biologia plantarum
an international journal for experimental botany

BIOLOGIA PLANTARUM (2023) 67: 28-35 DOI: 10.32615/bp.2022.039

Cucumber leaf necrosis caused by radiation with abrupt increase of far-red component

T. SHIBUYA[1,*], C. KATAOKA[2], K. NISHIO[2], R. ENDO[1], Y. KITAYA[3], Y. SHINTO[2], K.I. MISHIBA[4], and Y. IWATA[2]

[1] *Graduate School of Agriculture, Osaka Metropolitan University, Osaka 599-8531, Japan*
[2] *School of Life and Environmental Sciences, Osaka Prefecture University, Osaka 599-8531, Japan*
[3] *R&D Center for the Plant Factory, Osaka Metropolitan University, Osaka 599-8531, Japan*
[4] *Faculty of Agriculture, Ryukoku University, Otsu 520-2194, Japan*

[*]Corresponding author: E-mail: tshibuya@omu.ac.jp

Abstract

Plants exhibit morphological plasticity in response to changes in the proportion of far-red radiation (FR). However, little is known about the response to a sudden increase of FR component. Cucumber seedlings were acclimatized to radiation without FR (FR-) for 1 - 5 d after germination, and then transferred to radiation containing FR (FR+) at levels similar to those in natural sunlight. Other seedlings were acclimatized to FR- or FR+, which was maintained continuously. The sudden increase in FR damaged the cotyledons and the first true leaf, especially when radiation was changed from FR- to FR+ at days 3 or 4 after germination. Necrosis of the damaged leaves may have resulted from inhibition of water flow in leaf xylem, because wilting and decreased stomatal conductance were observed simultaneously with leaf necrosis. Plants in the treatment groups that showed the most frequent damage showed two peaks in cotyledon elongation. This suggests that the leaves that had been acclimatized to FR- were easily damaged by the sudden promotion of leaf expansion caused by FR+.

Keywords: leaf expansion, morphological plasticity, phytochrome, radiation quality.

Introduction

Plants can detect neighbouring vegetation by detecting changes in the proportion of far-red component in the radiation they receive, and can then change their morphology to tolerate or avoid the effects of shading (Smith and Whitelam 1997, Franklin 2008). This response results from a decrease in the proportion of active phytochrome under shading, which promotes stem elongation and leaf expansion (Ballaré *et al*. 1994, Gommers *et al*. 2013) in a shade-avoidance response. It is not well known that plants exhibit morphological plasticity in response to changes in the proportion of far-red radiation (FR) (Schmitt *et al.* 1995, Huber *et al*. 2021). However, the response to a sudden increase in the proportion of FR is little known, probably because associated sudden shading rarely occurs in nature except for partial and temporal radiation-quality change caused by sunflecks (Kaiser *et al.* 2018, Sellaro *et al.* 2019), whereas sudden increases in radiation availability due to, for example, gap formation are far more frequent (Naidu and DeLucia 1997, Yamashita *et al*. 2000, Oguchi *et al*. 2006). A sudden increase in the proportion of FR can occur in plant production and in plant experiments under controlled environments.

In a recent study (unpublished data), we observed that cucumber (*Cucumis sativus*) seedlings experienced significant leaf necrosis when the seedlings, which had been germinated under radiation with little FR, were transferred to radiation with rich FR, equivalent to the proportion in natural sunlight. This unexpected observation led us to hypothesize that a sudden increase in the proportion of FR resulted in physiological or morphological changes that led to high necrosis of leaves. To replicate our finding and test this hypothesis, we investigated the effect of timing of

---

Received 18 May 2022, *last revision* 8 September 2022, *accepted* 14 October 2022.
Abbreviations: FR - far-red radiation (+ present, - absent); PSS - phytochrome photostationary state.
Acknowledgements: This research was supported by a Japan Society for the Promotion of Science Grant-in-Aid for Scientific Research (B) (KAKENHI 18H02307, KAKENHI 21H02322).
Conflict of interest: The authors declare that they have no conflict of interest.

the sudden increase in FR on leaf morphology (Expt 1), and the effect of the direction of radiation quality change on leaf morphology (Expt 2) under LED irradiance. In addition, we investigated the effect of the sudden increase in FR on stomatal conductance (Expt 3) and cell enlargement (Expt 4) to determine the factors related to the leaf necrosis.

