Polyphenol Increases in Safflower and Cucumber Seedlings Exposed to Strong Visible Light with Limited Water

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To assess effects of the environmental stress on polyphenol compounds (polyphenols) in plants, the polyphenol contents were investigated in the seedlings of safflower (Carthamus tinctorius L.) and cucumber (Cucumis sativus L.) grown under three types of growth conditions: control; light stress, irradiated with strong light in the visible wavelength range; and light/water stress, irradiated with strong visible light with a limited water supply. The total polyphenol contents and the amounts of the major polyphenols, especially luteolin 7-O-glucoside in safflower cotyledons, and luteolin 7-O-glucoside and luteolin in safflower foliage leaves, increased in response to both stresses. The polyphenol increasing effect of light/water stress was clearly observed in safflower compared to cucumber, suggesting that plants that are resistant to these stresses can accumulate substantial amounts of polyphenols compared to the plants which respond weakly to the stresses.

Key words: safflower (Carthamus tinctorius L.); cucumber (Cucumis sativus L.); polyphenol; light stress; light/water stress

Recently, polyphenol compounds, which are ubiquitous in the plant kingdom have received much attention for their antioxidative and disease-preventing activities, and further for their contribution to health maintenance. For example, catechins, the major polyphenol compounds in green tea, are focused on for their antioxidative,1,2) antitumor,3) and antimutagenic4) activities. Therefore, effective production of polyphenols in plants has been considered to be important to supply a lot of polyphenols.

Many studies have indicated that plants accumulate polyphenols in response to UV-B (280–315 nm) radiation.5–7) However, little is known about the correlation between the accumulation of polyphenols in the plants and the environmental stresses imposed, except for UV-B radiation.

In this report, we investigated the effects of strong light stress in the visible wavelength region and water stress on the accumulation of polyphenols in safflower and cucumber seedlings in order to select the most suitable conditions for accumulating a high content of polyphenol in plants. Safflower leaves have been used as tea in many countries8) and their polyphenols were reported to have activities that suppress allergies9) and oxidation in vivo;10) therefore, the development of a method to increase polyphenols in safflower leaves is of great interest. Cucumber seedlings were investigated in comparison with safflower, because the cucumber has been recognized as a stress-sensitive plant.

Materials and Methods

Plant materials and reagents. Safflower seeds (Carthamus tinctorius L.) were purchased from the Sakata Seed Corp. (Yokohama, Japan). Cucumber seeds (Cucumis sativus L.) were kindly supplied by Dr. Hideaki Nakano. Kaempferol, luteolin, myricetin, quercetin, and rutin of HPLC grade were purchased from Funakoshi Co., Ltd. (Tokyo, Japan), and caffeic acid, p-coumaric acid, ferulic acid, and gallic acid from Nacarai Tesque Co., Ltd. (Kyoto, Japan). Chlorogenic acid, 3,5-dicaffeoyl quinic acid, and luteolin 7-O-glucoside were isolated in our laboratory. The chemical structures of each isolated compound were confirmed by measuring the 1H- and 13C-NMR spectra. The chromatographic solvents of HPLC grade and all other chemicals of reagent grade were purchased from Wako Pure Chemical Industries Co., Ltd. (Osaka, Japan).
Growth conditions. Seeds were sown on a mixture of vermiculite and compost (1:1) in an aluminum tray and kept in the dark for 48 h to cause germination. After germination, the seedlings were transferred to a chamber (FLI-301NH-L, Tokyorikakikai Co., Tokyo, Japan) and cultivated with a 12-h light (5000 lux) and 12-h dark cycle at a temperature of 25°C. The growth light in this period was supplied by fluorescent light tubes (FL40S-W, 40 W, Mitsubishi/ Osram Co., Kanagawa, Japan), and the illumination intensities were measured with an illuminometer (LX-1330, Custom Co., Tokyo, Japan).

The 7-day-old seedlings were divided into three groups, and grown for 7 more days under the following experimental conditions: 1) Control condition, the light intensity was 6000 lux and a total of 170 ml of water per day was supplied 2 or 3 times daily; 2) Light stress condition, the light intensity was 45000 lux and the same volume of water was supplied as described for the control condition; 3) Light/water stress condition, the light intensity was the same as that under the light stress condition and a total of 100 ml of water per day was supplied daily. In order to obtain the strong light illumination, a high pressure sodium lamp (NH360L, Iwasaki Electric Co., Ltd., Tokyo, Japan) was combined with the fluorescent light tubes. Under the control condition, the light illumination was partially covered with several sheets of wiping paper to suppress the light intensity without a significant change in the light quality. The experiments for the control condition were duplicated in order to estimate the difference between the same experimental conditions.

