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Original article

Expression profiles of genes for mitochondrial respiratory energy-dissipating systems and antioxidant enzymes in wheat leaves during de-etiolation

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ABSTRACT

Mitochondrial respiratory components participate in the maintenance of chloroplast functional activity. This study investigates the effects 48 h de-etiolation of spring wheat seedlings (*Triticum aestivum* L., var. Irgina) on the expression of genes that encode energy-dissipating respiratory components and antioxidant enzymes under continuous light conditions. The expression of *AOX1a* following the prolonged darkness exhibited a pattern indicating a prominent dependence on light. The expression of other respiratory genes, including *NDA2*, *NDB2,* and *UCP1b*, increased during de-etiolation and dark-to-light transition; however, changes in the expression of these genes occurred later than those in *AOX1a* expression. A high expression of *NDA1* was detected after 12 h of de-etiolation. The suppression of *AOX1a*, *NDA2*, *NDB2*, and *UCP1b* was observed 24 h after de-etiolation when the photosynthetic apparatus and its defence systems against excess light were completely developed. The expression patterns of the respiratory genes and several genes encoding antioxidant enzymes (*MnSOD*, *Cu-ZnSOD*, *t-APX*, *GR*, and *GRX*) were quite similar. Our data indicate that the induction of nuclear genes encoding respiratory and antioxidant enzymes allow the plants to control reactive oxygen species (ROS) production and avoid oxidative stress during de-etiolation.

1. Introduction

At present it is clear that the functions of chloroplasts and mitochondria are closely coordinated in the light, and that the components in the respiratory chain in the mitochondria participate in maintaining the functional activity of chloroplasts (Noguchi and Yoshida, 2008). The mitochondrial ETC (mETC) in plants, in contrast to mammalian ETC, consists not only of a phosphorylating cytochrome pathway (CP), but also of several non-phosphorylating pathways that are not coupled with the extrusion of protons from the matrix to the intermembrane space and hence, bypassing ATP synthesis. The additional enzymes include the cyanide-insensitive alternative oxidase (AOX), transferring electrons from the ubiquinone pool to oxygen and producing water (Vanlerberghe and McIntosh, 1997), and the rotenone-insensitive internal and external type II NAD(P)H dehydrogenases (Rasmusson et al., 2008). The AOX pathway bypasses the proton-pumping complexes III and IV and reduces the energy yield of respiration by at least two thirds. The type II NAD(P)H dehydrogenases (NDs) bypass Complex I. In addition, in the plant mETC there is a system similar to animal mitochondria that uncouples electron transport from ADP phosphorylation, regulated by the uncoupling proteins (UCP). UCP in the presence of free fatty acids facilitates the re-entry of protons into the mitochondrial matrix, dissipating the electrochemical proton gradient as heat (Borecky et al., 2006). These energy-dissipating systems (AOX, NDs, and UCP) allow a kind of fine-tuning of the mitochondrial membrane potential, and decrease reactive oxygen species (ROS) production caused by mETC over-reduction (Noguchi and Yoshida, 2008).

Among the non-phosphorylating pathways, the electron flow through the AOX pathway plays a crucial role in a photosynthesising cell (Noguchi and Yoshida, 2008; Dinakar et al., 2010; Zhang et al., 2010; Garmash et al., 2015; Vishwakarma et al., 2015). AOX

Abbreviations: AOX, alternative oxidase; AP, alternative respiratory pathway; t-APX, thylakoid-bound ascorbate peroxidase; Asc, ascorbate; COX, cytochrome oxidase; CAT, catalase; CP, cytochrome respiratory pathway; Cyt, cytochrome c; DHA, dehydroascorbate; DHAR, dehydroascorbate peroxidase; GPX, glutathione peroxidase; GR, glutathione reductase; Guaiacol PX, quaiacol peroxidase; MDA, monodehydroascorbate; MDAR, monodehydroascorbate reductase; NDA and NDB, rotenone-insensitive internal and external type II NADH dehydrogenases respectively; mETC, mitochondrial electron transport chain; NBT, nitrotetrazolium blue chloride; PAGE, polyacrylamide gel electrophoresis; qRT-PCR, Real-Time quantitative Reverse Transcription PCR; ROS, reactive oxygen species; SOD, superoxide dismutase; UCP, uncoupling protein; VDAC, voltage dependent anion channel