Materials and methods

Plants and growth conditions: In all experiments, cucumber (*Cucumis sativus* L.) cv. Hokushin seeds were sown in plastic pots (60-mm diameter, 55-mm height) at one seed per pot containing vermiculite. The seeds were germinated and then grown in a growth chamber maintained at an air temperature of 28 °C, a relative humidity of 50 %, and a vapor pressure deficit of 1.9 kPa (except for Expt 4). The CO_2 concentration was not rigorously controlled but was maintained at approximately 400 µmol mol^{-1}. The pots stood in nutrient solution 5 to 10 mm deep (the A-type recipe of *OAT Agrio Co.*, Tokyo, Japan). Irradiation was provided by LED panels (*LED Sun Light Z4*; *Taiwan Hipoint Co*., Kaohsiung, Taiwan) containing blue, green, red, and FR sources. The photosynthetic photon flux at the leaf surface was maintained at 300 ± 15 µmol(photon) m^{-2} s^{-1} during plant growth by adjusting the distance between the LED panels and the canopy surface daily. The 16-h photoperiod was throughout the experiment. The LED panels were installed on the different shelves in the growth chamber. The air in the chamber was circulated with fans to maintain approximately uniform atmospheric conditions.

In all experiments, we provided radiation without FR (FR-) or radiation containing FR at levels similar to those in natural sunlight (FR+) using the LED panels. In addition, radiation with a larger proportion of FR than in sunlight (FR++) was provided in Expt 2. The blue radiation (400 - 500 nm) accounted for 30 % of the photosynthetically active radiation (400 - 700 nm), green (500 - 600 nm) for 35 %, and red (600 - 700 nm) for 35 %, with no detectable far-red component. These percentages are approximately the same as those in sunlight. The red to far-red ratio of FR+ and FR++ was 1.2 and 0.5, respectively. The photon flux density of far-red of FR+ and FR++ was 88 µmol(photon) m^{-2} s^{-1} and 210 µmol(photon) m^{-2} s^{-1}, respectively. The spectra of the different treatments (Fig. 1) were measured using an *SS-110 spectroradiometer* (*Apogee Instruments*, Logan, UT, USA), and their phytochrome photostationary state (PSS), which represents the ratio of active phytochrome to total phytochrome, was calculated according to the method of Sager *et al*. (1988). The PSS of FR-, FR+ and FR++ were 0.87, 0.73. and 0.53, respectively.

Effects of timing of the sudden increase in far-red radiation on leaf morphology (Expt 1, Table 1): Some of the seedlings were acclimatized to FR- for 1 - 5 d after germination, and then were placed under FR+. The remaining seedlings were acclimatized to FR- or FR+ continuously without changing radiation quality. Fifteen seedlings were grown in each treatment for 12 d after germination. The leaves were observed once a day. We visually evaluated leaf damage as a percentage of the total cotyledon area which showed necrosis. We determined the relative necrosis area of cotyledons using the medians (0, 12.5, 37.5, 62.5, and 87.5 %) of five ranges (0, 0 - 25, 25 - 50, 50 - 75, and 75 - 100 %) that was visually evaluated for each seedling. We then calculated the elongation rate of the cotyledons by comparing the cotyledon length with the length measured on the previous day. We counted the number of seedlings with leaf damage 12 d after germination. The necrotic area was destructively measured on the same date on an image scanner by *LIA image analysis software* (K. Yamamoto, Nagoya University, Nagoya, Japan). The border plants were included in all measurements. This experiment was performed twice, but the cotyledon elongation rate was measured only once.

Effects of radiation quality changes on leaf morphology (Expt 2): In an independent experiment, the seedlings were acclimatized to FR- or FR+ for 4 d. Then the seedlings in each treatment group were distributed among the FR-, FR+, and FR++ (six combinations as shown in Table 2). Fifteen seedlings were grown in each treatment. The number of seedlings with leaf damage was counted 11 d after germination.

Effects of the sudden increase in FR radiation on stomatal conductance (Expt 3): The seedlings were acclimatized to FR- for 4 d after germination, and then were placed under FR+. The other seeds were germinated under FR- and were maintained continuously under these conditions. Twenty seedlings were grown in each treatment. The stomatal conductance of the tip region of cotyledons was measured by *SC–1 porometer* (*Decagon*

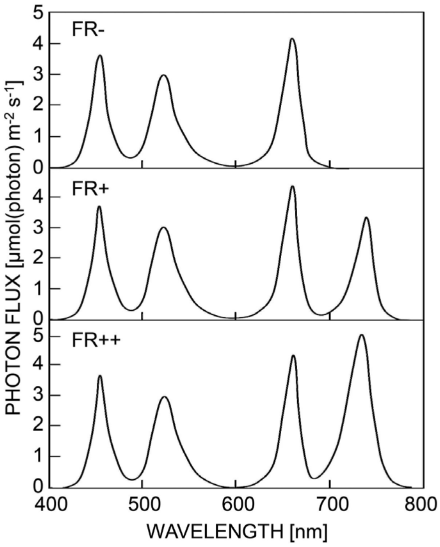

Fig. 1. The measured photon-flux spectrum of radiation without far-red radiation (FR-), radiation containing far-red radiation at approximately the same proportion as in sunlight (FR+), and radiation containing far-red radiation with a higher proportion than in sunlight (FR++).

