS

Leaf area. In the control pots the areas of the third leaves of the ON population increased 5-fold during cultivation period, but those of the BD population reached a maximum 1 week after the start of the control treatment. When UV-B was irradiated at 0.7 $W \cdot m^{-2}$ (3.92 $kJ \cdot m^{-2} \cdot d^{-1}$ of $UV\text{-}B_{BE}$) the growth of the leaves was retarded in both populations. There was no significant difference in the extent of growth retardation between the two populations. The extent of growth retardation was correlated with the dose of UV-B radiation in both populations at 1 week after supplementary radiation had begun. This correlation was also observed in seedlings irradiated for 2 weeks.

Fresh weight. The fresh weight of seedlings increased 3 to 4 – fold during the control treatment in both populations. When UV-B was irradiated at $0.7 \cdot W \cdot m^{-2}$ (3.92 $kJ \cdot m^{-2} \cdot d^{-1}$ of $UV\text{-}B_{BE}$), the increase in fresh weight in the BD population became inhibited only after 2 weeks of irradiation.. In the ON population a large degree of inhibition was observed after 1 week of irradiation, but by 2 weeks irradiation the plants had recovered from this inhibition to reach fresh weights approaching those in the BD population. The extent of inhibition of fresh weight was not correlated with the dose of radiation in BD population.

Anthocyanin content. The control treatment did not conspicuously change the content of anthocyanins. UV-B radiation resulted in extensive accumulation of anthocyanins in both populations. The ON population accumulated more anthocyanins after 1 week of radiation than did the BD population. By 2 weeks of radiation both populations had accumulated similar amounts of anthocyanins. The accumulation of anthocyanins in seedlings of the ON population was well correlated with the dose of UV-B radiation after 1 week of radiation. The correlation was also observed after 2 weeks of radiation, but the extent of the responses to UV-B doses was less than that seen at 1 week. In the BD population the correlation was not clear.

DISCUSSION

The growth of the third leaves was retarded by UV-B irradiation in both *S. virgaurea* populations, and the dose response of the inhibition differed little between the two populations, even though the UV-B intensities of their habitats differed [6]. These results suggest that the process of

growth inhibition of leaves by UV-B radiation may be similar between the two populations. Murase et al. [7] reported that UV-B-induced growth inhibition of cucumber first leaves could be related to a decrease in sensitivity of the plants to phytohormones. As UV-B radiation affected neither photosynthetic nor respiratory activity in our study, a process such as reduced sensitivity to plant hormones may also participate in the UV-B-induced growth inhibition of *S. virgaurea* leaves.

Sullivan et al. [4] classified wild plants growing in Hawaii by their change in biomass during a 12-week UV-B irradiation. Among the 29 species they tested, two populations growing at different altitudes were included for three species. They found that populations of *Plantago lanceolata* and *Oenothera stricta* growing above 1500 m were tolerant to UV-B. Our results indicate greater UV-B-induced inhibition of fresh weight growth in the ON population than in the BD population when they were irradiated UV-B for 1 week, suggesting that *S. virgaurea* growing in the Mt. Hakusan area may respond to UV-B radiation differently from the Hawaiian plants.

After 1 week of UV-B irradiation the ON population had accumulated more anthocyanins than the BD population had. The level was highly correlated with the dose of UV-B, although the difference between the two populations disappeared after 2 weeks of irradiation. These results suggest that the rate of biosynthesis of flavonoids in the ON population is higher than that in the BD population during the first week of irradiation, and that the rate is dependent on the intensity of UV-B. As flavonoids such as anthocyanins have been shown to act as UV protectors in higher plants, the ON population may have be more able to protect itself from UV-B radiation [1-3]. Therefore, ON population may be better able to adapt to increased global levels of UV-B radiation. It has been speculated that UV-B might induce the production of superoxide radicals that damage the structure of proteins and membranes in plant cells [7-9]. Murai et al. [5] reported that a population of *S. virgaurea* growing at a high altitude on Mt. Hakusan was more tolerant than plants grown at a lower altitude to a herbicide that induces superoxide radicals. This information supports the above supposition.

REFERENCES

1. Li, J. and T.-M. Ou-Lee (1993) *Arabidopsis* flavonoid mutants are hypersensitive to UV-B irradiation, *Plant Cell* **5**, 171-179.

2. Lois, R. and B.B. Buchanan (1994) Severe sensitivity to ultraviolet radiation in an *Arabidopsis* mutant deficient in flavonoid accumulation. II. Mechanisms of UV-resistance in *Arabidopsis*, *Planta* **194**, 504-509.

3. Lois, R (1994) Accumulation of UV-absorbing flavonoids induced by UV-B radiation in *Arabidopsis thaliana*, I. Mechanisms of UV-resistance in *Arabidopsis*, *Planta* **194**, 498-503.

4. Sullivan, J.H., A.H. Teramura and L.H. Ziska (1992) Variation in UV-B sensitivity in plants from 3,000-m elevation gradient in Hawaii, *Am. J. Botany* **79**, 737-743.

5. Murai, R., M. Yamagishi, S. Takumi and K. Murai (1997) Differential activities and transcripts levels of superoxide dismutase in two *Solidago virgaurea* populations from different altitudes, *Bull. RIAR, Ishikawa Agr. Coll.* **5**, 9-16.

6. Aoshima, F., M. Okano, H. Kon and T. Yoshitake (1999) The observation of UV-B at the different 2 altitude. *Trans. Jpn. Forestry Soc.* **2**, 1057-1058. [in Japanese]

7. Murase, N., N. Kondo, H. Shimizu, N. Nakajima, T. Izuta and T. Totsuka (1997) Effects of UV-B irradiation on growth and physiological activities of cucumber (*Cucumis sativus* L.) first leaves. Influence of nutrition. *J. Jpn. Soc. Atmos. Environ.* **32**, 38-45.

8. Takeuchi, R. Fukumoto, H. Kasahara, T. Sakaki and M. Kitao (1995) Peroxidation of lipids and growth inhibition induced by UV-B irradiation, *Plant Cell Reports* **14**, 566-570.

9. Green, R., R. Fluhr (1995) UV-B-induced PR-1 accumulation is mediated by active oxygen species, *Plant Cell* 7, 203-212.