Plant Physiology and Biochemistry 123 (2018) 160–169

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Research article

Primary carbon metabolism-related changes in cucumber exposed to single and sequential treatments with salt stress and bacterial infection

Joanna Chojak-Koźniewska[a,b,1], Elżbieta Kuźniak[a,*,1], Anna Linkiewicz[b], Sławomir Sowa[b]

[a] Department of Plant Physiology and Biochemistry, Faculty of Biology and Environmental Protection, University of Lodz, Banacha 12/16, 90-237 Łódź, Poland
[b] Genetically Modified Organisms Controlling Laboratory, Plant Breeding and Acclimatization Institute – National Research Institute, Radzików, Poland

ARTICLE INFO

Keywords:
Biotic stress
Salinity
Stress interactions
Photosynthetic carbon metabolism
C4 enzymes
P. syringae pv *lachrymans*

ABSTRACT

This study examines how salt stress interacts with bacterial infection at the metabolic level. We measured chlorophyll *a* fluorescence as well as profiles of phosphoenolpyruvate carboxylase (PEPC), NADP-malic enzyme (NADP-ME), NADP-isocitrate dehydrogenase (NADP-ICDH) and fumarase activities, malic and citric acids contents and the expression of *NADP-ICDH* and *NADP-ME* in the organ-dependent (root vs leaves) response of cucumber plants exposed to individual or sequential action of salt stress (50 mM or 100 mM NaCl) and *Pseudomonas syringae* pv *lachrymans* (Psl). NaCl treatment, Psl infection and the combination of these stresses caused disturbances in the activity of photosystem II which were suggested to specifically transmit the stress signals. PEPC and NADP-ME were induced in cucumber plants under stress, confirming that in C3 plants they function in defence responses. The profiles of malate and citrate contents, PEPC as well as NADP-ICDH and NADP-ME activities and gene expression in response to a combination of salt and pathogen stresses differed from those provoked by individual stress with respect to the direction, intensity and timing. The results indicated that the most pronounced defence response related to the readjustment of the carbon metabolism was observed in the leaves of plants exposed to combined stress. Intense activity changes of NADPH-generating enzymes, NADP-ICDH and NADP-ME, characterized the tailored response to combined stress and could be important for the integration of defence mechanisms between organs.

1. Introduction

The global climate change is leading to the emergence of new combinations of environmental stresses that adversely affect plants, and those between abiotic stressors and pathogens have been recognized important yield-limiting interactions (Suzuki et al., 2014). Plants often adjust to the constantly changing environmental conditions by metabolic flux (Doubnerová and Ryšlavá, 2011). Unfortunately, most studies of the plant metabolic adjustments focus on the effects of a single stressor (Krasensky and Jonak, 2012; Miller et al., 2017). Although interactions between salinity, one of the most important abiotic stresses affecting plant productivity worldwide, and pathogens have been recognized (Al-Sadi et al., 2010; Chojak-Koźniewska et al., 2017; Nostar et al., 2013), little is known about how their combination affects plant metabolism.

The reactions induced in plants exposed to abiotic stresses and pathogen attack are interconnected and the acclimation to concurrent stress is characterized by a combination of shared and tailored responses (Pandey et al., 2015). At the metabolic level, acclimation is associated with increased demand for carbon skeletons, energy and reducing equivalents to fuel defence while simultaneously maintaining growth and development (Bolton, 2009). The profile of primary metabolic responses in maize exposed to multiple abiotic stress differed from those caused by the individual stressors (Sun et al., 2016). In plants coping with multiple environmental stresses sensed by roots and leaves, this metabolic plasticity may allow to activate an appropriate defence response. Irrespective of the primary target for a given stressor, the information is transmitted throughout the entire plant (Baxter et al., 2014). Thus, the communication between roots and leaves is important for integrating the whole-plant acclimation response. Understanding these mechanisms is crucial for the development of high-yielding multiple stress-tolerant crops.

