Plant Physiology and Biochemistry 82 (2014) 209–217

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

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

Research article

Modulation of auxin content in Arabidopsis confers improved drought stress resistance

Haitao Shi [a, 1], Li Chen [b, 1], Tiantian Ye [a, c], Xiaodong Liu [d], Kejian Ding [b], Zhulong Chan [a, *]

[a] Key Laboratory of Plant Germplasm Enhancement and Specialty Agriculture, Wuhan Botanical Garden, Chinese Academy of Sciences, Wuhan 430074, China
[b] College of Plant Protection, Anhui Agricultural University, Hefei 230036, China
[c] University of Chinese Academy of Sciences, Beijing 100039, China
[d] College of Agronomy, Xinjiang Agricultural University, Urumqi, Xinjiang 830052, China

ARTICLE INFO

Article history:
Received 21 March 2014
Accepted 13 June 2014
Available online 20 June 2014

Keywords:
Abscisic acid
Arabidopsis
Auxin
Drought stress
Reactive oxygen species
Metabolite

ABSTRACT

Auxin is a well-known plant phytohormone that is involved in multiple plant growth processes and stress responses. In this study, auxin response was significantly modulated under drought stress condition. The *iaaM-OX* transgenic lines with higher endogenous indole-3-acetic acid (IAA) level and IAA pre-treated wild type (WT) plants exhibited enhanced drought stress resistance, while the *yuc1yuc2yuc6* triple mutants with lower endogenous IAA level showed decreased stress resistance in comparison to non-treated WT plants. Additionally, endogenous and exogenous auxin positively modulated the expression levels of multiple abiotic stress-related genes (*RAB18*, *RD22*, *RD29A*, *RD29B*, *DREB2A*, and *DREB2B*), and positively affected reactive oxygen species (ROS) metabolism and underlying antioxidant enzyme activities. Moreover, auxin significantly modulated some carbon metabolites including amino acids, organic acids, sugars, sugar alcohols and aromatic amines. Notably, endogenous and exogenous auxin positively modulated root architecture especially the lateral root number. Taken together, this study demonstrated that auxin might participate in the positive regulation of drought stress resistance, through regulation of root architecture, ABA-responsive genes expression, ROS metabolism, and metabolic homeostasis, at least partially.

1. Introduction

Drought is one of the major environmental stresses that drastically limit crop growth and production worldwide (Harb et al., 2010; Shi et al., 2012). To respond to drought stress, plants have evolved complex biochemical and physiological strategies to allow them adapt to sudden environmental changes (Cutler et al., 2010; Harb et al., 2010; Hirayama and Shinozaki, 2010; Krasensky and Jonak, 2012; Qin et al., 2011; Shi et al., 2013a, b). Most of these adaptation responses are regulated by several secondary messengers including some plant hormones (Cutler et al., 2010; Du et al., 2013a,b; Harb et al., 2010; Hirayama and Shinozaki, 2010; Qin et al., 2011). For example, plant endogenous abscisic acid (ABA) is largely activated in response to drought stress, and the induced ABA can trigger stomatal closure and modulate the expression of downstream genes and physiological changes (Cutler et al., 2010; Harb et al., 2010; Hirayama and Shinozaki, 2010; Qin et al., 2011; Seki et al., 2007).

As a well-known plant phytohormone, auxin is an important regulator of several diverse processes including seed dormancy, tropistic growth, root patterning, cell differentiation, flower organ development (Zhao, 2010; Zhao et al., 2001). In recent years, accumulating evidence suggests the potential link between auxin response and abiotic and biotic stresses, especially the crosstalks between auxin and other plant hormones such as salicylic acid (SA) and ABA (Kazan and Manners, 2009; Wang et al., 2007). For biotic stress response, increasing evidence indicates that auxin and SA act individually or via antagonistic crosstalk, suggesting the fine balance between them may be critical for plant–pathogen interaction