Table 1. Effect of the timing of sudden change of radiation composition on the proportions of cucumber seedlings whose cotyledons or first true leaves were damaged during the treatment (Expt 1). Values are percentages of damaged seedlings in 15 seedlings. FR-: radiation without far-red radiation. FR+: radiation containing far-red radiation at approximately the same proportion as in sunlight. Values within a column labeled with the same letter are not significantly different at $P < 0.05$ (pairwise comparisons using Fisher's exact test).

Time after germination [d] when FR- changed to FR+	Period of FR+ after germination [d]	Damaged seedlings [%]	
		Replicate 1	Replicate 2
Continuous FR+	12	13 ab	7 b
1	11	33 ab	20 ab
2	10	20 ab	33 ab
3	9	67 a	67 a
4	8	67 a	67 a
5	7	53 a	33 ab
Continuous FR-	0	0 b	0 b

Devices, Pullman, WA, USA) once a day from 3 to 11 d after germination. The average of stomatal conductance in two cotyledons of each seedling was considered as one biological replicate. We counted the number of seedlings with leaf damage once a day during the growing period.

Effects of radiation quality change on cell enlargement (Expt 4): In another independent experiment, the germinated seedlings were acclimatized to FR- for 4 d after germination, and then to FR+ for 7 d. Other seedlings were germinated and continuously maintained under FR- or FR+ for the full 11 d. Imprints of the abaxial surfaces of the undamaged cotyledons were obtained from three seedlings in each treatment by spreading a liquid adhesive (*Ekiban A*; *Taihei Yakuhin Co.,* Tokyo, Japan) onto the leaf surface, allowing it to dry, and then peeling it from the leaf. The imprints were observed under a *VHX-1000* digital microscope (*Keyence Corp.*, Osaka, Japan) at 800× magnification, which provided a field of view that covered 0.13 mm^2, and the numbers of epidermal cells were counted in three randomly selected fields of view on each leaf and averaged. The epidermal cell size was calculated by dividing the view field area by the number of cells. The number of cells per cotyledon was calculated by dividing the total cotyledon area by the average cell size.

Flow cytometry analysis was performed to verify whether cell expansion was accompanied by endoreduplication, based on the experiments for epidermal cell number and cell size described above. The cotyledons of the seedlings that were acclimatized to FR- (days 0 - 4) followed by FR+ (days 5 - 11) and those of seedlings that were continuously grown under either FR- or FR+ (days 0 - 11) were sampled at 4 and 11 d after germination. The ploidy distribution in each cotyledon was then determined by means of flow cytometry as in previous studies (Amijima *et al.* 2014). The chopping method (Galbraith *et al.* 1983) was performed with tissues derived from a cotyledon using *CyStain UV precise P* reagent (*Partec,* Münster, Germany). The suspension of nuclei was analysed by flow cytometry on a *CyFlow Space cytometer* (*Partec*) by *FloMax* software (*v.* 2.6, *Partec*) to determine the relative nuclear DNA content. The "cycle value" (Barow and Meister 2003) was determined to quantify the mean number of endoreduplication cycles per nucleus for each sample as:

$$\text{Cycle value} = (0\, n_2\text{C} + 1\, n_4\text{C} + 2\, n_8\text{C} + 3\, n_{16}\text{C} \cdots) / (n_2\text{C} + n_4\text{C} + n_8\text{C} + n_{16}\text{C} \cdots)$$

where n_2C, n_4C, n_8C ⋯ represent the numbers of nuclei with the corresponding C values (2C, 4C, 8C ⋯).

Statistical analysis: In Expt 1, we determined the effect of the radiation treatment on the proportion of the cotyledon area that showed necrosis and the area of necrosis per seedling 12 d after germination using a generalized linear mixed model with replication as a random factor. In Expt 1 and Expt 2, we determined the effect of the radiation treatment on the proportion of damaged seedlings by pairwise comparisons using Fisher's exact test (with significance at $P < 0.05$). In Expt 3, we analyzed the relationships between the stomatal conductance and the proportion of damaged seedlings using a generalized linear model. In Expt 4, we determined the effects of the radiation treatment on cell size (area) and cell number using analysis of variance (*ANOVA*) followed by the Tukey-Kramer test. All analyses were performed in *R v. 3.6.1 software* (www.r-project.org).

Results

Necrosis was observed on the cotyledons (Fig. 2*A*) and true leaves (Fig. 2*B*) in all treatment groups except the seedlings maintained under FR- throughout the whole period (Fig. 3, Table 1). There was a clear boundary between the living and dead tissues in the damaged cotyledons. No chlorosis was observed in cotyledons before the necrosis. Some true leaves, which did not show necrosis, did not expand normally (poorly expanded, deformed, or distorted) (Fig. 2). Leaves with necrosis and leaves that did not expand normally were both regarded as damaged.

