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ARTICLE

Putrescine Enhances Seed Germination Tolerance to Heat Stress in *Arabidopsis thaliana*

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ABSTRACT

Putrescine (Put) as the compound of plant polyamines is catalyzed by arginine decarboxylase (ADC), which is encoded by two members, ADC1 and ADC2 in Arabidopsis, and ADC2 is mainly responsible for Put biosynthesis. Accumulated evidence demonstrates the important function of Put in plant growth and development, but its role in regulating seed germination under high temperature (HT) has not been reported yet. SOMNUS (*SOM*) is the negative regulator for seed germination thermoinhibition by altering downstream gibberellin (GA) and abscisic acid (ABA) metabolism. In this study, we found exogenous application of Put obviously alleviated the inhibition effect of HT on seed germination. Whereas pharmacological inhibition of endogenous Put level reduced seed germination under HT. Consistently, HT induced the rapid accumulation of Put level, and the *adc2* mutant deficiency in Put biosynthesis also showed more sensitivity to HT stress. Furthermore, we found that the Put signal suppressed the expression of *SOM* and changed the transcriptional patterns of genes associated with GA/ABA metabolism. Genetic analysis also revealed *SOM* was epistatic to *ADC2* to alter GA/ABA metabolism. Collectively, our finding reveals the novel function of Put in controlling seed germination under HT through *SOM*, and provides the possibility to develop Put as the innovational regulator for uniform seed germination under HT stress.

KEYWORDS

Putrescine; seed germination; heat stress; *SOM*; *ADC2*

1 Introduction

Seed germination and dormancy is the vital process for plant life span, which is strictly controlled by endogenous phytohormones and environmental cues [1–8]. Among different phytohormones, gibberellin acid (GA) and abscisic acid (ABA) are two important hormones to determine seed germination or dormancy status, GA promotes seed germination whereas ABA induces seed dormancy [5,6]. Genetic screen experiment also showed a series of mutants deficiency in GA and ABA biosynthesis presented defect in seed germination [9–12]. Besides GA and ABA biosynthesis, the components for GA/ABA signal transduction also affect seed germination, for example, the various transcriptional factors *ABI3*, *ABI4* and *ABI5* perceive the ABA stimulation to control germination at different layers [13–17], The DELLAs protein including GIBBERELLIN INSENSITIVE (GAI), REPRESSOR OF GAL-3 (RGA), RGA-LIKE1 (RGL1), RGL2, and RGL3, are nucleus-localized negative factors for GA signal that inhibit

GA response, including seed germination, flowering time and stem elongation, etc. GA triggers the degradation of DELLAs protein by SLY1 ubiquitin E3 ligase in conjunction with GA receptors GID1a, GID1b and GID1c [18–20]. Besides GA and ABA, other phytohormones, such as auxin, ethylene and JA also coordinate seed germination or dormancy directly or indirectly through altering GA and ABA metabolism [5,21,22]. Apart from the genetic background and hormone signal, environmental factors, mainly light and temperature, also affect seed germination or dormancy process [3,5]. The phytochrome phyB in seed perceives the red-light irradiation to stimulate seed germination. The CCCH-type zinc-finger protein SOMNUS negatively regulates light-dependent seed germination downstream of PIF1 in Arabidopsis [23,24]. Temperature is another environmental cue to affect seed germination, cold treatment under low temperature, called as stratification, breaks the seed dormancy through the bHLH transcription factor *SPATULA* [25]. Ambient high temperature (HT) also induces seed dormancy, also named seed germination thermoinhibition, to prevent seed germination under unfavorable environments [26]. HT can induce the expression of *NCDE4* to induce the accumulation of ABA to repress seed germination in lettuce [27,28]. Our previous study also shows that SOMNUS associates with ABI5 to induce seed dormancy under HT [29], and MADs-box transcriptional factor *AGL67* recruits histone mark reader EBS to epigenetic activation of *SOM* expression during HT stress [30]. However, the underlying mechanism still needs further investigation.

