Biochemical and Biophysical Research Communications 437 (2013) 502–508

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

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

Putrescine regulating by stress-responsive MAPK cascade contributes to bacterial pathogen defense in *Arabidopsis*

Su-Hyun Kim [a,1], Sun-Hwa Kim [a,1], Seung-Jin Yoo [a], Kwang-Hyun Min [a], Seung-Hee Nam [b], Baik Ho Cho [a], Kwang-Yeol Yang [a,*]

[a] Department of Plant Biotechnology, College of Agriculture and Life Sciences, Chonnam National University, Gwangju 500-757, Republic of Korea
[b] Jeonnam Agricultural Research & Extension Services, Jeonnam, Naju 520-715, Republic of Korea

ARTICLE INFO

Article history:
Received 5 June 2013
Available online 2 July 2013

Keywords:
Putrescine
ADC
Arabidopsis MPK3/MPK6
Pst DC3000
PR-1

ABSTRACT

Polyamines in plants are involved in various physiological and developmental processes including abiotic and biotic stress responses. We investigated the expression of *ADCs*, which are key enzymes in putrescine (Put) biosynthesis, and roles of Put involving defense response in *Arabidopsis*. The increased expression of *ADC1* and *ADC2*, and the induction of Put were detected in *GVG-NtMEK2^{DD}* transgenic *Arabidopsis*, whereas, their performance was partially compromised in *GVG-NtMEK2^{DD}/mpk3* and *GVG-NtMEK2^{DD}/mpk6* mutant following DEX treatment. The expression of *ADC2* was highly induced by *Pst* DC3000 inoculation, while the transcript levels of *ADC1* were slightly up-regulated. Compared to the WT plant, Put content in the *adc2* knock-out mutant was reduced after *Pst* DC3000 inoculation, and showed enhanced susceptibility to pathogen infection. The *adc2* mutant exhibited reduced expression of *PR-1* after bacterial infection and the growth of the pathogen was about 4-fold more than that in the WT plant. Furthermore, the disease susceptibility of the *adc2* mutant was recovered by the addition of exogenous Put. Taken together, these results suggest that *Arabidopsis* MPK3 and MPK6 play a positive role in the regulation of Put biosynthesis, and that Put contributes to bacterial pathogen defense in *Arabidopsis*.

1. Introduction

Mitogen-activated protein kinase (MAPK) cascades are major signal transduction pathways to translate extracellular stresses into intercellular responses in all eukaryotes. A MAPK cascade minimally consists of MAPKKKs, MAPKKs and MAPKs modules that are linked in various ways to upstream receptors and downstream targets [1]. In several species, including *Arabidopsis*, MAPK cascades are activated by a variety of stress stimuli, including pathogen attack, drought, cold, salt, wounding, osmotic shock and reactive oxygen species [2,3]. Approximately 20 different MAPKs can be identified in the *Arabidopsis* genome, and other plants are likely to have similar numbers of MAPKs [2]. A number of research groups demonstrated that especially MPK3 and MPK6, two *Arabidopsis* MAPKs, have been implicated in biotic and abiotic stress responses in *Arabidopsis* [1,4,5]. However, little is known about the downstream targets that are regulated by this MAPK cascade. According to the studies of Zhang and co-workers, MPK3 and MPK6 regulate *Botrytis cinerea*-induced ethylene production, and also, camalexin production in *GVG-NtMEK2^{DD} Arabidopsis* [5,6]. In tobacco, we demonstrated that the SIPK and WIPK, which are tobacco orthologs of MPK6 and MPK3, regulate polyamine (PA) synthesis through transcriptional regulation of the biosynthetic genes [7].

In plants, three PAs (putrescine, spermidine and spermine) are abundant and involved in mainly abiotic stresses as well as biotic stresses [8,9]. PA biosynthesis was started from ornithine or arginine to synthesize putrescine (Put) by the activation of ornithine decarboxylase (ODC) or arginine decarboxylase (ADC), respectively. Produced Put was converted into spermidine (Spd) by spermidine synthase (SPDS) and spermdine was catalyzed into spermine (Spm) by spermine synthase (SPMS) [9]. However, there are no intact or degrade ODC sequences in the model plant *Arabidopsis* genome, and no ODC expressed sequences tags. As ODC is a key enzyme in PA biosynthesis, *Arabidopsis* is reliant on the additional ADC pathway [10]. In *Arabidopsis*, there are two genes of *ADC*, *ADC1* and *ADC2*. The duplicated genes in *Arabidopsis* seem to be related to the differential regulation of gene responsiveness. The differential expression of *ADCs* has been mostly reported under abiotic stress conditions. *ADC2* expression is strongly induced by several abiotic stresses like drought, high salinity, and mechanical injury, while *ADC1* is mainly induced by cold [9,11,12]. In many plants, including *Arabidopsis*, the overexpression of *ADC* that accumulates Put confers plant tolerance against abiotic stresses

* Corresponding author. Fax: +82 62 530 0207.
E-mail address: kyyang@chonnam.ac.kr (K.-Y. Yang).
[1] These authors contributed equally to the paper.

0006-291X/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.06.080

[13–15]. By contrast, the *Arabidopsis* mutant defective Put biosynthesis (*adc1*, *adc2*, *adc2-1*) displayed more sensitivity to salt and low temperature stresses than that of WT plants [16,17]. However, an expression of *ADCs* and roles of Put in plant defense against pathogens still remains elusive in *Arabidopsis*. In this paper, we report that *Arabidopsis* MPK3 and MPK6 are involved in regulating Put synthesis through transcriptional regulation of both *ADC1* and *ADC2* genes. The expression of *ADC2* was induced by bacterial pathogen infection, and the *adc2* mutant defective in Put biosynthesis was more susceptible to pathogen infection than the WT plant. The disease susceptibility of the *adc2* mutant was correlated with a reduced expression of the *PR-1* gene.

