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Expression profiling of ABA pathway transcripts indicates crosstalk between abiotic and biotic stress responses in Arabidopsis

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ABSTRACT

Recent breakthrough on identification and characterization of PYR/PYLs as ABA receptors enables us to better understand the perception, signaling and transportation of ABA in plant. Based on publicly available microarray data, transcriptional levels of ABA signaling pathway core components were compared after stress and phytohormone treatments, including these involved in ABA metabolism, signal transduction, and catabolism. The results showed that both abiotic and biotic stress treatments increased the expression levels of ABA key metabolism and catabolism transcripts. The expression levels of PYR/PYLs were down-regulated and these of PP2Cs and ABFs were uniformly up-regulated after exogenous ABA application and under stress conditions. The results indicated that the increased ratio of PP2Cs:PYR/PYLs might be required for activation of the downstream ABA signal pathway under both abiotic and biotic stress conditions. We concluded that abiotic and biotic stress responses shared ABA signal pathway in Arabidopsis.

1. Introduction

The plant hormone ABA regulates many key processes in plants and serves as an endogenous messenger in biotic and abiotic stress responses [1–3]. Abiotic stress such as drought and high salinity results in strong increases of ABA levels accompanied by a major change in gene expression [4–7]. To date, many ABA signaling components have been identified. ABA receptors have until recently remained either elusive or contested. A family of novel START domain proteins, known as PYR/PYLs (also known as RCARs), were identified as ABA receptors by separate research groups. The results showed that several PYR/PYLs interact with and inhibit clade A PP2Cs [8–11]. Seventy-six Arabidopsis genes were identified as PP2C-type phosphatase candidates [12,13] and six of the nine PP2Cs in clade A have been identified as negative regulators of ABA response [14–19]. In contrast, SnRK2s act as positive signaling components in ABA signaling [3,20–23]. The default state of the SnRK2 protein kinases is an autophosphorylated, active state, and that the SnRK2 kinases are kept inactive by the PP2Cs through physical interaction and dephosphorylation [10]. PYR/PYLs interact with and are able to inactivate the PP2Cs after binding ABA. The ABA-bound receptors also disrupt or decrease the interaction between the PP2Cs and the SnRK2s, thus

preventing the PP2C-mediated dephosphorylation and thereby relieving inhibition of the SnRK2s [10]. Accumulation of phosphorylated SnRK2s leads to subsequent phosphorylation of the basic leucine zipper (bZIP) transcription factors called ABFs/AREBs [24,25]. The ABFs then bind to ABA-responsive promoter elements (ABRE) to induce the expression of ABA-responsive genes [26].

ABA response eventually leads to changes in gene expression. Exogenous ABA as well as conditions that increase endogenous ABA redirect the expression of part of Arabidopsis genome [4,5,27,28]. However, it is not clear how short-term and long-term stress and phytohormone treatments affect expression levels of ABA pathway core components. Recently, two groups found that ABA and abiotic stress conditions or treatments altered the relative levels of PYR/PYL/RCAR and PP2C family members and increased the PP2Cs:PYR/PYLs ratio [11,29]. These results indicated that higher PP2Cs:PYR/PYLs ratio would lead to a desensitization of the ABA response. However, these data were obtained from one specific time point and only three abiotic stress conditions were applied in one study. Furthermore, only several ABA signaling transcripts were included in these manuscripts. Considering at least 252 different receptor/PP2C/SnRK2 complexes (14 receptors × 6 PP2Cs × 3 SnRK2s) in Arabidopsis, it is important to understand how different stresses and ABA treatment change the expression of the whole ABA pathway transcripts and thus alter the sensitivity and plasticity of the response. In addition, the high dynamic range of ABA levels intrigues us to check dynamic changes of these transcripts under stress conditions.

In this study, we reported transcriptional profiling of Arabidopsis ABA pathway core components after abiotic stress, biotic stress and plant hormone treatments based on publicly available microarray

data. The aims of this manuscript are as follows: (1) to provide information to understand how abiotic and biotic stress conditions and phytohormone treatments affect the expression levels of ABA metabolism, signaling transduction, and catabolism pathway related transcripts; (2) to further understand dynamic changes of these transcripts under stress conditions; and (3) to propose a possible model for the fine-tuning of hormone responses.