Besides its major function in photoassimilate production, the photosynthetic apparatus acts as highly sensitive sensor of stresses because the excitation pressure influencing the photosynthetic light energy capture and carbon fixation is a common result of most, if not all, adverse environmental conditions (Bechtold et al., 2005; Berger et al., 2007; Kuźniak et al., 2010). Under stress, the balance between light

* Corresponding author.
E-mail addresses: j.chojak@ihar.edu.pl (J. Chojak-Koźniewska), elzbieta.kuzniak@biol.uni.lodz.pl (E. Kuźniak).
[1] J. Chojak-Koźniewska and E. Kuźniak take responsibility for the integrity of the work as a whole.

https://doi.org/10.1016/j.plaphy.2017.12.015
Received 30 November 2017; Accepted 6 December 2017
Available online 07 December 2017
0981-9428/

energy absorbed through photosystem II (PSII) and the photosynthetic electrons final consumption through metabolic sinks is disturbed (Kuźniak et al., 2016; Wituszyńska et al., 2013). This deregulation of the chloroplast metabolism initiates signals which are important for the integration of diverse environmental stresses in central metabolism and for elicitation of the systemic acclimation/defence response (Wulff-Zottele et al., 2010; Karpiński et al., 2013).

Phosphoenolpyruvate carboxylase (PEPC) and NADP-malic enzyme (NADP-ME), key enzymes in the C4 and CAM (Crassulacean Acid Metabolism) photosynthetic carbon cycle, have different non-photosynthetic roles in C3 plants (Doubnerová and Ryšlavá, 2011). The cytosolic NADP-ME supplies NADPH for biosynthetic pathways, regulates malate concentration, intracellular pH and turgor pressure in the guard cells whereas the chloroplastic NADP-ME fulfils positive function in lipid biosynthesis (Hýskova and Ryslava, 2013). PEPC supports carbon–nitrogen interactions by replenishment of the tricarboxylic acid (TCA) cycle with oxaloacetate, supplies carbon skeletons for ammonium assimilation, contributes to the fixation of respired CO_2, to the homeostasis of malate and guard cell metabolism during stomatal opening (O'Leary et al., 2011; Ryšlavá et al., 2003).

The enhanced activities of PEPC and/or NADP-ME have been found in C3 plants encountering environmental stresses (Doubnerová and Ryšlavá, 2011), indicating that the β-carboxylation-related processes could be involved in the acclimatory reprogramming of plant metabolism. Under stress, when the stomata are closed and photosynthesis is reduced, the activity of PEPC re-fixing the respired CO_2 can be important for activation of plant defence mechanisms. Plants overexpressing PEPC were characterized by increased activities of NADP-ME and NADP-isocitrate dehydrogenase (NADP-ICDH) and total contents of pyruvate as well as glutamate and aspartate, which may indicate that the TCA cycle and the subsequent amino acid synthesis were enhanced in these plants (Miyao and Fukayama, 2003). Cytosolic NADP-ICDH producing NADPH has been shown to contribute to redox homeostasis and to regulation of pathogen responses in *Arabidopsis* leaves (Mhamdi et al., 2010).

In C3 plants, PEPC, NADP-ME and NAD-malate dehydrogenase form a metabolic cycle which can be advantageous under stress. Besides providing CO_2 for Calvin cycle, it can deliver oxaloacetate for TCA cycle where it is converted to 2-oxoglutarate which can be used in nitrogen assimilation and in biosynthesis of osmotically active compounds such as proline. Moreover, this cycle produces NADPH for detoxification of stress-induced reactive oxygen species (ROS) via the ascorbate-glutathione cycle, for membrane repair processes and for biosynthesis of secondary metabolites contributing to plant defence against pathogens (Doubnerová and Ryšlavá, 2011).

Organic acid metabolism is of fundamental importance for several biochemical pathways, including energy production as well as formation of precursors for amino acid biosynthesis and it is also implicated in plant acclimation to environmental stress (López-Millán et al., 2000). Malate plays an important role in the transport of reducing equivalents to the cytosol by an oxaloacetate-malate shuttle at the inner membrane of chloroplasts and mitochondria. In C3 plants, malate has been proposed to be an important respiratory substrate, such that a significant fraction of pyruvate enters the TCA cycle via the combined reactions of PEPC, malate dehydrogenase (MDH) and NAD-malic enzyme. PEPC-derived malate imported into the mitochondria replenishes the TCA cycle intermediates (O'Leary et al., 2011). Citrate can act not only as a metabolite of the reactions associated with the TCA cycle, but also as an important acetyl donor for acetyl–CoA synthesis (Popova and Pinheiro de Carvalho, 1998). In CAM plants, malate and citrate are internal reservoirs of both CO_2 and reducing equivalents for later use in the Calvin cycle. At excess irradiance during the day, when the stomata are closed, citrate decarboxylation contributes more to the accumulation of CO_2 for re-assimilation by RubisCO than malate. As the energy demand for the turnover of citrate is higher than for malate, the accumulation of citrate has been suggested to be advantageous for the harmless use of energy under excess excitation pressure (Duarte et al., 2013). The potential significance of this mechanism in other plants, however, remains to be elucidated.

