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Biochar supplementation altered the expression of antioxidant proteins in rice leaf chloroplasts under high-temperature stress

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Abstract

In order to identify the key antioxidant defense systems used to cope with high-temperature stress in rice leaf chloroplasts following biochar supplementation, the present study compared the expression levels of chloroplast proteins related to antioxidant defense in high-temperature stressed rice leaves between without (C0) and with biochar supplementation (C40; 40 g biochar kg^{-1} soil). A total of sixteen differentially expressed antioxidant chloroplastic proteins were identified. Among them, three antioxidant enzyme proteins and eight thioredoxin proteins were 62–123% and 37–225% higher under the C40 treatment compared to C0, respectively. These results suggest that both antioxidant enzymes and the thioredoxin system are central to the biochar-mediated protection of rice leaves exposed to high-temperature stress.

Keywords Antioxidant proteins, Biochar, Chloroplasts, High temperature stress, Rice

Introduction

Rice is one of the most important cereals in the world and serves as a staple food for more than half of the world's population [2]. Global rice demand is projected to increase by 30% by 2050 [19]. Meanwhile, increasing temperature due to climate change poses a threat to rice production. Global temperature is projected to increase by 2 °C by 2050 [1], while global rice yield is projected to be reduced by 3% with a global increase in temperature of 1 °C [22]. Therefore, it is necessary to develop proactive strategies to alleviate the negative effects of global warming on rice production.

Biochar is a carbon (C)-rich solid product formed by the pyrolysis of organic matter in the absence or limited supply of oxygen [8]. The supplementation of biochar to croplands has received wide attention because it offers an option to sequester C in soils and consequently mitigate global warming [9, 18], while also affecting soil properties and crop performance [4, 7, 12]. Our recent study suggests that biochar supplementation can mitigate the negative effects of high-temperature stress on the growth of rice plants [5], but the mechanism by which biochar improves tolerance to high temperature remains little known. Thus, there is a need to elucidate the fundamental understanding of the physiological mechanisms governing this mitigative effect of biochar supplementation.

High-temperature stress induces excessive reactive oxygen species (ROS) production in plant cells and consequently damages cellular and molecular components (e.g., cellular proteins, lipids, and DNA), leading to cell injury or even death [16]. ROS is largely produced in the chloroplast upon high-temperature stress and is

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mitigated by antioxidant pathways [3, 13, 20]. Antioxidant enzymes such as superoxide dismutase, peroxidase, and glutathione reductase are the most important components for defense against oxidative stress [15]. The thioredoxin system, comprised of thioredoxin, nicotinamide adenine dinucleotide phosphate, and thioredoxin reductase, provides electrons to thiol-dependent antioxidant enzymes (e.g., peroxidase and peroxiredoxin) to remove ROS at high reaction rates and hence is crucial for alleviating oxidative stress-induced cell damage [11]. In the present study, we determined the effect of biochar supplementation on the expression levels of antioxidant proteins (antioxidant enzyme and thioredoxin system proteins) in the chloroplasts of rice leaves under high-temperature stress. Our objective was to identify the key antioxidant defense systems used to cope with high-temperature stress in rice leaf chloroplasts following biochar supplementation.

Materials and methods

The data were obtained from an experiment conducted in 2020. Briefly, a high-yielding hybrid rice variety, Longliangyouhuazhan, was grown in pots (length × width × height = 20 cm × 15 cm × 25 cm) without (C0) and with biochar supplementation (C40, 40 g biochar kg^{-1} soil). Six consecutive days (6–11 days after transplanting) of daily mean temperatures (35–36 °C) beyond the critical high temperature for rice tillering (33 °C; [6]) were monitored during the experimental period. The experimental details can be found in [5].

The uppermost fully-expanded leaves were sampled from three pots (replicates) for each treatment. The samples were placed in plastic bags and kept cool on ice during transport to the laboratory, after which they were hulled and stored at −80 °C until proteins were extracted. Total proteins were extracted using the cold acetone method, and then measured via protein DC assay (Bio-Rad) and calculated according to a bovine serum albumin protein standard curve. A proteomic analysis was carried out using iTRAQ (isobaric tags for relative and absolute quantitation) coupled LC-MS/MS (liquid chromatography-mass spectrometry/mass spectrometry). The details of protein extraction and proteomic analysis are provided in Supplementary File S1.

The functions of the identified proteins were defined according to the Gene Ontology (GO) database (http://www.geneontology.org). The differentially expressed proteins related to antioxidant defense (antioxidant enzyme and thioredoxin system proteins) in chloroplasts were selected for further evaluation. The differentially expressed proteins were defined as those that were significantly different ($p < 0.05$) in the expression level between C40 and C0. The details of bioinformatics analysis are provided in Supplementary File S1.

Results and discussion

A total of sixteen differentially expressed antioxidant proteins in chloroplasts were identified, including five antioxidant enzyme proteins and eleven thioredoxin system proteins (Table 1). These differentially expressed antioxidant proteins had molecular weights of 18.4–60.3 kDa. The sequence coverage of these differentially expressed antioxidant proteins varied between 10.6 and 45.8% and the number of peptides was 2–19.