Plant polyamines, including Put (Put), spermidine and spermine, belong to low molecular weight compounds with nitrogen-containing aliphatic structure, and particulate into a series of basic physiological and development processes, such as cell division, rhizogenesis, leaf senescence, zygotic, embryogenesis, etc. [31–34]. There exist two pathways for Putbiosynthesis in *planta*, one pathway depends on arginine decarboxylase (ADC), the other is dependent on Orn decarboxylase (ODC) [31,35]. The ADC pathway is catalyzed by three consecutive enzymes including ADC, agmatine iminohydrolase (AIH), and N-carbamoyl Putamidohydrolase (CPA). The generated Put is then degraded to spermidine and spermine by spermidine synthetase (SPDS) or spermine synthetase (SPMS) [36]. Because the ODC activity is not detectable in Arabidopsis, the ADC-dependent pathway is believed as the main route for Putbiosynthesis, there exist *ADC1* and *ADC2* encoding ADC enzyme in Arabidopsis genome, the expression of *ADC1* is stable but the expression of *ADC2* is frequently changed with environmental factor, and correlates with endogenous oscillated Put level, thus it is reasonable that ADC2 is mainly responsible for Put biosynthesis in response to environment stress [37,38].

Our previous study already found the small molecule compounds, such as carbon monoxide (CO), nitric oxide (NO) or gama-aminobutyric acid, regulate seed germination or dormancy [39–45]. Here we intend to screen more novel chemicals to enhance seed germination tolerance to ambient HT stress. In this study, we reported Put can obviously improve seed germination thermotolerance, and the ADC2 is the main enzyme responsible for Put biosynthesis in Arabidopsis. Furthermore, the combination of physiological, genetic and biochemical analysis indicates that ADC2-mediated Put signal enhances seed vigor through suppressing *SOM* expression and altering its downstream GA/ABA metabolism. Thus, our finding provides mechanistic evidence for developing Put as the potential growth regulator to uniform seed germination in modern agriculture production.

2 Materials and Methods

2.1 Arabidopsis Growths and Treatment

All of the used wild-type and mutant seeds are Columbia ecotype, including *adc2–1* (Salk_026916C) from ABRC (Arabidopsis thaliana Resource Center), the *som* and *SOM-GFP* line that we used before [30]. The seedling was germinated and grown on the 1/2 MS (Murashige and Skoog) medium for 2 weeks in plant incubator under white light condition (30 μmol m^{-2} s^{-1}, 16 h light/8 h dark, 22°C), and then the plant was cultivated in the soil in the greenhouse (50 μmol m^{-2} s^{-1}, 16 h light/8 h dark, 22°C)

and the seeds was harvested in 10–14 weeks of growth. The crossed *adc2/som* and *SOM-GFP/adc2* line were obtained by artificial pollination. The seeds were harvested at the same time in each batch for seed germination or dormancy assay. For Put treatment, the filter-sterilized Putas indicated concentration was added into the medium after autoclaving. For HT treatment, the seeds harvested within one or two months were used, the seed was soaked in the water for 3 h for imbibition, and then the seeds were surfaced sterilized and sowed on the 1/2 MS medium, and placed in the growth cabinet at indicated high temperature for 3 days, and the seed germination percentage was recorded.

2.2 Seed Germination Analyses

The freshly harvested seed was dried for one or two months by silica gel, and seed germination analysis was performed as previous methods [30,45]. In brief, the dried seed was imbibed for 3 h and surface sterilized with 5% (v/v) hypochlorite and 0.02% (v/v) Triton X-100 solution for 10–15 min, and the seeds were washed with sterilized water for three or five times, and then the sterilized seeds were sowed on the 1/2 MS germination medium supplement with 1% sucrose, and placed in the constant light (50 μmol m^{-2} s^{-1}) to initiate seed germination (using 22°C as the control, 28°C to 32°C for HT stress). Seed with radical protruded from the seed coat was recorded as germination. The germinated seeds were observed with stereoscope and the germination percentage was calculated. For each germination assay, at least three biological replicate experiments were performed. Data presented are the means ± SD of three independent assays with seeds from different plants.