2. Materials and methods

2.1. Plant materials and growth conditions

Arabidopsis thaliana ecotype Columbia, *GVG-NtMEK2^{DD}*, *GVG-NtMEK2^{DD}/mpk3*, *GVG-NtMEK2^{DD}/mpk6* and *adc2* mutant plants were grown at 22 °C in a growth room. Transgene expression was induced by spraying with dexamethasone (DEX, 1 μM). The *adc2* mutant was ordered from the ABRC. T-DNA specific primers and the gene specific primers were used for determining the position of SALK T-DNA tags [17]. Homozygous plants were identified by PCR.

2.2. Bacterial pathogen inoculation

Pseudomonas syringae pv. *tomato* DC3000 (*Pst* DC3000) were streaked out from −80 °C glycerol stock onto king's B agar medium with 25 μM/ml rifampicin for 2 days at 28 °C. 4-week-old *Arabidopsis* plants are used for infiltration (1×10^5 CFU/ml) or dipping (4×10^8 CFU/ml) inoculation. To test bacterial growth, diluted leaf extracts were spotted on king's B agar medium containing the appropriate antibiotics. The plate was incubated at 28 °C for 2, and 4 days, and the number of colonies were counted. For complementation, 2 μM Put was infiltrated into leaves 1 day before infiltration with *Pst* DC3000. For the TLC and HPLC analysis of PAs, plants were dipped with *Pst* DC3000 in 10 mM $MgCl_2$ with 0.01% silwet L-77.

2.3. Reverse transcription-polymerase chain reaction (RT-PCR) and quantitative real-time polymerase chain reaction (qRT-PCR)

Reverse transcription was then performed following the protocol described in [7]. qRT-PCR with the QuantiTect SYBR Green RT-PCR kit (JMC R&D) was conducted using a Rotor-Gene 2000 real-time thermal cycling system (Corbett Research). After normalization to an *Actin* control, the relative levels of gene expression were calculated. The primer pairs are listed in Supplemental Table 1.

2.4. Protein extraction, immunoblot analysis and in-gel kinase activity assay

Total protein was extracted from leaf tissue and was stored at −80 °C. The protein concentration was determined by the Bio-Rad protein assay kit using bovine serum albumin standard. Immunoblot analysis of proteins was carried out as described previously [7]. In-gel kinase activity assay was performed with myelin basic protein as a substrate for kinase as described previously [7].

2.5. Polyamine analysis

The PAs were extracted by homogenizing the powdered plant samples (100 mg) with 5% cold perchloric acid (PCA) as described by Marcé et al. [18]. The dansylamines were separated on a TLC plate using 4:1 (V/V) chloroform/triethylamine as the developing solvent [19]. The samples dissolved in acetonitrile were then analyzed by reversed phase high-performance liquid chromatography (HPLC, Agilent Technologies 1200) [18].

3. Results and discussion

3.1. Polyamine content and the expression of polyamine synthetic genes in GVG-NtMEK2^{DD} transgenic Arabidopsis

Our previous study has shown that the NtMEK2-SIPK/WIPK cascade regulates polyamine (PA) synthesis, especially putrescine (Put) synthesis, through transcriptional regulation of biosynthetic genes in tobacco [7]. To fully understand the interactions between PA regulated by MAPK cascade and defense response, we decided to use the model plant system of *Arabidopsis*, because the knock-out mutants were available. Therefore we used gain-of-function *GVG-NtMEK2^{DD}* transgenic *Arabidopsis* plants, which had already been used in a number of other studies [5,6]. To investigate whether a stress-responsive MPK3/MPK6 MAPK cascade is involved in PA biosynthesis in *Arabidopsis*, we monitored the transcript levels of PA synthetic genes such as *ADC1*, *ADC2*, *SPDS1*, *SPDS2*, *SPDS3* and *ACL5* using RT-PCR in *GVG-NtMEK2^{DD}* plants after DEX treatment. The both *ADC1* and *ADC2* genes involved Put biosynthesis were highly up-regulated. By contrast, the mRNA levels of *SPDS1*, *SPDS2* and *SPDS3* involving Spd biosynthesis and *ACL5* involving Spm biosynthesis were not induced or some genes were down-regulated (Fig. 1A). Then, we examined the expression of *ADC1* and *ADC2* in *GVG-NtMEK2^{DD}/mpk3* and *GVG-NtMEK2^{DD}/mpk6* mutant using real-time qRT-PCR. The activation of NtMEK2 and knock-out of either MPK3 or MPK6 in *GVG-NtMEK2^{DD}*, *GVG-NtMEK2^{DD}/mpk3* and *GVG-NtMEK2^{DD}/mpk6* plants were evaluated by an in-gel kinase assay using myelin basic protein as a substrate and immunoblot analysis using anti-Flag, anti-MPK3 and anti-MPK6 (Supplementary Fig. S1). The expression of *ADC1* and *ADC2* was partially compromised in *GVG-NtMEK2^{DD}/mpk3* and *GVG-NtMEK2^{DD}/mpk6* plants compared to that in *GVG-NtMEK2^{DD}* plants. Especially, the transcripts of *ADC1* were dramatically reduced in *GVG-NtMEK2^{DD}/mpk3* plants (Fig. 1B). It was suggested that MPK3 and MPK6 play positive roles in the full expression of *ADC* genes in *Arabidopsis*. Several previous researches have represented that MPK3 and MPK6 of *Arabidopsis* are induced by different biotic and abiotic stress stimuli such as pathogen infections, wounding, reactive oxygen species, high and low temperatures, drought, hyper-osmolarity [6,20–24]. Also both *ADC1* and *ADC2* expression is strongly induced by several stresses like drought, high salinity, mechanical injury, cold, and virus infection [9,11,12,25]. Taken together, the induction of *ADC1* and *ADC2* by several stresses is regulated by activation of the stress-responsive MPK3/MPK6 cascade in *Arabidopsis*.

