DIFFERENTIAL PHOTOOXIDATIVE DAMAGE OF RICE PLANTS IN RESPONSE TO 5-AMINOLEVULINIC ACID AND OXYFLUORFEN

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SUMMARY

Oxyfluorfen (OF), an herbicidal toxicity not only human but also ecology, is widely using in forestry and agriculture. 5-aminolevulinic acid (ALA) is a precursor of chlorophyll and heme biosynthetic pathway. We compare the herbicidal effect of ALA and OF on rice seedlings *in vitro* and *in vivo*. Leaf tissues of rice plants *(Oryza sativa* cv. Dongjin) incubated with various concentrations of ALA and OF showed the increase of conductivity under illumination with earlier and much more increasing in ALA incubated tissues. Foliar application of ALA and OF on rice seedlings also caused photodynamic damage on treated plants with different oxidative stress symptom on treated leaves. The formation of malondialdehyde (MDA) and hydrogen peroxide H_2O_2 increased whereas the maximum/potential quantum efficiency of photosystem II (F_v/F_m), the maximum primary yield of photochemistry of photosystem II (F_v/F_o) and chlorophyll and carotenoid content decreased in both treated plants with a greater in ALA treated plants. Overall ALA acted as herbicide and caused greater photodynamic damage on treated plants than OF did. ALA can be used as a safe substitute for highly toxic herbicide OF.

Key words. 5-aminolevulinic acid, cellular leakage, efficiency of photosystem II, oxyfluorfen, photodynamic damage

Abbreviates: ALA: 5-aminolevulinic acid, HAI: hours after illumination, H_2O_2: hydrogen peroxide, MDA. malondialdehyde, OF: oxyfluorfen, Proto IX: protoporphyrin IX, Protox: Protogen oxidase, ROS: reactive oxygen species.

INTRODUCTION

Tetrapyrroles play vital roles in various biological processes, including photosynthesis and respiration. The biosynthesis of tetrapyrroles in all living cells occurs through several steps where the formation of 5-aminolevulinic acid (ALA) is the first committed intermediate and protoporphyrin IX (Proto IX) is the last intermediate in the common pathway before separating into heme and chlorophyll branch. The biosynthesis of porphyrin is tightly regulated at several levels to coordinate apoprotein synthesis and to avoid the accumulation of intermediate tetrapyrroles. All intermediate tetrapyrroles are potent photosensitizes. Their accumulations lead to produce reactive oxygen species (ROS) such as singlet oxygen, hydrogen peroxide (H_2O_2), which destroys vital protein such as the photosystem I, II as well as membrane lipids and pigments and ultimately lead to cell death (Chakraborty and Tripathy, 1992).

Oxyfluorfen (Fig. 2) is used to control a large number of broadleaf and grassy weeds in both forestry and agriculture. It is classified as a highly toxic and persistent herbicide, which persists in soil and accumulates in terrestrial plants and certain aquatic environments through runoff. It is toxic to humans also, including carcinogenicity, reproductive and developmental toxicity, neurotoxicity, and acute toxicity. Oxyfluorfen is a contact herbicide and light is required for its herbicidal activity on plants. The target of oxyfluorfen is protoporphyrinogen oxidase (Protox) which catalyze the formation of Protoporphyrin IX from Protogenporphyrin IX in tetrapyrrole pathway (Figure 1). This inhibition leads to accumulate intermediate of tetrapyrrole which are potent photo sensitizer. These access intermediate of tetrapyrrole will absorb light energy that is used in detrimental reactions in which energy or electrons are subsequently transferred to oxygen, resulting in the formation of highly reactive oxygen species, causing peroxidation of membrane lipids and cell death (Lee et al., 1993).

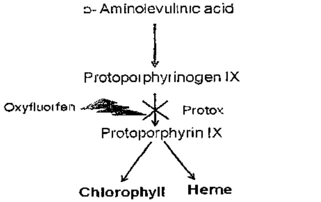

Figure 1. Tetrapyrrole biosynthetic pathway. Two main tetrapyrrole products are chlorophyll and heme. Protox: protogen oxidase, the enzyme catalyze the formation of Protoporphyrin IX from Protogenporphyrin IX.