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participates in oxidising chloroplast-generated reductants imported to the mitochondria, thereby unloading the photosynthetic ETC (Raghavendra and Padmasree, 2003; Dinakar et al., 2010). Light is thought to contribute to the regulation of AOX via direct photoreceptor control and indirect photosynthesis-dependent variations in metabolites (Tepperman et al., 2004; Rasmusson and Escobar, 2007; Igamberdiev et al., 2014). NDs are also important in a photosynthesising cell (Michalecka et al., 2003; Rasmusson and Escobar, 2007) because internal NDs (NDA, NDC) are involved in the oxidation of matrix NAD(P)H (Rasmusson et al., 2008). It is considered that NDA proteins oxidize NADH produced from photorespiration (Svensson and Rasmusson, 2001; Gardeström et al., 2002). NDC1 is thought to contribute to malate oxidation (Elhafez et al., 2006). External NDs (NDB) allow the cytosolic NAD(P)H to be oxidized (Elhafez et al., 2006; Rasmusson et al., 2008). In different plant species NDs genes are induced by light (Svensson and Rasmusson, 2001; Escobar et al., 2004; Elhafez et al., 2006), and by analysing light quality and photoreceptor mutants, *NDA1* and *NDC1* light regulation was shown to be a direct response mediated by photoreceptors (Rasmusson and Escobar, 2007). In Arabidopsis, *NDA2*, *NDB2*, and *AOX1a* were up-regulated in a coordinated manner in various light treatments, potentially forming a complete respiratory chain capable of oxidising matrix and cytosolic NAD(P)H (Elhafez et al., 2006; Clifton et al., 2006).

The role of UCP in illuminated leaves is still unclear. In Arabidopsis, two UCP genes (*UCP1* and *UCP5*) showed light-induction (Yoshida and Noguchi, 2009). Also, in the leaves of *UCP1*-deficient Arabidopsis, the rate of the oxidation of photorespiratory glycine and the rate of photosynthesis were suppressed (Sweetlove et al., 2006), suggesting that the main physiological role of *UCP1* in Arabidopsis leaves is the maintenance of the redox ability of the mitochondrial electron transport chain to facilitate photosynthetic metabolism.

Regardless, the mechanisms of light-responsive gene expression for energy-dissipating respiratory components, sequence of engagement, and coordination of AOX, NDs and UCP in illuminated leaves are still not fully elucidated. Meanwhile, it has been shown that the expression of genes encoding the mitochondrial respiratory components in Arabidopsis leaves under high light (400 μmol m^{-2} s^{-1}) has differential regulation, suggesting they have separate roles in plant metabolism mediation (Escobar et al., 2004; 2006; Yoshida and Noguchi, 2009). In particular, *AOX1a* and *NDB2* was mainly induced by chemical inhibition of the respiratory chain; *NDA1* expression was affected by photosynthesis-related ROS, and several genes encoding energy-dissipating respiratory components (*AOX1a, NDA1, NDB1, UCP1, UCP5*) were induced by elevated photosynthesis as a result of high level of CO_2 and light simultaneously, suggesting their dependence on the modulation in cellular carbon status (Yoshida and Noguchi, 2009). On the other hand, a weak light responsiveness in *AOX1a* expression was observed after 4 h and 12 h of low light exposure (10 μmol m^{-2} s^{-1}) in leaves of 5-d-old etiolated Arabidopsis seedlings (Escobar et al., 2004). Concurrently, *NDA1* and *NDC1* were significantly induced by light. However, the addition of sucrose to growth medium had no effect on gene expression in the light, suggesting that carbon status does not play a large part in the regulation of these genes. In other studies, strong and specific light dependence of *NDA1* transcription was also measured in 40-d old Arabidopsis leaves when plants were grown in 100 μmol m^{-2} s^{-1} light (Michalecka et al., 2003); whereas there was no light effect on *NDC1* expression in the same conditions (Escobar et al., 2006). These results show that induction and regulation of the energy-dissipating respiratory components in a photosynthesising cell are highly dependent on light level, physiological status of the plant, and the overall growth conditions.