Since a stress-responsive MPK3/MPK6 cascade was found to regulate Put biosynthetic genes, we extracted PAs from *GVG-NtMEK2^{DD}* before and after DEX treatment and separated them by thin-layer chromatography (TLC). At 24 h, the *GVG-NtMEK2^{DD}* plants were found to have a significantly higher Put content (Fig. 2A). Then we quantitatively measured PAs content in *GVG-NtMEK2^{DD}* and *GVG-NtMEK2^{DD}/mpk3* and *GVG-NtMEK2^{DD}/mpk6* plants before and after DEX treatment using HPLC. HPLC assay showed that the Put level in the *GVG-NtMEK2^{DD}* plants 24 h after DEX treatment was increased to about 8 times that before DEX treatment. Partially compromised Put induction by loss of either MPK3 or MPK6 is correlated with the reduced *ADC1* and *ADC2* gene expression in *GVG-NtMEK2^{DD}/mpk3* and *GVG-NtMEK2^{DD}/mpk6* plants. Despite changes of Put content, Spd and Spm content are

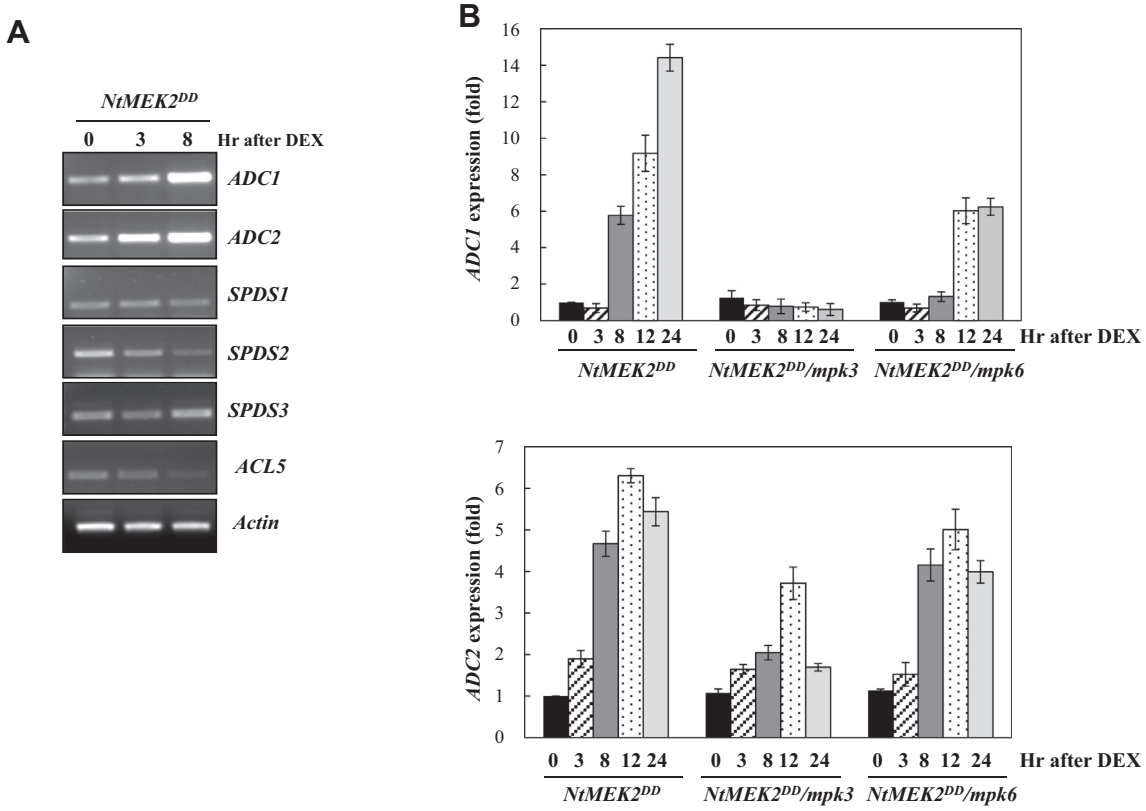

Fig. 1. RT-PCR and qRT-PCT analysis of the PA synthetic genes in *GVG-NtMEK2^{DD}* transgenic *Arabidopsis* plants, *GVG-NtMEK2^{DD}/mpk3* and *GVG-NtMEK2^{DD}/mpk6* mutant. (A) Both *ADC1* and *ADC2* genes involving Put biosynthesis are up-regulated in *NtMEK2^{DD}* transgenic plants after DEX treatment. RT-PCR analysis of *GVG-NtMEK2^{DD}* transgenic plants was performed to observe the expression patterns of two Put synthetic genes (*ADC1* and *ADC2*), three Spd synthetic genes (*SPDS1*, *SPDS2* and *SPDS3*) and the Spm synthetic gene (*ACL5*) in *Arabidopsis*. The *Actin* gene was used as a control to confirm the integrity of the RNA samples. (B) The expression of *ADC1* and *ADC2* is partially compromised in *GVG-NtMEK2^{DD}/mpk3* and *GVG-NtMEK2^{DD}/mpk6* mutant. Transcripts levels were determined by qRT-PCR analysis. Levels of *Actin* transcript were used to normalize the different samples. Data shown are means ± SE of samples conducted in triplicate.

almost unchanged (Fig. 2B). This means that the levels of Spd and Spm in the cells are under tight homeostatic regulation [14]. Taken together, these results demonstrate that the stress-responsive MPK3/MPK6 cascade involved in PA biosynthesis, especially Put biosynthesis, through transcriptional regulation of the biosynthetic genes in *Arabidopsis*.

