

Are antioxidants induced by UV-B exposure in a leaf of spinach transported into another leaf ?

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Seedlings of spinach (*Spinacia oleracea* L. “King of Denmark” and “Minsterland”) were grown with or without supplemental UV-B under field conditions for five or nine days when their fifth or sixth leaves were expanding. Except two leaves which were just expanded and expanding, all other leaves of each seedling were removed before tested. One of these two leaves was exposed to UV-B irradiation and the other leaf was covered with lumirror film (no transmission below 320 nm) to prevent it from UV-B exposure. 1,1-diphenyl-2-picrylhydrazyl scavenging activities (antioxidative activity) in leaves without covers were increased according to UV-B doses they were exposed to. And removal of UV-B exposure with lumirror decreased foliage antioxidative activities. This increase or decrease of activities in leaves did not dependent on the UV-B doses their paired leaves were exposed to in both two cultivars. The results demonstrated that spinach foliage antioxidants induced by UV-B exposure were not transported into another leaf .

Key words : ultraviolet-B, antioxidant, spinach

INTRODUCTION

Many enzymes and secondary compounds of higher plants have been demonstrated to protect against oxidative damage by the radical formation induced by penetration of UV-B into mesophyll¹⁾. In experiments performed in our laboratory, enhanced UV-B irradiation increased 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging

activities (antioxidative activity) in leaves of spinach seedlings grown in 10.5cm vinyl pots with the population of 50 plants/m². Whereas the enhanced UV-B irradiation had no effects on their antioxidative activities in their harvested leaves when grown until harvest in the field with the population of 66.7 plants/m² (not published). The higher population and the larger leaves in the latter which shaded their leaves by each other and prevented them from UV-B

exposure might cause the difference of UV-B effectiveness. These results indicated that foliage antioxidants induced by UV-B exposure could not be transported into other parts of plants not exposed to UV-B. The objective of this study is to estimate whether antioxidants induced in a leaf by UV-B exposure are transported into another leaf of spinach seedling.

MATERIALS AND METHODS

UV-B radiation was provided by Q-panel UVB-313 (Q panel Inc., Cleveland, OH, USA) sunlamps suspended above plants. UV sunlamps were filtered with 0.13 mm thick cellulose diacetate (transmission down to 290 nm) or lumirror (no transmission below 320 nm) following the procedure outlined in Nouchi et al ²⁾.

Spinach "King of Denmark" and "Minsterland" were sown one seed per 3 cm square hole of plug tray filled with a standard greenhouse potting mixture. Seedlings were nursing in a greenhouse for four weeks and potted into 10.5 cm pot filled with the same mixture. Seedlings of "King of Denmark" were treated when their fifth leaves were expanding. Except fourth and fifth leaves, all other leaves of seedlings were removed. Of one third of the seedlings, fourth leaves were exposed to UV-B irradiation and the upper sides of fifth leaves were covered with lumirror (no transmission below 320 nm) prevented from UV-B exposure. Of another one third, fourth leaves were covered with lumirror and fifth leaves were not covered. Of the rest seedlings, no leaves were covered. They were grown for

five days with or without the supplemental UV-B irradiation under field conditions with three replications. Seedlings of "Minsterland" were treated when their sixth leaves were expanding in the similar way and grown for nine days with three replications in the field. The plant population in each plot was 50 plants/m².

After the treatment, 12 leaf disks of 6 mm ϕ punched from each leaf of them were homogenized with 20 mL of 80 % methanol. After standing in a dark place, the supernatant was collected and its DPPH radical-scavenging activity was measured following the outlined in Suda ⁴⁾ to determine its antioxidative activity.

RESULTS AND DISCUSSION

Solar radiations and biologically-effective UV-B(UV-B_{BE}) ³⁾ doses during the experiments were shown in Table 1. The proportions of UV-B_{BE} to solar radiation under the supplemental UV-B conditions were about three times greater than in the sunlight.

Leaves of which upper sides covered with lumirror were exposed to less than 1 % of UV-B_{BE} which non-covered leaves were exposed to both on their upper and lower side

Concerning "King of Denmark", mean antioxidative activities in covered leaves, in non-covered leaves exposed to solar UV-B without or with supplemental UV-B were 260, 430 or 560 μ mol Trolox eq. /100g Fw respectively (Table 2). These results were not dependent on leaf positions. The experiment where "Minsterland" were tested showed the similar result. Average antioxidative activities

in covered leaves, in non-covered leaves exposed to solar UV-B without or with supplemental UV-B were 260, 400 or 500 $\mu\text{mol Trolox eq. /100g Fw}$ respectively (data not shown).

Enhanced UV-B irradiation increased antioxidative activities in leaves of both two spinach cultivars significantly. And removal of UV-B exposure with lumirror decreased their foliage antioxidative activities significantly. This increase or decrease of activities in leaves was independent of the UV-B doses their paired leaves were exposed to in both two cultivars. These results demonstrated that the antioxidants induced by UV-B

irradiation in a leaf were slightly transport into another leaf of spinach cultivars tested.

Table 1. Solar radiation and UV-B irradiation during the experiments

Cultivar	period of experiments		mean daily solar radiation (MJ/m ² /day)	UV-B Supplementation	mean daily UV-BBE (KJ/m ² /day)
King of Denmark	2002 3/12~3/16	solar*	15.9		2.1
		plots*	10.3	without with	1.47 (1.0) 4.95 (3.4)
Minsterland	2002 3/17~3/25	solar	14.6		1.98
		plots	9.4	without with	1.39 (1.0) 3.77 (2.7)

Value of UV-B_{BE} in () indicated the relative value standardized on The values without supplementation.

*) solar : radiation measured in a open space

plots: radiation measured under the lamp flames

Table 2. Transport of antioxidants in a spinach leaf into the other leaf

Treatment and leaf positions of leaves measured	Solar UV-B	Solar + supplemental UV-B
	Antioxidative activity ($\mu\text{mol Trolox eq./100g}$)	
Two leaves were exposed to UV-B		
The fourth leaf : exposed to UV-B	460 ± 20	580 ± 20
The fifth leaf : exposed to UV-B	480 ± 20	590 ± 20
The fourth leaf was covered		
The fourth leaf : not exposed to UV-B	270 ± 20	280 ± 20
The fifth leaf : exposed to UV-B	400 ± 20	510 ± 20
The fifth leaf was covered		
The fourth leaf : exposed to UV-B	380 ± 20	550 ± 20
The fifth leaf : not exposed to UV-B	220 ± 20	280 ± 10

cv : King of Denmark Treated duration: five days

Each value is mean of 15 leaves \pm SE

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