2.3 RT-QPCR

The germinated seeds after different treatments were used for total RNA extract using TRIzol reagent (Invitrogen). RT-qPCR experiment was manipulated as described method [30]. In brief, the first strand cDNA was synthesized using 0.5 μg of DNase-treated RNA, moloney murine leukemia virus reverse transcriptase (Fermentas) and oligo (dT) 18 primer in 20 μL reaction system. Prepared cDNA was diluted at a different concentration from 2 to10 ng/μL as the templates. The qPCR experiment was prepared in the SYBR Green I Master Mix on a Roche Light Cycler 480 real-time PCR machine according to the manufacturer's instructions. All RT-qPCR experiments were independently performed in triplicate, and representative results were shown. *PP2A* was used as an internal control. The information of gene specific primers information for RT-qPCR is listed as Supplemental Table 1, and gene expression data were normalized to the expression of *PP2A*.

2.4 Putcontent Analyses

The Put content was measured using the previous method with minor modifications [36]. After different treatments, about 100 mg germinated seeds were collected and homogenized in 1 mL of 5% HClO/2N NaOH solution in chilled mortars and pestles. After the homogenates were incubated in ice-cold water for 1 h, 10 μL benzoyl chloride was added and vortexes for 10 s, and then incubated for 20 min at room temperature after adding 2 mL saturated NaCl, 10 μL benzoyl chloride, vortexed for 10 s, and incubation for 20 min at room temperature, we added 2 mL saturated NaCl. The benzoyl-polyamines were extracted using 2 mL diethyl ether. After centrifugation at 1 500 g × 5 min, 1 mL of the ether phase was collected, evaporated to dryness under a stream of warm air, and re-dissolved in 100 μL methanol for assay immediately. Aliquots of sample were diluted 5 to 20 fold and injected into HPLC, with excitation at 350 nm and emission at 495 nm. The solvent system consisted of methanol: water, run isostatically at 60% to 65% methanol, at a flow rate of 1 mL/min. Standards were treated in a similar way.

2.5 Statistical Analysis

The obtained results were analyzed using GraphPad Prism8 software. The mean values were calculated, and statistically significant differences were evaluated using ANOVA analysis followed by Tukey's test for

germination assays and qRT-PCR analysis (*$P < 0.05$; **$P < 0.01$). Standard deviation (±SD) was also provided to indicate the variations associated with the particular mean values.

3 Results

3.1 Ambient HT Induces the Accumulation of Put in Imbibed Seed

To understand the role of Put in seed germination under HT stress, we first treated the imbibed seeds with gradient HT stress, and then measured the content of Put in the seeds. As shown in Fig. 1A, we found increased HT from 28°C to 34°C treatment gradually increased the content of Put in the germinated seeds after 24 h of treatment, and reached the maximum level after 32°C treatment, and then obviously dropped down once the treatment temperature was over 34°C, probably such temperature showed lethal for seed vigor. The time-course of temperature on Put accumulation in the seed was also measured (Fig. 1B), we found HT treatment quickly induced the accumulation of Put during the first 24 h and then dropped down during the following 36 h, suggesting the dynamic biosynthesis of Put in the seed during HT treatment.

