Journal of Environmental Sciences Vol.17, No.6, pp.1038—1041, 2005

ISSN 1001—0742
CN11—2629/X

Article ID: 1001-0742(2005)06-1038-04 **CLC number**: X17 **Document code**: A

Responses of antioxidant enzyme and photosynthesis in rape seedling to the combined stresses of acid rain and ultraviolet-B radiation

LIANG Chan-juan[1], HUANG Xiao-hua[2], TAO Wen-yi[1], ZHOU Qing[1,3,*]

(1. The Key Laboratory of Industrial Biotechnology, Ministry of Education, Southern Yangtze University, Wuxi 214036, China. E-mail: zhouqeco@yahoo.com.cn; 2. College of Chemistry and Environmental Science, Nanjing Normal University, Nanjing 210097, China; 3. School of Biotechnology, Southern Yangtze University, Wuxi 214036, China)

Abstract: Effects of the simulated acid rain(AR) and ultraviolet-B(UV-B, 280—320 nm) radiation with a single or two ways simultaneously (AR + UV-B) on the antioxidant enzyme and photosynthesis of the rape seedlings were investigated by the hydroponic culture. The results of static experiment indicated that the tolerance of rape seedling to single stress(AR or UV-B) is stronger than that to dual stresses(AR + UV-B). Furthermore, the dual stresses had additive effect on catalase activity, and a synergistic effect on MDA content, net photosynthesis rate, water use efficiency as well as intercellular CO_2 concentration. Meanwhile, it has an independent effect on chlorophyll content, stomatal conductance, and transpiration rate as well as membrane permeability. During 64 h restoration course, the dynamic change in the curves of physiological and biochemical indices were not identical, and none of them show a simple linear variation. According to the static and dynamic experiments, it was found that a responsive sequence of catalase activity, membrane permeability, MDA content and photosynthetic characteristics to the above-mentioned stresses was as follows: AR + UV-B > UV-B > AR.

Keywords: combined stresses; elevated UV-B radiation; simulated acid rain; rape seedling; CAT activity; photosynthesis

Introduction

The effects of solar ultraviolet-B(UV-B, 280—320 nm) radiation on plants have been studied intensively over the last two decades in connection with researches on the biological impacts of stratospheric ozone depletion, and there are a large number of reports about the effects of UV-B radiation on visual symptoms, leaf ultrastructure and anatomy, photosynthesis pigments, UV-B absorbing compounds, photosynthesis, growth and development, yield and protective mechanisms of plants etc.(Kakani, 2003; Heman, 2002; Correia, 1999). Acid deposition(acid rain) is another major environmental problem, known as a stress factor affecting plants, it has been studied intensively(Menz, 2004; Feng, 2000). Multiple stresses play an important role in the growth of plant, since the environmental stresses rarely act on the biotic isolation system. Therefore, it is essential to study the effect of two major stresses on the early stage of plants growth. However, few reports relate to the interaction between UV-B radiation and acid rain(AR). Moreover, they are often focused to access the sensitivity of plants to acid rain and supplemental UV-B radiation(Paoletti, 1998; Sun, 2003). It is well acknowledged that the accumulation of biomass greatly depends on photosynthesis and antioxidant enzymes scavenging reactive oxygen species(ROS) caused by stress, both of them play important roles in plants growth. The aims of our present study were to determine the effects of combined stresses of UV-B radiation and AR(both under the detrimental threshold) on photosynthesis and catalase(CAT) activity in rape seedlings, which is one of important oil crop planted in large parts of China, as well as to obtain the information of different physiological processes to single and dual stresses. Because the impact of stress is a temporal function, the recovering process of photosynthesis and CAT activity in rape seedling has been observed synchronously at different time after being treated with UV-B radiation and AR. It is beneficial to evaluate objectively the effects of combined stresses on physiological, biochemical metabolism and self-restoration in plants.

