The effects of Enhanced UV-B Radiation on Root Morphology and Secretion Content of Rice (*Oryza sativa* Linn.)

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**Abstract.** A hydroponic experiment was carried out to study the effects of UV-B radiation (5.0kJ/m²) on root morphology and root exudates in the local rice variety Baijiaolaoping in a terraced field in the Yuanyang region of China. It was observed that UV-B radiation had the following impact on rice physicochemical properties (1) significant inhibition in both root biomass and the whole biomass of rice, (2) significant increase in the free amino acid and total phenol content secreted by roots on the 40th and 60th day of growth, (3) significant increase in the soluble sugar content in all three growth phases (4) significant increase in oxalic acid and succinic acid, (5) significant decrease in malic acid. The content of tartaric acid was not significantly impacted by the two treatments (natural light 0 kJ/m², UV-B radiation (5.0kJ/m²). Therefore, UV-B enhancement significantly affects the content of low molecular organic acids in rice, which is closely related to its altered root morphology.

1. Introduction

Increase in incident ultraviolet (UV-B) radiation (280-315 nm) because of human activity has caused damage to the stratosphere and depletion of the ozone layer, and become a global environmental issue, especially in the context of agricultural production. The effect of UV-B radiation has attracted heightened interest in recent years [1]. UV-B radiation is known to have a wide range of effects on terrestrial ecosystems, and a clear understanding of the nature and mechanism of the influence of UV-B radiation on the interactions of plants, pathogen and related microorganisms remains limited[2]. Low doses of UV-B radiation promote plant root growth and increase root activity, while higher doses of UV-B radiation inhibit the root growth of seedlings. Increase in UV-B radiation affects the root metabolism as well as that of the whole plant by influencing the metabolism of the aboveground part of the plant[3]. Rice is a major global food crop, and impact to rice production from abiotic stressors such as UV-B radiation, therefore, has significant economic implications. In recent years, researchers worldwide have studied the influence of UV-B radiation on growth, and morphology of modern rice varieties. Studies have focused on the effects of photosynthesis, UV-absorbing substances, antioxidant systems, endogenous hormone regulation, lodging resistance, disease resistance and yield [4-5]. The inhibition of UV-B radiation along the main root length of rice seedlings is extremely critical as it has a major influence on the inhibition of root number and root biomass[6]. Previous studies have reported the effects of UV-B radiation on the shoot section of rice, but studies on root growth and exudates are conspicuous by their absence. Plant roots are an important metabolic connection between the aboveground part of the Earth, and the rhizosphere, between which they facilitate the exchange of water, nutrients and metabolites. The carbon fixed and converted into carbohydrates during photosynthesis is...
translocated to the rhizosphere microenvironment through root exudates, providing nutrients for the soil microorganisms [7].

2. Materials and methods

2.1. Test materials
A local variety of rice (Oryza sativa L.), Baijiaolaojing, grown in a Yuanyang terraced field, was used as the variety of choice for the current study.

2.2. Experimental design and UV-B radiation treatment
The rice seeds were disinfected with 3% hydrogen peroxide solution, germinated and seeded. Two weeks later, the seedlings were transplanted them in pots (9 seeds in one pot, each pot 54*39*30cm) and a nutrients solution of International Rice was applied, which was changed once a week to maintain the PH between 5.5 and 6.0. Two treatments, one with natural light (0 kJ/m$^2$) and the other with UV-B radiation (5.0 kJ/m$^2$) were used in the present study. Ten pots of rice seedlings were selected and five 40W UV lamps (Beijing, UV308, spectrum 280-320 nm) were suspended 40cm above them. After transplanting from seedlings to greening, 92 days from July to October, the plants were irradiated daily for nine hours from 09:00 to 17:00 hrs (except 7 rainy days).

2.3. Treatment and Sampling
The root exudates were collected by hydroponic method [8]. The first sample was collected on the 20th day treatment. The adsorbed material on the root surface was washed with ultrapure water, and the roots were dried with absorbent paper. For exudates on the surface, the roots of 2-3 seedlings were immersed in 25 mL of ultrapure water in a test tube, and the test tubes were wrapped with aluminum foil to prevent penetration of light. After two hours, the samples were removed and the solution in the test tube was diluted to 25 mL with ultrapure water. A 0.45μm membrane was stored at -20 °C for later use. A total of three root exudate samples were collected by repeating the above steps on days 40 and 60, respectively. By using the samples collected as above, the content of low molecular weight organic acids, total sugars, amino acids and total phenols commonly found in root exudates were determined.

Determination of root growth in rice: The roots of rice were plated on an automated root scanning device (Perfection V700 Photo, Seiko Epson Corp, Japan) equipped with WinRHIZO software (version 2009a, Regent Instruments, Quebec, Canada) to determine root length, total number of roots, number of root tips and root surface area. The biomass of the roots and aerial parts of rice were estimated separately.