ALA (Fig. 1, 2) formation was the major regulatory point in tetrapyrrole pathway and it was subject to multiple regulatory mechanisms (Tanaka & Tanaka, 2007). Low concentration of ALA acts as growth stimulate factor which lead to increase the growth and yield of radish, kidney beans, barley, potatoes, and garlic by 10–60% in treated plants (Hotta et al., 1997), increased salt and cold temperature tolerance (Hotta and Watanabe 1999) and in photodynamic therapy. But high concentration of ALA act as herbicide and lead to accumulate high level of intermediate tetrapyrroles and severely damage treated plant when expose to light (Chakraborty and Tripathy, 1992).

Our study compares the differential physiological photodynamic induced-oxidative stress of rice plants (*Oryza sativa* cv. Dongjin) in response to high concentration of ALA and oxyfluorfen-a peroxidizing herbicide.

MATERIALS AND METHODS

Materials

Plant material: Three-week-old of wild-type Korean rice plants (*Oryza sativa* cv. Dongjin) were used for experiment They were grown in green house at 28°C in 14/10 h light/dark cycle.

Chemical: 5-aminolevulinic acid (Fluka), commercial oxyfluorfen (Goal®) and other chemical for analysis experiment were ordered from Sigma or Fluka branch.

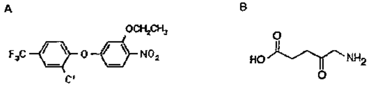

Figure 2. Structure formula of oxyfluorfen (A) and 5-aminolevulinic acid (B)

Methods

Measurement of conductivity (Cellular leakage). The leaves tissues of rice plant were incubated with various concentrations of herbicide following method of Lee et al., 1995. Cellular leakage was determined periodically by the detection of electrolyte leakage into the bathing medium using a conductivity meter (Cole - Parmer Instruments, USA). Because of differences in the background conductivity of different treatment solutions, the results were expressed as changes in conductivity upon exposure to light.

Lipid peroxidation: Lipid peroxidation was estimated by the level of MDA production using a slight modification of the thiobarbituric acid method described by Buege and Aust (1978).

Pigment extraction and analysis: Chlorophylls and carotenoids concentration were measured spectrophotometrically by the method of Lichtenthaler (1987).

In vivo detection of H_2O_2 in plant: H_2O_2 was visually detected in the leaves of plants by using 3, 3-diaminobenzidine as the substrate (Thordal-Christensen et al., 1997).

Photosynthesis activity measurement: Fluorescence parameters variable ($F_v = F_m - F_o$), minimal (F_o) and maximal (F_m) of rice leaves were measured using chlorophyll fluorometer (Handy PEA chlorophyll fluorometer, Handsatech instrument, England) according to the manufacturer's instruction.

We used Microsoft Excel for data analysis. The data represents the mean ± S.E. of three replicates.

RESULTS AND DISCUSSION

Effect on cellular leakage

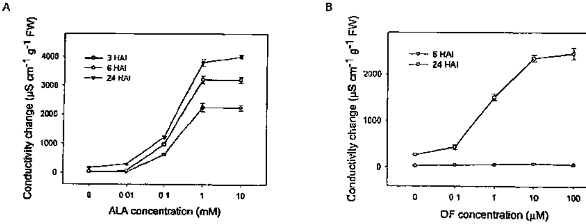

Figure 3. Effect of ALA (A) and OF (B) on cellular leakage from leaf squares of rice seedlings. The data represents the mean ± S.E. of three replicates. In some cases, error bar is obscured by symbol. HAI: hours after illumination.

Rice leaf tissues were incubated with different concentrations of 5-aminolevulinic acid (ALA) or oxyfluorfen (OF) for 12 h under the dark. After illumination under continuous light, the leaf tissues exhibited necrotic lesions in both treated leaf tissues. The necrosis level and the magnitudes of the leakages were in proportion to herbicide concentrations and treated times. The leakage reached maximum at concentration 1 mM ALA and 10 µM OF (Figure 3A, B). Electrolyte leakage greatly increased in ALA incubated leaf dishes at 3 h after illumination, whereas significant electrolyte leakage change was not detected in OF incubated leaf dishes at 6 h after illumination. It indicated that ALA had stronger effect on incubated leaves tissue than OF did. At 24 h after illumination following 12 h dark incubation, the leakage greater increased in ALA treated leaf tissues than in OF treated leaf tissues (Figure 3A, B). Together it suggest that ALA and OF led to exude electrolytes in incubated leaf tissues and ALA caused stronger damage than OF did.

