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Research article

Plastoquinone redox state modifies plant response to pathogen

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ABSTRACT

The role of PQ (plastoquinione) redox state in establishment of response to pathogen infection (*Botrytis cinerea*) was tested along the regulation of main antioxidative enzymes (superoxide dismutase – SOD, catalase – CAT) and photochemistry of PSII (photosystem II) in *Mesembryanthemum crystallinum* plants performing C_3 and CAM (Crassulacean acid metabolism) carbon metabolism. The redox state of PQ was modified by two inhibitors of photosynthetic electron transport resulting in a more oxidised (3-(3,4-dichlorophenyl)-1,1-dimethylurea; DCMU) or reduced (2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone; DBMIB) PQ redox state simulating darkness and high light conditions, respectively. Irrespective of the type of treatment (mock inoculation or pathogen inoculation) SOD activity depended on the PQ pool. Our results suggest that regarding changes in infection-induced CAT activity, plants developed response that is vital for hypersensitive-like (HR-like) response establishment only when PQ pool generated signal was similar to that in light presence (DBMIB pre-treatment). When PQ pool generated signal was similar to darkness, CAT activity response remained stress-independent, similarly to SOD. Fluorescence parameters of PSII, Qp (photochemical quenching coefficient) and NPQ (non-photochemical quenching) were affected only in the tissues treated with DCMU in stress-independent manner. We suggest that in case of abiotic and biotic stresses signals emerging from PQ pool indirectly orchestrate plant response and carbon metabolism affects this regulatory pathway.

1. Introduction

Plants are sessile organisms and they routinely experience a combination of different stresses (Baker et al., 2000; Wiese and Kranz, 2004). Plants sense their environment to acclimate to constantly changing conditions. There are several systems to perceive environmental signals and to orchestrate an adequate response. The common background of different stresses is related to changes in the prooxidant/antioxidant equilibrium. Reactive oxygen species (ROS) generated by biotic and abiotic stresses can elicit oxidative damage, however at low concentrations they act as signalling molecules inducing defence responses (Foyer and Noctor, 2009; Shetty et al., 2008; Vranová et al., 2002). The involvement of ROS in oxidative modifications of several proteins that play a crucial role in plant acclimation to stress was also shown (Møller et al., 2007), and they are known as molecules involved in regulation of plant development processes, organogenesis, senescence and defence against pathogens (Matamoros et al., 2003).

Light intensity and photosynthetic metabolism are among the most important factors affecting plant response to stress. It was shown that β-carboxylating CAM plants are better adapted to different stresses in comparison to C_3 plants (Nyman et al., 1990). Our earlier research (Kuźniak et al., 2010) indicated that response of *Mesembryanthemum crystallinum* to *Botrytis cinerea* depended, at least partly, on the type of photosynthetic metabolism. In both C_3- and CAM-performing plants infection resulted in the development of HR-like defence reaction or soft-rot spreading lesions depending on the post-inoculation light conditions. Light was shown to be necessary for plant resistance and HR-like lesions were co-localized with H_2O_2 accumulation. At low light, fungal development was not

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successfully limited and soft-rot spreading lesions developed. It was suggested that light-dependent processes related to CAM and H_2O_2 accumulation were parallel with resistance to *B. cinerea*. However, it remains unclear whether plant reactions depend on carbon metabolism *per se* or on differences in the activity of photosynthetic electron transport (PET) chain connected with carbon metabolism (Kuźniak et al., 2010).

Despite detailed knowledge on plant–pathogen interactions, the influence of light and photosynthesis on pathogen colonization and plant defence still requires clarification (Chandra-Shekara et al., 2006; Scharte et al., 2005). Antioxidants and redox signals are central to most stress responses and it was found that signals essential for host defence were sent from chloroplasts and especially from the plastoquinone (PQ) pool (Karpinski et al., 1997; Kuźniak and Skłodowska, 2005; Pfannschmidt, 2003; Wiese and Kranz, 2004). As to abiotic stressors, the involvement of PQ redox state in regulation of plant defence has been unequivocally confirmed (Mühlenbock et al., 2008). Later it was found that tobacco plants accumulate PQ in response biotic, as well abiotic stressors (Bajda et al., 2009). In this scheme, PQ redox state acts as a master switch that regulates a multi-component response to excess excitation energy (EEE) including changes in prooxidant–antioxidant balance, stomatal conductance, ethylene production and signalling (Mühlenbock et al., 2008). As PET undergoes feed-back regulations resulting from many other processes, it is not possible to predict if PQ effect on resistance in C_3- and CAM-performing plants is similar. DCMU (3-(3,4-dichlorophenyl)-1,1-dimethylurea) and DBMIB (2,5-dibromo-3-methyl-6-isopropylbenzoquinone) are two known inhibitors of PET. DCMU blocks PET moving PQ pool to more oxidised state simulating dark conditions, while DBMIB binds to cyt b_6f and moves PQ pool toward more reduced state simulating light conditions. As we found that *M. crystallinum* – *B. cinerea* interaction depends on light (Kuźniak et al., 2010), we simulated dark and light conditions with PET inhibitors to study the possible involvement of PQ redox state in regulation of *M. crystallinum* antioxidant defence, in relation to the type of photosynthetic carbon metabolism and to the impact on response to *B. cinerea* infection.