Determination of low molecular weight organic acids: The collected root exudates were passed through a 0.45 μm membrane and analyzed for oxalic acid, succinic acid, malic acid and tartaric acid. The analysis was performed by High Performance Liquid Chromatography (HPLC). An Agilent 1100 chromatograph with a flow rate of 0.6 mL/min, an injection volume of 15 μL, a column temperature of 30 °C, and an analysis time of 10 min was used for analysis of the above samples. The total sugar content in root exudates was determined by fluorenone colorimetric method, while the amino acid content was determined by acid ninhydrin colorimetry, and the phenolic compound content was determined by Folin-Ciocalteau colorimetry (760 nm)[9].

3. Results

3.1. Effects of enhanced UV-B radiation on rice biomass
The effect on rice biomass following treatment by enhanced UV-B, is presented Table 1. The aboveground biomass of rice decreased significantly during each one of the three collection periods. The root biomass of the irradiated rice significantly increased after the first 20 days(P<0.05). There was no significant difference between the two treatments on the 40th day after treatment. The root biomass decreased significantly on the 60th day (Table 1). It was also observed that during the 60-day cycle of
enhanced UV-B radiation, the aboveground biomass of rice decreased significantly, the biomass of roots first increased and then decreased, and the biomass of the entire plant also decreased significantly.

### Table 1. Effect of UV-B Radiation on Rice Biomass

| Hydroponic time | Treatment          | Biomass (g/ Strain) |          |          |          |          |
|-----------------|--------------------|---------------------|----------|----------|----------|----------|
|                 |                    | Above ground        | Root     | Whole plant biomass |
| Day 20          | Natural lighting   | 0.35±0.010*        | 0.07±0.002 | 0.43±0.012 |
|                 | UV-B radiation     | 0.33±0.003*        | 0.08±0.00* | 0.42±0.008 |
| Day 40          | Natural lighting   | 1.38±0.184*        | 0.30±0.020 | 1.68±0.20* |
|                 | UV-B radiation     | 0.81±0.078         | 0.27±0.030 | 1.09±0.108 |
| Day 60          | Natural lighting   | 2.50±0.006*        | 0.58±0.10* | 3.09±0.11* |
|                 | UV-B radiation     | 1.76±0.009         | 0.42±0.084 | 2.18±0.174 |

Note:* and ** Significant differences and significant differences, *Express P<0.05, **Express P<0.01, n=3.

3.2. Effects of UV-B radiation on rice root morphology

The effect of UV-B light on rice root morphology is presented in Table 2. Under UV-B radiation treatment, root length, root surface area and root volume increased significantly on the 20th as well as on the 40th day of treatment. On the 60th day following treatment, the root length decreased significantly, but the root surface area and root volume did not show a significant change.

### Table 2. Effect of UV-B Radiation on Rice Root morphology

| Hydroponic time | Treatment          | Root length (L, cm) | Root surface area (SA, cm²) | Root volume (V, cm³) |
|-----------------|--------------------|---------------------|------------------------------|----------------------|
| Day 20          | Natural lighting   | 192.00±4.00         | 24.00±0.87                   | 0.26±0.02            |
|                 | UV-B radiation     | 227.00±1.00*        | 33.00±6.4*                   | 0.53±0.15*           |
| Day 40          | Natural lighting   | 262.00±22.00        | 34.00±0.29                   | 0.37±0.13            |
|                 | UV-B radiation     | 698.00±42.00*       | 81.00±2.6*                   | 0.79±0.08*           |
| Day 60          | Natural lighting   | 1782±150.2*         | 190.00±230                   | 1.68±0.08            |
|                 | UV-B radiation     | 1281.00±51.00       | 176.00±110                   | 2.06±0.12            |

The enhancement of UV-B radiation leads to changes in the secretion of low molecular weight organic acids in rice leaves. On the 20th day of the experiment, the concentrations of acetic acid, tartaric acid and malic acid were not significantly different under the two treatments, while the concentrations of oxalic acid and succinic acid were significantly higher. On the 40th day, the succinic acid and acetic acid concentration increased significantly, while the level of malic acid decreased significantly. There was no significant change in tartaric acid content on day 60, but oxalic acid and succinic acid levels were significantly increased. Tartaric acid content remained unchanged, but malic acid decreased significantly (Fig1.).
Fig. 1. Effect of UV-B Radiation on Secretion of Low Molecular Weight Organic Acids in Rice Root Exudate

3.3. Effects of UV-B radiation on soluble sugar, free amino acids and total phenol secretion in rice roots
UV-B radiation increased the soluble sugar content in each of the three periods, by 7.41% - 24.09%. The free amino acid and total phenolic content did not change significantly on the 20th day, but increased significantly on the 40th and 60th days by 15.68% - 27.14% and 25.37% - 31.5% respectively (Figure 2).