3.2. Pathogen-induced expression patterns of the ADC1 and ADC2 genes and Put level in the adc2 mutant

To understand the expression of *ADCs* in plant defense responses against bacterial pathogen, we analyzed the expression of *ADC1* and *ADC2* transcript levels in WT plants after inoculation with the virulent bacteria *Pst* DC3000. WT plants inoculated with *Pst* DC3000 exhibited a greater increase in *ADC2* transcript levels at 48 h, which remained until 72 h, while *ADC1* transcript levels were slightly induced upon inoculation with bacterial pathogen (Fig. 3A). There are lots of research data to define the different expressions of *ADC1* and *ADC2* in *Arabidopsis* after abiotic stress treatment, but just one previous report has mentioned biotic stress as far as we know. *ADC2* expression is strongly induced by several abiotic stresses, such as drought, high salinity, and mechanical injury, while *ADC1* is mainly induced by cold [8,9,11–13]. In contrast, both *ADC1* and *ADC2*, which were up-regulated during cucumber mosaic virus (CMV)-elicited hypersensitive response (HR) [25]. In this study, we demonstrate that *ADC1* and *ADC2* transcript levels showed different transcriptional expression upon bacterial infection. This observation might be associated with potential differences of ADC1 or ADC2 promoter activity under specific stress conditions. Several putative *cis*-acting regulatory elements such as STRE (stress response element), SURE (sucrose-responsive elements) and DRE (dehydration-responsive element) showed contrasting patterns of distribution between ADC1 and ADC2 promoters [26]. The monocotyledonous rice also has two ADC genes which contain different putative *cis*-acting elements, and mRNA analysis showed the two rice genes are differently regulated by abiotic stresses [27].

To investigate the roles of Put in plant defense response, we obtained the mutant of a pathogen-inducible gene for *ADC2*, *adc2* (SALK_073977, named as *adc2*) from ABRC. T-DNA insertion was detected by genomic DNA PCR in *adc2* mutant. The T-DNA insertion was positioned at the exon of *ADC2* gene (Fig. 3B). Under normal growth conditions, there was no difference in development between the *adc2* mutant and the WT plant. RT-PCR analysis with RNA samples infected with *Pst* DC3000 demonstrated that the *ADC2* transcripts were completely abolished in the *adc2* mutant. However, the expression of *ADC1* gene was not affected in the *adc2* mutant compared to the WT plants (Fig. 3C). To evaluate the PAs content in *adc2* mutant missing the *ADC2* gene, PA levels were measured by HPLC. In the WT plants, Put content increased over 3-fold under *Pst* DC3000 inoculation, but not Spd and Spm. In the *adc2* mutant, Put content were about 60% that of the WT plants before *Pst* DC3000 inoculation (Fig. 3D). The 60% Put content of WT plants were detected in *adc2* mutant, which could be associated with presence of *ADC1* gene in the *adc2* mutant. However, in *adc2* mutant, Put content was a little increased under *Pst* DC3000 inoculation. This observation is consistent with the slightly induced expression of *ADC1* upon inoculation with the

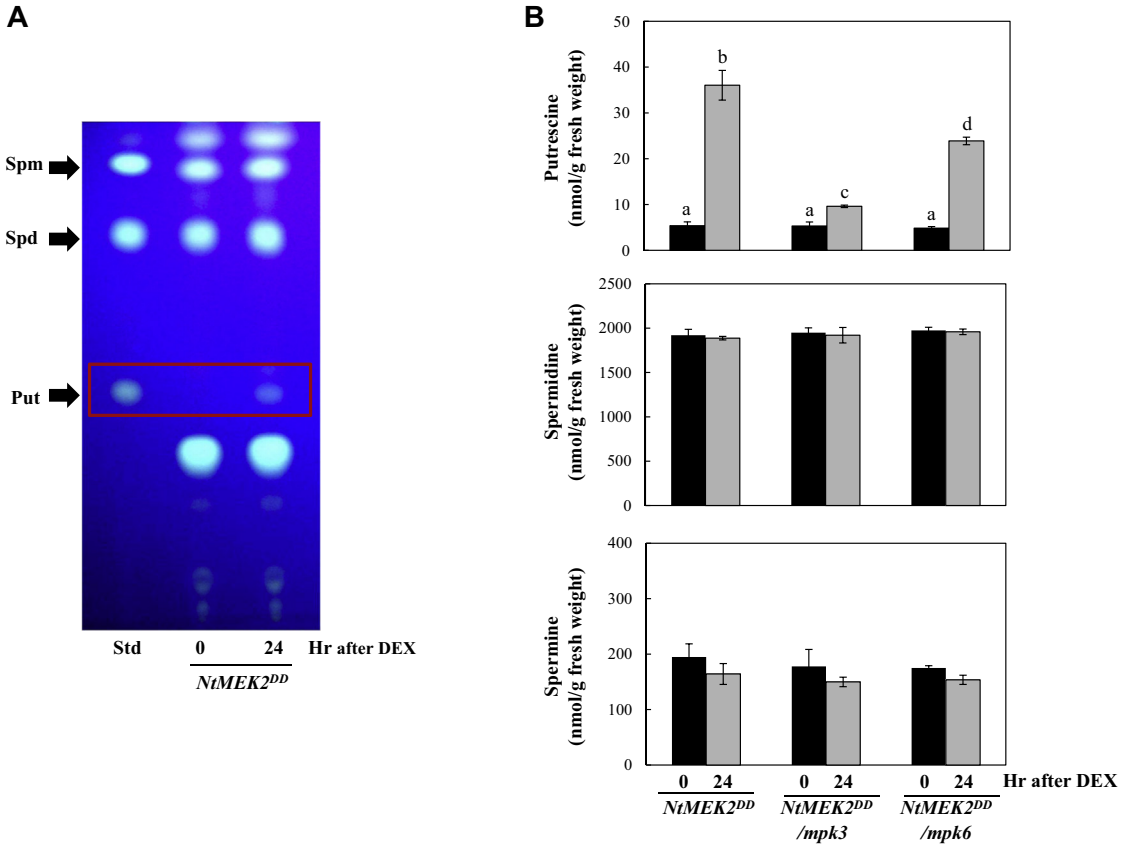

Fig. 2. TLC and HPLC analysis of PA biosynthesis in *GVG-NtMEK2^{DD}* transgenic *Arabidopsis* plants, *GVG-NtMEK2^{DD}/mpk3* and *GVG-NtMEK2^{DD}/mpk6* mutant. (A). Elevated levels of Put in *NtMEK2^{DD}* transgenic plants following treatment with DEX. Three different dansylated PAs were then separated and evaluated by TLC after being developed using a solvent system that contained PA standards that included Put, Spd, and Spm. (B) Loss of MPK3 or MPK6 partially compromised Put induction in *GVG-NtMEK2^{DD}/mpk3* and *GVG-NtMEK2^{DD}/mpk6* mutant. The samples were quantitatively measured by HPLC. Different letters among treatments indicate significant differences according to Duncan's multiple range test, $P < 0.05$. Data shown are means ± SE of samples conducted in triplicate.

