Journal of Plant Physiology 205 (2016) 93–96

Contents lists available at ScienceDirect

Journal of Plant Physiology

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

Abiotic stress and phytohormones affect enzymic activity of 1-*O*-(indole-3-acetyl)-β-D-glucose: *myo*-inositol indoleacetyl transferase from rice (*Oryza sativa*)

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ARTICLE INFO

Article history:
Received 24 May 2016
Received in revised form 19 July 2016
Accepted 19 July 2016
Available online 13 September 2016

Keywords:
IAA
IAA ester conjugates
IAInos synthase
SCPL acyltransferase
Oryza sativa

SUMMARY

Indole-3-acetic acid (IAA) conjugation is a part of mechanism regulating free auxin concentration. 1-*O*-(indole-3-acetyl)-β-D-glucose: *myo*-inositol indoleacetyl transferase (IAInos synthase) is an enzyme involved in IAA-ester conjugates biosynthesis. Biotic and abiotic stress conditions can modulate auxin conjugates formation in plants. In this study, we investigated effect of plant hormones (IAA, ABA, SA and 2,4-D) and abiotic stress (drought and salt stress: 150 mM NaCl and 300 mM NaCl) on expression level and catalytic activity of rice IAInos synthase. Enzymic activity assay indicated that all tested phytohormones affected activity of IAInos synthase, but only ABA had inhibiting effect, while IAA, SA and 2,4-D activated the enzyme. Drought and salt stress induced with lower NaCl concentration resulted in decreased activity of IAInos synthase, but 300 mM NaCl had no effect on the enzyme. Despite observed differences in enzymic activities, no changes of expression level, tested by semiquantitative RT-PCR and Western blot, were detected. Based on our results it has been supposed that plant hormones and stress conditions affect IAInos synthase activity on posttranslational level.

1. Introduction

Free indole-3-acetic acid (IAA) concentration in plants is tightly regulated by several processes occurring in response to some developmental and environmental signals. One of the mechanisms responsible for auxin homeostasis is formation of IAA conjugates (Bajguz and Piotrowska, 2009). The conjugated IAA is thought to function as auxin storage and also to be involved in auxin transport, protection against peroxidative degradation and auxin excess detoxification (Woodward and Bartel, 2005). Depending on the character of the molecule and bond via which it is conjugated to auxin, IAA conjugates are divided in two groups: ester and amide conjugates (Korasick et al., 2013). Predominant form of IAA conjugates in monocots are its ester conjugates with sugars or *myo*-inositol (Ludwig-Müller, 2011). Endosperm of maize (*Zea mays*) kernels contains 97–99% of IAA in the form of ester conjugates (Jensen and Bandurski, 1994), half of which consists of IAA-*myo*-inositol or IAA- *myo*-inositol glycosides (Ueda and Bandurski, 1974). In rice (*Oryza sativa*), the amount of IAA ester conjugates is estimated as 62–70% of total endogenous IAA (Hall, 1980).

The first stage of IAA ester conjugates formation is synthesis of 1-*O*-indole-3-acetyl-β-D-glucose (IAGlc) (Michalczuk and Bandurski, 1980) by IAGlc synthase according to following reaction:

IAA + UDP-glucose → 1-*O*-IAGlc + UDP

In maize and rice, the second stage of IAA ester conjugates synthesis is formation of indole-3-acetyl-*myo*-inositol (IAInos) via transfer of IAA moiety from 1-*O*-IAGlc to *myo*-inositol (Kęsy and Bandurski 1990):

1-*O*-IAGlc + *myo*-inositol → IAInos + glucose

This reaction is catalyzed by 1-*O*-(indole-3-acetyl)-β-D-glucose: *myo*-inositol indoleacetyl transferase (IAInos synthase) which enzymic activity has only been detected in maize and rice so far (Kęsy and Bandurski 1990; Ciarkowska et al., 2013). Based on amino acid sequence analysis this enzyme has been classified as a member of serine carboxypeptidase-like (SCPL) acyltransferases family, a group of glycosylated enzymes which show homology to serine carboxypeptidases (Kowalczyk et al., 2003). Enzymes belonging to this family catalyze transfer of an acyl moiety from energy-rich 1-*O*-β-