Fig. 2. Effects of UV-B Radiation on Soluble Sugar, Free Amino Acid and Total Phenol Production in Rice Roots

3.4. Correlation coefficient between root morphology and root exudates
There was a significant positive correlation between root length and oxalic acid, malic acid and tartaric acid contents (P<0.01), and a significant positive correlation with succinic acid and acetic acid content was observed. Root surface area and root volume were significantly positively correlated with root exudates (Table 3), while there was a significant positive correlation between root exudates and root morphology.

| Type of secretion | Root length (L, cm) | Root surface area (S, cm²) | Root volume (V, cm³) |
|------------------|---------------------|---------------------------|---------------------|
| oxalic acid      | 0.918**             | 0.952**                   | 0.935**             |
| Succinic acid    | 0.895*              | 0.956**                   | 0.980**             |
| Malic acid       | 0.967**             | 0.959**                   | 0.901**             |
| Tartaric acid    | 0.951**             | 0.968**                   | 0.932**             |
| Acetic acid      | 0.883*              | 0.949**                   | 0.979**             |

4. Discussion

4.1. Response of rice root growth to UV-B radiation pairs
In this study, the plant dry weight, root length, root surface area and root volume of rice under UV-B radiation treatment under hydroponic conditions increased in the earlier part of seedling stage, which was studied with Chen [10]: UV-B radiation has a significant inhibitory effects on the root length and
dry weight of rice seedlings. Several factors related to the growing environment may impact the above observations, especially rice cultivation methods (hydroponics versus soil culture), the intensity of UV-B radiation, and the length of treatment. While rice roots may be facilitate rapid nutrient absorption in the hydroponic growing environment; The leaves on the upper part of the rice shoots were the first to be affected by UV-B radiation enhancement treatment. The photosynthesis system was inhibited, resulting in a decrease in the amount of photosynthetic products, and UV-B radiation also inhibited the growth of the shoots, thereby forcing an increase in function of the roots. This may be explained in terms of adaptation of rice to stress by environmental factors. The results of this experiment are in agreement with those reported by Hu [11], as well as observations related to the overall root activity and rooting rate of rice treated by UV-B radiation.

4.2. Response of rice root exudates to UV-B radiation pairs
Plants subject to environmental stress would elicit a variety of responses[12], regulating the rhizosphere microenvironment by increasing root exudates[13]. In this study, it was found that when UV-B radiation was enhanced, the content of oxalic acid and succinic acid in low molecular weight organic acids increased significantly, while the content of malic acid had a significant decrease. There was no significant difference in tartaric acid content, He [4]. In this study, UV-B radiation caused the secretion of oxalic acid and succinic acid in rice roots to increase, while the secretion of tartaric acid and malic acid decreased. These difference may be partially attributable to different culture methods, collection periods, treatment methods and other factors. The soluble sugar content in the root exudates increased significantly, which was related to the low photosynthetic enzyme activity induced by UV-B radiation, which, in turn, affected the distribution of photosynthetic products, leading to changes in the composition and quantity of plant root exudates. The free amino acid content increased significantly, the concentrations observed after 60 days were lower than that after the 40th day, which may be related to changes in energy metabolism of proteins and initiation resistance of protein content secreted by roots. The change in total phenolic content in roots was similar to that of free amino acids, which may be due to stress from other biotic and abiotic factors. When UV-B radiation is enhanced, rice is known to secrete more phenolic substances to coordinate this stress. There was a significant positive correlation between root morphology and root exudates.

4.3. Effect of UV-B radiation on secretion in root systems and the indirect effects on soil environment.
The influence of UV-B radiation on the morphology and growth of plant roots also have an effect on root exudates [14]. The net photosynthetic products of plants are transferred to the lower part of the ground through root exudates, which has a significant effect on soil active organic carbon. One of the sources of soil organic carbon is the underground root exudates of plants, which influences the composition and distribution of soil microorganisms. Oxalic acid in root exudates plays an important role in the cycling of soil organic carbon, and promotes net C loss by accelerating soil organic microbial mineralization (SOM)[15]. Therefore, this study observed that low-molecular-weight organic acids, soluble saccharides and amino acids secreted by the roots increased significantly after UV-B irradiation, which is because low molecular weight substances secreted by roots of rice, can not only have a positive influence on root zone microbial community, but also have an effect on the composition and content of soil organic carbon. In short, the decrease in the content of low molecular weight organic acids is one of the reasons for the decline in rice biomass. This study can partially reveal the mechanism of UV-B affecting the underground part of rice.

5. Conclusion
The enhancement of UV-B radiation significantly inhibited overall rice biomass. Root morphology was significantly promoted in the early stage of radiation, and subsequently inhibited in the later stage. UV-B radiation significantly increased the secretion of oxalic acid and succinic acid, and significantly reduced the concentrations of malic acid in the root exudate. No significant effect was observed on tartaric acid. The soluble sugar, free amino acid and total phenol content increased significantly after
UV-B irradiation.

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