2. Materials and methods

2.1. Plant materials and stress/hormone treatment

The *Arabidopsis thaliana* ecotype Columbia-0 was grown in a growth chamber under the light intensity of 100 mmol photons $m^{-2} s^{-1}$, and a 14 h light/10 h dark photoperiod at 22/18 °C. Arabidopsis seeds were imbibed at 4 °C for 4 days in the dark to promote the uniformity of seed germination, and planted on half MS medium plate. Fourteen-day-old seedlings were then transferred to the same half MS medium plate containing 40% PEG, 100 mM NaCl, or 10 μM ABA for indicated time. For cold treatment, 14-day-old seedlings were put into 4 °C for 24 h. For drought treatment, rosettes of 14-day-old seedling were detached and put at room temperature for 3 h. All of above materials were collected for RNA isolation and real time PCR analysis.

2.2. Affymetrix microarray data analysis

The Arabidopsis Genome Initiative (AGI) numbers of Arabidopsis ABA signaling pathway core components were obtained from TAIR (http://www.arabidopsis.org), including 10 of 14 PYR/PYLs (data for PYL10 to PYL13 are not available from Affymetrix ATH1 microarray), 9 clade A PP2Cs, 10 SnRK2s, and 3 ABFs. The expression levels of 5 genes involved in ABA biosynthesis [30–32], 4 genes involved in ABA catabolism [33,34], and 12 ABA-responsive genes were also checked in this study and some of them were used as positive controls. All AGI IDs were input into The Bio-Array Resource for Plant Biology (http://bar.utoronto.ca/) using the tool of "e-Northerns w. Expression Browser" [35]. AtGenExpress datasets [36], including stress, hormone, and pathogen series, were selected for transcriptional profiling analyses. All raw data were downloaded and saved as text files.

2.3. Cluster analysis

Hierarchical cluster analyses were performed on selected sets of genes using the CLUSTER program (http://bonsai.ims.u-tokyo.ac.jp/~mdehoon/software/cluster/) [37] by the uncentred matrix and

Table 1
Primer sequences used for real time PCR.

Primer name	Primer sequence
Tubulin-qF	AACCTACACCAACCTCAACC
Tubulin-qR	GTGGATTCTTGGGTATGGGAC
PYR1-qF	GCGAACACATCAACGGAAAG
PYR1-qR	CCAGATCCGATTCTCTTTCTCG
PYL1-qF	ACAGCCAGAGAATCTCAACAC
PYL1-qR	TTGATTGGGAGAGTTGGGTG
PYL3-qF	GGACGAGTTCTCAACACTAGAC
PYL3-qR	CGGTGTGCTATGAGTGATGTG
PYL7-qF	CAGTGTACCTCTGTTCTCGTC
PYL7-qR	GGTTTGTATTTCTGCGGCTG
ABI1-qF	TGAGATGGCAAGGAAGCGGATTCT
ABI1-qR	GGCTTCAAATCAACCACCACCACA
ABI2-qF	ACACGTGGCAAGAGAAGTGGAAGA
ABI2-qR	CCGCAATTCGCGACAAAGATGTGA
PP2CA-qF	AAGATCGGTACGACGTCGGTTTGT
PP2CA-qR	TCTGCACTTCTCCGCAACATGAGA
SnRK2.2-qF	TGCCGGACGGTTTAGTGAAGATGA
SnRK2.2-qR	AGGTGCCGGACTTCCATCTAACAA
SnRK2.3-qF	TTACGAGCGGATTTGCAATGCAGG
SnRK2.3-qR	AGGAGCAGGACTTCCATCCAACAA
SnRK2.6-qF	TTTGTTGCTGACCCTGCAAAGAGG
SnRK2.6-qR	TCTTCTATGCTTTGGCCCGGTTGA
SnRK2.10-qF	CCTCTCCCGTATATTTGTCACC
SnRK2.10-qR	AATGTCGGGTTCTCTTTCCTG

complete linkage method. Resulting tree figures were displayed using the software package Java Treeview (http://jtreeview.sourceforge.net/) as described by Chan et al. (5).

