Response of cucumber plants to increased UV-B radiation under water stress

YANG Zhi-min[1],   ZHENG Shao-jian[2],   HU Ai-tong[1],   ZHENG You-fei[3],   YAN Jing-yi[3]

(1. College of Science, Nanjing Agricultural University, Nanjing 210095, China; 2. College of Natural Resources and Environmental Sciences, Nanjing Agricultural University, Nanjing 210095, China; 3. Department of Applied Meteorology, Nanjing Institute of Meteorology, Nanjing 210044, China)

Abstract: Three days old cucumber (*Cucumis sativus* L.) was grown in a factorial design under two levels of water condition (well watered and drought) and two supplemental levels of ultraviolet-B(280—320 nm) irradiance(0, 0.24, 0.42 W/m^2) for 30 days. Plants were grown in a greenhouse under visible light with 400 $\mu mol/(m^2.s)$, day-night temperature 32/20℃ and relative humidity 70 ± 10%. The combination of UV-B radiation and water stress resulted in the decrease in plant height, leaf area ratio and relative growth rate, and increase in specific leaf mass and net assimilation rate. There was almost no effect of ultraviolet-B radiation on the contents of chlorophyll a and b under water stress. However, interactive effects of ultraviolet-B radiation and water conditions on the content of anthocyanin were much great. UV-B irradiance with water stress also caused a reduction in net photosynthesis, apparent quantum efficiency, gas exchange and stomatal resistance, and an increase in evaporation.
Key words: cucumber, UV-B, water stress, interaction.
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Introduction

During the past several decades numerous investigations on productivity, physiology and ecology of plants subjected to the increased UV-B radiation have been documented(Yang, 1994). However, most of them have examined the effects of single UV-B stress on plants. It is common for plants to experience two or more stresses simultaneously under natural conditions. Increased solar UV-B radiation reaching the earth's surface and coming global climate changes which further give rise to the changes of environmental factors(such as temperature, water, solar radiation) will exert interactive effects on plants(Yang, 1994; Zheng, 1995, Teramura, 1993). Therefore, it is very important to characterize their combined biological effects.

Water stress is one of the most common limiting factors to plant production. It affects both quantity and quality of the yields adversely (Boyer, 1982). Some researchers have begun to examine the interactions between UV-B radiation and drought(Sullivant, 1990; Zheng, 1996). However, such studies have been limited and the mechanism for them has not been clearly demonstrated. This communication was to evaluate the interactions between chronic water stress and enhanced UV-B radiation and further examine the effects of these stresses on growth, physiology and biochemistry of cucumber plants.

1   Materials and methods

1.1   Culture conditions

Cucumber(*Cucumis sativus* L. cv Jing ni No. 4) seeds were surface-sterilized, washed and

soaked overnight. Soaked seeds were planted in plastic pots($35 \times 25cm$) containing a mixture of garden soil and sand($2:1$). When the seedlings emerged, 8 seedlings of uniform size were retained in each pot and on day third the following experimental treatment were started and continued until day thirty. Plants were grown in a greenhouse with PAR intensity of 400 $\mu mol/(m^2 \cdot s)$ by supplementary lighting, day-night temperature 32/20℃ and relative humidity $70 \pm 10\%$.

1.2   UV set up and experimental design

Ultraviolet-B radiation was supplied by preaged UV-emitting fluorescent lamps UV-B 313, Q-Panel and filtered through cellulose acetate film to remove ultraviolet-C radiation and clear polyester film(optically equivalent to Maylar D absorbing almost all radiation < 320nm) for the control treatment. The spectral irradiance was weighted with the generalized plant response action spectrum(Caldwell, 1971) and normalized at 300 to obtain biologically effective UV-B. Three levels of UV-B irradiance were placed as following: UV_0(UV-B, 0 W/m^2), UV_1(UV-B, 0.24 W/m^2), UV_2(UV-B, 0.42 W/m^2), while lamps were suspended above the plants and adjusted in height. The plants received UV-B radiation for 10h from 7:00 am to 17:00 pm. Two treatments of water (W_{400}:400 ml per por daily, well-watered; W_{100}:100 ml per pot daily, water stress) were combined with UV-B irradiation treatments. This resulted in a total of six treatments in a factorial design.