Necrotic lesions and higher conductivity change may have been due to the accumulation of intermediate tetrapyrroles in cytoplasm, which subsequently generates reactive oxygen species (ROS) with light activation, causing rapid peroxidation of the cell membrane and ultimately, lethal cell damage. These data are in agreement with previous reports from Jung and Back (2005), Jung and Kuk (2007).

Photodynamic stress upon foliar application of ALA and OF

In vitro application is more sensitive than *in vitro* application, so 5 mM ALA and 50 µM OF were used for foliar application in three-week-old rice seedlings. Both treated plants showed full photodynamic symptom at 30 h after illumination. Foliar application of 50 µM OF caused many brow necrosis and little desiccation on fully developed mature leaves. Whereas foliar application of 5 mM ALA caused white necrosis and little desiccation at 30 h after illumination (Figure 4A).

In vitro detection of H_2O_2 also indicated the present of ROS on treated leaves upon foliar application. Different damage symptom observed from ALA and OF treated leaves. The damage of ALA was expressed by large brown area on the leaves whereas OF caused brown spots damage to the leaves comparing with control leaves (Figure 4B).

Figure 4. Photodynamic symptom expressed on control plants, ALA and OF treated plants (C, ALA and OF, respectively) A: Herbicidal effected symptom. B: *In vivo* detect the present of H_2O_2 on the treated leaves.

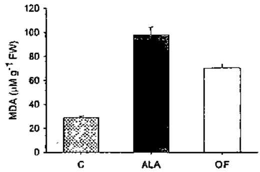

Figure 5. Effect of ALA and OF on malondialdehyde (MDA) production. MDA content was determined in control plants, ALA and OF treated plants (C, ALA and OF respectively). Each data point is the mean ± S.E. of three replicates.

Effect on lipid peroxidation

Malondialdehyde (MDA) production is an index of peroxidation of unsaturated membrane lipids. The formation of MDA radical is an indicator of oxidative stress induced damage to membrane. Both ALA and OF foliar application caused significantly enhancement in MDA production, these data are in agreement with previous reports from Chakraborty and Tripathy (1992), Jung and Back (2005), Jung and Kuk (2007). However, white necrosis ALA treated leaves caused greater increasing in lipid peroxidation of unsaturated membrane lipids than brown necrosis OF treated leaves did (Figure 5). It demonstrated that ALA caused stronger damage than OF did on treated plants.

Loss of chlorophylls and caroteniods

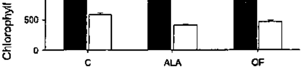

Figure 6. Effect of ALA and OF on chlorophyll and carotenoid content in control plants, ALA and OF treated plants (C, ALA and OF respectively). Each data point is the mean ± S.E. of three replicates.

Chlorophyll and carotenoid are two main pigments of photosynthesis apparatus which has important role in photosynthesis system. Chlorophyll molecules are specifically arranged in and around photosystem that are embedded in the thylakoid membranes of chloroplasts. The function of the vast majority of chlorophyll absorb light and transfer that light energy by resonance energy transfer to a specific chlorophyll pair in the reaction center of the photosystem. Carotenoid have two key roles in plant, they absorb energy using in photosynthesis and protect chlorophyll from damage. Foliar application of ALA or OF led to the decrease of the total chlorophylls and carotenoid level in both treated plants comparing with untreated plants (Figure 6), it demonstrated the damage in photosynthesis system, these data are in agreement with previous reports from Chakraborty and Tripathy (1992), Jung and Back (2005), Jung and Kuk (2007). However total chlorophylls and carotenoid caused the decrease in ALA treated plants more than in OF treated plants. It indicated that ALA caused stronger photodynamic damage than OF did on photosynthesis system of treated plants.