1 Materials and methods

1.1 Plant culture

The rape(*Brassica juncea* L.) seeds of "Shilifeng" were sterilized for 10 min by $HgCl_2$ (0.1%), and washed three times with deionized water. After being soaked for 4 h, seeds were placed in the dish underlaid with three pieces of filter paper and germinated in the incubator at 25 ± 1℃. When the length of hypocotyl was about 2 cm, seedlings were planted in plastic pots(diameter 10 cm, five plants per pot) filled with deionized water under the illumination of 8 klx(12 h/d), which were aired twice every day. After the first leaf occurred, the seedlings were cultured in Arnon + Hoagland solution. The nutrient solution was renewed every 3 d for the pH stabilization. The seedlings were cultured for 5 weeks before being treated with UV-B radiation and AR.

1.2 Treatment

Enhanced UV-B radiation was performed with 40W UV-B lamps(Nanjing Lamp Factory, China), which were hanged perpendicularly over the plants cultivated in the lab. Radiant intensity was 0.45 W · m^2 (Zheng, 2002), which was measured by ultraviolet radiac (made by photo-electricity instrument factory of Beijing Normal University, China). Seedlings were irradiated for 6 h from 9:00 to 15:00, for a total of 5 d. The height of lamps over the plants was adjusted to maintain consistent radiation intensity.

Simulated acid rain(SAR) solution was confected with H_2SO_4 : HNO_3 = 4.7 : 1 in equivalent(pH 1.0). Then the solution was diluted with deionized water to pH 3.5, which is the damage threshold value of rape seedlings under simulated acid rain stress(Tang, 1996), and sprayed evenly on the leaves until drops began to fall. The same amount of distilled water was applied to another set as the CK. Rape seedlings were treated with AR twice at the interval of 24 h.

Half of the rape seedlings treated with AR were placed immediately under UV-B lamps, and were then treated with the methods written above.

Foundation item: The National Natural Science Foundation of China(No. 20471030) and the Foundation of State Planning Committee(No. GFZ040628); * Corresponding author

There were 4 sample sets in our experiments: controls (sprayed with deionized water), AR(sprayed with SAR at pH 3.5), UV-B (irradiated with UV-B and sprayed with deionized water), AR + UV-B(sprayed with SAR and then irradiated with UV-B). There were 3 replicates for each set and 3 pots for per treatment.

1.3 Methods of measurement

Cell membrane permeability(E%), MDA content, CAT activity and chlorophyll content were measured according to literature (Zhang, 1990). Net photosynthesis rate (Pn), transpiration rate (En), leaf conductance to water vapour (Gs) and intercellular CO_2 concentration(Ci) were measured under the ambience at 25℃ with a portable photosynthetic system(CIRAS-1, PP Systems, UK). PFD were 300 μmol/(m^2 · s), and there were 320 mg/L CO_2 provided by the CIRAS-1 photosynthesis system. Water utilizing efficiency (WUE) was expressed with the ratio of Pn to En.

All data were analyzed with LSD test($P < 0.05$).

2 Results and discussion

2.1 Instant responses of photosynthesis and CAT activity in rape seedling to various stresses

2.1.1 AR, UV-B and AR + UV-B stresses on CAT activity

As a terminal oxidase, CAT prevents the production of excessive intercellular reactive oxygen species(ROS). MDA, the product of membrane lipid peroxidation, and membrane permeability(E%) are indicators characterizing detrimental rate under adverse stress. As shown in Table 1, when rape seedlings were treated with AR, CAT activity, membrane permeability and MDA content respectively increased by 28.8%, 8.1% and 11.5%, compared to those of control. There was no significant difference in membrane permeability, and MDA content between AR treatment and CK, but the difference in CAT between AR treatment and CK was remarkable. These results indicate that the induced CAT activity in rape seedling is high enough to scavenge ROS caused by AR and then attenuates its injury to cell membrane system. The CAT activity, membrane permeability and MDA content in rape seedlings with UV-B treatment were respectively 85.0%, 18.1% and 21.6% higher than those of control. The differences were pronounced, but those did not occur to the samples with AR treatment. It shows that the increase of CAT activity in rape seedling treated with UV-B radiation did not resist membrane lipid peroxidation triggered by UV-B stress. The above three indices of rape seedling with AR + UV-B treatment respectively increased by 113.8%, 26.0% and 66.7%, and the differences between them are all significant. According to the comparison among AR, UV-B and AR + UV-B treatments, it can be concluded that the tolerance of rape seedling to single stress is stronger than to dual stresses. The responsive sequence of protective enzyme system to different stresses was AR + UV-B > UV-B > AR. Further analyzing the datum in Table 1, we found that dual stresses have an additive effect on CAT activity, a synergistic effect on MDA content and an independent effect on membrane permeability in rape seedlings.

