Plant Physiology and Biochemistry 127 (2018) 553–560

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

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

Research article

Genome-wide transcriptomic analysis of BR-deficient Micro-Tom reveals correlations between drought stress tolerance and brassinosteroid signaling in tomato

Jinsu Lee[a,1], Donghwan Shim[b,1], Suyun Moon[a], Hyemin Kim[a], Wonsil Bae[a], Kyunghwan Kim[a], Yang-Hoon Kim[c], Sung-Keun Rhee[c], Chang Pyo Hong[d], Suk-Young Hong[e], Ye-Jin Lee[e], Jwakyung Sung[e,**], Hojin Ryu[a,*]

[a] Department of Biology, Chungbuk National University, Cheongju 28644, Republic of Korea
[b] Department of Forest Genetic Resources, National Institute of Forest Science, Suwon 16631, Republic of Korea
[c] Department of Microbiology, Chungbuk National University, Cheongju, 28644, Republic of Korea
[d] TheragenEtex Bio Institute, Suwon 16229, Republic of Korea
[e] Division of Soil and Fertilizer, National Academy of Agricultural Science, RDA, Wanju, 27715, Republic of Korea

ARTICLE INFO

Keywords:
Brassinosteroids
Phytohormone
Drought stress
NGS
Tomato

ABSTRACT

Brassinosteroids (BRs) are plant steroid hormones that play crucial roles in a range of growth and developmental processes. Although BR signal transduction and biosynthetic pathways have been well characterized in model plants, their biological roles in an important crop, tomato (*Solanum lycopersicum*), remain unknown. Here, cultivated tomato (WT) and a BR synthesis mutant, Micro-Tom (MT), were compared using physiological and transcriptomic approaches. The cultivated tomato showed higher tolerance to drought and osmotic stresses than the MT tomato. However, BR-defective phenotypes of MT, including plant growth and stomatal closure defects, were completely recovered by application of exogenous BR or complementation with a *SlDWARF* gene. Using genome-wide transcriptome analysis, 619 significantly differentially expressed genes (DEGs) were identified between WT and MT plants. Several DEGs were linked to known signaling networks, including those related to biotic/abiotic stress responses, lignification, cell wall development, and hormone responses. Consistent with the higher susceptibility of MT to drought stress, several gene sets involved in responses to drought and osmotic stress were differentially regulated between the WT and MT tomato plants. Our data suggest that BR signaling pathways are involved in mediating the response to abiotic stress via fine-tuning of abiotic stress-related gene networks in tomato plants.

1. Introduction

Sessile plants face challenging conditions for survival as they are vulnerable to surrounding environmental changes at all stages of their growth and development. The ability to respond rapidly to a range of changing conditions is crucial for the survival of individual plants and successive generations. Abiotic/biotic stresses lead to various changes in morphological, biochemical, and physiological cellular responses in plants (Bass and Lazar, 2016; Zhu, 2016). To cope with the diverse range of environmental stresses, terrestrial plants have developed integrated approaches involving internal and external signaling pathways (Liu and He, 2017; Qi et al., 2017). Plant hormones are a critical part of the internal signaling network, and play central roles in the coordinated regulation of plant growth and development in response to external signal cues (Liu and He, 2017; Qi et al., 2017). In particular, plant hormones mediate flexible responses to environmental changes via a suite of biosynthetic and signaling responses (Suzuki, 2016).

Brassinosteroid (BR) is a unique plant steroid hormone that plays key roles in plant growth and development during early seedling development, flowering, root development, vascular development, and

* Corresponding author.
** Corresponding author.
E-mail addresses: jinsulee90@gmail.com (J. Lee), shim104@korea.kr (D. Shim), sooym21@gmail.com (S. Moon), gpalsrla93@gmail.com (H. Kim), wsbae80@gmail.com (W. Bae), kyungkim@chungbuk.ac.kr (K. Kim), kyh@chungbuk.ac.kr (Y.-H. Kim), rhees@chungbuk.ac.kr (S.-K. Rhee), changpyo.hong@theragenetex.com (C.P. Hong), syhong67@korea.ac.kr (S.-Y. Hong), leeyj418@korea.ac.kr (Y.-J. Lee), jksung@korea.kr (J. Sung), hjryu96@chungbuk.ac.kr, hjryu96@gmail.com (H. Ryu).
[1] These authors contributed equally to this work as first authors.