The proportion of seedlings whose leaves were damaged was significantly higher in the seedlings that had been acclimatized to FR- for 3 and 4 d after germination followed by transfer to FR+ than in the seedlings that had been maintained under FR- or FR+ throughout the whole

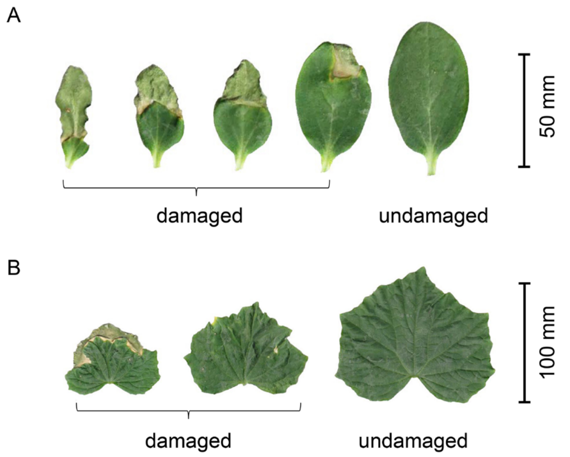

Fig. 2. Different degrees of damage in (*A*) cotyledons and (*B*) true leaves of cucumber seedlings that had been acclimatized to radiation without far-red radiation (FR-) from 0 - 4 d after germination and then placed under radiation with far-red radiation at levels similar to those in natural sunlight (FR+) for 8 d (Expt 1).

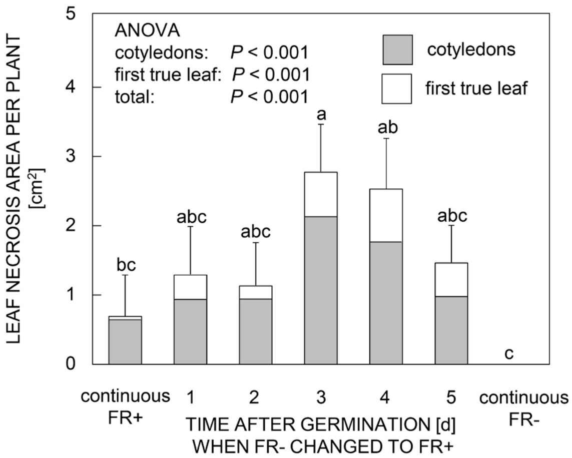

Fig. 3. Effect of increased duration of far-red radiation on leaf necrosis area of cucumber seedlings in Expt 1 (means of two replicates, $n = 15$ seedlings in each replicate). FR-: radiation without far-red radiation. FR+: radiation containing far-red radiation at approximately the same proportion as in sunlight. Data were analyzed using a generalized linear mixed model with replication as a random factor. *Bars* labeled with the same letter are not significantly different at $P < 0.05$ (*ANOVA* followed by Tukey-Kramer test) in the total (cotyledons plus first true leaf) necrotic area.

Table 2. Effect of the direction of sudden change of radiation composition on the proportions of cucumber seedlings whose cotyledons or first true leaves were damaged during treatment (Expt 2). Values are percentages of damaged seedlings in 15 seedlings. FR-: radiation without far-red radiation. FR+: radiation containing far-red radiation at approximately the same proportion as in sunlight. FR++: radiation containing far-red radiation with a higher proportion than in sunlight. Values labeled with the same letter are not significantly different at $P < 0.05$ (pairwise comparisons using Fisher's exact test).

Radiation treatment		Damaged seedlings [%]
0 - 4 d after germination	5 - 11 d after germination	
FR-	FR-	0 b
	FR+	60 a
	FR++	87 a
FR+	FR-	0 b
	FR+	0 b
	FR++	47 ab

experiment (Table 1). The effect of radiation treatment on the necrotic area was significant in both cotyledons and true leaves (Fig. 3). The total area of necrosis (cotyledons and first true leaf) was significantly higher in the seedlings that had been acclimatized to FR- for 3 and 4 d followed by FR+ than in the seedlings that had been continuously maintained under FR-. Cotyledon necrosis was observed 7 - 8 d after germination in all affected treatment groups, and necrosis by the end of the growing period was significantly greater after 3 to 4 d under FR- followed by FR+ than it was under continuous FR+ or FR- (Fig. 4*A*). Wilting of cotyledons was observed simultaneously with necrosis, or at most 1 - 2 d after necrosis began (Fig. 4*A*).

The leaf elongation rate tended to peak 1 - 2 d after germination and then to decrease in all treatment groups (Fig. 4*B*). However, it tended to increase again to near its maximum value within 1 or 2 d when the radiation quality was changed from FR- to FR+ at days 3 or 4 after germination.

The leaf damage occurred most frequently in the treatments where the proportion of FR was increased during the growing period (Table 2). The change from FR- to FR++ caused more frequent leaf damage than the change from FR- to FR+, but the difference was not significant. Leaf damage was also observed when radiation quality was changed from FR+ to FR++.