The effects of UV-B exclusion. To assess the effect of UV-B from the illumination lights, the conditions of both the control and the light stress were examined in a UV-B exclusion setup using a UV-B cut film (≤ 315 nm, Sixrightclean, Mitsubishi Chemical MVK Co., Ltd., Tokyo, Japan). The films were papered on the inside surfaces on the growth chamber. The other experimental procedures were the same as those mentioned above.

Sampling of leaves. The cotyledons and foliage leaves of seedlings grown for 7 days under each growth condition were collected and immediately weighed. In each sampling, about 30 leaves were combined and analyzed in order to diminish the variations between the individual leaves. Small parts of each leaf, about 1/8 of a whole leaf, was cut off for the chlorophyll (Chl) measurements. The remaining parts were freeze-dried and weighed; the ratios of the fresh weights to dry weights (fresh/dry ratios) were calculated and used as an indicator of water stress.

Chlorophyll measurement. The fresh parts of the leaves were ground with quartz sand and then 5 ml of 80% (v/v) acetone was added. The homogenates were centrifuged at 1500 × g for 5 min. The supernatants were diluted 10 times with 80% acetone and spectrophotometrically measured. The Chl a/b ratios were obtained by the method of Arnon.

Polyphenol extraction. The freeze-dried leaves (25 mg) were ground in a mixture of quartz sand and 5 ml of dimethyl sulfoxide (DMSO). The mixtures were centrifuged at 1500 × g for 10 min. After the supernatants were collected, the precipitates were mixed with the same volume of DMSO and centrifuged. The combined supernatants were stored at −80°C until the polyphenol analyses.

Measurement of total polyphenol. The concentrations of the total polyphenols in the DMSO extracts were measured by the method of Folin and Denis with a slight modification. Samples (200 μl) in a test tube (15 ml) were mixed with 2 ml of Folin-Denis reagent. After 3 min, 2 ml of 10% (w/v) sodium carbonate was added and well mixed. The mixtures were centrifuged at 1500 × g for 10 min after leaving them for 60 min at room temperature. The absorbances at 760 nm of the supernatants were measured with a spectrophotometer (UV-1600 Shimadzu, Kyoto, Japan). The contents of the total polyphenols were calculated as the gallic acid equivalent mg/g of fresh weight.

HPLC analysis. The HPLC analysis was done using an LC-VP series HPLC system (Shimadzu, Kyoto, Japan). A column of Develosil C-30-UG-5 (250 × 4.6 mm i.d., Nomura Chemical Co., Aichi, Japan) was used, and the column temperature was kept at 25°C. A linear gradient of 0–100% of solvent B (acetonitrile-H2O = 40:60, v/v) in solvent A (acetic acid-acetonitrile-H2O = 1:5:94, v/v) over the course of 180 min at a flow rate of 0.8 ml/min was used. Five μl of the DMSO extracts passed through a 0.2-μm pore filter was used for the analysis. Polyphenols were monitored by the absorbance at 330 nm. The calibration curve for p-coumaric acid, which was measured under the same HPLC conditions as that for the DMSO extracts, showed linearity in the injected amounts between 1.5 and 49 nmol, indicating that the HPLC conditions used are adequate for quantitative analysis.

Results

Effects of UV-B exclusion

In these experiments, fluorescent light tubes and a high pressure sodium lamp were used as the light sources. Although the high pressure sodium lamp supplies little emission below 400 nm, the fluorescent light does include a small amount of UV-B light. The effects of UV-B from the lights on the growth of see-
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Table 1. Effects of Light and Light/Water Stresses on the Fresh Weights of a Leaf and the Ratios of the Fresh/Dry Weights