2.4. Quantitative real time PCR

Total RNA was extracted and purified using a QIAGEN-RNeasy Mini Kit (QIAGEN, Valencia, CA, USA) according to guidelines specified by the manufacturer. Two biological replicates from different growth chambers were prepared for each treatment. RNA was quantified using a Nanodrop spectrophotometer (ND-1000, NanoDrop Technologies, Wilmington, DE, USA) and RNA quality was assessed using a 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA) according to the manufacturer's protocol. Aliquots of 5 μg RNA were reverse transcribed using a SuperScript® III Reverse Transcriptase Kit (Invitrogen, NY, USA) under the conditions suggested by the manufacturer. Reverse transcription products were diluted 10 times in water prior to quantitative real time quantitative PCR (qRT-PCR). Aliquots of cDNA were used as template for qRT-PCR. Reactions were set up with Power SYBR® Green PCR Master Mix (Applied Biosystems) according to the manufacturer's instructions in a total
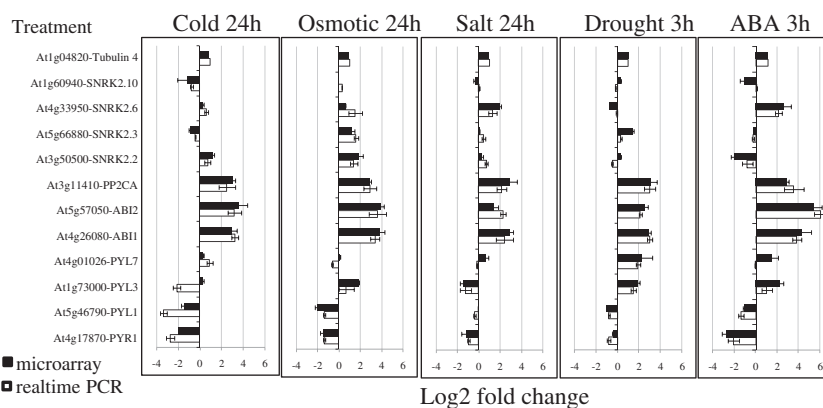

Fig. 1. Verification of expression of ABA pathway core components by stress and ABA treatments using qRT-PCR. Four PYR/PYL genes, three PP2C genes and four SnRK2 genes were selected. Two-week-old seedlings were transferred to 1/2MS plate containing 40% PEG, 100 mM NaCl, or 10 μM ABA or 4 °C for indicated time. For cold treatment, 14-day-old seedlings were put into 4 °C for 24 h. For drought treatment, rosettes of 14-day-old seedling were detached and put at room temperature for 3 h. Primers used for qRT-PCR were listed as in Table 1.
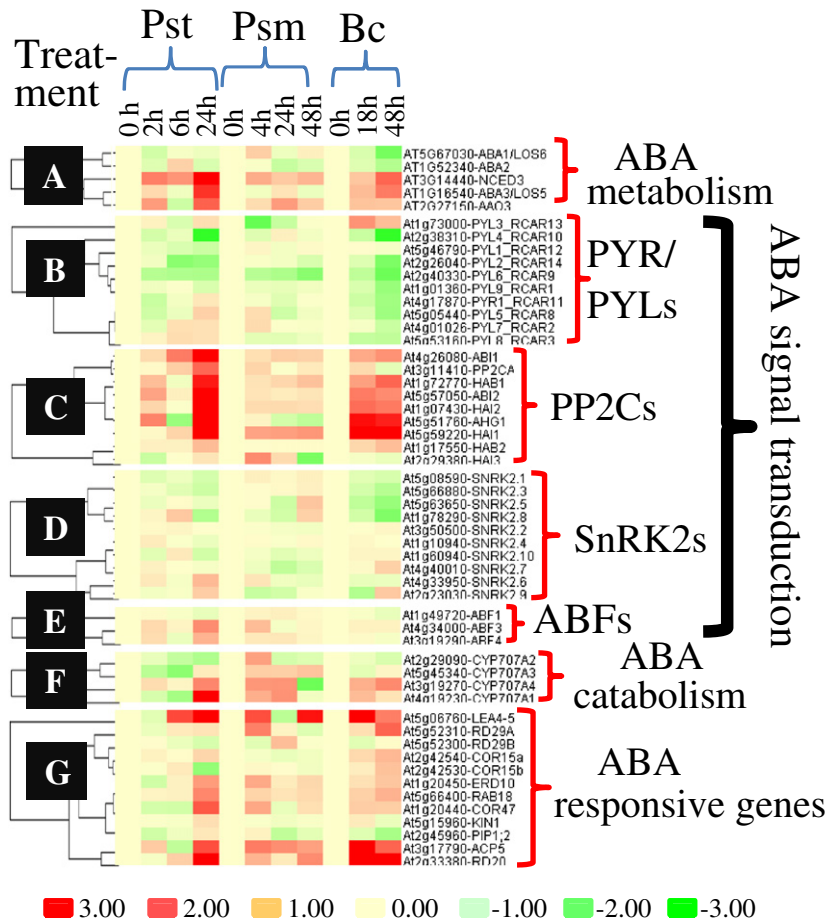
Fig. 2. Transcriptional expression of ABA signal core components in Arabidopsis shoot after abiotic stress treatment. The expression data were obtained from The Bio-Array Resource for Plant Biology (http://bar.utoronto.ca/welcome.htm) [35]. The original microarray data were from AtGenExpress and described by Kilian [36]. Cold: 4 °C; osmotic: 300 mM mannitol; salt: 150 mM NaCl; drought: dry air stream; oxidative: 10 μM methyl viologen; heat: 38 °C.