The stomatal conductance of the seedlings that had been acclimatized to FR- followed by transfer to FR+ increased for 2 d after changing radiation quality (4 d after germination), and then drastically decreased to 55 % within one day (Fig. 5*A*). No such reduction was observed in the seedlings that had been continuously maintained under FR-, whereas the stomatal conductance tended to peak 8 d after germination. The proportion of damaged seedlings

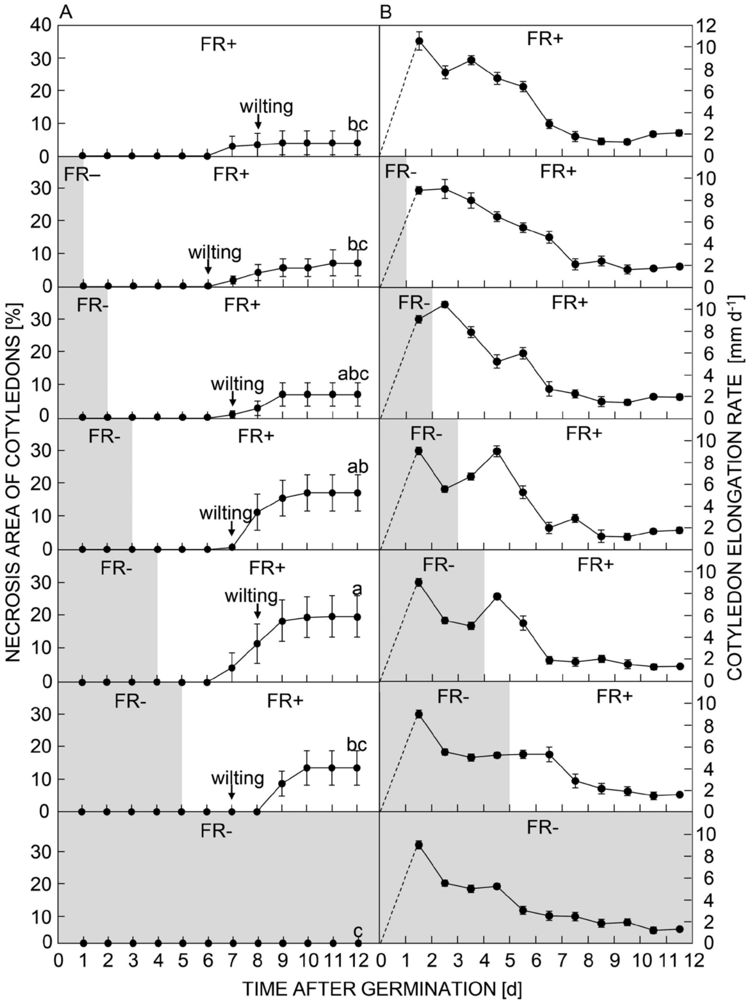

Fig. 4. Changes in (*A*) necrotic area and (*B*) elongation rate of cucumber cotyledons during the radiation treatment in Expt 1. Values of necrotic area are means of two replicates ($n = 15$ seedlings in each replicate). Values of elongation rate are means of 15 samples (with no replication; $n = 15$). FR-: radiation without far-red radiation. FR+: radiation containing far-red radiation at approximately the same proportion as in sunlight. *Shaded areas* represent the duration of the FR- treatment. *Arrows* indicate the day when wilting of cotyledons was observed in each treatment group. Values of necrotic area were analyzed using a generalized linear mixed model with replication as a random factor. Plots labeled with the same letter on day 12 are not significantly different at $P < 0.05$ (*ANOVA* ollowed by Tukey-Kramer test).

increased from 3 d after changing radiation quality (7 d after germination), and then reached to 85 % by the end of the growing period (Fig. 5*B*). We found a significant negative relationship ($P < 0.001$) between the stomatal conductance and the proportion of damaged seedlings in the seedlings that had been acclimatized to FR- followed by transfer to FR+.

There was no significant difference in the cell size between seedlings maintained continuously under FR- and those acclimatized to FR- followed by FR+ (Table 3). However, the cell sizes in both treatments were significantly smaller than that of seedlings maintained continuously under FR+. The number of cells per cotyledon did not differ significantly among treatments. The shape of cells in the cotyledons acclimatized to FR- followed by FR+ tended to be deformed and rather irregular compared with cells in the other treatments (Fig. 6).

The frequencies of 8C, 16C, and 32C nuclei in the cotyledon cells increased from day 4 to day 11 in both treatments (Fig. 7). The ploidy distribution was similar in the seedlings acclimatized to FR- and then transferred to FR+ and in those maintained continuously under FR+. The cycle value, which indicates the mean number of endoreduplication cycles per nucleus, did not differ significantly between treatments at 4 or 11 d after germination, but increased significantly from day 4 to day 11 in both treatments (Fig. 7).