Abbreviations: ABA, abscisic acid; BSA, bovine serum albumin; CAT, catalase; DW, dry weight; EL, electrolyte leakage; FW, fresh weight; GC-TOF-MS, gas chromatography time-of-flight mass spectrometry; GR, glutathione reductase; GSH, reduced glutathione; GSSG, oxidized glutathione; H_2O_2, hydrogen peroxide; IAA, indole-3-acetic acid; MS, Murashige and Skoog; MU, 4-methylumbelliferone; MUG, 4-methylumbelliferyl-β-glucuronide; $O_2^{\bullet-}$, superoxide radical; POD, peroxidase; ROS, reactive oxygen species; SA, salicylic acid; SOD, superoxide dismutase; WT, wild type.

* Corresponding author. Tel.: +86 27 87510823; fax: +86 27 87510251.
E-mail address: zhulongch@wbgcas.cn (Z. Chan).

[1] Equal contributors: these authors contributed equally to this work.

http://dx.doi.org/10.1016/j.plaphy.2014.06.008
0981-9428/

(Kazan and Manners, 2009; Wang et al., 2007). For abiotic stress response, Du et al. (2012, 2013b) found that the change in auxin homeostasis could influence ABA synthesis, and that the balance of auxin and ABA homeostasis played a crucial role in diverse stress responses. Additionally, several transgenic plants (*PDS-RNAi* transgenic rice, *OsGH3-2* over-expressing rice, *AtYUCCA6* over-expressing Arabidopsis plants, and *AtYUCCA6* over-expressing potato plants) with modulated endogenous endogenous indole-3-acetic acid (IAA) level exhibited auxin-related developmental phenotypes together with affected drought stress resistance (Du et al., 2012, 2013b; Kim et al., 2013; Lee et al., 2012). However, the direct link between auxin and drought stress response remains unclear, and the underlying physiological and molecular mechanisms are also unknown.

In this study, the transgenic plants with modulated IAA level were used to dissect the potential involvement of auxin in drought stress response in Arabidopsis. Additionally, the physiological, molecular, and metabolic mechanisms modulated by endogenous and exogenous auxin were also partially revealed. This study will provide some insights into understand the physiological and molecular mechanisms of auxin-mediated drought stress resistance in Arabidopsis.

2. Materials and methods

2.1. Plant materials and growth conditions

Arabidopsis thaliana seeds of Columbia ecotype were sterilized with 10% (w/v) NaClO and then washed with deionized water. After stratification at 4 °C for 3 days in darkness, the seeds were then sown on Murashige and Skoog (MS) medium plate containing 1% (w/v) sucrose or in soil in the growth chamber. The growth chamber was controlled at 23 °C and at an irradiance of about 150 μmol quanta m^{-2} s^{-1}, with 65% relative humidity under 16 h light and 8 h dark cycles. Nutrient solution was watered twice every week to keep the well growth condition. The transgenic lines of *iaaM-OX* and the *yuc1yuc2yuc6* triple mutant have been described previously (Cheng et al., 2006; Liu et al., 2013; Mai et al., 2011; Qin et al., 2005).

2.2. Plant drought stress treatment and stress resistant assay

For drought stress treatment, 14-day-old Arabidopsis plants in pots were subjected to control condition (well-watering) and drought condition (withholding water) in the soil for 21 days. To investigate the effect of exogenous IAA on drought stress resistance, 7-day-old Arabidopsis plants were watered with 1 μM IAA for 7 days before drought stress treatment. More than three pots from each variety were used in each independent experiment, and all these pots of plants were rotated daily during drought stress treatment to minimize the environment effect. The electrolyte leakage (EL) of plant leaves from different lines was determined under control and drought stress conditions as Shi et al. (2012, 2013a, b,c, 2014a,b) described. The survival rate of stressed plants was recorded after 4 days of recovery from 21 days of drought stress treatment. All experiments were repeated three times.