bacterial pathogen in Fig. 3A. However, Spd and Spm content were not change in the *adc2* mutant compared to the WT plants, which suggests that the levels of Spd and Spm in the cells are under tight homeostatic regulation [28]. These results suggest that *ADC2* gene is responsible for the major route of Put biosynthesis induced by inoculation with *Pst* DC3000.

The amount of PAs derived from *Pst* DC3000 was undetectable on TLC when we extracted from a 1×10^7 cell culture of *Pst* DC3000 (data not shown). This result is consistent with a study conducted by Yoda et al. [29], in which a small amount of Spd derived from *P. cichorii* was detected, while those of Put and Spm derived from *P. cichorii* were undetectable in tobacco plants. These results indicate that the accumulation of PAs in pathogen infected leaves resulted from the host's metabolic compounds, and PAs derived from pathogens were negligible in estimating the total PA content in pathogen-inoculated leaves. Taken together, these results suggest that the expression of *ADC2* gene is important for full Put biosynthesis under normal conditions, and also for pathogen-inducible Put biosynthesis under *Pst* DC3000 infection in *Arabidopsis*.

3.3. Increased susceptibility to Pst DC3000 and suppression of PR-1 expression in the adc2 mutant

To evaluate whether reduced Put content in the *adc2* mutant have effect on bacterial pathogen response, WT plants and *adc2* mutant were inoculated through infiltration with the *Pst* DC3000 and disease progression was investigated. As shown in Fig. 4A, the *adc2* mutant developed more disease symptoms than WT plants. The bacterial growth was monitored for 4 days, and that in the *adc2* mutant was approximately 4-fold higher than that in WT plants (Fig. 4B). These results indicate that the reduced Put in the *adc2* mutant resulted in the suppression of defense against *Pst* DC3000, and Put plays an important role in plant defense against the bacterial pathogen. To investigate that a supply of Put is recovered for plant defense response, we performed chemical complementation analysis in the *adc2* mutant. 2 μM Put was infiltrated into leaves of the *adc2* mutant exogenously. One day later after Put treatment, *adc2* mutant were inoculated with *Pst* DC3000. The WT plants were then infiltrated with water 1 day before *Pst* DC3000 inoculation as a control. After 4 days after *Pst* DC3000 inoculation, the exogenous Put-treated *adc2* mutant showed recovered symptoms and reduced bacterial growth on WT plants treated with *Pst* DC3000 (Fig. 4A and B). These results indicate that Put is required for defense resistance to *Pst* DC3000 in *Arabidopsis* plants.

We checked the expression of defense related gene *PR-1* after *Pst* DC3000 inoculation both WT and *adc2* mutant. However, the transcripts of *PR-1* showed different kinetics to WT plants after bacterial infection in the *adc2* mutant. The transcript levels of *PR-1* in the *adc2* mutant were slow, and less induction was observed 24 h and 48 h after inoculated with *Pst* DC3000 compared to that in WT plants (Fig. 4C). Because *PR-1* is a biomarker for the defense hormone salicylic acid (SA), this result suggested that Put might play a role in the increased plant defense resistance related to the SA signaling pathway. Alcázar et al. [30] demonstrated that a potential cross-talk occurred between Put and gibberellin (GA) in transgenic *Arabidopsis* plants with increased levels of *ADC2* transcript and elevated Put content. The transgenic plants showed dwarfism and late-flowering, and the phenotype was