M. crystallinum, C_3/CAM intermediate plant is a well known model plant used to compare C_3 and CAM metabolism. $C_3 \rightarrow$CAM transformation can be accelerated by several abiotic stressors, e.g. salinity, chilling, high light etc. Light is necessary to perform transformation and it was noted that CAM can be induced only if light intensity reached the threshold level around 260 μmol m^{-2} s^{-1} (Miszalski et al., 2001). During CAM induction, increase in activity of main antioxidative enzymes (superoxide dismutase – SOD, catalase–CAT) prevents excessive ROS production (Castillo, 1996; Miszalski et al., 1998; Niewiadomska and Borland, 2008). It was also observed that *M. crystallinum* appeared to be resistant to fungal (*B. cinerea*) and bacterial (*Pseudomonas syringae*) pathogens. Changes in SOD and CAT activities in leaves infected with those pathogens are related to the type of photosynthetic metabolism of the stressed plants (Kuźniak et al., 2010; Libik-Konieczny et al., 2011).

Since scarce data concerning the role of photosynthesis in biotic stress responses are available, and recent reports revealed the participation of PQ redox state in the orchestration of abiotic stress response (Mühlenbock et al., 2008), we studied possible connection between PQ pool as a master switch in regulation of plant response to stress and antioxidant enzymes as executors of this reaction. The involvement of PQ pool in regulation of SOD and CAT activities during *M. crystallinum–B. cinerea* interaction was characterized with respect to C_3 and CAM types of photosynthetic carbon metabolism.

2. Material and methods

2.1. Plant material

Plants of *M. crystallinum* L. were grown in soil culture, in a growth chamber under 12 h photoperiod at 25/17 °C (day/night), 60/80% RH and 250–300 μmol quanta m^{-2} s^{-1} (PAR). Two weeks after sowing seedlings were transferred to individual pots. Six week-old plants were divided into two groups: one irrigated with tap water (C_3) and the other irrigated with 0.4 M NaCl to induce CAM (CAM). After next 12–14 days (eight week-old plants) CAM occurrence was confirmed in NaCl-treated plants by estimation of diurnal Δ-malate concentration in the leaf cell sap (data not shown) with a reflectometer (RQflex 10, Merck®), according to the manufacturer's instruction.

2.2. Pre-treatment with PET inhibitors

Application of inhibitors to intact leaves was performed according to the method described by Karpinski et al. (1999). The 100 mM DCMU (3-3,4-dichlorophenyl-1,1-dimethyl urea) and 50 mM DBMIB (2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone) stock solutions were prepared in ethanol and dimethylsulphoxide, respectively. The plants were sprayed with 500 μM water solution of DCMU or DBMIB with a hand sprayer. Solution volume of 15 mL was sprayed over plate handling 15 plants (~1 mL of solution per plant). Pre-treatment was provided always 2 h after the beginning of the light phase, which started at 6:00 a.m. The concentrations of DCMU and DBMIB were selected on the basis of preliminary screening experiments (data not shown). The inhibitor-treated plants were kept 24 h in a growth chamber before pathogen inoculation.

2.3. B. cinerea infection

B. cinerea isolate (nr 1631) was provided by the Bank of Plant Pathogens (Poznan, Poland). Inoculation solution was prepared according to the procedure previously described by Kuźniak et al. (2010). It contained 2×10^6 mL^{-1} spores of *B. cinerea* in 5 mM glucose and 2.5 mM KH_2PO_4 solution. The inoculation solution was syringe-infiltrated into leaves of 8-weeks old plants. One injection

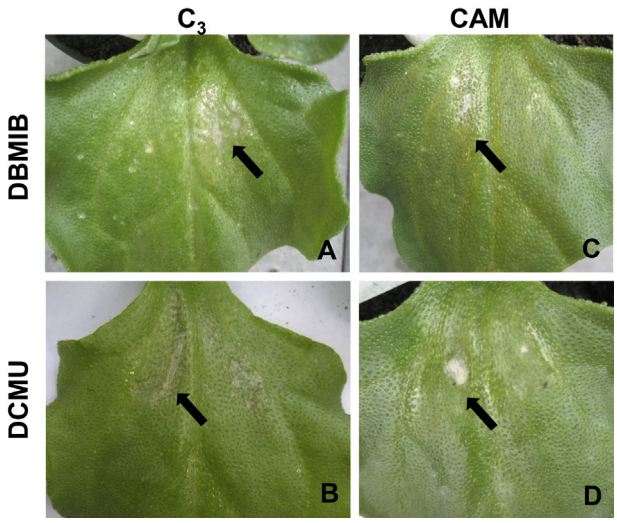

Fig. 1. *Botrytis cinerea* infection symptoms on DBMIB and DCMU-treated C_3 (**A**, **B**) and CAM (**C**, **D**) leaves of *Mesembryanthemum crystallinum*. Symptoms were recorded 2 days post-inoculation. Arrows indicate infection-induced necrotic spots.