Reduced photosynthesis efficient

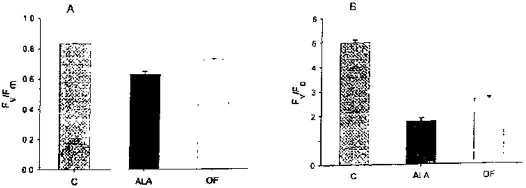

Figure 7. Effect of ALA and OF on photosynthesis efficient. The maximum potential quantum efficiency of PS II (F_v/F_m) (A) and the maximum primary yield of photochemistry of PS II (F_v/F_o) (B) was determined in control plants, ALA and OF treated plants (C, ALA and OF respectively). Each data point is the mean ± S.E. of three replicates.

Measurements of chlorophyll fluorescence parameters provides useful information about photosystem II (PSII) activity and changes in photosynthetic metabolism of stress plants. Both of maximum/potential quantum efficiency of PS II (F_v/F_m) and the maximum primary yield of photochemistry of PS II (F_v/F_o) are related with photosynthetic efficiency of plant leaves (Shangguan et al., 2000).

The photodynamic damage caused by ALA and OF foliar application led to reduce F_v/F_m and F_v/F_o with greater in ALA treated plants than in OF treated plants (Fig. 7). It indicated that ALA and OF caused damage on photosynthesis system with a greater in ALA treated plants. Together with MDA and *in vivo* H_2O_2 level, it suggested that ALA caused greater photooxidative stress than OF did on treated plants.

CONCLUSION

In conclusion, both ALA and OF caused the photodynamic induced oxidative stress on treated rice seedlings. The damage were elucidated by the present H_2O_2-a product of ROS (Fig. 4B), which increased conductivity in leaf disks (Fig. 3), destroyed membrane lipids (Fig. 5), pigments (Fig. 6); and led to reduce efficient of photosystem II (Fig. 7) with a greater in ALA treated plants, demonstrating markedly rapid herbicidal effect of ALA compared to that of OF. ALA can be used as a safe substitute for highly toxic herbicide OF.

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SO SÁNH ẢNH HƯỞNG CỦA HAI LOẠI THUỐC DIỆT CỎ 5- AMINOLEVULINIC

AXÍT VÀ OXYFLOURFEN TRÊN CÂY LÚA

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TÓM TẮT

Oxyfluorfen (OF) là một trong những loại thuốc diệt cỏ được sử dụng phổ biến trong nông lâm nghiệp để phòng trừ cỏ dại. Tuy nhiên loại thuốc này gây ảnh hưởng không tốt tới sức khoẻ con người cũng như môi trường sinh thái. 5 - aminolevulinic axit (ALA) là cơ chất quan trọng đầu tiên trong con đường sinh tổng hợp chất diệp lục ở sinh vật quang hợp và heme ở mọi cơ thể sống. Trong nghiên cứu này chúng tôi tiến hành đánh giá tác động của hai loại thuốc diệt cỏ trên đến mức độ hư hại lên cây lúa và mô lá lúa. Kết quả thí nghiệm cho thấy, dưới tác động của ánh sáng, ALA làm hư hại và rò rỉ ion từ mô lá ra dịch ủ nhanh và mạnh hơn OF. Biểu hiện hư hại khác nhau cũng xảy ra ở cây lúa được phun xịt với hai loại thuốc này. Đồng thời sự tạo thành của các gốc tự do tăng cao ở màng tế bào, hiệu quả của hệ thống quang hợp II giảm, hàm lượng các sắc tố quang hợp cũng giảm với mức độ hư hại mạnh hơn khi xử lý bằng ALA. Kết quả nghiên cứu chỉ ra rằng ALA với cơ chế hoạt động như thuốc diệt cỏ nhưng có tác động mạnh hơn cả loại thuốc diệt cỏ thông dụng OF. Như vậy ALA có thể được dùng thay thế OF như là thuốc diệt cỏ thân thiện với môi trường.

Từ khóa: 5-aminolevulinic acid, cellular leakage, hiệu quả của hệ quang hợp II, oxyfluorfen, quang hư hại.

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