Table 1 Effects of AR, UV-B and AR + UV-B on membrane permeability, MDA content and CAT activity of rape seedling

Treatments	E, %	MDA, mg/g	CAT, mg/g
CK	31.46 ± 0.47 a (0.0)	0.30 ± 0.01 a (100.0)	19.85 ± 0.29 a (100.0)
AR	39.56 ± 0.99 a (8.1)	0.36 ± 0.03 a (111.5)	25.56 ± 0.56 b (128.8)
UV-B	49.56 ± 0.36 b (18.1)	0.39 ± 0.03 b (121.6)	36.72 ± 0.32 c (185.0)
AR + UV-B	57.46 ± 0.79 c (26.0)	0.54 ± 0.05 c (166.7)	42.43 ± 1.60 d (213.8)

Notes: Values followed by the same letter in the same column were not significantly different ($P < 0.05$)

2.1.2 AR, UV-B and AR + UV-B stresses on photosynthesis

As shown in Table 2, AR reduced Chl content, as well as Gs, Pn, En and WUE in rape seedlings comparing with those of control. Unlike chlorophyll content, Pn in leaves between AR treatment and CK was significantly different. It suggests that the decrease in Pn occurs earlier than Chl, and the former is more sensitive to AR stress than the latter, the result is consistent with the previous studies(Zhou, 1997). The decrease of Gs indicates the increase of stomatal resistance which is one of restricting factors leading to the reduction in photosynthesis rate and transpiration rate. En decreased less than Pn. Thereby, AR notably reduced WUE. The increase in Ci was related to the decrease in Pn and Gs.

Chl content, Gs, Pn, En and WUE in rape seedlings under UV-B stress also decreased. Moreover, their decrease rates were larger than those of AR treatment. The difference of Chl content between treatments UV-B and CK was significant, that did not occur to the samples with AR treatment. The fall in stomatal conductance was one of the reasons that intercellular CO_2 concentration increases, photosynthetic rate steeply decreases and transpiration rate drops. WUE in leaves treated with UV-B radiation also decreases. It was because descending rate of Pn was larger than that of En.

Table 2 Effects of AR, UV-B and AR + UV-B on photosynthesis in rape seedlings

Treatments	Chl, mg/g	Pn, μmol/(m^2 · s)	En, mmolH_2O/(m^2 · s)	WUE, μmol/mmol H_2O	Ci, μl/L	G_S, mmolH_2O/(m^2 · s)
CK	1.33 ± 0.06a(100.0)	5.6 ± 0.3a(100.0)	1.05 ± 0.08a(100.0)	5.46 ± 0.39a(100.0)	227 ± 2 a(100.0)	174.5 ± 2.3a(100.0)
AR	1.11 ± 0.07a(83.6)	4.0 ± 0.4 b(71.4)	1.02 ± 0.01ab(97.1)	3.94 ± 0.24b(72.2)	248 ± 2 a(109.3)	112.3 ± 5.3b(64.4)
UV-B	0.68 ± 0.04b(51.4)	3.2 ± 0.1c(57.1)	0.92 ± 0.01ab(87.6)	3.44 ± 0.05b(63.0)	251 ± 6 a(110.6)	108.0 ± 0.8b(61.9)
AR + UV-B	0.57 ± 0.05b(42.9)	1.2 ± 0.2d(21.4)	0.87 ± 0.02b(85.3)	1.39 ± 0.20c(25.5)	287 ± 5 b(126.4)	96.5 ± 2.1b(55.3)