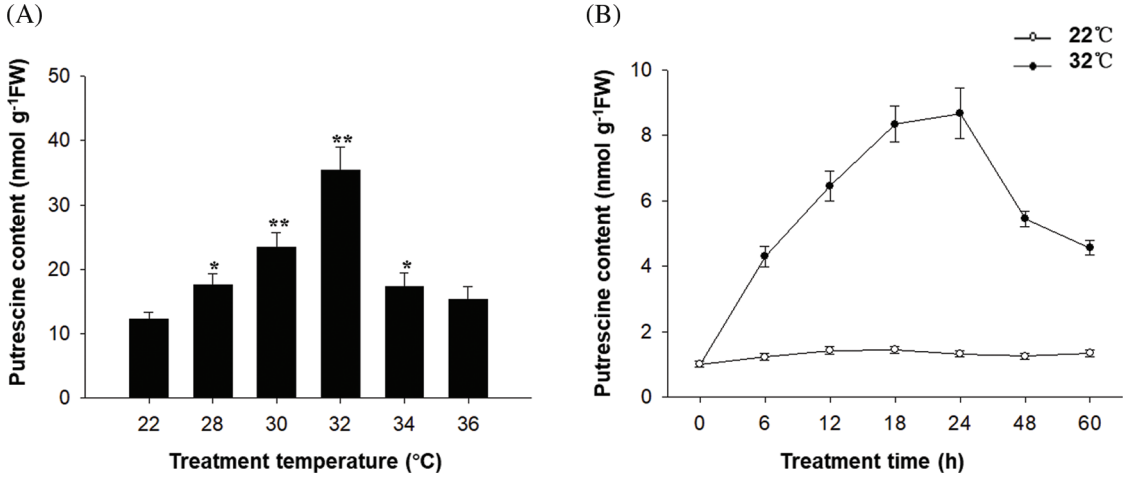

Figure 1: HT induced the accumulation of Put biosynthesis in germinated seeds. (A) Effects of gradient ambient temperature on the content of Put in germinated seeds. The germinated seeds of wild-type col line were incubated under the indicated temperature for 24 h, and the content of Put was measured. Quantitative analysis is the average of three repeats ± standard error. Asterisks indicate statistical differences between Col under 22°C and other temperature gradient, as determined using student's *t*-test (*$P < 0.05$, **$P < 0.01$). (B) Time-course effect of Put content under HT stress at 22°C and 32°C. Germinated seeds were incubated under 22°C and 32°C for indicated time and the content of Put was determined. Quantitative analysis is the average of three repeats ± standard error

3.2 Exogenous Put Treatments Enhance the Seed Germination Tolerance to Ambient HT Stress

To explore the possible function of Put in controlling seed germination under HT stress, we pretreated the imbibed seeds with exogenous Put, and then found additional Put obviously enhanced seed germination under HT stress at 32°C (Fig. 2A). The exogenous Put concentration at 1 μM particularly showed more efficient to promote seed germination, while the promoting effect of Put over 1 μM partially was not so obvious as that at 1 μM, though still accelerating seed germination under HT stress (Fig. 2B). ADC is the main enzyme responsible for Put biosynthesis in *planta*. DMFA and D-arginine were previously reported as the special inhibitor of ADC enzyme, which suppressed the biosynthesis of Put. Here we also treated the imbibed seeds of Col with DMFA and D-arginine, and compared the germination rate of seeds supplemented with Put, as shown in Fig. 2C, DMFA and D-arginine treatment obviously suppressed the

seed germination percentage compared with the those seeds without inhibitor treatment under 28°C or 30°C, these pharmacological results support the conclusion that Put positively regulates seed germination under HT stress.