https://doi.org/10.1016/j.plaphy.2018.04.031
Received 2 February 2018; Received in revised form 18 April 2018; Accepted 24 April 2018
Available online 27 April 2018

photomorphogenesis, and is also involved in multiple stress tolerance responses (Clouse, 2011; Nolan et al., 2017). Extensive genetic and molecular studies in model plants identified the main components of BR-related responses and their interactions with other signaling pathways (Hao et al., 2013). BR signaling is initiated by BR binding directly to the BRASSINOSTEROID-INSENSITIVE1 (BRI1) on the cell surface (Kinoshita et al., 2005). Subsequent activation of two key transcription factors, BRASSINAZOLE-RESISTANT1 (BZR1) and BRI1-EMS-SUPPRESSOR 1 (BES1), leads to dynamic transcriptional reprogramming (Wang et al., 2006). Despite well-defined canonical BR signaling pathways, it remains unknown how the simple BR signaling cues can modulate diverse physiological processes during whole plant cycles. Recent studies showed that crosstalk with plant hormones including auxin, salicylic acid, jasmonic acid, ethylene, and ABA produced a range of physiological effects associated with BR signaling (Wang et al., 2014). Numerous studies also described the critical roles of BR in enhancing tolerance to a wide range of biotic and abiotic stresses in terrestrial plants (Gruszka et al., 2016; Li et al., 2016a). However, to date, most studies have focused on the physiological effects of exogenous BR on plant stress tolerance rather than on the genetic underpinnings of the BR response. Exogenous application of BR to induce plant immunity produces conflicting resistance phenotypes against pathogens in different organs (Hao et al., 2013), suggesting the existence of distinct tissue-specific roles for BR in plant growth and stress responses. Thus, the molecular mechanisms of BR-mediated developmental networks and their simultaneous interactions with a broad range of stress signaling pathways remain poorly understood.

Tomato (*S. lycopersicum* L.) is one of the most important crops in the world as well as an important model in plant science. The tomato genome sequence has recently been published, and its protein-coding genes have been well characterized and validated in a variety of databases (Consortium and The toma, 2012). To date, the effects of several hormones on tomato growth and development have been well studied by genome-wide global transcriptome analysis (Wang et al., 2013; Li et al., 2016b), but only rarely the molecular details of the effect of BR. Micro-Tom (MT), a dwarf cultivar of tomato, responded to exogenous application of epi-brassinolide (BL) and GAs with restored normal phenotypes. Map-based cloning revealed that MT has a mutation in the *DWARF* gene (d) leading to mis-splicing and the generation of a truncated DWARF protein (Martí et al., 2006). The *DWARF* gene encodes a cytochrome P450 enzyme catalyzing the C-6 oxidation of 6-deoxocastasterone (6-deoxoCS) to castasterone (CS) in BR biosynthesis (Bishop et al., 1999). Although these results indicate that the dwarf tomato cultivar, MT, has defects in BR biosynthetic pathways, the molecular characteristics of the BR defected responses remain unclear. Genome-wide transcriptome analysis has emerged as a useful tool for the exploration of global gene expression profile changes under diverse circumstances. Gene ontology (GO) enrichment analysis and building network models through protein-protein interactions and co-expression patterns can also be used for functional profiling of differentially expressed genes (DEGs) among different biological samples (Consortium and Gene ont, 2015). Recently, various advanced transcriptome analyses were applied in numerous organisms, enabling this technique to be widely used in identifying key transcriptomic changes, leading to improved understanding of the interplay between internal and external signaling cues during growth and developmental processes (Wang et al., 2013; Li et al., 2016b; Cheng et al., 2016; Gao et al., 2016).