<table>
<thead>
<tr>
<th>growth conditions</th>
<th>fresh weights (g)</th>
<th>fresh/dry</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cotyledon</td>
<td>foliage leaf</td>
</tr>
<tr>
<td>in safflower</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.185/0.192b (0.186)b</td>
<td>0.151/0.131 (0.164)</td>
</tr>
<tr>
<td>Light stress</td>
<td>0.213 (0.181)</td>
<td>0.307 (0.302)</td>
</tr>
<tr>
<td>Light/water stress</td>
<td>0.204</td>
<td>0.199</td>
</tr>
<tr>
<td>in cucumber</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.271/0.227 (0.245)</td>
<td>0.300/0.226 (0.308)</td>
</tr>
<tr>
<td>Light stress</td>
<td>0.307 (0.306)</td>
<td>0.547 (0.610)</td>
</tr>
<tr>
<td>Light/water stress</td>
<td>0.237</td>
<td>0.237</td>
</tr>
</tbody>
</table>

*: Fresh weights per leaf, calculated from the numbers and the total weights of the combined and analyzed leaves.

b: Values in the duplicated experiments.

The seedlings of safflower and cucumber were grown under three different conditions (control, light stress, light/water stress). Table 1 shows the fresh weights of a leaf and the ratios of the fresh weights to dry weights of a leaf. In safflower, both stresses increased about 10% of the fresh weights of the cotyledons. The fresh weights of the safflower foliage leaves increased over 2-fold due to the light stress and about 1.5-fold due to the light/water stress, in comparison with those under the control condition. In cucumber, the light stress similarly increased the fresh weights; however, no significant increase in the fresh weights was observed under the light/water stress.

The fresh/dry ratios of the leaves decreased due to the two stresses for both safflower and cucumber. The light stress decreased about 30% of the ratio in safflower and about 50% in cucumber. The additional water stress, i.e., light/water stress, further decreased the ratios by about 10%.

The values in the parentheses of Table 1 represent the fresh weights per leaf and the fresh/dry ratios in the experimental setup of UV-B exclusion using the UV-B cut film. By comparison with the mean values of the duplicated experiments under the control condition, the changes in the fresh weights by the UV-B exclusion were less than 20% in both the safflower and cucumber. The UV-B exclusion had little effect also on the fresh weights under the light stress condition. The fresh/dry ratios of the seedlings grown in the UV-B exclusion setup are coincident within less than 10% deviation even at maximum compared to those under the conditions without UV-B exclusion.

<table>
<thead>
<tr>
<th>growth conditions</th>
<th>cotyledon</th>
<th>foliage leaf</th>
</tr>
</thead>
<tbody>
<tr>
<td>in safflower</td>
<td>2.74/2.86b (2.70)b</td>
<td>3.08/3.21 (3.04)</td>
</tr>
<tr>
<td>Light stress</td>
<td>2.86 (2.88)</td>
<td>3.52 (3.35)</td>
</tr>
<tr>
<td>Light/water stress</td>
<td>2.98</td>
<td>3.51</td>
</tr>
<tr>
<td>in cucumber</td>
<td>2.75/2.78 (2.72)</td>
<td>3.01/2.94 (2.89)</td>
</tr>
<tr>
<td>Light stress</td>
<td>2.86 (2.86)</td>
<td>3.52 (3.30)</td>
</tr>
<tr>
<td>Light/water stress</td>
<td>2.98</td>
<td>3.51</td>
</tr>
</tbody>
</table>

*: Values in the duplicated experiments.

Chlorophyll a/b ratios

The Chl a/b ratios were calculated in order to assess the influence of the stresses on the photosynthetic apparatus (Table 2). In safflower, both stresses increased the Chl a/b ratios in the cotyledons about 5% and that in the foliage leaves about 20%. The Chl a/b ratios in cucumber were similarly increased by both stresses. The data in the UV-B-excluded experiments, which are shown in the parentheses of Table 2, agreed with those in the experiments without UV-B exclusion with about 5% deviation.

Total polyphenol contents

Figure 1 shows the contents of the total polyphenols in the seedlings of safflower (a) and cucumber (b). The values of the control groups indicate the means of the data obtained in the duplicated experiments, which were coincident within less than 10% deviation. The polyphenol contents in the leaves were increased by the two stresses in both safflower and cucumber. In safflower, the polyphenol contents of the foliage leaves were increased 1.7-fold by the light stress and 2.6-fold by the light/water stress. In cucumber, although the increase in polyphenol contents of the foliage leaves due to light stress was...
Fig. 1. Polyphenol Contents of Cotyledons and Foliage Leaves.