Discussion

The sudden increase in FR damaged the cotyledons and true leaves of cucumber seedlings, causing wilting and necrosis. The necrosis may be due to the inhibition of water flow in leaf xylem that prevented water supply to the tips of the leaves, because wilting was generally observed at the same time as leaf damage, and there was a clear boundary between the living and dead tissues. The negative relationship between the stomatal conductance and the proportion of damaged seedlings supports this hypothesis. However, we could not determine the direction of causality in this study; the less conductance may be due to less transpiration in the damaged tissue. The decrease in stomatal conductance after sudden FR increase may be related to the decrease in water conductivity in xylem due to FR radiation (Ouedraogo *et al.* 1986, Casal *et al.* 1994). Although FR possibly affects stomatal conductance through the development of leaves and stomata, it is unlikely that FR directly affects stomatal aperture (Weraduwage *et al.* 2022). The necrosis was observed during leaf development and was not preceded by chlorosis, suggesting that it was not due to senescence.

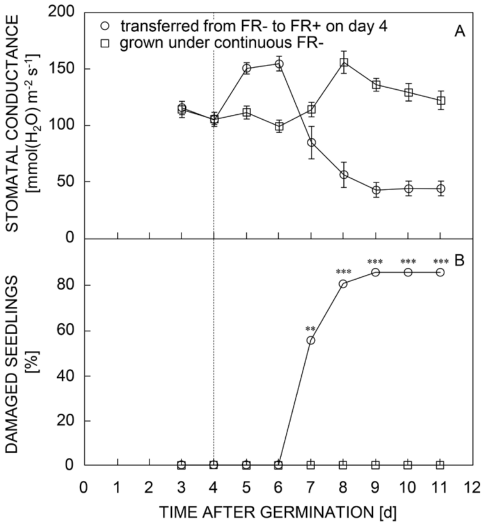

Fig. 5. *A* - Changes in stomatal conductance of the seedlings that had been acclimatized to radiation without far-red radiation (FR-) for 4 d followed by radiation containing far-red radiation at approximately the same proportion as in sunlight (FR+), and the seedlings that had been maintained continuously under FR-. *B* - proportion of the damaged seedlings in each treatment groups (Expt 3). Values of stomatal conductance are means of $n = 20$ samples (with no replication). Significance (pairwise comparisons using Fisher's exact test): ** - $P < 0.01$; *** - $P < 0.001$.

The fact that leaf damage occurred when the proportion of FR increased suggests that a similar problem may occur when sunlight is blocked by neighbors. However, compared with the sudden decrease in the proportion of FR due to the formation of canopy gaps in vegetation (Lee 1987), a sudden increase in the proportion of FR (*i.e.*, sudden shading) would be rare except for partial and temporal radiation-quality change caused by sunflecks (Kaiser *et al.* 2018, Sellaro *et al.* 2019).

The elongation rate of the cotyledons acclimatized to FR- increased after the sudden increase in the proportion of FR, indicating that the seedlings respond to the increased FR by growing faster. In the treatment groups that showed two peaks of cotyledon elongation rate (seedlings acclimatized to FR- for 3 or 4 d followed by FR+), seedlings were damaged more frequently and more severely than in the other treatments, indicating that the leaf damage may have resulted from the sudden promotion of leaf expansion by FR+. The leaf damage was observed even when radiation quality was held constant in the FR+ treatment, probably owing to the increased proportion of FR due to mutual shading and self-shading during the growing period.

Although the mechanism of the leaf damage was not studied, we can propose a hypothesis that should be tested in future research: that wilting and necrosis of the

Table 3. Average cell area and number of cells per cotyledon in cucumber seedlings acclimatized to different radiation composition (Expt 4). Three cotyledons were measured in each treatment. FR-: radiation without far-red radiation. FR+: radiation containing far-red radiation at approximately the same proportion as in sunlight. Values labelled with the same letter are not significantly different at $P < 0.05$ (*ANOVA* followed by Tukey-Kramer test).

Radiation treatment		Cell area [µm^2]	Cell number per cotyledon [×10^3 cells]
0 - 4 d after germination	5 - 11 d after germination		
FR-	FR-	1072 b	872 a
FR-	FR+	1068 b	909 a
FR+	FR+	1441 a	948 a
ANOVA		$P = 0.015$	$P = 0.914$

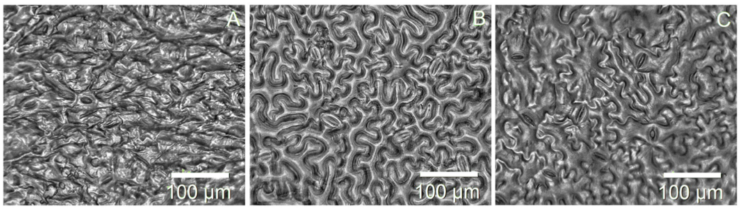

Fig. 6. Microscopic images of the abaxial surfaces of cucumber cotyledons that had been acclimatized to radiation without far-red radiation (FR-) for 4 d followed by radiation containing far-red radiation at approximately the same proportion as in sunlight (FR+) (*A*), to continuously to FR+ (*B*), and to continuously FR- (*C*) (Expt 4).