volume of 20 μl and 0.3 μM of each primer. The ABI StepOne Plus Real Time PCR System (Applied Biosystems) was used to detect amplification levels and was programmed for an initial step at 95 °C for 2 min, followed by 40 cycles of 15 s at 95 °C and 1 min at 60 °C. All reactions were run in duplicate or triplicate and average values were calculated. Quantification was performed with at least two independent experiments. The housekeeping tubulin 4 gene (AT1g04820) was used as an endogenous control. Relative expression levels of target genes and SD values were calculated using the $2^{-\Delta\Delta CT}$ method [38]. The

sequences of forward and reverse primers for each gene are listed in Table 1.

3. Results

3.1. Verification of microarray data using qRT-PCR

qRT-PCR was carried out to verify microarray data downloaded from The Bio-Array Resource for Plant Biology (http://bar.utoronto.ca/).

Fig. 3. Transcriptional expression of ABA signal pathway core components in Arabidopsis after hormone treatment. The expression data were obtained from The Bio-Array Resource for Plant Biology (http://bar.utoronto.ca/welcome.htm) [35]. ACC: 10 μM; IAA: 1 μM; ABA: 10 μM; GA3: 1 μM.

Fig. 4. Transcriptional expression of ABA signal pathway core components in Arabidopsis after pathogen inoculation. The expression data were obtained from The Bio-Array Resource for Plant Biology (http://bar.utoronto.ca/welcome.htm) [35]. Pst: 100 μg/ml lipopolysaccharides from *Pseudomonas syringae* pv. *tomato* DC3000; Psm: *Pseudomonas syringae* pv. *maculicola* ES4326 with unknown concentration; Bc: 5×10^5 spores/ml conidiospores of *Botrytis cinerea*.

In total, 4 PYR/PYLs, 3 PP2Cs, and 4 SnRK2s were randomly selected for qRT-PCR experiment. Altogether, the expression ratios measured by microarray and by qRT-PCR were highly correlated ($r > 0.95$). The trends of both increased and decreased expression for the comparison were similar except PYL3 and PYL7 under several stress conditions (Fig. 1). In addition, expression levels of NCED3 (At3g14440), ABA3/LOS5(At1g16540), and CYP707A1(At4G19230) were significantly up-regulated by abiotic stress and ABA treatments (Fig. 2D; Supplementary Fig. 1D). The results were consistent with previous observations, which showed that expression of these genes was enhanced by ABA and abiotic stress [30–32]. Furthermore, expression levels of most ABA-responsive genes were up-regulated under stress conditions or by ABA treatment (Figs. 2G, 3G, and 4G; Supplementary Fig. 1G). These data indicated that publicly available microarray data are reliable for further analyses.

3.2. Regulation of ABA metabolism transcripts by stress and exogenous ABA

NCED3, ABA3/LOS5 and AAO3 are key enzymes in the biosynthesis of ABA. Expression levels of these genes were up-regulated by abiotic stress (except heat and oxidative stress) (Fig. 2A), ABA (Fig. 3A) and biotic stress treatments (Fig. 4A). Cold (12 h), osmotic (12 h), and drought (1 h) stress treatments significantly enhanced the expression of NCED3 gene by 51.0-, 88.4-, and 116.0-fold respectively (Fig. 2A). The NCED3 transcript was also up-regulated significantly after 24 h inoculation of *Pseudomonas syringae* (41.7-fold) and slightly after 48 h inoculation of *Botrytis cinerea* (3.5-fold) (Fig. 4A). The same trends were observed in roots after stress or ABA treatment (Supplementary Fig. 1). Expression level of ABA1/LOS6 showed slight

increase after exogenous ABA and stress treatment except heat and biotic stress (Figs. 2A, 3A, and 4A).