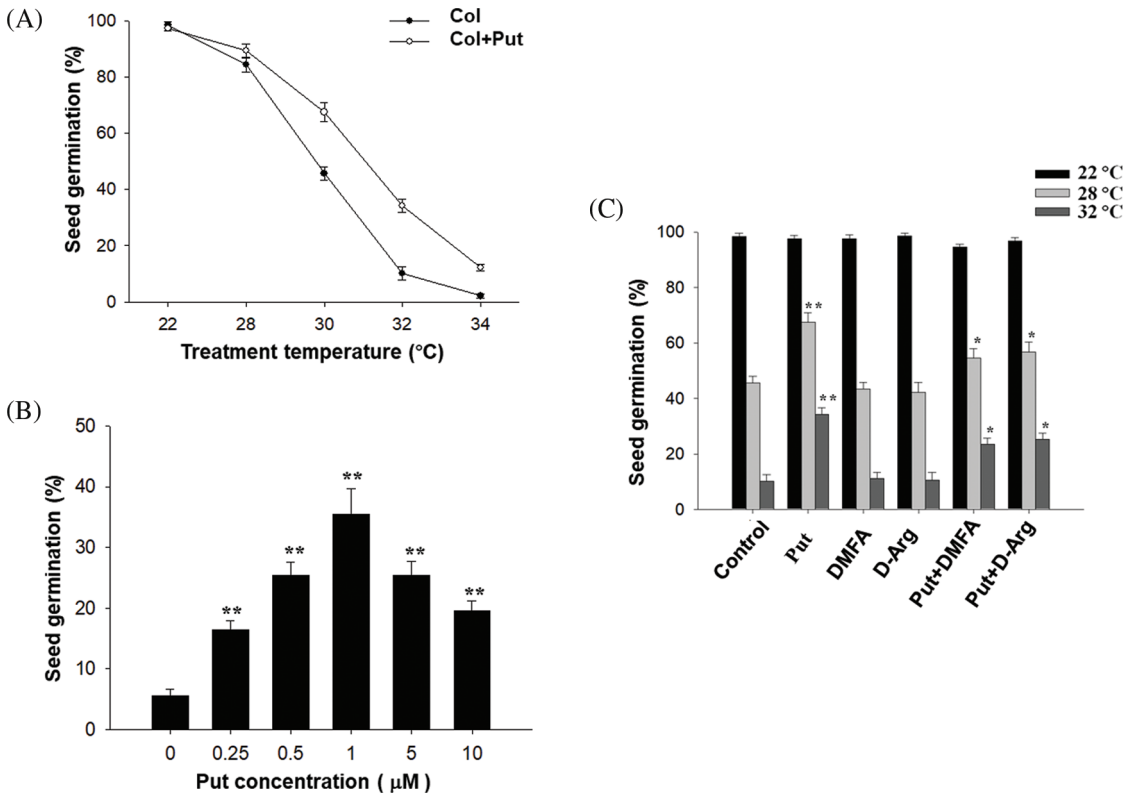

Figure 2: Put positively regulated seed germination under HT. (A) Additional Put enhanced seed germination under HT. Imbibed seeds were treated with or without 1 μM Put for 3 days, and the germination percentage was recorded. Quantitative analysis is the average of three repeats ± standard error. (B) Dose effect of put on seed germination under HT stress. Wild-type Col seed was treated with the indicated concentration of Put and incubated under HT at 32°C for 3 days, and seed germination percentage was calculated. Quantitative analysis is the average of three repeats ± standard error. Asterisks indicate statistical differences between col and that at different Put concentration, as determined by using student's *t*-test (**$P < 0.01$). (C) Pharmacological analysis of seed germination under HT. Imbibed seeds were treated with Put (1 μM), DMFA (100 nM) or D-arginine (1 μM) for 3 days, and seed germination percentage was calculated. Quantitative analysis is the average of three repeats ± standard error. Asterisks indicate statistical differences between col at 22°C, 28°C, 32°C and col with different additions, as determined using student's *t*-test (*$P < 0.05$, **$P < 0.01$)

3.3 The Mutant Deficiency in Put Biosynthesis Shows Low Seed Germination under HT at 32°C

Both *ADC1* and *ADC2* are the important genes responsible for Put biosynthesis in Arabidopsis [38]. To test their roles during seed germination under HT, we firstly checked the transcriptional levels of *ADC1* and *ADC2* in imbibed seeds after HT stress by RT-qPCR analysis. HT treatment indeed induced the expression of *ADC2* but not for *ADC1* (Fig. 3A), which is in accordance with the previous result that ADC2 is the inducible enzyme, and suggests that *ADC2* is more important in regulating seed germination under HT stress. To confirm such hypothesis, we obtained the *adc2*-null mutant of random T-DNA insertion pools in ABRC.

