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Perturbations of carotenoid and tetrapyrrole biosynthetic pathways result in differential alterations in chloroplast function and plastid signaling

Joon-Heum Park, Sunyo Jung*

School of Life Sciences and Biotechnology, BK21 Plus KNU Creative BioResearch Group, Kyungpook National University, Daegu 41566, South Korea

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ABSTRACT

In this study, we used the biosynthetic inhibitors of carotenoid and tetrapyrrole biosynthetic pathways, norflurazon (NF) and oxyfluorfen (OF), as tools to gain insight into mechanisms of photooxidation in rice plants. NF resulted in bleaching symptom on leaves of the treated plants, whereas OF treatment developed a fast symptom of an apparent necrotic phenotype. Both plants exhibited decreases in photosynthetic efficiency, as indicated by F_v/F_m . NF caused severe disruption in thylakoid membranes, whereas OF-treated plants exhibited disruption of chloroplast envelope and plasma membrane. Levels of Lhca and Lhcb proteins in photosystem I (PSI) and PSII were reduced by photooxidative stress in NF- and OF-treated plants, with a greater decrease in NF plants. The down-regulation of nuclear-encoded photosynthesis genes *Lhcb* and *rbcS* was also found in both NF- and OF-treated plants, whereas plastid-encoded photosynthetic genes including *Rbcl*, *PsaC*, and *PsbD* accumulated normally in NF plants but decreased drastically in OF plants. This proposes that the plastids in NF plants retain their potential to develop thylakoid membranes and that photobleaching is mainly controlled by nuclear genes. Distinct photooxidation patterns between NF- and OF-treated plants developed differential signaling, which might enable the plant to coordinate the expression of photosynthetic genes from the nuclear and plastidic genomes.

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1. Introduction

Carotenoids and tetrapyrroles are required for many indispensable functions in photosynthesis. Tetrapyrroles are essential metabolites for energy transfer, signal transduction, redox reaction, and detoxification of reactive oxygen species (ROS) and xenobiotics [1,2], while carotenoids are essential components of photosynthetic membranes, assist in harvesting light energy, and function as photo-protectants and antioxidants [3,4]. Their branched metabolic pathways of synthesis and degradation are tightly controlled to provide adequate amounts of each metabolite (carotenoids/tetrapyrroles) and to prevent accumulation of photoreactive intermediates (tetrapyrroles).

Colored carotenoids protect chloroplasts against ROS-induced photooxidation by quenching triplet chlorophyll and singlet oxygen (1O_2) [5]. Bleaching herbicide norflurazon (NF) blocks phytoene

desaturation, accumulating phytoene at the expense of colored cyclic carotenoids [6]. It prevents the formation of enough carotenoids to ensure efficient photoprotection of the photosynthetic apparatus and inhibits plastid biogenesis. As a consequence, chlorophyll is degraded, depending on the intensity of illumination, which leads to the typical bleaching symptoms [6].

The tetrapyrrole biosynthetic pathway leads to the synthesis of a number of important products including the chlorophylls and hemes. Protoporphyrinogen IX oxidase (PPO) catalyzes the oxidation of protoporphyrinogen IX (Proto IX) to protoporphyrin IX (Proto IX) [7]. Diphenyl ether herbicide oxyfluorfen (OF) inhibits PPO in the chloroplast membrane, and the accumulated Proto IX leaks from the plastid [8]. Then it is converted to Proto IX, which is a potent photodynamic compound and promotes oxidative stress, photo-bleaching and non-enzymatic lipid peroxidation, by herbicide-insensitive enzyme in the cytoplasm [9,10].

Besides their well-known functions in light harvesting and photo-protection, carotenoids can play a role through their non-enzymatic oxidation in the sensing and signaling of ROS and photooxidative stress [11,12]. Intermediates in the tetrapyrrole

* Corresponding author.

E-mail address: sjung@knu.ac.kr (S. Jung).