1.3   Growth and morphological responses

Plant height was counted and leaf area was determined from each treatment with LiCor-3100 area meter. Leaves, stems and roots were weighted after drying at 70℃ for 48h. Specific leaf mass (SLM), leaf area ratio(LAR), relative growth rater(RGR) and net assimilation rate(NAR) were calculated based on the formula of Gardner et al. (Gardner, 1985):

$$SLM(g/m^2) = (LM/A),$$
$$LAR(m^2/kg) = (A/M),$$
$$RGR(g/d) = (Ln\ M_2\text{-}LnM_1)/(T_2 - T_1),$$
$$NAR[g/(m^2 \cdot d)] = (M_2 - M_1/A_2 - A_1) \times LnA_2 - LnA_1/T_2 - T_1.$$

Where M, A and T are leaf day weight, leaf area and time respectively.

1.4   Physiological and biochemical determinations

Measurements of photosynthetic photon flux density (PPFD), net photosynthetic CO_2 assimilation, gas exchange, evaporation and stomatal resistance of leaf 2 were made with LiCor-6200 photosynthetic analytic system in a large growth chamber maintained at 27℃ ($\pm$ 1℃). Apparent quantum efficiency was calculated as the rate between net photosynthesis and PPFD.

Chlorophyll measurements: Several samples of leaves were ground with acetone. The homogenate was clarified by centrifugation and filtration. The absorptivity of the clear extracts was measured at 645 and 663 nm. Chlorophyll content of the extracts was calculated by using the MacKinney coefficients(MacKinney, 1941).

Anthocyanin measurements were made following the methods of Lindoo et al. (Lindoo, 1987): the dry leaf samples were placed in a vial with the mixture of methanol water concentrated HCl(80:20:1) and put on a shaker in the dark at 2℃. After 48h, the extract was filtered through filter paper and the A was read at 530 and 657 nm. Anthocyanin concentration was determined by the formula(A) = A530-1/3A657 and was given a A/g weight of leaf tissue.

1.5   Statistical analysis

Individual plants were assigned at random to each combined treatment of light and watering. For the data on plant height, leaf area and day weight of leaves, stems and roots, at least 8 samples

from separate plants were analyzed. For biochemical and physiological determinations, 4 samples were used. The values were statistically tested by using Student Newman-Keuls multiple and the significance was assumed at a 95% level.

2   Results

2.1   Growth responses

Under single drought-stress condition, the plant height was reduced by 27%, while 0.42 W/m^2 UV-B radiation under well-watered condition caused a 21% decrease and 33% decrease combined with water stress(Table 1). Specific leaf mass(SLM) and net assimilation rate (NAR) increased in response to UV-B radiation, but significant differences were observed only in the treatments of W_{400} + UV_1 and W_{400} + UV_2 in SLM(Table 1). In well-watered plants, the relative growth rate(RGR) was significantly reduced by supplemental UV-B radiation while it was unaffected in the influence of drought. Leaf area ratio (LAR) varied under the different interactions between UV-B radiation and watered stress.

Table 1   Responses of cucumber plants to the combination of UV-B radiation and water stresses

Parameters	W_{400}			W_{100}		
	UV_0	UV_1	UV_2	UV_0	UV_1	UV_2
Height, cm	14.51a	13.92a	11.50b	10.60b	10.11b	9.68b
SLM, g/m^2	28.11b	33.67a	32.32a	33.33a	33.46a	34.22a
LAR, m^2/kg	140.6a	117.8c	111.9c	132.1b	117.0c	110.8c
NAR, $g/(m^2 \cdot d)$	11.45a	11.84a	12.28a	9.82b	9.77b	10.16b
RGR, g/day	0.22a	0.14b	0.13b	0.14b	0.13b	0.13b

Notes: Means in each line followed by the same letter are not significantly different at $P = 0.05$ according to Student Newman-Keuls multiple range test

2.2   Chlorophyll and anthocyanin

The supplemental UV-B radiation and drought treatment separately caused the reductions in the accumulation of Chl. a and Chl. b(Table 2). In normally watered condition, total chlorophyll was reduced by 36%—40% by UV-B irradiances and UV-B radiation under drought situation had little effects on it. The anthocyanininformation was significantly promoted by supplemental UV-B radiation under either well watered or drought conditions(Table 2).

Table 2   Changes of chlorophyll and anthocyanin concentrations under different water conditions and UV-B radiation intensity

Parameters	W_{400}			W_{100}		
	UV_0	UV_1	UV_2	UV_0	UV_1	UV_2
Chl., mg/g FW	1.09a	0.69c	0.65c	0.91b	0.89b	0.88b
Chl. a/chl. b	1.55a	1.26b	1.31b	1.56a	1.55a	1.49a
Anthocyanin, A/g FW	0.30b	0.41a	0.44a	0.31b	0.43a	0.48a

Notes: Means in each line followed by the same letter are not significantly different at $P = 0.05$ according to Student Newman-Keuls multiple range test.