Notes: Values followed by the same letter in the same column were not significantly different ($P < 0.05$)

The variation of each index in rape seedlings treated with AR + UV-B was similar to treatments AR and UV-B. The fierce decline of Gs and Pn led to significant increase of Ci. It shows that the variation of indices in rape seedlings

under dual stresses is more dramatic than single stress. The responsive sequence of photosynthesis in rape seedlings to the three stresses was also AR + UV-B > UV-B > AR, which is coincident with that of antioxidative enzyme system. Further analyzing indicated that the response of different physiological processes or a certain physiological process to stresses were not similar. The dual stresses have a synergistic effect on Pn, WUE and Ci, in agreement with the previous reports (Sun, 2003), and also have an independent effect on Chl content, Gs and En.

2.2 Restoration of CAT and photosynthesis in rape seedling under various stresses

2.2.1 AR, UV-B and AR + UV-B stresses on CAT

After being treated with AR, UV-B and AR + UV-B, the various tendency of CAT activity and membrane permeability reflects some information about the self-restoration in plants from Fig. 1. The dynamic variation of CAT activity is displayed in Fig. 1a. The data at 0 h indicate that CAT activity in leaves with three treatments were notably higher than that of the control. The irritability response of CAT to stresses benefits to scavenge excessive ROS caused by the stresses, consequently alleviate the injury to plants. The differences in the increase rate of CAT activity between treatments AR, UV-B and AR + UV-B hinted that the intensity sequence of free radical reaction was AR + UV-B > UV-B > AR. The dynamic curves also show that the inflexion of CAT activity with AR + UV-B treatment occurred at 16 h, later than the other two stresses. Moreover, it was still markedly different from CK at 64 h. The results suggest that the self-restoration of rape seedlings under combined stresses is more tardigrade than that under single stress.

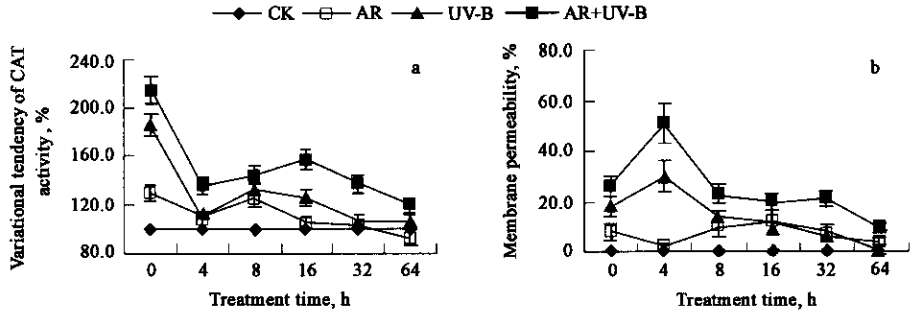

Fig. 1 Restoration of CAT activity(a) and membrane permeability(b) of rape seedling under AR, UV-B and AR + UV-B stresses

Fig. 1b shows the process of self-restoration of membrane permeability in leaves under the three stresses. Firstly, when measured at 0 h, membrane permeability in leaves with AR, UV-B and AR + UV-B treatments increased by 8.1%, 18.1% and 26.0% respectively, compared to CK. The difference of membrane permeability among the treatments of UV-B, AR + UV-B and CK was pronounced, but it was not significant between the treatment of AR and CK. It indicated that the injurious effect of AR on the plant is lagged. Secondly, during 4—64 h E% increased initially and then decreased with treatment of AR, but that with the treatments of UV-B and AR + UV-B declined consistently. It was also found that the variation of membrane permeability with treatment of AR reached its maximum at 16 h, but the maximal membrane permeability with treatments UV-B and AR + UV-B occurred at 4 h. The results denote that the injurious effect of AR on the plant occurs late. Finally, no difference in membrane permeability among treatments AR, UV-B, AR + UV-B and CK was observed at 64 h. This indicates that the injury of the three stresses to membrane permeability is basically restored. However, the extent of restoration in membrane permeability under dual stresses is lower than those under single stress during the experiment. It suggests that plants have stronger resistance to single stress than to dual stresses.