The seedlings of safflower (a) and cucumber (b) were grown under the growth conditions of three types: CT, control; LS, light stress; LWS, light/water stress. The open bars show the polyphenol contents of cotyledons and the closed bars those of foliage leaves. The values in the control group represent the mean of duplicated experiments, and the error bars indicate the deviations of the two control experiments. The values were expressed as mg of the gallic acid equivalent per g of fresh weights.

HPLC analysis of polyphenol compounds

We tested several HPLC conditions to separate and identify polyphenol compounds. As a standard solution, a mixture of 11 authentic polyphenol compounds, consisting of cinnamic acid derivatives, flavones and flavonols, was analyzed. These compounds are the typical polyphenol components of plant polyphenols. Under the selected HPLC condition, a satisfactory separation pattern of these polyphenol compounds was obtained (Fig. 2).

Figures 3 and 4 show the HPLC elution profiles of the DMSO extracts of the safflower and cucumber seedlings, respectively. The peaks were numbered according to the retention times. In safflower, the heights of each peak were greatly different between the seedlings grown under the control condition and those grown under the light/water stress condition. The difference was greater in the foliage leaves (Figs. 3(c) and (d)) than in the cotyledons (Figs. 3(a) and (b)). Increments in several peak heights due to the stresses were also observed in the cucumber as well as in the safflower. The polyphenols of peaks #6 and #11 in the safflower leaves (Figs. 3(c) and (d)) were identified to be luteolin 7-O-glucoside and luteolin, respectively, by coinjection with authentic samples. The major peak #4 in the cucumber (Fig. 4) showed the UV absorption spectrum which is characteristic of polyphenols (UV λmax (MeOH) nm: 267, 320) suggesting that this peak can be used as an indicator of polyphenol affected by stresses. In the foliage leaves of cucumber, the increasing magni-
Fig. 3. HPLC Elution Profiles of DMSO Extracts of Safflower Cotyledons (a and b) and Foliage Leaves (c and d).
HPLC conditions were described in Methods. The peaks with the same retention times are expressed as the same peak numbers: a and c, control; b and d, light/water stress. The peaks #6 and #11 were identified as luteolin 7-O-glucoside and luteolin by cochromatography with authentic samples.

Fig. 4. HPLC Elution Profiles of DMSO Extracts of Cucumber Cotyledons (a and b) and Foliage Leaves (c and d).
HPLC conditions were described in Methods. The peaks with the same retention times are expressed as the same peak: a and c, control; b and d, light/water stress.

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tudes due to both stresses were similar; peak area #4 particularly increased by about 5-fold due to both stresses.

Luteolin 7-O-glucoside and luteolin in safflower
The contents of luteolin 7-O-glucoside (peak #6 in Fig. 3) and luteolin (peak #11 in Fig. 3) in the safflower leaves grown under the three growth conditions are summarized in Table 3. The contents of luteolin 7-O-glucoside and luteolin were measured by HPLC using the calibration curves of each purified compound corresponding to the two flavonoids. Luteolin was not found in the cotyledons, but it was found in the foliage leaves along with luteolin 7-O-glucoside. In the cotyledons, luteolin 7-O-glucoside increased about 1.4-fold due to the light stress and 2-fold due to the light/water stress. In the foliage leaves, the contents of each flavonoid significantly
differed between the duplicated experiments under the control conditions. However, the sums of the contents of these two flavonoids in the each control experiment were 0.634 mg/g and 0.663 mg/g, respectively, and were coincident within less than 5% deviation. The sums of both flavonoids increased 2.1-fold respectively, and were coincident within less than 5% deviation. The sums of both flavonoids increased 2.1-fold due to the light stress and 2.7-fold due to the light/water stress.

The contents of luteolin 7-O-glucoside and luteolin in the seedlings grown under the UV-B exclusion setup are shown in the parentheses of Table 3. In the cotyledons, the UV-B exclusion decreased about 30% from the luteolin 7-O-glucoside contents under the control condition and reversely increased about 30% of those under the light stress condition. In the foliage leaves, although the UV-B exclusion decreased about 10% of the sums of these two flavonoids under the control condition, the sums of the contents of luteolin 7-O-glucoside and luteolin under the light stress condition in the UV-B exclusion setup almost agreed with those in the experiments without UV-B exclusion.