PCR analysis using special primers indicated the T-DNA was located in the first exon to abolish the functional transcript of *ADC2* to inactivate ADC enzyme activity (Fig. 3B).

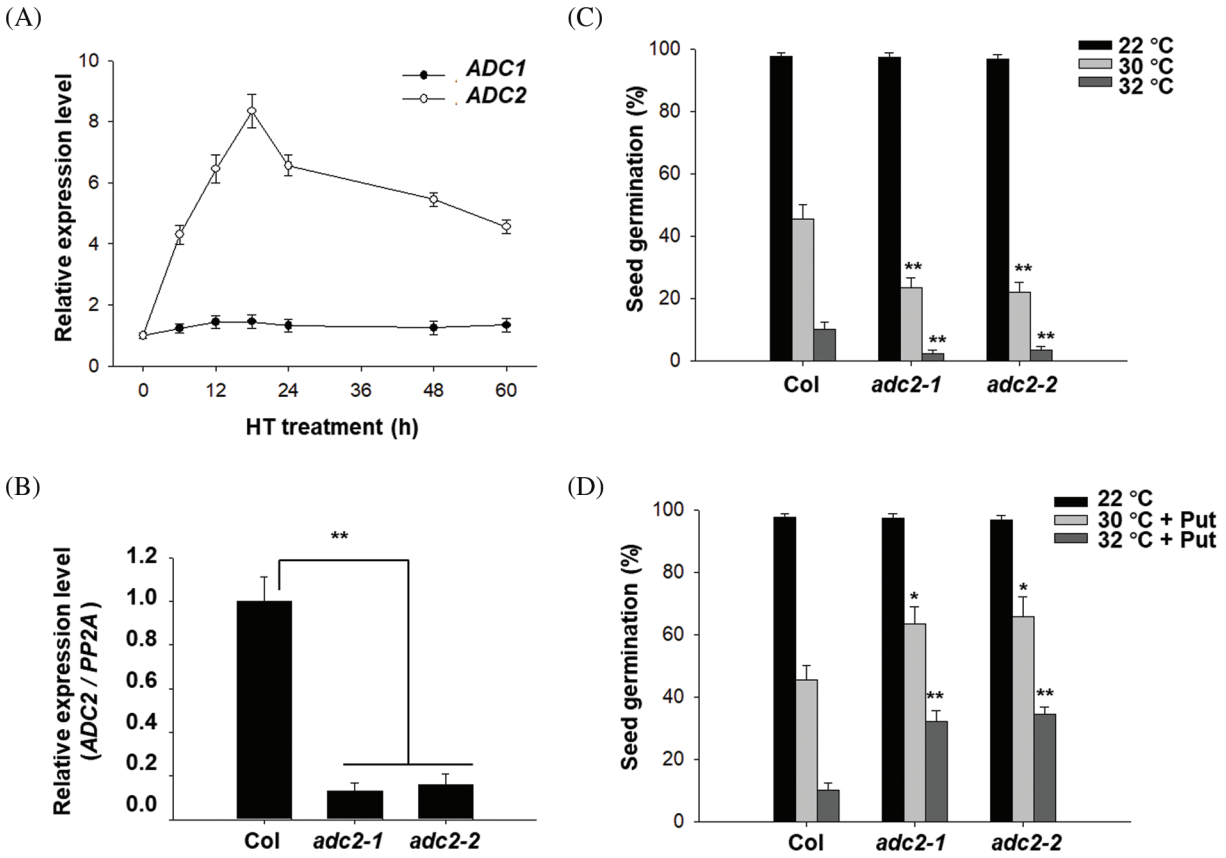

Figure 3: Evaluating the function of *ADC2* in regulating seed germination under HT stress. (A) Time-course effect of HT on the transcriptional levels of *ADC1* and *ADC2* under HT stress. Imbibed seeds were incubated under HT at 32°C for the indicated time, and the transcriptional levels of *ADC1* and *ADC2* were measured by RT-QPCR analysis. Quantitative analysis is the average of three repeats ± standard error. (B) Validating the T-DNA insertion of *adc2-1* and *adc2-2* by RT-QPCR analysis. The expression level of *ADC2* was quantitated by RT-QPCR analysis. *PP2A* was used for the loading control. (C) HT seriously suppressed seed germination of *adc2*-null mutants. Imbibed seeds of Col, *adc2-1/2* mutants were incubated under different temperatures as indicated for 3 days, the seed germination was calculated. Quantitative analysis is the average of three repeats ± standard error. Asterisks indicate statistical differences between Col and *adc2-1*, *adc2-2* at different temperatures by student's *t*-test (***P* < 0.01). (D) Put treatment rescued the low germination rate of *adc2* mutant under HT stress. The imbibed seeds of Col and *adc2-1/2* were treated with or without Put at 1 μM and incubated under 32°C for 3 days, and the seed germination percentage was calculated. Quantitative analysis is the average of three repeats ± standard error. Asterisks indicate statistical differences between Col, *adc2-1*, *adc2-2* under 22°C and 30°C + Put, 32°C + Put, as determined by student's *t*-test (**P* < 0.05, ***P* < 0.01)