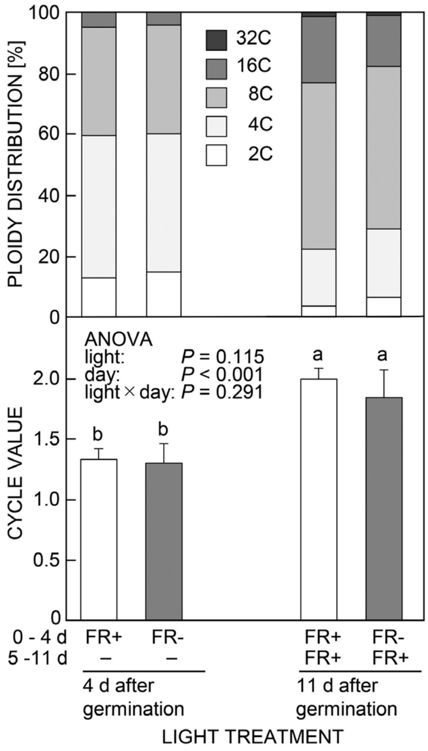

Fig. 7. Changes in ploidy distribution and cycle value of cucumber cotyledons during the radiation treatments based on flow cytometry (Expt 4). Values are means of 10 samples (with no replication; *n* = 10). FR-: radiation without far-red radiation. FR+: radiation containing far-red radiation at approximately the same proportion as in sunlight.

leaves were caused by antagonistic interactions among the responses to multiple factors that determine leaf growth under FR. For example, if factors such as cotyledon growth are more plastic than factors such as xylem development, which can be regulated by radiation (Casal *et al.* 1994, Falcioni *et al.* 2018, Bantis *et al.* 2021), the antagonism between these factors, which is possibly generated by the sudden increase in FR, may cause the leaf damage. The factors that potentially determine leaf size include cell wall extensibility (Sasidharan *et al.* 2008), pressure potential (Lockhart 1965, Passioura and Fry 1992), cell division rate (Donnelly *et al.* 1999, Tardieu *et al.* 1999), and cytoplasm size.

The epidermal cell size and cell number of the cotyledons that were acclimatized to FR- followed by FR+ were similar to those in the seedlings maintained under continuous FR-, although the cotyledon elongation rate was temporarily increased by the sudden increase in FR radiation in two of the treatments (*i.e.*, after 3 and 4 d of acclimatization under FR-). These results also suggest that there are antagonistic responses during cell expansion when the FR radiation suddenly increases. The deformed epidermal cells in the cotyledons acclimatized under FR- followed by FR+ may support this hypothesis. To test this hypothesis, the dynamics of leaf anatomical structure should be investigated. We observed endoreduplication, which can contribute to cell expansion (Cookson *et al.* 2006), during leaf expansion. However, because the frequency of ploidy did not differ between treatment groups, endoreduplication does not appear to be directly related to the leaf damage, although it may be indirectly related if it causes a mismatch between cell size and the vascular support for each cell (*i.e.*, insufficient water transport to the leaf tips).

In conclusion, we found that cucumber seedlings that had been acclimatized to a low proportion of FR responded negatively to sudden increases in FR. By clarifying the factors that control this response, we may obtain clues to the limits of plant strategies for adaptation to changes in their radiation environment. In addition, these limits may influence plant growth when the radiation quality changes drastically during plant production and during plant experiments in a controlled environment.

References

Amijima, M., Iwata, Y., Koizumi, N., Mishiba K.I.: The polar auxin transport inhibitor TIBA inhibits endoreduplication in dark grown spinach hypocotyls. - Plant Sci. **225**: 45-51, 2014.

Ballaré, C.L., Scopel, A.L., Jordan, E.T., Vierstra, R.D.: Signaling among neighboring plants and the development of size inequalities in plant populations. - Proc. nat. Acad. Sci. USA **91**: 10094-10098, 1994.

Bantis, F., Panteris, E., Dangitsis, C., Carrera, E., Koukounaras, A.: Blue light promotes vascular reconnection, while red light boosts the physiological response and quality of grafted watermelon seedlings. - Sci. Rep. **11**: 1-14, 2021.

Barow, M., Meister A.: Endopolyploidy in seed plants is differently correlated to systematics, organ, life strategy and genome size. - Plant Cell Environ. **26**: 571-584, 2003.

Casal, J.J., Ballaré, C.L., Tourn, M., Sánchez, R.A.: Anatomy, growth and survival of a long-hypocotyl mutant of *Cucumis sativus* deficient in phytochrome B. - Ann. Bot. **73**: 569-575, 1994.