2.2.2 AR, UV-B and AR + UV-B stresses on photosynthesis

The dynamic curves of chlorophyll content in rape seedlings with three treatments are shown in Fig. 2a. With respect to AR treatment, chlorophyll content ceased to decrease at 32 h, and then increased closely to CK, similar to the variation of UV-B and AR + UV-B stresses. It indicated that the stress does not exceed the range in which plants can bear, and that when the injury of the stress to plants occurs, the activity of antioxidative enzyme is induced to prevent heavier injury to plants. The declining rate in chlorophyll content with the treatment of AR + UV-B was the biggest and the treatment of AR was the smallest. It shows that the decrease rate in chlorophyll content and the extent of final restoration are associated with the intensity and pattern of stress.

The variation of net photosynthesis rate (Pn) in rape seedlings under AR stress was semblable with that under AR + UV-B stress, and they all increased initially and then decreased(Fig. 2b). The results indicates that the injury of AR and AR + UV-B stresses on plants is not restored, and the injurious extent of the latter is so serious that the system is about to break down. In addition, compared with the variation of Chl content with the treatment of AR, Pn with the same treatment illuminates that Pn is more sensitive to AR, and the fall of Chl is not the only cause to the decrease in Pn. The variation of Pn with treatment UV-B was contrary to other treatments from 32 to 64 h(Fig. 2c), and the damage by UV-B stress is lightened gradually due to the light-repairation in plants(Shi, 2001).

The dynamic curves of En under different stresses were all alike to "W" during 0—64 h(Fig. 2c). The decrease in En was due to the increase in stomatal resistance at the beginning of stress. Lately, Gs gradually increased, and consequently the exchange of water enhanced and En rose.

The dynamic curves of WUE, determined by the ratio of Pn to En, were all analogous to "M" under different stresses. As Pn decreased less than En, this meant that En is the pivotal factor influencing the variation of WUE (Fig. 2d).

Stomatal conductance (Gs) has an effect on Pn and En by restricting the transfers of CO_2 and H_2O_2. It decreased sharply initially and then slightly during 64 h (Fig.2e). The variation of Ci, influenced by the variation of Gs and Pn, is shown in Fig.2f. During 0—64 h, the dynamic curves of Ci were also homologous to "W". Analyzing the relation among Pn, Ci and Gs, we found that although both Pn and Gs decreased, the drop of Pn is the critical factor leading to the increase of Ci.