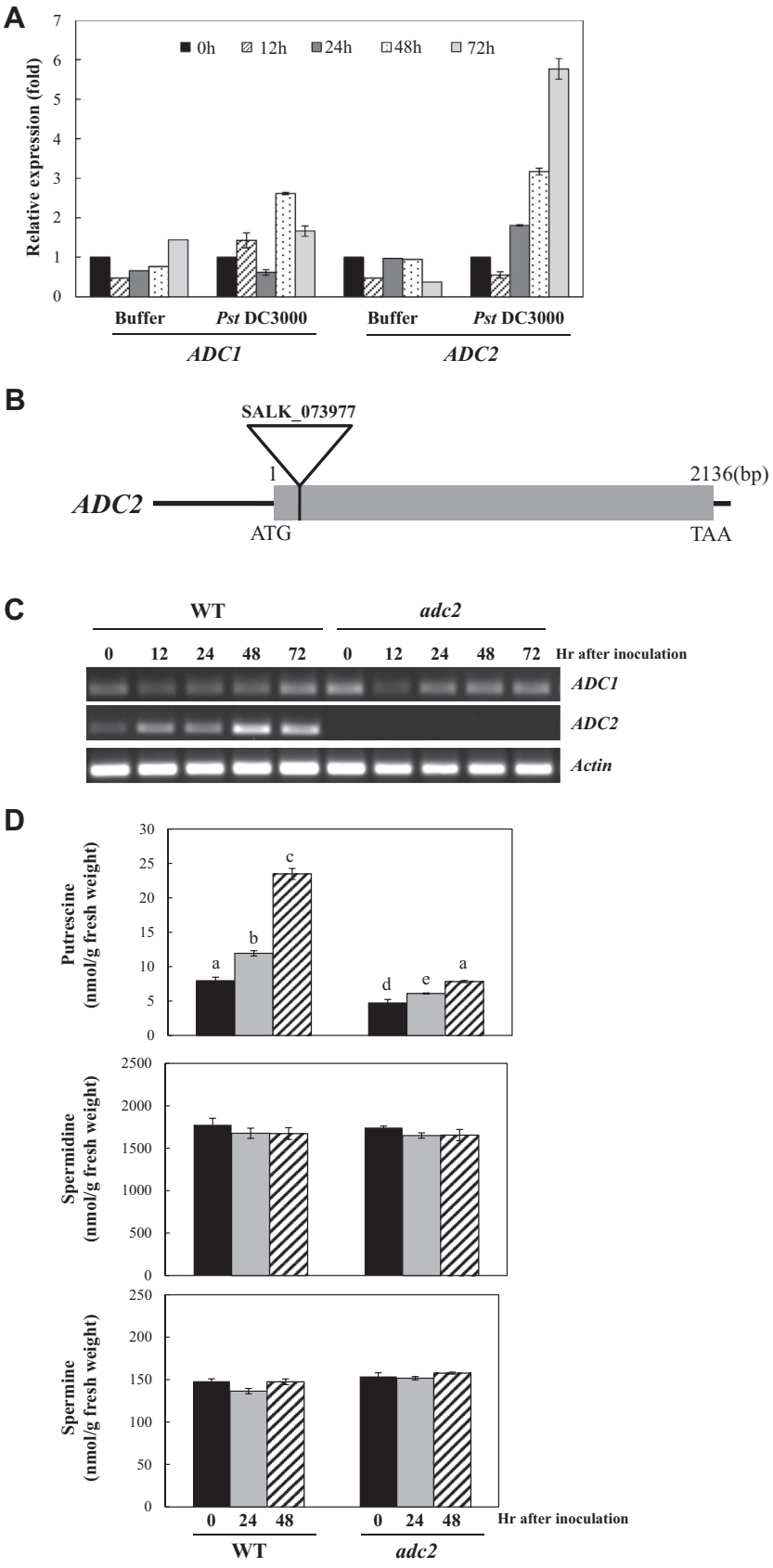

Fig. 3. Expression of *ADC2* gene is important for the pathogen-inducible Put biosynthesis under *Pst* DC3000 infection. (A) The expression of *ADC2* is strongly induced after *Pst* DC3000 infection in WT plants. Transcripts levels were determined by qRT-PCR analysis. Levels of *Actin* transcript were used to normalize different samples. (B) Schematic representation of the T-DNA insertion site in the *ADC2* gene. The triangle showed the T-DNA insertion site of the *adc2* mutant. (C) RT-PCR analysis of *ADC1* and *ADC2* of WT plants and *adc2* mutant after *Pst* DC3000 infection. (D) Loss of *ADC2* partially compromised Put induction in WT plants and *adc2* mutant after *Pst* DC3000 infection. The samples were quantitatively measured by HPLC. Different letters among treatments indicate significant differences according to Duncan's multiple range test, $P < 0.05$. Data shown are means ± SE of samples conducted in triplicate.