Among the five antioxidant enzyme proteins, expression levels of two glutathione reductase proteins (A2XAX5 and A2XCU8) were 25–72% lower under C40 conditions compared to C0, whereas those of another three proteins of peroxidase (B8AJE7), superoxide dismutase (B8B8G3), and peroxiredoxin (P0C5D4) were 62–123% higher in C40 leaves compared to C0 (Fig. 1A; Table 1). This result demonstrates that peroxidase, superoxide dismutase, and peroxiredoxin are important components of the chloroplastic antioxidant defense system against high-temperature stress in rice leaves upon biochar supplementation.

Among the eleven thioredoxin system proteins, eight thioredoxins (A2WY84, A2XVT3, A2XYX7, A2YL83, A2YUQ6, B8AEW4, B8AG42, and B8BNI2) were 37–225% higher in the expression level under C40 than under C0, while only two thioredoxins (B8ALJ3 and B8B186) and one thioredoxin reductase (B8B1A9) were 23–29% lower under C40 conditions compared to C0 conditions (Fig. 1B; Table 1). These results highlight the prominence of thioredoxin proteins in high temperature-responsive antioxidant activity in the chloroplasts of rice leaves supplemented with biochar.

It has been well documented that nitric oxide (NO) can boost the antioxidant activity in plants under abiotic stress conditions [17]. The synthesis of NO in plants includes two pathways: (1) the enzymatic pathway (nitrate reductase, nitric oxide-like synthase, nitrite-NO reductase, and xanthine oxidase); and (2) the non-enzymatic pathway (nitrification and de-nitrification) [14]. As both these two pathways of NO synthesis are related to nitrogen (N) supply, several studies have shown that N application can increase leaf antioxidant enzyme activities and reduce yield losses in rice under high temperature conditions [10, 21]. In this study, we observed that biochar supplementation increased shoot N uptake and up-regulated leaf N assimilation and transport proteins in rice (see [5] for details), indicating that biochar supplementation may alter the synthesis of NO and in turn regulate the antioxidant defense system in the chloroplasts of rice leaves exposed to high temperature (Fig. 2). However, further investigations are required to confirm this possibility.

The findings of this study suggest that the combined expression of antioxidant enzymes (peroxidase,

Table 1 Identified differentially expressed proteins related to antioxidant defense in chloroplasts of rice leaves exposed to high temperature between without and with biochar supplementation

Protein accession	Molecular weight (kDa)	Sequence coverage (%)	Number of peptides	Protein description
Antioxidant enzyme				
A2XAX5	60.2	34.4	12	Glutathione reductase(predicted) OS = *Oryza sativa* subsp. *indica* OX = 39,946 GN = OsI_09408 PE = 3 SV = 1
A2XCU8	60.3	45.4	19	Glutathione reductase OS = *Oryza sativa* subsp. *indica* OX = 39,946 GN = OsI_10133 PE = 3 SV = 1
B8AJE7	49.1	38.3	16	PEROXIDASE_4 domain-containing protein OS = *Oryza sativa* subsp. *indica* OX = 39,946 GN = OsI_07630 PE = 3 SV = 1
B8B8G3	19.6	35.5	4	Sod_Cu domain-containing protein OS = *Oryza sativa* subsp. *indica* OX = 39,946 GN = OsI_25396 PE = 4 SV = 1
P0C5D4	23.7	45.2	10	Putative peroxiredoxin Q, chloroplastic OS = *Oryza sativa* subsp. *indica* OX = 39,946 GN = OsI_22010 PE = 3 SV = 1
Thioredoxin system				
A2WY84	19.7	39.0	6	Thioredoxin domain-containing protein OS = *Oryza sativa* subsp. *indica* OX = 39,946 GN = OsI_04888 PE = 4 SV = 1
A2XVT3	19.1	19.4	3	Thioredoxin domain-containing protein OS = *Oryza sativa* subsp. *indica* OX = 39,946 GN = OsI_16748 PE = 4 SV = 1
A2XYX7	19.1	25.0	4	Thioredoxin domain-containing protein OS = *Oryza sativa* subsp. *indica* OX = 39,946 GN = OsI_17911 PE = 4 SV = 1
A2YL83	32.0	45.8	11	Thioredoxin domain-containing protein OS = *Oryza sativa* subsp. *indica* OX = 39,946 GN = OsI_25975 PE = 4 SV = 1
A2YUQ6	20.5	15.9	3	Thioredoxin-like protein CITRX, chloroplastic OS = *Oryza sativa* subsp. *indica* OX = 39,946 GN = OsI_29059 PE = 3 SV = 1
B8AEW4	20.6	10.6	2	Thioredoxin domain-containing protein OS = *Oryza sativa* subsp. *indica* OX = 39,946 GN = OsI_09468 PE = 4 SV = 1
B8AG42	18.6	13.9	2	Thioredoxin domain-containing protein OS = *Oryza sativa* subsp. *indica* OX = 39,946 GN = OsI_08226 PE = 4 SV = 1
B8ALJ3	25.8	38.9	9	Thioredoxin-like protein AAED1, chloroplastic (predicted) OS = *Oryza sativa* subsp. *indica* OX = 39,946 GN = OsI_11064 PE = 4 SV = 1
B8B186	18.4	13.4	3	Thioredoxin domain-containing protein OS = *Oryza sativa* subsp. *indica* OX = 39,946 GN = OsI_24031 PE = 4 SV = 1
B8B1A9	38.2	19.3	4	Thioredoxin reductase OS = *Oryza sativa* subsp. *indica* OX = 39,946 GN = OsI_22780 PE = 3 SV = 1

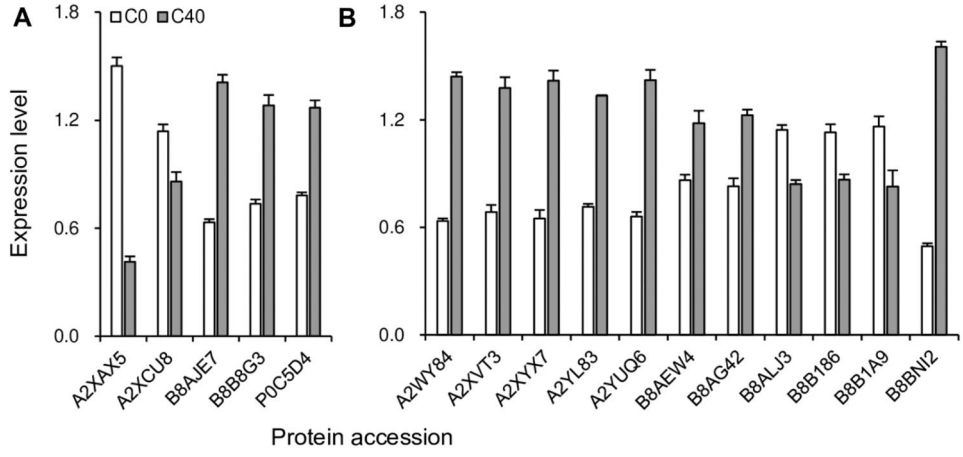

Fig. 1 Expression levels of antioxidant enzyme proteins (**A**) and thioredoxin system proteins (**B**) in chloroplasts of rice leaves exposed to high temperature without (C0) and with biochar supplementation (C40, 40 g biochar kg^{-1} soil). Columns and bars represent the means and standard deviations for three replicates, respectively. Protein descriptions are provided in Table 1

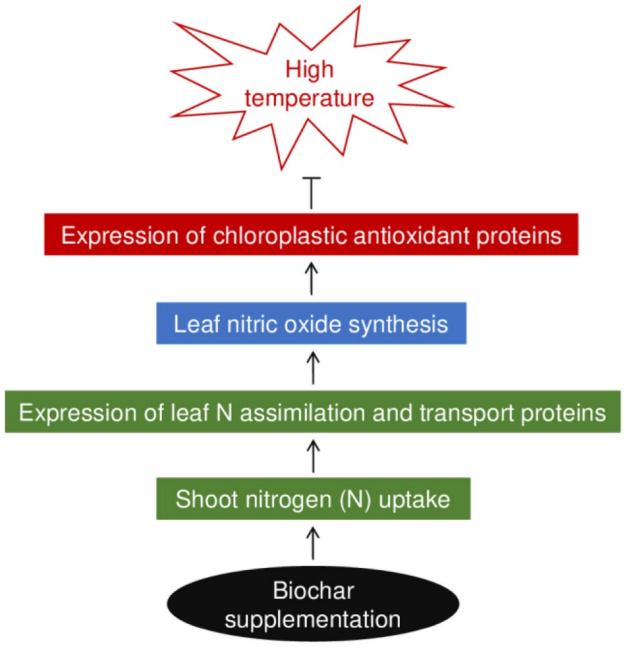

Fig. 2 A potential process underlying the effect of biochar supplementation on antioxidant defense systems in rice leaf chloroplasts exposed to high temperature

superoxide dismutase, and peroxiredoxin) and the thioredoxin system are central to the biochar-mediated protection of rice leaves exposed to high-temperature stress. This study provides insight into the physiological mechanisms by which biochar supplementation mitigates the negative effect of high-temperature stress on rice plants.

Abbreviations

C	Carbon
C0	The treatment without biochar supplementation
C40	The treatment with supplementation of 40 g biochar kg^{-1} soil
N	Nitrogen
NO	Nitric oxide
ROS	Reactive oxygen species

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13765-024-00911-9.

Supplementary Material 1

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Author contributions
MH conceived the study, analysed the data, and wrote the paper; XY, JC and FC performed the experiment.

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Data availability
The data used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest
The authors declare that they have no competing interests.

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