To study the metabolic interactions between salinity and biotic stress responses, we exposed cucumber plants to single and combined treatments with NaCl and bacterial pathogen. We focused on the biochemical processes linked to carboxylate metabolism in plants exposed to a sequential action of NaCl and *Pseudomonas syringae* pv *lachrymans* (Psl) causing the angular leaf spot, the second most severe disease of cucumber (Olczak-Woltman et al., 2009). We measured chlorophyll *a* fluorescence as well as organ-dependent (root vs leaves) profiles of PEPC, NADP-ME, NADP-ICDH and fumarase activities, malic and citric acids contents and the expression of *NADP-ICDH* and *NADP-ME*.

2. Materials and methods

2.1. Plant material and experimental design

Cucumber plants ('Cezar F1', PlantiCo Zielonki, Poland) were grown as described by Chojak-Koźniewska et al. (2017). Three-week-old plants were first exposed to salt stress and irrigated for seven days with 50 mM or 100 mM NaCl. Thereafter NaCl application was ceased and the fully expanded leaves were inoculated with *P. syringae* pv *lachrymans* (Psl; strain No IOR, 1990 obtained from the Bank of Plant Pathogens of the Institute of Plant Protection in Poznań, Poland) according to Kuźniak et al. (2013). After inoculation, all plants were irrigated with water. The experimental variants were as follows: control; Psl (infected plants); 50 mM NaCl; 100 mM NaCl (salt-treated, non-infected plants); 50 mM NaCl + Psl and 100 mM + Psl (salt-treated and infected plants). Analyses were performed in leaves and roots seven days after salt treatment, i.e. at the time point of inoculation (T0) and 2, 5, 7 days after Psl inoculation (T2, T5, T7), respectively.

2.2. Measurements

2.2.1. Chlorophyll fluorescence

Chlorophyll fluorescence in leaves of cucumber plants adapted to dark for 20 min was measured by Handy FluorCam FC 1000-H (Photon System Instruments, Brno, Czech Republic). Leaves imaging process was performed via FluorCam 7.0 (version 1.0.27.0) according to the protocol of multiple exposure saturating light in mode of pulse amplitude (PAM). Minimum and maximum fluorescence yields F_0 and F_m, were obtained before and after a saturating pulse, respectively. Fraction of photosystem (PS) II centres that are open (qL) was automatically measured. Maximum PSII quantum yield (F_v/F_m) was calculated by the formula: $F_v/F_m = (F_m - F_0)/F_m$, non-photochemical quenching was quantified by the parameter $NPQ = (F_m\ F_m^{Lss})/F_m^{Lss}$, and 'vitality index' as $R_{Fd} = (F_m - F_t)/F_t$.

2.2.2. Determination of citric and malic acids

Citric (CA) and malic (MA) acids contents were analysed using enzymatic test kits (Boehringer Mannheim, Germany). Tissues (0.5 g) were homogenized in 4% $HCLO_4$ or in triple distilled water, respectively for CA or MA determination. After centrifugation, the supernatant was adjusted to pH 8.0 and used to determine the contents of CA and MA (mg g^{-1} FW) according to the manufacturer's recommendations.

2.2.3. Enzymes assays

Fresh tissues (0.5 g) were homogenized in 2.5 ml of extraction buffer containing 0.05 M Tris-HCl (pH 7.6), 1 mM EDTA, 1 mM $MgCl_2$, 10 mM β-mercaptoethanol, 1 mM dithiothreitol and 0.5% polyvinylpyrrolidone. After centrifugation (15 min, 15 000 g, 4 °C), the supernatant was used for determination of NADP-ICDH (EC 1.1.1.42) and PEPC (EC 4.1.1.31) activities. For NADP-ME (EC 1.1.1.42) activity assay, samples were prepared in 0.1 M Hepes-KOH (pH 8.0) containing: