Journal of Photochemistry and Photobiology B: Biology 128 (2013) 64–69

Contents lists available at ScienceDirect

Journal of Photochemistry and Photobiology B: Biology

journal homepage: www.elsevier.com/locate/jphotobiol

Near-UV radiation acts as a beneficial factor for physiological responses in cucumber plants

Makiko Mitani-Sano [a,1], Takafumi Tezuka [a,b,*]

[a] Division of Biological Mechanisms and Functions, Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya 464-8601, Japan
[b] Department of Complex Systems Science, Graduate School of Information Science, Nagoya University, Nagoya 464-8601, Japan

ARTICLE INFO

Article history:
Received 26 February 2013
Received in revised form 19 July 2013
Accepted 31 July 2013
Available online 20 August 2013

Keywords:
Cell volume
Cucumis sativus
Leaf thickness
Near-UV radiation
Photosynthesis
Pyridine nucleotides [nicotinamide coenzymes: NAD(H), NADP(H)]

ABSTRACT

Effects of near-UV radiation on the growth and physiological activity of cucumber plants were investigated morphologically, physiologically and biochemically using 3-week-old seedlings grown under polyvinyl chloride films featuring transmission either above 290 nm or above 400 nm in growth chambers. The hypocotyl length and leaf area of cucumber seedlings were reduced but the thickness of leaves was enhanced by near-UV radiation, due to increased upper/lower epidermis thickness, palisade parenchyma thickness and volume of palisade parenchyma cells. Photosynthetic and respiratory activities were also promoted by near-UV radiation, associated with general enhancement of physiological/biochemical responses. Particularly, metabolic activities in the photosynthetic system of chloroplasts and the respiratory system of mitochondria were analyzed under the conditions of visible light with and without near-UV radiation. For example, the activities of NAD(P)-dependent enzymes such as glyceraldehyde-3-phosphate dehydrogenase (G3PDH) in chloroplasts and isocitrate dehydrogenase (ICDH) in mitochondria were elevated, along with levels of pyridine nucleotides (nicotinamide coenzymes) [NAD(H) and NADP(H)] and activity of NAD kinase (NADP forming enzyme). Taken together, these data suggest that promotion of cucumber plant growth by near-UV radiation involves activation of carbon and nitrogen metabolism in plants. The findings of this research showed that near-UV radiation reaching the Earth's surface is a beneficial factor for plant growth.

1. Introduction

Many aspects of the inhibitory effects of UV radiation on photosynthetic activity and the growth of plants have been reported [1–8]. In our previous studies [9–13], we described that natural solar UV radiation (near-UV, wavelength; 300–400 nm) reaching the Earth's surface and the near-UV radiation from near-UV lamps cause not inhibition but rather enhancement of growth (so-called physiological activation) in higher plants such as tomato, radish, eggplant, soybean and pea plants. In other words, near-UV (300–400 nm) or UV-A (320–400 nm) radiation, whose wavelengths lie within the natural solar UV (290–400 nm) spectral region, led to growth promotion and physiological activation of plants [9–14].

According to Fox and Caldwell [16], many plants have specific response to UV radiation. Namely, shoot biomass of *Medicago sativa*, *Alyssum alyssoides* and *Poa pratensis* was enhanced to be respectively 100%, 83% and 33% by supplementary UV-B radiation in the field under the solar radiation. On the contrary, the biomass of *Geum macrophyllum*, *Amaranthus retroflexus* and *Boromus tectrorum* was suppressed to be respectively 56%, 50%, and 8%. Ref. [7] reported that the suppressive effects of UV-B radiation on plant biomass are improved by UV-A radiation under the field condition. In short, the field under the condition of natural solar radiation with near UV and visible light is probably proper for the plant growth (biomass). Ref. [17] reported that the action spectrum for physiological responses such as DNA damage lowers predicted impact of UV radiation by ozone depletion.

We have measured the wavelength of solar radiation reaching three sites on the Earth's surface: Nagoya University (137°E/35°11′N, altitude; 50 m); *Mt.* Norikura-dake [137°34′E/36°8′N, altitude: 2770 m near the summit (3060 m) in the central part of Japan; and the Lake Pumayum Co (90°30′E/28°32′N, altitude; 5070 m)] in the southeastern part of the Tibetan Plateau, in China. As a result, we could not detect wavelengths shorter than 300 nm at the three sites [13,15,19,20], although it is generally believed that the wavelength of solar UV radiation reaching the Earth's surface is in the region of 290–400 nm [18]. This discrepancy may depend on differences in the quality and quantity of air

---

* Corresponding author at: Division of Biological Mechanisms and Functions, Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya 464-8601, Japan. Tel./fax: +81 52 789 5376.
E-mail address: shubunjp@yahoo.co.jp (T. Tezuka).
[1] Present address: Ishihara Sangyo Co. Ltd., Kusatsu City 525–0025, Japan.

1011-1344/$ - see front matter © 2013 Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.jphotobiol.2013.07.025

pollution in the stratosphere/troposphere between the periods measured by us and [18]. Many papers regarding effects of UV radiation on the growth of plants seem to describe the effects of UV-B radiation and UV-A radiation on the growth and physiological responses of plants by paying no attention to spectral balance of UV radiation and visible light under natural solar radiation. Namely, the suppression of the growth of plants under UV radiation described in these many papers seems to make an incorrect estimation for effects of near-UV radiation. If the estimation of UV effects on plants described in these papers is appropriate, crops in agricultural lands will surely decrease, but in fact those are not.

Near-UV radiation also resulted in enhancement of growth (cell division) in *Chlorella* [19–21] as well as freshwater phytoplankton [22], when exposure was for a long period. The wide variety of plants is clearly adapted to the environment of natural solar radiation (i.e., near-UV radiation and photosynthetically active radiation (PAR)) reaching the Earth's surface.

Forster et al. [23] described that the total effects of UV radiation on the growth of plants depend on the balance among UV-B, UV-A, and PAR. Thus far, studies of effects of UV radiation on plants in growth chambers have been mostly carried out under abnormal light conditions with UV/PAR ratios different from those in natural solar radiation.

The spectral balance [near-UV/visible light (PAR); (300–400 nm)/(400–800 nm) ratio] within the natural solar spectrum on the Earth's surface was shown to be approximately 7% (natural conditions) in summer [19,20]. Bearing this spectral balance (7%) in mind, in the present study, we therefore examined the morphological, physiological and biochemical responses of cucumber plants cultured under several conditions with variation in the near UV/PAR ratio using combinations of near-UV and fluorescent lamps in a growth chamber to ascertain relationships between the balance of near-UV/visible light (PAR) radiations and plant growth in order to define the problem of plant cultivation under the light condition in greenhouses from an agricultural point of view.

2. Materials and methods

2.1. Plant materials

Seeds of cucumber (*Cucumis sativus* L. cv. Chikanarisanto) plants were sown in vinyl-pots (8.5 cm in diameter, 8 cm in depth) containing peat moss (Griendtsveen: Regeling Handels Potgronden, Netherlands) and sand (3:1, v/v) and placed in our own specially designed growth chamber. After emergence, seedlings were cultured with a 500-fold diluted nutritious solution (50 mL pot^{-1}) of Hyponex (Murakami Bussan Co. Ltd., Tokyo, Japan) every 2nd day. At three weeks after sowing, the first leaves were used for morphological, physiological and biochemical analyses.