To confirm the function of *ADC2* in promoting seed germination under HT, we compared the seed germination percentage of *adc2-1*, *adc2-2* and Col line under gradient HT stress. As shown in Fig. 3C, the seed germination of *adc2-1* and *adc2-2* was relatively lower than that of wild-type Col line under gradient HT stress, and probably inactivated the ADC enzyme activity to reduce endogenous Put level.

Furthermore, we pretreated *adc2-1/2* with Put, and found additional Put increased the seed germination of *adc2-1/2* under HT (Fig. 3D). Thus, these experiments suggest that ADC2 mediates the seed germination under HT through suppressing Put biosynthesis.

3.4 Put Treatment Suppresses the Expression of SOM and Downstream ABA Biosynthesis

It is reported that *SOM* acts as the critical regulator to control seed germination under HT [30]. Here we also monitored the *SOM* expression in *adc*-null mutants and Col seed under HT. HT treatment rapidly induced the expression of *SOM* in the imbibed Col seeds, and such effect was relatively higher in the *adc2-1* and *adc2-2* mutant seeds. Consistently, additional Put treatment also suppressed HT-induced *SOM* expression in the imbibed *adc2-1* and *adc2-2* mutant seeds (Fig. 4A). These data suggest that *ADC2*-dependent Put mediates *SOM* expression under HT stress.