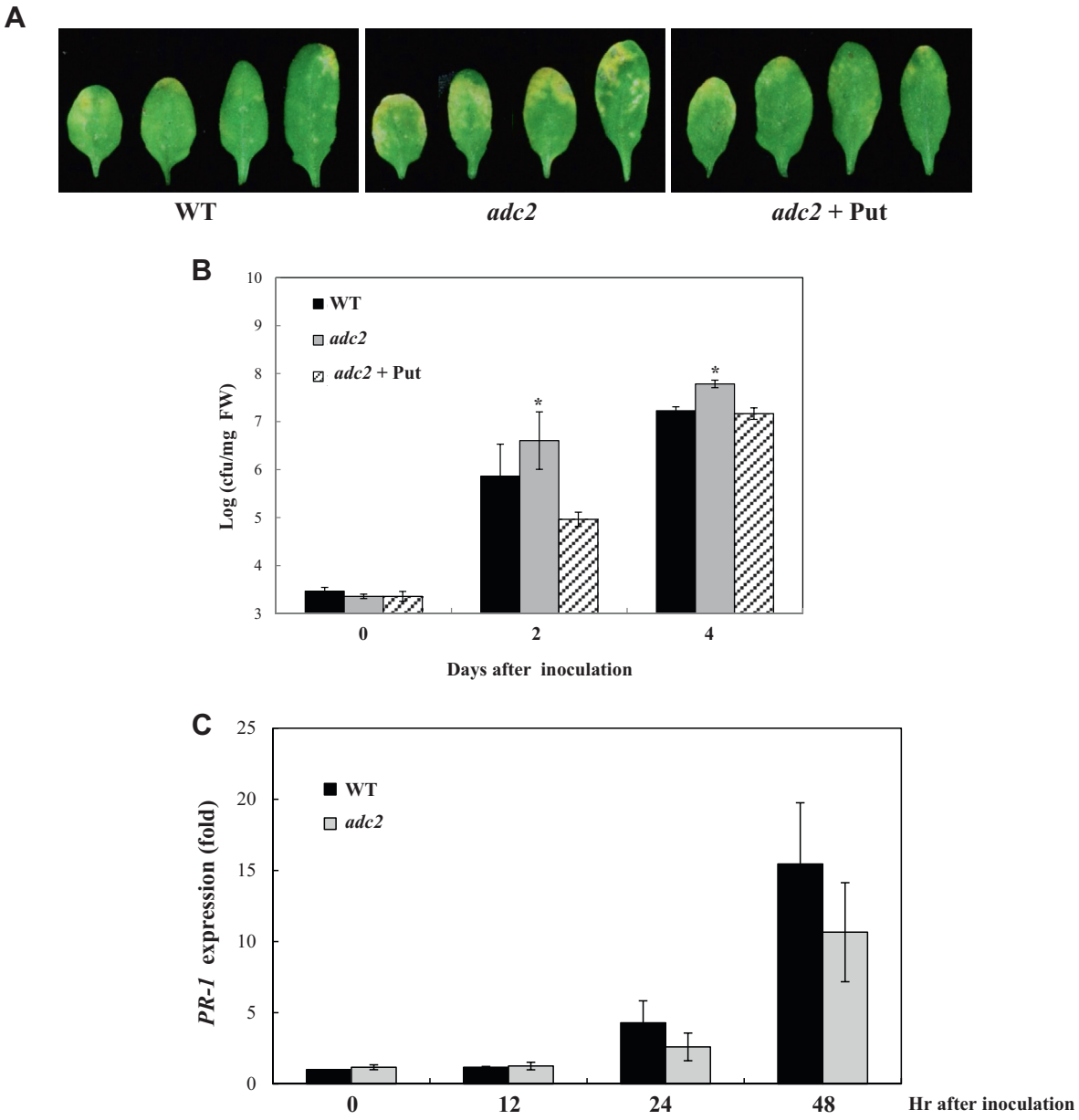

Fig. 4. The reduced Put in *adc2* mutant shows the suppression of defense against *Pst* DC3000. (A and B) The *adc2* mutant developed more disease symptoms and showed higher bacterial growth than WT plants. The exogenous supply of Put is recovered for plant defense against bacterial pathogen. (C) The expression of *PR-1* is impaired in *adc2* mutant. Transcript levels were determined by qRT-PCR analysis. Levels of *Actin* transcript were used to normalize different samples. Statistical differences from the WT are indicated by an asterisk (*); Duncan's multiple range test, $P < 0.05$. Data shown are means ± SE of samples conducted in triplicate.

rescued by gibberellin A_3 (GA_3) application. In addition, these authors represented that *PR-1* gene was increased in the transgenic *ADC2* overexpressor line using microarray analysis. This suggests that alterations in Put levels may affect different stress signaling pathways including GA, SA and possibly other hormones. Recently, Marco et al. [31] suggested that the elevation of endogenous PA actively participated in stress signaling, through intricate cross-talk with abscisic acid (ABA), GA and other hormones, including ethylene (ET), Jasmonic acid (JA) and SA using MAPMAN analysis of *Arabidopsis* plants with increased Put levels.

To our knowledge, the interplay of PA, especially Put, and SA in response to plant defense response is less discussed. Lots of results from many authors has represented that PA, especially Spm, tetraamine thermospermine (TSpm), and their degradation product, hydrogen peroxide, were one of the key elements to induce host and nonhost HRs and defense against pathogens in higher plants. However, Spd and Spm efficiently induced the production of hydrogen peroxide and concomitant cell death in *Arabidopsis*, tobacco and rice plants, whereas Put did not [29]. This means that further mechanisms govern the roles of Put in plant and pathogen interactions. In this study, we demonstrated that the other role of Put in disease response is related to plant defense hormones, SA, at least partially. However, we still do not fully understand how Put was related to SA signaling in *Arabidopsis*. Therefore, further works needs to be done to determine the defense mechanism involving in Put and plant hormones such as SA.

Acknowledgments

This study was financially supported by the Basic Science Research Program through the National Research Foundation of Korea [grant No. KRF-2007-331-F00022 and 2009-0076547], as

funded by the Korean Government, and by the Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry and Fisheries, Ministry for Food, Agriculture, Forestry and Fisheries, Republic of Korea.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2013.06.080.