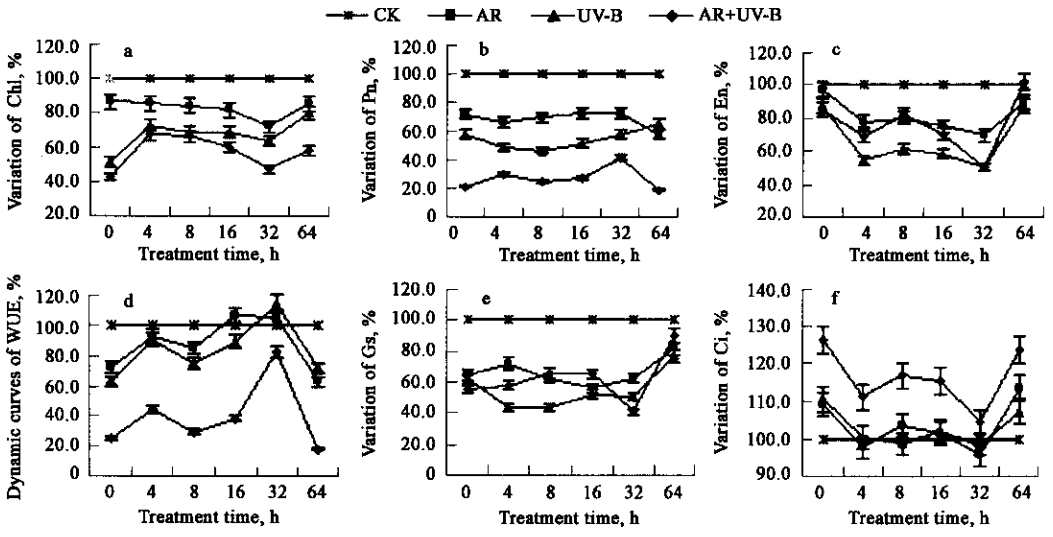

Fig.2 Restoration of photosynthesis of rape seedling under AR, UV-B and AR + UV-B stresses

3 Conclusions

The static results show that the responsive sequences of CAT, E%, MDA and photosynthetic parameters to the three stresses in rape seedlings are all in the order of AR + UV-B > UV-B > AR.

The effects of combined stress on different physiological processes in rape seedlings are complicated. AR + UV-B stress shows an additive effect on CAT activity, a synergistic effect on the MDA, Pn, WUE and Ci, and an independent effect on Chl content, Gs, En and E%.

The results by dynamic measuration indicate that the variation of photosynthesis and protective enzyme in rape seedlings with the three treatments are not identical, and none of them show the simple linear variation. The responsive sequences of CAT, E%, MDA and photosynthetic parameters to the three stresses are also AR + UV-B > UV-B > AR.

References:

Correia C M, Torres-Pereira M S, Torres-Pereir M G, 1999. Growth, photosynthesis and UV-B absorbing compounds of Portuguese Barblea wheat exposed to ultraviolet-B radiation[J]. Environmental Pollution, 104: 384—388.

Feng Z W, 2000. Impact and control strategies of acid rain deposition on terrestrial ecosystems in China[J]. Engineering Science, 2(9): 5—11.

Hernan C, Susana M G, Maria L T, 2002. Effect of UV-B radiation on antioxidant defense system in sunflower cotyledons[J]. Plant Science, 162: 939—945.

Kakani V G, Reddy K R, Zhao D *et al.*, 2003. Field crop response to ultraviolet-B radiation: a review[J]. Agricultural and Forest Meteorology, 120: 191—218.

Menz F C, Seip H M, 2004. Acid rain in Europe and the United Stated: an update[J]. Environmental Science & Policy, 7: 253—265.

Paoletti E, 1998. UV-B and acid rain effect on beech(*Fagus sylvatica* L.) and holmoak (*Quercus ilex* L.) leaves[J]. Chemosphere, 36(4-5): 835—840.

Shi S B, Ben G Y, Zhao X Q *et al.*, 2001. Effects of supplementary UV-B radiation on net photosynthetic rate in the alpine plant *Gentiana straminea* [J]. Acta Phytoecologica Sinica, 25(5): 520—524.

Sun Z L, Peng C L, Sun Z J *et al.*, 2003. Photosynthetic response of plants to supplementary UV-B radiation and sulfite and their resistances[J]. Acta Phytoecologica Sinica, 27(3): 285—292.

Tang H S, 1996. Effect of simulated acid rain on rape[J]. Agro-Environmental Protection, 15(6): 261—263.

Zhang Z L. 1990. Guide to experiments of plants physiology[M]. Beijing: High Educational Press. 59: 88.

Zheng Y F, He Y H, Gan S J, 2002. Feature of micro-climate in wheat field under enhanced UV-B radiation[J]. Agro-Environmental Protection, 21(5): 406—409.

Zhou Q, Huang X H, Wang D Y *et al.*, 1997. Effects of rare earth La on the winters weet harmed by simulated acid rain[J]. Chinese Journal of Ecology, 16(6): 59—61.

(Received for review February 16, 2005. Accepted March 30, 2005)