Cookson, S.J., Radziejwoski, A., Granier, C.: Cell and leaf size plasticity in *Arabidopsis*: what is the role of endoreduplication? - Plant Cell Environ. **29**: 1273-1283, 2006.

Donnelly, P.M., Bonetta, D., Tsukaya, H., Dengler, R.E., Dengler, N.G.: Cell cycling and cell enlargement in developing leaves of *Arabidopsis*. - Dev. Biol. **215**: 407-419, 1999.

Falcioni, R., Moriwaki, T., De Oliveira, D.M., Andreotti, G.C., De Souza, L.A., Dos Santos, W.D., Bonato, C.M., Antunes, W.C.: Increased gibberellins and light levels promotes cell wall thickness and enhance lignin deposition in xylem fibers. - Front. Plant Sci. 9: 1391, 2018.

Franklin, K.A.: Shade avoidance. - New Phytol. Wiley Online Library **179**: 930-944, 2008.

Galbraith, D.W., Harkins, K.R., Maddox, J.M., Ayres, N.M., Sharma, D.P., Firoozabady E.: Rapid flow cytometric analysis of the cell cycle in intact plant tissues. - Science **220**: 1049-1051, 1983.

Gommers, C.M., Visser, E.J., St Onge, K.R., Voesenek, L.A., Pierik, R.: Shade tolerance: when growing tall is not an option. - Trends Plant Sci. **18**: 65-71, 2013.

Huber, M., Nieuwendijk, N.M., Pantazopoulou, C.K., Pierik, R.: Light signalling shapes plant-plant interactions in dense canopies. - Plant Cell Environ. **44**: 1014-1029, 2021.

Kaiser, E., Morales, A., Harbinson, J.: Fluctuating light takes crop photosynthesis on a rollercoaster ride. - Plant Physiol. **176**: 977-989, 2018.

Lee, D.W.: The spectral distribution of radiation in two

Neotropical rainforests. - Biotropica **19**, 161-166: 1987.

Lockhart, J.A.: An analysis of irreversible plant cell elongation. - J. theor. Biol. **8**: 264-275, 1965.

Naidu, S.L., De Lucia, E.H.: Acclimation of shade-developed leaves on saplings exposed to late-season canopy gaps. - Tree Physiol. **17**: 367-376, 1997.

Oguchi, R., Hikosaka, K., Hiura, T., Hirose, T.: Leaf anatomy and light acclimation in woody seedlings after gap formation in a cool-temperate deciduous forest. - Oecologia **149**: 571-582, 2006.

Ouedraogo, M., Hubac, C., Monard, J.F.: Effect of far red light on root growth and on xylem sap in cotton (*Gossypium hirsutum* L.). - Plant Cell Physiol. **27**: 17-24, 1986.

Passioura, J.B., Fry, S.C.: Turgor and cell expansion: beyond the Lockhart equation. - Funct. Plant Biol. **19**: 565-576, 1992.

Sager, J.C., Smith, W.O., Edwards, J.L., Cyr K.L.: The use of spectral data to determine photosynthetic efficiency and phytochrome photoequilibria. - Trans. amer. Soc. Agr. Eng. **31**:1882-1889, 1988.

Sasidharan, R., Chinnappa, C.C., Voesenek, L.A., Pierik, R.: The regulation of cell wall extensibility during shade avoidance: a study using two contrasting ecotypes of *Stellaria longipes*. - Plant Physiol. **148**: 1557-1569, 2008.

Schmitt, J., McCormac, A.C., Smith, H.: A test of the adaptive plasticity hypothesis using transgenic and mutant plants disabled in phytochrome-mediated elongation responses to neighbors. - Amer. Natur. **146**: 937-953, 1995.

Sellaro, R., Smith, R.W., Legris, M., Fleck, C., Casal, J.J.: Phytochrome B dynamics departs from photoequilibrium in the field. - Plant Cell Environ. **42**: 606-617, 2019.

Smith, H., Whitelam, G.C.: The shade avoidance syndrome: multiple responses mediated by multiple phytochromes. - Plant Cell Environ. **20**: 840-844, 1997.

Tardieu, F., Granier, C., Muller, B.: Modelling leaf expansion in a fluctuating environment: Are changes in specific leaf area a consequence of changes in expansion rate? - New Phytol. **143**: 33-43, 1999.

Weraduwage, S.M., Frame, M.K., Sharkey, T.D.: Role of guard cell-or mesophyll cell-localized phytochromes in stomatal responses to blue, red, and far-red light. - Planta **256**: 1-10. 2022.

Yamashita, N., Ishida, A., Kushima, H., Tanaka, N.: Acclimation to sudden increase in light favoring an invasive over native trees in subtropical islands, Japan. - Oecologia **125**: 412-419, 2000.