2.3   Physiological changes

A summary of effects of UV-B radiation and watered stresses on net photosynthesis, quantum efficiency, gas exchange, evaporation and stomatal resistance is presented in Table 3. On a leaf area basis net photosynthesis was significantly decreased in plants grown under a combination of UV-B radiation and drought. Quantum efficiency showed the same decrease as net photosynthesis. The stomatal resistance under UV-B radiation performed differently under different water

conditions.

Table 3   Physiological changes of cucumber seedlings under different water conditions and UV-B radiation intensity

Parameters	W_{400}			W_{100}		
	UV_0	UV_1	UV_2	UV_0	UV_1	UV_2
Net photosynthesis, μmol $CO_2/(m^2.s)$	23.25a	18.11b	14.61c	18.65b	15.50c	14.09c
Quantum efficiency, mmol CO_2 μmol/PPFD	58.13a	45.28b	36.00c	46.63b	38.75c	35.23c
Gas exchange, μmol/$(m^2.s)$	0.92a	0.78b	0.81b	0.81b	0.64c	0.60c
Evaporation, mmol $H_2O/(m^2 \cdot s)$	39.25a	35.11b	33.81b	29.28c	34.88b	38.66a
Stomatal resistance, s/cm^2	0.43b	0.56a	0.57a	0.57a	0.59a	0.58a

Notes: Means in each line followed by the same letter are not significantly different at $P=0.05$ according to the Student Newman-Keuls multiple range test

3   Discussion

Both drought and UV-B radiation had a significant effect on cucumber plants. But no additional reductions in plant growth were observed when they grown in the combination of stressed. UV-B radiation under well watered conditions significantly reduced the plant height, leaf enlargement and biomass accumulation of cucumbers while the UV-B effectiveness under drought were limited.

Among the growth parameters, the most significant response to the combined stresses was the increase in SLM. An increased SLM is generally thought to be correlated to the decrease in leaf area or increase in leaf thickness (Bornman, 1991). This would be interpreted to provide an increased plant protection from UV-B damage by minimizing the potential area or increasing spongy mesophyll cells to alleviate UV penetration (Vu, 1982) . Although the increase in SLM is a characteristic response of plants to UV-B radiation(Mirali, 1986), it is possible that drought may also make condition to it as the results were presented in this experiment, therefore the effectiveness of UV-B radiation may be reduced.

Under well watered conditions both levels of supplemental UV-B radiation significantly reduced the total chlorophyll, but its effectiveness was alleviated by drought (Table 2). This imply that resistance of plants to UV-B exposure under drought situation was induced, and the beneficial effect may be associated with increased anthocyanin production of seedling leaves (Table 2). Accumulation of anthocyanin and other UV-absorbing compounds in the upper epidermis could act as solar screens by absorbing ultraviolet before it reaches sensitive targets of organelles (Flint, 1983), and therefore both anatomical and biochemical response in leaf tissues to UV-B radiation and drought reveals increased protective mechanisms from damage by either stress.

Net photosynthesis was significantly reduced owing to the combined stresses. This may result in the reduction in dry weight of the seedlings. The apparent quantum efficiency (AQE) was consistent with net photosynthesis under the combination of stresses. However, the reductions in them caused by UV-B radiation exceed those by water stresses(Table 3). Previous studies have shown that drought and UV-B radiation may damage electron transport in PSII(Iwanzik, 1983).

And in this study, 0.24 W/m^2 UV-B radiation reduced AQE by 22% under normal watered condition and 33% under drought-stressed plants.

The reduction in net photosynthesis could be ascribed to a stomatal limitation(Sullivan, 1990; Yang, 1996). Studies on soybeans indicated that the combination of UV-B radiation and drought significantly affected stomatal limitations on photosynthesis(Teramura, 1983). In this way, our results were consistent with the previous findings. Teramura(Teramura, 1983) noted that UV-B radiation may results on an inhibition of stomatal closure and produced localized water loss in leaves, which therefore may result in reduced turgor for cell expansion and growth. However, as the mechanism of the UV-B irradiation-induced changes in diffusion resistance is unknown, there is no evidence for it.

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