2.3. Quantification of GUS activity

GUS activity was quantified as Jefferson et al. (1987) described. Briefly, 0.2 g of *DR5::GUS* plants (14-day-old plants under control and drought stress conditions for 0, 5, 15, 20 days, respectively) were homogenized in liquid nitrogen and the extracts were suspended with 1 ml of extraction buffer [50 mM sodium phosphate (pH 7.0), 0.1% (m/v) sodium lauryl sarcosine, 10 mM Na_2EDTA, 10 mM 2-mercaptoethanol, and 0.1% (v/v) Triton X-100]. After centrifugation at 12,000 rpm for 10 min at 4 °C, the supernatants were used to assay GUS activity based on the obtained amount of 4-methylumbelliferone (MU) from the substrate 4-methylumbelliferyl-β-glucuronide (MUG) with known concentrations of MU as a standard using Infinite M200 Microplate reader (Tecan, Männedorf, Queensland). The protein concentration of the extracts was assayed using the Bradford method (Bradford, 1976) with bovine serum albumin (BSA) as a standard.

2.4. Assays of relative leaf water loss rate in vitro

Relative leaf water loss rate was determined as Shi et al. (2012, 2013b, c) described, and was expressed as % change in leaf fresh weight (FW) *in vitro*.

2.5. Determination of hydrogen peroxide (H_2O_2) content and superoxide radical ($O_2^{\bullet -}$) content

H_2O_2 content and $O_2^{\bullet -}$ content was quantified using the titanium sulfate regent that formed peroxide-titanium complex and plant $O_2^{\bullet -}$ ELISA Kit (10-40-488, Bejing Dingguo, China) based on antibody-antigen-enzyme-antibody complex, respectively, according to Shi et al. (2012, 2013a, b,c, 2014a,b) described.

2.6. Determination of antioxidant enzymes activities

Four antioxidant enzymes activities including catalase (CAT, EC 1.11.1.6), superoxide dismutase (SOD, EC 1.15.1.1), peroxidase (POD, EC 1.11.1.7) and glutathione reductase (GR, EC 1.6.4.2) were assayed using CAT Assay Kit (S0051, Haimen Beyotime, China), Total SOD Assay Kit (S0102, Haimen Beyotime, China), Plant POD Assay Kit (A084-3, Nanjing Jiancheng, China) and GR Assay Kit (S0055, Haimen Beyotime, China), respectively, according to the manufacturer's instructions as Shi et al. (2012, 2013a, b, c, 2014a, b) described.

2.7. RNA isolation and quantitative real-time PCR

Total RNA was isolated using TRIzol reagent (Invitrogen, California, USA) according to the manufacturer's instruction. After digestion with RQ1 RNase-free DNase (Promega, Wisconsin, USA) to avoid possible genomic DNA contamination, 2 μg of total RNA was used to synthesis first-strand cDNA using reverse transcriptase (TOYOBO, Osaka city, Japan). Quantitative real-time PCR was performed using CFX96™ Real Time System (BIO-RAD, California, USA) with iQ™ SYBR® Green Super mix (BIO-RAD, California, USA) as Shi et al. (2013b, c) described. The gene expression levels were standardized with *ubiquitin 10* (*UBQ10*) using comparative ΔΔCT method, and the specific primers were listed in Supplementary Table S2.

2.8. Quantification of metabolites

Plant metabolite extraction and metabolite derivatization were performed as Lisec et al. (2006) described. Then the derivatizated extracts was injected into a DB-5MS capillary (30 m × 0.25 mm × 0.25 μm, Agilent J&W GC column, California,USA) using gas chromatography time-of-flight mass spectrometry (GC-TOF-MS) (Agilent 7890A/5975C, California, USA) as Shi et al. (2014b) described. The metabolites were identified based on retention time index specific masses and reference spectra in mass spectral libraries (NIST 2005, Wiley 7.0), and the quantification of metabolites was performed based on the pre-