2.2. Growth chambers

Two growth chambers were each partitioned with shelves to get three (upper, middle and lower) compartments. Both left-hand and right-hand walls of each compartment (120 × 90 × 60 cm) were equipped with a lightproof fan (12 × 12 cm). The inside of the compartments was covered with a layer of poly (vinyl chloride) film (0.1 mm thick), either PVC-290 or PVC-400 (C.I. Kasei Co., Ltd., Tokyo), allowing the passage of light of wavelength longer than 290 or 400 nm, respectively [12,19–21]. Each ceiling of five compartments (+UV) was equipped with 12 fluorescent lamps (FL-20SS-BRN, Toshiba, Tokyo) as a source of visible light (PAR) and two near-UV lamps (black-light fluorescent lamp; FL-20S-BLB, Toshiba, Tokyo) emitting light within the near-UV range (305–400 nm) with a peak at 352 nm. The spectral energy distribution curves determined at the plant level in the respective compartments are described previously [12,19,20]. The ceiling of one compartment (−UV) was equipped with 12 fluorescent lamps and two dummy near-UV lamps, that is only PAR without near-UV irradiation. The near-UV lamps were partly wrapped with aluminum foil for fluence regulation as described below. The outside of each chamber was covered with PVC-400. Both growth chambers were set in an air-conditioned room at 25 °C. Plants were grown under a cycle of light (10 h) and dark (14 h), and the temperature in each compartment was kept at 25 °C (light) and 20 °C (dark). The fluence rates of irradiance in each compartment were measured at plant level with one radiometer (Li-188B Integrating quantum, radiometer, photometer, Li-Cor., USA) for PAR and another radiometer (Topcon UVR-1/UVR-36, Tokyo Optical Co. Ltd., Tokyo, Japan) for near-UV radiation. The fluence rate of PAR without near-UV radiation was 32.5 W m^{-2} at plant level and the fluence rates of near-UV radiation were 0, 0.25, 0.5, 1.0, 1.4 and 2.8 W m^{-2} at plant level.

2.3. Morphological analysis

The hypocotyl length (cm) and the first leaf area (cm^2) of three-week-old cucumber plants cultured under the conditions of PAR with or without near-UV radiation were measured. After determining first leaf area, the central portion of each leaf was cross-sectioned to measure the total leaf thickness, the thicknesses of the upper and lower epidermis as well as the palisade and spongy parenchyma, and the volumes of palisade and spongy parenchyma cells. Thickness was measured using a microscope with a built-in micrometer and cell volumes were estimated by the method of [24].

2.4. Measurement of photosynthesis and respiration

Gas exchange of the cucumber leaves for photosynthesis (O_2 evolution) and dark respiration (O_2 uptake) was measured with an O_2 gas analyzer using a Clark-type oxygen electrode by the method of [12]. Two leaf disks (1 cm in diameter) were cut into segments (about 3 mm square) with a razor blade and soaked in 2 ml of aqueous medium of the oxygen electrode containing 50 mM HEPES (pH 7.2) and 0.5 mM $CaSO_4$. After a few minutes, the gas exchange was started by an addition of 0.625 M Na_2CO_3. Photosynthesis of leaf segments was measured with illumination from a projector (Master Hi-Lux, Rikagaku Seiki, Tokyo). After measuring photosynthesis, respiration was assessed under dark conditions. Data were all calculated on the basis of leaf area.

2.5. Measurement of chlorophyll content

Pairs of leaf disks (1 cm in diameter) were obtained from the first leaves of three-week-old cucumber seedlings and chlorophyll was extracted as described previously [25,26]. Content was estimated according to the method of [27], a modified version of the method of [28], and was expressed on the basis of leaf area.

2.6. Isolation of chloroplasts and mitochondria

First leaves (10 g) of three-week-old cucumber seedlings were homogenized using a glass homogenizer containing 30 mL of grinding medium consisting of 25 mM TES [N-tris (hydroxymethyl) methyl-2-amioethanesulfonic acid], 5 mM Na_2-EDTA (disodium ethylenediaminetetraacetate), 0.4 M sucrose, 5% PEG (polyethylene glycol), 1 mM DTT (dithiothretol) and 1% soluble PVP (polyvinylpyrrolidone). Crude homogenates were squeezed through four layers of gauze and centrifuged at 1500*g* for 5 min to obtain pellets for preparation of chloroplasts and