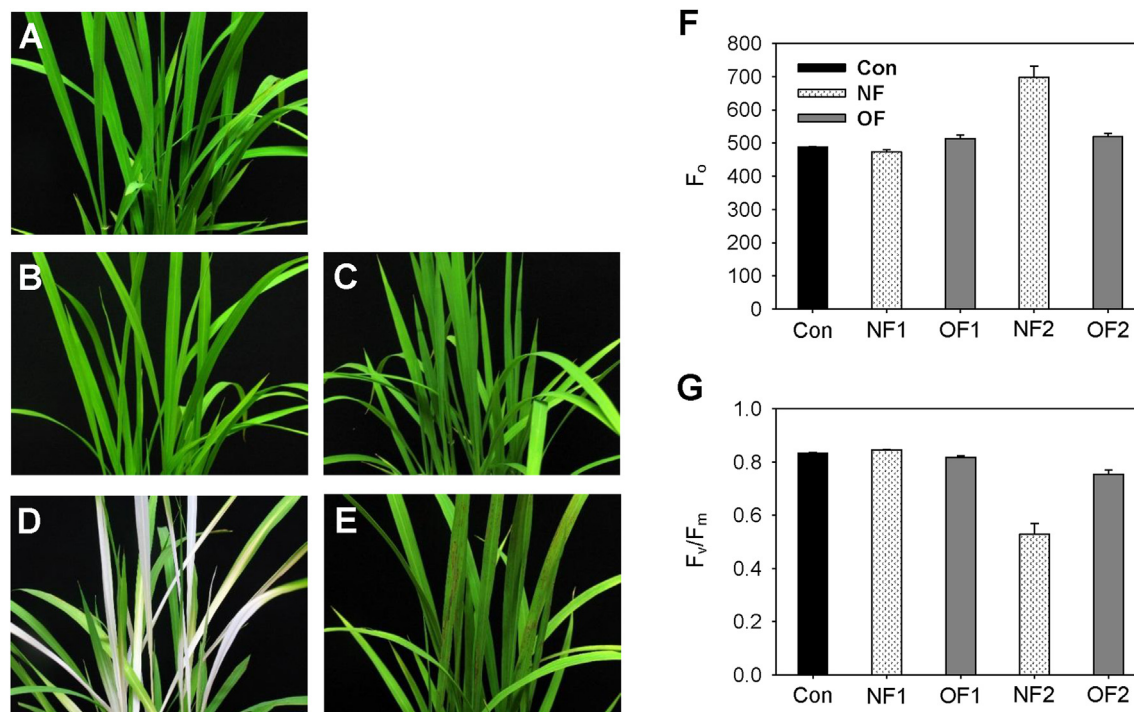


Fig. 1. Photooxidative phenotype (A–E) and photosynthetic parameters, F_0 (F) and F_v/F_m (G), of rice plants with the foliar application of NF and OF. The efficiency of PSII photochemistry, F_v/F_m , was used to assess the functional damage to the plants. Three-week-old rice plants were sprayed with either 50 μM NF or 50 μM OF, placed in darkness for 12 h to allow absorbance, and then illuminated. Con, untreated control; NF1 and OF1, 16 h after NF and OF treatment, respectively; NF2 and OF2, 40 and 88 h after NF and OF treatment, respectively. The data represent the mean \pm S.E. of three replicates.

biosynthetic pathway may also serve as signaling molecules that communicate the status of the pathway to the transcriptional machinery in the nucleus of the cell, thereby regulating levels of proteins that require chlorophyll for their function [13,14]. It appears that the biosynthesis of chlorophyll is intimately linked to the presence and/or synthesis of the light-harvesting chlorophyll (Lhc)-binding proteins [15]. Retrograde communication coordinates the expression of nuclear genes encoding organellar proteins with the metabolic and developmental state of the plastid and mitochondria [16,17] through signals emitted from the organelles that regulate nuclear gene expression. Several different plastid processes involving products of plastid protein synthesis, $^1\text{O}_2$, and intermediates of tetrapyrrole biosynthesis produce these signals [18,19] and plastid-to-nucleus communication appears to be of particular importance during plant stress responses.

In this study, photooxidation induced by perturbation in carotenoid and tetrapyrrole biosynthetic pathways was investigated by using their biosynthetic inhibitors, NF and OF, in rice plants. A direct comparison of the two modes of photooxidation, which exhibit bleaching and necrosis, has not been presented yet. To address these questions, we compared ultrastructure as well as functions of the photosynthetic complexes between NF- and OF-treated plants. We also compared the expression of photosynthetic genes, encoded in plastid and nuclear genomes, to elucidate plant signaling under photooxidative stress. Our results demonstrate that distinct photooxidation patterns by NF and OF coordinate the expression of nuclear and plastid genes, altering structure and function of photosynthetic apparatus.

2. Materials and methods

2.1. Plant material and herbicide treatments

The germinated seeds of rice (*Oryza sativa* cv Dongjin) plants

were sown in pots and grown in greenhouse and maintained with day/night temperatures of 30/25 $^\circ\text{C}$, relative humidity of 65–80%, 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetically active radiation, and a 14/10 h day/night period. Three week-old rice plants were treated with 50 μM oxyfluorfen (OF) (KyungNong Inc.) or 50 μM norflurazon (NF) (Supelco Inc.), placed in darkness for 12 h to allow absorbance, and then illuminated. Leaf samples for NF1 and OF1 plants were taken 16 h after NF and OF treatments. For NF2 and OF2 plants, leaf samples were taken 40 and 88 h after NF and OF treatment, respectively. Newly developed leaves in NF plants were taken for 88-h sample since the typical bleaching by NF was not due to a photo-dependent destruction of developed leaves but inhibition of pigment synthesis in newly developing leaves. Technical-grade NF and OF were dissolved in 30% acetone with 0.1% Tween 20.

2.2. Measurement of chlorophyll *a* fluorescence

Chlorophyll *a* fluorescence was measured *in vivo* using a pulse amplitude modulation fluorometer (Handy PEA; Hansatech Instruments) after dark adaptation for 20 min. The minimal fluorescence yield, F_0 , was obtained upon excitation with a weak measuring beam from a pulse light-emitting diode. The maximal fluorescence yield, F_m , was determined after exposure to a saturating pulse of white light to close all reaction centers. The ratio of F_v to F_m , representing the activity of PSII, was used to assess the functional damage to the plants.

2.3. Ultrastructure of chloroplast by TEM observations

Samples from differently treated leaves were fixed by vacuum infiltration with 2% paraformaldehyde and 2% glutaraldehyde in 0.05 M sodium cacodylate buffer, pH 7.2. After washing, they were fixed for 2 h in osmium tetroxide, in the same buffer, dehydrated through an ethanol series and embedded in Spurr resin by

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