3.3. Regulation of ABA signal transduction by stress and exogenous ABA

In general, expression levels of most PYR/PYLs were down-regulated in both shoot (Fig. 2B) and root tissues (Supplementary Fig. 1B) after cold, osmotic, salt, and drought treatments, whereas these of most clade A PP2Cs were uniformly up-regulated in both shoot (Fig. 2C) and root tissues (Supplementary Fig. 1C). Further analysis indicated that exogenous ABA treatment and pathogen inoculation also down-regulated expression levels of most PYR/PYLs and up-regulated these of PP2Cs (Figs. 3B and C and 4B and C). There were no such significant changes for both PYR/PYLs and PP2Cs after oxidative, heat stress and other phytohormones treatment (Figs. 2B and C and 3B and C; Supplementary Fig. 1B and C).

Osmotic and salt stresses mainly increased the expression levels of most SnRK2 genes, especially for SnRK2.1, 2.6 and 2.7 (24 h treatment) in shoot tissue (Fig. 2D). Expression levels of SnRK2.6, 2.7 and 2.9 also increased after ABA treatment and *P. syringae* colonization (Figs. 3D and 4D). No significant trends were observed for SnRK2s under other stress conditions. However, exogenous ABA treatment and stress conditions except *B. cinerea* infection activated expression of ABFs (Figs. 2E, 3E, and 4E). Expression level of ABF1 increased up to 43.2-fold under cold stress condition and that of ABF3 increased up to 16.5-, 12.4-, and 8.5-fold by osmotic stress, salt stress and exogenous ABA treatment, respectively (Fig. 2E).
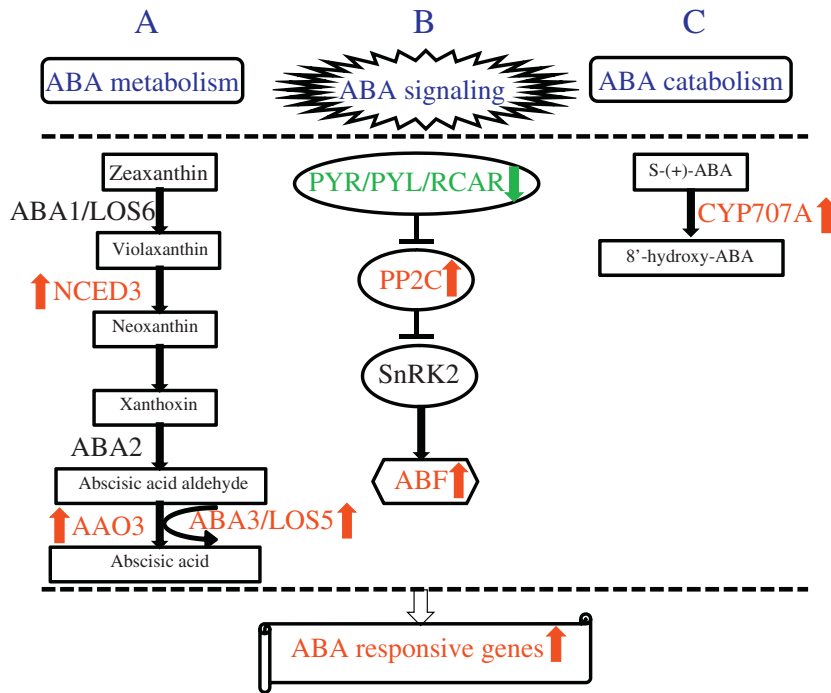
Fig. 5. Exogenous ABA as well as conditions that increase endogenous ABA regulates ABA signal pathway core components. ABA key metabolism and catabolism transcripts generally increased after treatments. Expression levels of ABA receptors were down-regulated, while these of clade A PP2C were uniformly up-regulated after stress or ABA treatment. The downstream ABA signal component, ABFs, and most ABA-responsive genes were activated in most cases.