Abbreviations: 2,4-D, 2,4-dichlorophenoxyacetic acid; ABA, abscisic acid; GA_3, gibberellic acid; *GH3*, *Gretchen Hagen3*; IAA, indole-3-acetic acid; IAGlc, 1-*O*-indole-3-acetyl-β-D-glucose; IAInos, indole-3-acetyl-*myo*-inositol; JA, jasmonic acid; MeJA, methyl jasmonate; NAA1, naphtalene acid; SA, salicylic acid; SCPL, serine carboxypeptidase-like; UDP, guridine diphosphate glucose.

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http://dx.doi.org/10.1016/j.jplph.2016.07.018
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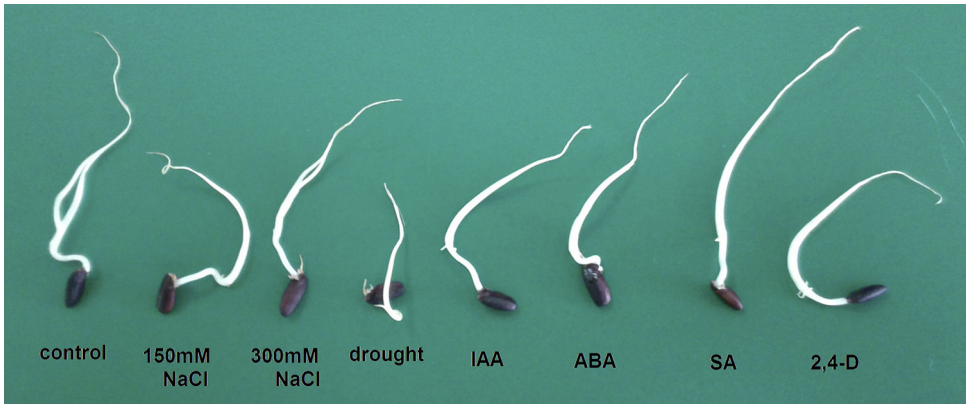

Fig. 1. 6-day old rice seedlings after 24 h incubation under stress conditions or in presence of phytohormone.

glucose esters to nucleophilic group of acceptor molecule (Mugford and Milkowski, 2012). SCPL acyltransferases play important role in plant secondary metabolism pathways, such as biosynthesis of sinapate esters (Lehfeldt et al., 2000; Shirley et al., 2001; Stehle et al., 2009) and formation of auxin conjugates (Kowalczyk et al., 2003; Starzyńska and Kowalczyk 2012). They are also involved in regulation of defense responses against biotic and abiotic stress (Liu et al., 2008; Mugford et al., 2009).

In our previous studies we have identified IAInos synthase activity in rice seedlings (Ciarkowska et al., 2013). The activity of this enzyme was easily detectable in 6-days old seedlings, but extremely low in younger plants. We have also found cDNA sequence corresponding to IAInos synthase from rice in UniProt database. Using heterologous expression system we have confirmed that this sequence encodes catalytically active IAInos synthase (unpublished results). In this study we describe how subjecting to abiotic stress (salt stress, drought) and phytohormones: IAA, abscisic acid (ABA), salicylic acid (SA) and 2,4-dichlorophenoxyacetic acid (2,4-D) affects expression and activity of IAInos synthase in 6-days old rice (*Oryza sativa*) seedlings.