### Discussion

There are many studies on the effects of increased UV-B radiation on the polyphenol content in higher plants.\(^{12,14}\) Most of the studies report an increase in the polyphenol contents due to UV-B radiation; the polyphenol increase induced by UV-B is supposed to be ascribed to the protective effect of plants against UV-B light.\(^{15,17}\) In our experiments, the strong light irradiation also caused an increase in the total polyphenol contents in the safflower and cucumber seedlings; however, the polyphenol increase under the strong light condition was observed even when the UV-B light was excluded using the UV-B cut film. Therefore, it is confirmed that the increase in the polyphenol contents in the seedlings grown under strong light conditions does not depend on the light in the UV-B wavelength range but on that in the visible wavelength range. It should be noted that, only in the cucumber foliage leaves, did the strict UV-B exclusion from the growth light significantly suppress the increasing effect on the total polyphenol contents due to the light stress. The UV-B light may partially function to increase the polyphenol compounds in cucumber seedlings.

The irradiation of UV-A (315–400 nm) also has been reported to have increasing effects on photosynthetic activities and polyphenol contents\(^{15–17}\) as well as the UV-B irradiation. In these experiments, we used the fluorescent light tubes and a high pressure sodium lamp as the light sources: in the light from the fluorescent light tubes, the percentage of the light intensity in the UV-A wavelength range was 2.5% of the total light intensity below 700 nm, but the irradiation light from the high pressure sodium lamp contained negligible UV-A light. Because the fluorescent lights contributed 24% of the whole growth light, the ratio of the UV-A light contained in the growth light below 700 nm was estimated to be very slight, approximately 0.6%. Thus, the effects of the strong light illumination that we observed in this study should be mainly attributed to the light in the visible wavelength range, although this was not confirmed by experiments.

The effects of the stress conditions on a plant's photosynthetic apparatus were evaluated by measuring the Chl a/b ratio.\(^{18,19}\) While the changes in the cotyledon Chl a/b ratios due to both stresses were negligible, the Chl a/b ratios of the foliage leaves in the safflower and cucumber seedlings were increased by about 20% due to both stresses (Table 2). However, no large decrements in the total Chl contents were observed in these foliage leaves (data not shown). These results indicate that no severe photodamage occurred in the photosystems of the seedlings under both stress conditions and that the polyphenol contents of the plant leaves can be increased by exposing the plants to strong light radiation.

In order to examine the effects of the light stress and the light/water stress on the drought extent of the leaves, the fresh/dry weight ratios of the leaves were measured. The light stresses in the presence of much water (170 ml) and of a small amount of water (100 ml) suppressed the fresh/dry ratios to the level of 60–70% and 50–60% of the control leaves, respectively, indicating that the seedlings under the light stress conditions suffer not only light stress but also water stress. Though water culturing of seedlings makes it possible to avoid water stress even in the

<table>
<thead>
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<th>growth conditions</th>
<th>cotyledon</th>
<th>foliage leaf</th>
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<tbody>
<tr>
<td></td>
<td>luteolin 7-O-glucoside (mg/g)</td>
<td>luteolin 7-O-glucoside (mg/g)</td>
</tr>
<tr>
<td>Control</td>
<td>0.294/0.274(^{+}) (0.220)</td>
<td>0.606/0.242 (0.403)</td>
</tr>
<tr>
<td>Light stress</td>
<td>0.412 (0.550)</td>
<td>1.24 (1.36)</td>
</tr>
<tr>
<td>Light/water stress</td>
<td>0.575</td>
<td>1.19</td>
</tr>
</tbody>
</table>

\(^{+}\) Milligrams of each flavonoid per g of fresh weights, measured using each purified compound corresponding to the flavonoids as a standard.

\(^{\circ}\) Values in the duplicated experiments.

\(^{\circ}\) Values in the parentheses were obtained in the UV-B exclusion setup.
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The presence of strong light irradiation, soil culture was chosen in this study, because soil culture is more similar to cultivation in natural fields. To confirm the effects of water stress on the accumulation of polyphenols, an additional water stress was added to the light stress condition. The additional water stress was then evaluated by comparing the difference in the fresh/dry ratio of the leaves between the seedlings exposed to the light stress and to the light/water stress. Under the light/water stress condition, the fresh/dry weight ratios of the leaves decreased more than those under the light stress (Table 1), ascertaining that the additional water stress caused an increase in the drought extent of the seedlings. The fresh weights as well as the fresh/dry weight ratios were decreased by the additional water stress. These results indicate that the additional water stress induced growth suppression under the light/water stress condition, although photodamage was not very severe under these conditions, as has been mentioned.

It is known that cucumber seedlings are very sensitive to water stress. Tevini et al. reported that the water stress caused decreases in the fresh weights and leaf areas in cucumber seedlings. Similar observations were also reported by Yang et al. In our results, the fresh weights of leaves in the cucumber seedlings greatly decreased due to the water stress that was added to the stress of the strong light radiation (Table 1), which is consistent with the results from these previous reports.

In safflower, the decrease in the fresh weights due to the additional water stress was smaller than that in cucumber; the safflower seedlings seem to be more tolerant than the seedlings of cucumber to water stress. On the other hand, the polyphenol-increasing effect by the additional water stress was more clearly observed in safflower than in cucumber when judged from the difference in the polyphenol contents between these two plant species. These results suggest that the plants resistant to water stress accumulate a higher amount of polyphenols and that plants may adapt not only to the strong visible light radiation but also to water stress by increasing their polyphenol contents.

The effects of the light stress and the light/water stress on the total polyphenol contents were more noticeable in the foliage leaves than in the cotyledons (Fig. 1). In higher plants, nutrients are stored in the cotyledons. The decomposition of the stored components in the cotyledons is accelerated after elongation of a foliage leaf. The cotyledons of the seedlings, which were analyzed for their components, are supposed to be in the decomposition stage. Therefore, the increase in the polyphenol contents due to both stresses might be more clearly observed in the foliage leaves than in the cotyledons.

The change in each peak area of the HPLC elution profiles, which is induced by the stresses, showed that individual polyphenols in plants seedlings respond variously to the stresses. For example, the areas of the #7 and #8 peaks, derived from the safflower cotyledons and shown in Figs. 3(a) and (b), were increased by the light stress and further by the light/water stress, but peak area #10 was decreased by both stresses to half of that under the control conditions. Peak area #7 was especially increased 4-fold due to the light/water stress. In the safflower foliage leaves, both stresses also greatly increased the areas of the #7 and #8 peaks. In the cucumber foliage leaves, although peak area #3 increased only twice due to both stresses, peak area #5 increased more than 9-fold. These results emphasize that the measurement of only total polyphenol contents may be insufficient to evaluate the effects of the environmental stress on plants; it is also necessary to identify the changes in the kinds of polyphenols which respond to the stress.

Although each of the contents of luteolin 7-O-glucoside and luteolin in the safflower seedling varied widely in each control experiment (Table 3), the other analyzed parameters except for the contents of luteolin 7-O-glucoside and luteolin were coincident in the two control experiments. Furthermore, the peak areas in the HPLC elution profiles were similar within about 10% deviation between the two control experiments, except for the peaks #6 to #11 in the safflower foliage leaves, and the sums of both the luteolin 7-O-glucoside and luteolin contents in the two control experiments were similar. It is assumed that the large deviation in either luteolin or luteolin 7-O-glucoside could then be attributed not to the experimental error but to some unknown factors concerned with the glycosylation of luteolin. We, therefore, used the sums of luteolin 7-O-glucoside and luteolin contents in order to estimate the effects of both stresses on the contents of the flavonoids contained mainly in the safflower seedlings.

The increases in the sum of luteolin and luteolin 7-O-glucoside contents indicated that the biosynthesis of these compounds might be stimulated by the light and light/water stresses. The antioxidant activities of luteolin 7-O-glucoside in the safflower leaf extract in vivo and luteolin in the artichoke leaf extract for human leukocytes have been reported. Our results indicate the possibility that luteolin and its glucoside function as antioxidants in plants to protect them from oxidative stress induced by the strong visible light radiation and the water stress.

This study has shown that strong visible light caused a significant increase in the polyphenol contents in the seedlings of safflower and cucumber and that additional water stress further increased the polyphenol contents. Further experiments are needed to find what kinds of polyphenols in safflower and in cucumber easily respond to each individual stress.
Acknowledgment

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References