3.4. Regulation of ABA catabolism by stress and exogenous ABA

Among the ABA catabolic pathways, the 8′-hydroxylation is reportedly the major regulatory step, which is catalyzed by a cytochrome P450, family 707(CYP707). Expression levels of four CYP707A transcripts were up-regulated after most abiotic stress (up to 135.0-fold) and exogenous ABA treatment (up to 13.4-fold) (Figs. 2F and 3F). After pathogen inoculation, CYP707A1 and CYP707A4 showed slight increase in the long-term treatment (Fig. 4F).

4. Discussion

ABA, as a key phytohormone, plays a vital role in plant stress response. ABA and stress treatments extensively changed the expression level of Arabidopsis genes [4,5,27,28]. The sites of ABA perception have intrigued plant biologists for many years. Recent breakthrough on identification and characterization of PYR/PYLs as ABA receptors enables us to better understand the perception, signaling and transportation of ABA in plant [8–11].

In this study, we compared how stress conditions and phytohormone treatments regulated transcriptional levels of ABA signaling pathway core components based on publicly available Affymetrix ATH1 microarray data. The results indicated that stress conditions and exogenous ABA treatment generally increased ABA key metabolism transcript levels (Fig. 5A). As a key gene regulates ABA metabolism, NCED3 gene expression was increased in several species, both in roots and in leaves by abiotic stress [30,31,39], and following compatible *P. syringae* infection [40]. These results together confirmed that both abiotic and biotic stress treatments promoted ABA biosynthesis. Indeed, levels of ABA increased rapidly during pathogen *B. cinerea* infection [41], bacterial colonization [42], as well as numerous abiotic stress conditions [43–45].

Interestingly, expression levels of PYR/PYLs were down-regulated, while these of clade A PP2C were uniformly up-regulated after stress or ABA treatment (Fig. 5B). These results (less ABA receptors and more PP2Cs) were somewhat opposite to what we expected.

According to the basic model of ABA signal pathway, in the presence of endogenous ABA, PYR/PYLs interact with PP2Cs and inhibit phosphatase activity, allowing SnRK2 activation and phosphorylation of target proteins. Therefore, it is reasonable if expression of ABA receptors increased in the presence of endogenous ABA. However, the results presented here indicated that interaction between PYR/PYLs and PP2Cs might not require transcriptional up-regulation of both PYR/PYLs and PP2Cs after stress or ABA treatment, but actually the increased ratio of PP2Cs:PYR/PYLs. We could not rule out the possibility that post-transcriptional modification played a role in ABA signal transduction pathway. Other possibilities included that (1) several PYR/PYLs form constitutive complexes with PP2Cs [8,9]; (2) some PP2C–SnRK2 interactions may be also constitutive, as ABI1 and SnRK2.3 interact both in the presence and absence of ABA [46,47]; (3) formation of PYR/PYLs homodimer/heterodimer and PYR/PYLs-PP2Cs complex may be important for activation of ABA downstream pathway [48]. Through these unknown mechanisms, the expression levels of downstream ABA signal component, ABFs, were actually activated in most cases except *B. cinerea* inoculation (Fig. 5B).

Since ABA is very important for plant growth and stress response, the homeostasis of endogenous ABA is vital after exogenous ABA treatment or under stress conditions. Cytosolic levels of the phytohormone ABA can range from the nanomolar to the micromolar range depending on environmental challenge and/or developmental stage [49,50], indicating that the balance between ABA metabolism and ABA catabolism is important for activation of stress response. In this study, transcripts involved in ABA catabolism were also up-regulated under stress conditions (Fig. 5C). The expression level of one key ABA catabolism transcript, CYP707A3, actually increased up to 135.0-, 17.5-, 4.6-, 12.4-fold by cold, osmotic, salt and drought stress, respectively (Fig. 2F). Another transcript, CYP707A1, was up-regulated by up to 12.5-fold after *P. syringae* colonization (Fig. 4F). Therefore, homeostasis of endogenous ABA is important for plant to activate both abiotic and biotic stress responses.

Taken together, our results showed that exogenous ABA as well as stress conditions that increased endogenous ABA regulated the

expression levels of ABA signaling pathway core components. Both abiotic and biotic stress treatment up-regulated the expression levels of ABA key metabolism and catabolism transcripts. The increased ratio of PP2Cs:PYR/PYLs might be required for activation of the downstream ABA signal pathway under stress conditions. These results indicated that abiotic and biotic stress response shared ABA signal pathway in Arabidopsis.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ygeno.2012.06.004.

Acknowledgments

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