2. Material and methods

2.1. Plant material

Black rice wholemeal (Bio Planet, Poland) was used as plant material. Rice (*Oryza sativa*) seeds were soaked in distilled water at 37 °C for 24 h. Plants were grown in darkness at 27 °C on Petri dishes. For the abiotic stress effects, 5-d-old seedlings were transferred to 150 mM or 300 mM NaCl solutions or exposed to drought conditions by transferring them to Petri dishes without water for additional 24 h. For the phytohormone effects, 5-d-old seedlings were incubated for 24 h in 10 μM IAA, 10 μM ABA, 10 μM SA or 0.05 μM 2,4-D solutions. Control seedlings were grown in distilled water.

2.2. Tissue homogenization

Rice seedlings were homogenized (1 g tissue: 1 mL buffer) with 50 mM HEPES buffer, pH 7.4, using mortar and pestle. The homogenates were centrifuged at 10,000 × *g* for 10 min at 4° C (Sigma Sartorius 3 K 30 Centrifuge, 12154 rotor, Germany). The supernatant fluid was used for analysis.

2.3. Enzyme activity assay

Enzymic activity of IAInos synthase was determined in a total volume of 8 μL containing 27.4 mM HEPES buffer, pH 7.4, 13.2 mM UDPG, 3.5 mM IAA, 0.016 μCi [2′-^{14}C]IAA (55 mCi mmol^{-1}), 13.2 mM *myo*-inositol, 19.4 mM D-gluconic acid lactone, 2.2 mM $MgCl_2$ and 5.5 μU of recombinant IAGlc synthase, with 3 μL of the supernatant fluid from tissue homogenates. The reaction was stopped after 2 h incubation in 30 °C by drying 4 μL of aliquots on Silica Gel F_{260} TLC plate (Merck). TLC was performed using ethyl acetate: *n*-butanone: ethanol: water (5: 3: 1: 1) as a solvent. For indole compounds visualization, the plate was stained with Van Urk-Salkowski reagent (Ehmann, 1977). Bands corresponding to IAInos were excised and placed in a vial with 2 mL EcoLite (+) scintillation fluid (ICN). Radioactivity level was measured in Wallac 1409 liquid scintillation counter (Turku, Finland). For each experiment we used 3 biological repetitions. Data are presented as mean ± standard deviation (SD).

2.4. RNA isolation and reverse transcription-PCR (RT-PCR) analysis

Total RNA was extracted from the rice seedlings using Gene MATRIX Universal RNA/miRNA Purification Kit (EURx). RNA samples were pretreated with RNA-se free DNase I (Thermo Scientific) to remove any contaminating genomic DNA. First-strand cDNA synthesis was performed using 1 μg of RNA with RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific) according to the manufacturer's instructions. PCRs of 20 μL with 1.2 μL of template cDNA were performed with 1.2 U of Maxima Hot Start *Taq* DNA Polymerase (Thermo Scientific). IAInos synthase gene (UniProt EEC73124.1) expression was analyzed using gene-specific primers: F: 5′-CGTGCAATGGGAAGTACTGG-3′; R: 5′-AGCAAGGCATGATCTCCACT-3′. Actin gene expression (control) was analyzed using following gene-specific primers: F: 5′-GGACTCTGGTGATGGTGTCA-3′; R: 5′-TGTGCTGAGAGATGCCAAGA-3′. Primers sequences were generated using Primer3. Thermo cycling was performed using T100™ Thermal Cycler (BIO-RAD) with the following cycling conditions: 95 °C for 4 min,

35 cycles of 94 °C for 45 s, 50.7 °C (IAInos synthase) or 53 °C (actin) for 45 s; 72 °C for 90 s, followed by final extension of 72 °C for 10 min. Samples obtained with RT-PCR were separated by electrophoresis in 1% agarose gel in TAE buffer using DELFIN electrophoresis apparatus (DNA Gdańsk).

2.5. SDS-PAGE and western blot analysis

SDS-PAGE was performed according to the method of Ogita and Markert (1979) in a Mini Protean II electrophoresis apparatus (Bio-Rad) using 12% (w/v) running gel and 6% (w/v) stacking gel. A supernatant fluid of rice seedling extract containing 20 μg of protein was subjected to the SDS-PAGE. The protein mixture