References

[1] H. Nakagami, A. Pitzschke, H. Hirt, Emerging MAP kinase pathways in plant stress signaling, Trends Plant Sci. 10 (2005) 346–393.
[2] S. Zhang, D.F. Klessig, MAPK cascade in plant defense signaling, Trends Plant Sci. 6 (2001) 520–526.
[3] A.K. Sinha, M. Jaggi, B. Raghuram, N. Tuteja, Mitogen-activated protein kinase signaling in plants under abiotic stress, Plant Signal. Behav. 6 (2011) 196–203.
[4] N.S. Mishra, R. Tuteja, N. Tuteja, Signaling trough MAP kinase networks in plants, Arch. Biochem. Biophys. 452 (2006) 55–68.
[5] D. Ren, Y. Liu, K.-Y. Yang, L. Han, G. Mao, J. Glazebrook, S. Zhang, A fungal-responsive MAPK cascade regulates phytoalexin biosynthesis in *Arabidopsis*, Proc. Natl. Acad. Sci. USA 105 (2008) 5638–5643.
[6] L. Han, G.-J. Li, K.-Y. Yang, G. Mao, R. Wang, Y. Liu, S. Zhang, Mitogen-activated protein kinase 3 and 6 regulate *Botrytis cinerea*-induced ethylene production in *Arabidopsis*, Plant J. 64 (2010) 114–127.
[7] E.-K. Jang, K.-H. Min, S.-H. Kim, S.-H. Nam, S. Zhang, Y.C. Kim, B.H. Cho, K.-Y. Yang, Mitogen-activated protein kinase cascade in the signaling for polyamine biosynthesis in tobacco, Plant Cell Physiol. 50 (2009) 658–664.
[8] R. Alcázar, F. Marco, J.C. Cuevas, M. Patron, A. Ferrando, P. Carrasco, A.F. Tiburcio, T. Altabella, Involvement of polyamines in plant response to abiotic stress, Biotechnol. Lett. 28 (2006) 1867–1876.
[9] S.S. Hussain, M. Ali, M. Ahmad, K.H.M. Siddique, Polyamine: natural and engineered abiotic and biotic stress tolerance in plants, Biotechnol. Adv. 29 (2011) 300–311.
[10] C. Hanfrey, S. Sommer, M.J. Mayer, D. Burtin, A.J. Michael, *Arabidopsis* polyamine biosynthesis: absence of ornithine decarboxylase and the mechanism of arginine decarboxylase activity, Plant J. 27 (2001) 551–560.
[11] M.A. Perez-Amador, J. Leon, P.J. Green, J. Carbonell, Induction of the Arginine decarboxylase *ADC2* gene provides evidence for the involvement of polyamines in the wound response in *Arabidopsis*, Plant Physiol. 130 (2002) 1454–1463.
[12] R. Wimalasekera, F. Tebartz, G.F.E. Scherer, Polyamines, polyamine oxidase and nitric oxide in development, abiotic and biotic stresses, Plant Sci. 181 (2011) 593–603.
[13] R. Alcázar, J. Planas, T. Saxena, X. Zarza, C. Bortolotti, J. Cuevas, M. Bitrián, A.F. Tiburcio, T. Altabella, Putrescine accumulation confers drought tolerance in transgenic *Arabidopsis* plants over-expressing the homologous *Arginine decarboxylase* 2 gene, Plant Physiol. Biochem. 48 (2010) 547–552.
[14] B.Q. Wang, Q.F. Zhang, J.H. Liu, G.H. Li, Overexpression of PtADC confers enhanced dehydration and drought tolerance in transgenic tobacco and tomato: effect on ROS elimination, Biochem. Biophys. Res. Commun. 413 (2011) 10–16.
[15] T. Capell, L. Bassie, P. Chrisou, Modulation of the polyamine biosynthetic pathway in transgenic rice confers tolerance to drought stress, Proc. Natl. Acad. Sci. USA 101 (2004) 9909–9914.
[16] K. Urano, Y. Yoshiba, T. Nanjo, T. Ito, K. Yamaguchi-Sinozaki, K. Shinozaki, *Arabidopsis* stress-inducible gene for arginine decarboxylase AtADC2 is required for accumulation of putresine in salt tolerance, Biochem. Biophys. Res. Commun. 313 (2004) 369–375.
[17] J.C. Cuevas, R. López-Cobollo, R. Alcázar, X. Zarza, C. Koncz, T. Altabella, J. Salinas, A.F. Tiburcio, A. Ferrando, Putrescine is involved in Arabidopsis freezing tolerance and cold acclimation by regulating abscisic acid levels in response to low temperature, Plant Physiol. 148 (2008) 1094–1105.
[18] M. Marcé, D.S. Brown, T. Capell, X. Figueras, A.F. Tiburio, Rapid high-performance liquid chromatographic method for the quantitation of polyamines as their dansyl derivatives: application to plant and animal tissues, J. Chromatogr. 666 (1995) 329–335.
[19] A.F. Tiburcio, R. Kaur-Sawhney, R.B. Ingersoll, A.W. Galston, Correlation between polyamines and pyrrolidine alkaloids in developing tobacco callus, Plant Physiol. 78 (1985) 323–326.
[20] G. Taj, P. Agarwal, M. Grant, A. Kumar, MAPK machinery in plants: recognition and response to different stresses through multiple signal transduction pathways, Plant Signal. Behav. 5 (2010) 1370–1378.
[21] S.H. Kim, D.H. Woo, J.M. Kim, S.Y. Lee, W.S. Chung, Y.H. Moon, Arabidopsis MKK4 mediates osmotic-stress response via its regulation of MPK3 activity, Biochem. Biophys. Res. Commun. 412 (2011) 150–154.
[22] A.K. Shiha, M. Jaggi, B. Raghuram, N. Tuteja, Mitogen-activated protein kinase signaling in plants under abiotic stress, Plant Signal. Behav. 6 (2011) 196–203.
[23] D. Tsugama, S. Liu, T. Takano, Drought-induced activation and rehydration-induced inactivation of MPK6 in *Arabidopsis*, Biochem. Biophys. Res. Commun. 426 (2012) 626–629.
[24] X.M. Liu, X.C. Nguyen, K.E. Kim, H.J. Han, J. Yoo, K. Lee, M.C. Kim, D.J. Yun, W.S. Chung, Phosphorylation of the zinc finger transcriptional regulator ZAT6 by MPK6 regulates *Arabidopsis* seed germination under salt and osmotic stress, Biochem. Biophys. Res. Commun. 430 (2013) 1054–1059.
[25] Y. Mitsuya, Y. Takahashi, T. Berberich, A. Miyazaki, H. Matsumura, H. Takahashi, R. Terauchi, T. Kusano, Spermine signaling plays a significant role in the defense response of *Arabidopsis thaliana* to cucumber mosaic virus, J. Plant Physiol. 166 (2009) 626–643.
[26] I. Hummel, G. Bourdais, G. Gouesbet, I. Couée, R.L. Malmberg, A.El. Amrani, Differential gene expression of arginine decarboxylase *ADC1* and *ADC2* in *Arabidopsis thaliana*: characterization of transcriptional regulation during seed germination and seedling development, New Phytol. 163 (2004) 519–531.
[27] A. Peremarti, L. Bassie, C. Zhu, P. Christou, T. Capell, Molecular characterization of the *Arginine decarboxylase* gene family in rice, Transgenic Res. 19 (2010) 785–797.
[28] P. Bhatnagar, R. Minocha, S.C. Minocha, Genetic manipulation of the metabolism of polyamines in poplar cells. The regulation of putrescine catabolism, Plant Physiol. 128 (2002) 1455–1469.
[29] H. Yoda, K. Fujimura, H. Takahashi, I. Munemura, H. Uchimiya, H. Sano, Polyamines as a common source of hydrogen peroxide in host- and nonhost hypersensitive response during pathogen infection, Plant Mol. Biol. 70 (2009) 103–112.
[30] R. Alcázar, J.L. Garcia-Martinez, J.C. Cuevas, A.F. Tiburcio, T. Altabella, Overexpression of *ADC2* in Arabidopsis induces dwarfism and late-flowering through GA deficiency, Plant J. 43 (2005) 425–436.
[31] F. Marco, R. Alcázar, A.F. Tiburcio, P. Carrasco, Interactions between polyamines and abiotic stress pathway responses unraveled by transcriptome analysis of polyamine overproducers, OMICS J. Integr. Biol. 15 (2011) 775–781.