In addition, it is generally accepted that AOX may act to reduce ROS generation through the mitochondrial ETC and thus, it is considered to be an important part of the cellular antioxidative defence system (Møller, 2001; Szal et al., 2009; Vishwakarma et al., 2015). The promoter region of *AOX1a* in Arabidopsis contains a number of regulatory elements, some of which are associated with light and stress responses (Ho et al., 2008). Similar to *AOX1a*, several genes encoding cellular redox-mediated enzymes in Arabidopsis were induced by high light treatment and/or suppressed in darkness (Yoshida and Noguchi, 2009). Also, Arabidopsis wild type plants treated with antimycin A (for the inhibitions of electron transfer by ferredoxin-PQ reductase in the chloroplast and by complex III in the mitochondrion) under light conditions had a significant increase in the transcription levels of *AOX1a* and higher expression of genes encoding antioxidant enzymes in contrast to *aox1a* knock-out mutants (Vishwakarma et al., 2015).

In the present study, we analysed the expression patterns of respiratory genes involved in the non-phosphorylating pathways (AOX, NDs, UCP) in the first leaves of wheat seedlings during de-etiolation in continuous light and discuss the ways in which expression of each gene was regulated during the development of a photosynthesising cell and their differences between the components. We chose 190 μmol m^{-2} s^{-1} as the level of illumination, which corresponds to the typical average value of light intensity in the habitat of the plant species (Garmash et al., 2013). Previously, we found that *AOX1a* expression, the respiratory activity, and AP capacity in leaves and mitochondria increased significantly during the first 6–12 h of continuous irradiation (Garmash et al., 2015). At this time, prolamellar bodies already converted into thylakoids and there is positive net-photosynthetic activity (Garmash et al., 2013). We have also studied the induction of nuclear genes encoding some important ROS-scavenging enzymes in addition to ROS production to provide additional clarity to the functional relationship between non-phosphorylating respiratory pathways and the reactive oxygen network component functions in the light. Considering that AOX is a target as well as a regulator for stress response (Van Aken et al., 2009), we present a hypothetical model for signalling pathways that induce the expression of *AOX* and other genes that encode respiratory non-phosphorylating enzymes in leaves during de-etiolation.

2. Materials and methods

2.1. Plant material and growth conditions

Plant material and growth conditions were similar to those described previously (Garmash et al., 2013, 2015). A spring wheat (*Triticum aestivum* L.) cutivar “Irgina” was used throughout the experiment. Three-day-old seedlings were transferred into 3-dm^3boxes with a half-strength Knop medium and were grown in the dark for 2 days in a growth chamber (KBWF 720, Binder, Tuttlingen, Germany) at 23 °C and 70% relative humidity. Thereafter, the etiolated seedlings were exposed to continuous light (190 μmol(photon) $m^{-2}s^{-1}$, photosynthetic active radiation) at 21 °C and allowed to green for 48 h. Nutrient solution was changed every day. The light was produced by luminescent lamps (TL-D 30W, Philips, Amsterdam, The Netherlands). All measurements were carried out on the first leaf blade, the top segment (1.5 cm) of which was removed and, from the rest; a segment of 2–3 cm in length was used in the experiment. The leaves exposed to light during 0, 1, 2, 4, 6, 12, 24, and 48 h were used. In a separate experiment, the seedlings after 6 h of the de-etiolation phase were put into dark for 18 h and thereafter again exposed to light for the next 24 h to analyze the light-dependent character of gene expression of mitochondrial and anti-oxidative proteins.

2.2. O_2 exchange and capacities of the CP and AP in leaf tissue

The O_2 uptake rate was measured using a Clark-type thermoelectrically controlled oxygen electrode (Oxytherm system, Hansatech Inst., Pentney, Norfolk, England) at 21 °C. Small leaf slices (0.015 g FW) were placed in the reaction vessels of the electrode unit containing 1.5 ml HEPES buffer (50 mM, pH 7.2). The O_2 uptake rate was measured in the presence of KCN (2 mM, inhibitor ofcytochrome respiration, CP) to measure alternative pathway respiration (AP) capacity (Valt),