In this study, we provide genetic evidence of critical physiological roles of BR in drought and osmotic stress tolerance in tomato plants. A BR-deficient MT cultivar with a loss-of-function mutation in a BR biosynthetic *DWARF* gene (Martí et al., 2006; Bishop et al., 1999) exhibited hypersensitivity to drought stress as a result of abolition of stress-induced stomatal closure. Using Illumina high-throughput mRNA sequencing (RNA-seq), we examined global transcriptional profiles in MT and a wild-type BGA cultivar tomato and identified GO enrichment networks involved in stress, plant development, and hormonal interactions. Our data provide a comprehensive picture of the transcriptomic changes during the BR-mediated stress and developmental programs in an important crop plant.

2. Materials and methods

2.1. Plant materials and growth conditions

Solanum lycopersicum cultivars BGA (WT) and MT were used. All plants were grown in a greenhouse at 23 °C under a long-day lighting regime (16 h light/8 h dark) with 60% humidity. Seedlings were grown in a plant growth chamber at 25 °C with the same lighting and humidity conditions. Surface-sterilized seeds were sown on Murashige and Skoog (MS) medium.

2.2. RNA extraction, cDNA synthesis, and qRT-PCR

Total RNAs were extracted from young leaf and shoot samples using a Total RNA extraction kit (Intron Biotechnology, Korea) according to the manufacturer's instructions. Total RNA concentration and quality were measured using a K5600 Micro-spectrophotometer (Shanghai Biotechnol Co., China). RNA OD 260/280 ratios were 1.9–2.1 and OD 260/230 ratios were 2.0–2.5. For RNA sequencing, total RNA samples were combined into two groups: WT and MT. Each sequencing sample contained RNA from three biological replicates. A first-strand synthesis KIT (Enzynomics, Korea) with oligo (dT) primers was used for cDNA synthesis from 2 μg of total RNA. The first-strand synthesis reaction was carried out at 50 °C for 60 min and then cooled on ice for 1 min. The resultant cDNA was then used for real-time quantitative PCR with a Quant Studio 3 (Applied Biosystems, USA) instrument using SYBR Green Real-time PCR Master Mix (Applied Biosystems). Primer sequences are listed in Table S6. Threshold cycle (Ct) values were used to calculate 2-ΔΔCt for expression analysis, where ΔΔCt for treated plants was determined as follows: (Ct target gene - Ct actin gene) - control plant (Ct target gene - Ct actin gene) (Livak and Schmittgen, 2001).

2.3. Sequence analysis

Raw data were deposited with the Accession Number RP130238 in the NCBI Short Read Archive database. Differential gene expression analysis was based on published protocols (Trapnell et al., 2012). Briefly, raw sequencing data were first evaluated using the FastQC program. Then the reads were 5′ trimmed according to quality score ($Q > 30$) and the adapter sequence was removed using the NGS QC toolkit (v2.2.3) (Patel and Jain, 2012). Mapping to the tomato reference genome (ensemble genome release 26) was performed using Tophat (Trapnell et al., 2009, 2012). Comparative analysis was conducted using cufflinks. Cufflinks (Trapnell et al., 2009) fpkm values for DEGs were used to generate plots. Comparative analysis and GSEA were performed using PlantGSEA (Yi et al., 2013). Annotated TAIR IDs were submitted to the tools as the query list, and suggested backgrounds were used as the selected references.

2.4. Physiological analysis of drought stress responses

To test for drought tolerance, watering was withheld from 4-week-old pot-grown MT and BGA plants for 2 weeks and plant deterioration was monitored. For leaf water loss assays, leaves were removed from fully developed 4-week-old tomato plants and placed under the same conditions. Leaf fresh weight was measured immediately after separation, then leaves were placed in Petri dishes and weight was measured over time. Cotyledon leaf epidermal peels were placed in cells of a 24-well culture plate and incubated for 30 min under light (4000 lux) in MS medium with or without 250 mM sorbitol and with or without 10 nM epi-brassinolide pH 5.7. Images of epidermal peels were taken using an inverted light microscope (Nikon, Korea), and the widths and