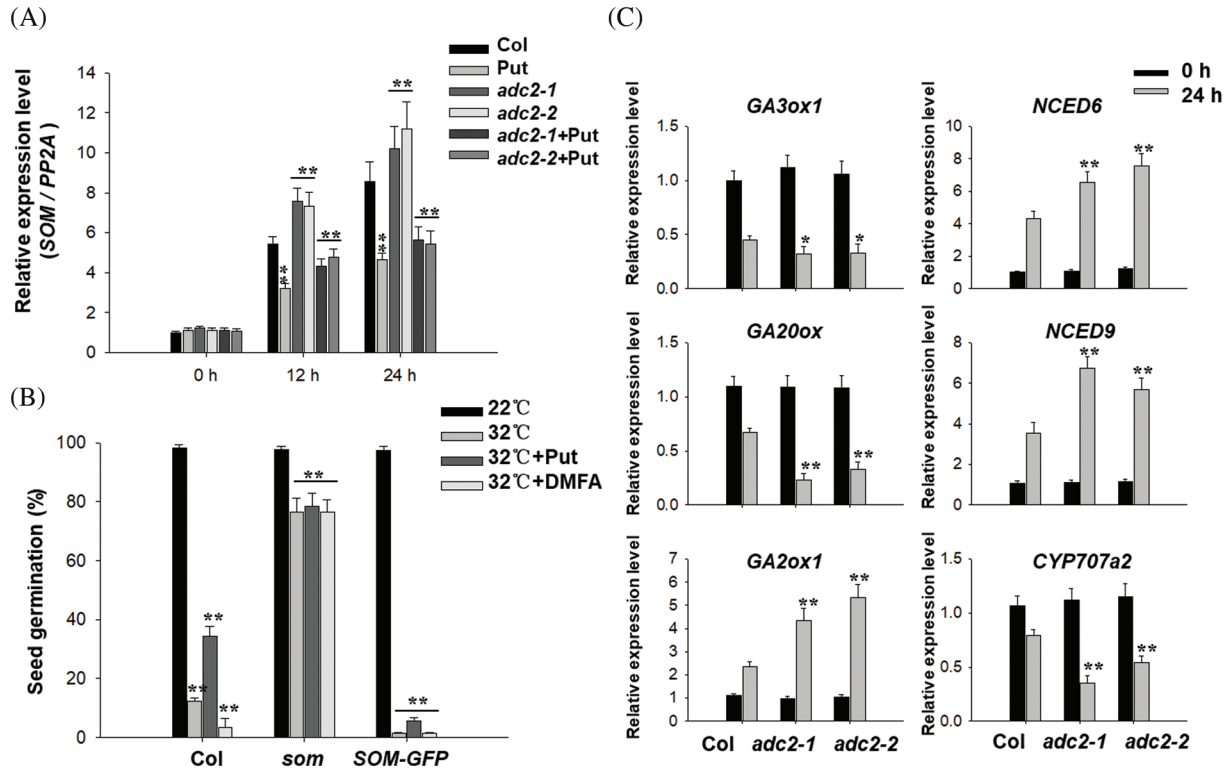

Figure 4: Put regulated seed germination under HT through *SOM*. (A) *ADC2*-mediated Put suppressed *SOM* expression under HT. The imbibed col, *adc2-1, adc2-2* seeds were treated with 1 μM Put under HT at 32°C for indicated time and the expression of *SOM* was measured by RT-qPCR analysis. The *PP2A* was used as the loading control. Asterisks indicate statistical differences between Col and *adc2-1*, *adc2-2* with and without Put at a different time point, as determined using student's *t*-test (**$P < 0.01$). (B) Effect of Put and its biosynthesis inhibitor on the expression of *SOM*. The Col seed was treated with Put (1 μM) and DMFA (100 nM) under 22°C or 32°C for 3 days, and seed germination percentage was calculated. Quantitative analysis is the average of three repeats ± standard error. Asterisks indicate statistical differences between Col, *som*, *SOM-GFP* under 22°C and 32°C at different treatments, as determined using student's *t*-test (**$P < 0.01$). (C) The different expression patterns of genes related to GA/ABA metabolism under HT. The imbibed seeds of Col, *adc2-1, adc2-2* were treated under HT 32°C for 24 h, and the expression levels of genes related GA metabolism (*GA3ox1, GA20ox1, GA2ox1*) and ABA metabolism (*NCED6, NCDE9* and *CYP707A2*) were measured by RT-qPCR analysis. Quantitative analysis is the average of three repeats ± standard error. Asterisks indicate statistical differences between relative transcript abundances in Col and *adc2-1*, *adc2-2* at different time point by student's *t*-test (*$P < 0.05$; **$P < 0.01$)

The balance of GA/ABA determines the seed germination status, and *SOM* regulates the expression of genes associated with GA/ABA metabolism to affect seed germination under HT stress [46]. Here we also measured the expressions of GA and ABA anabolic genes and GA/ABA catabolic genes included. As shown in Fig. 4C, HT triggered the expression of *GA2ox1, NCED6* and *NCED9*, as well as suppressed the expression of *GA3ox1, GA20ox1* and *CYP707A2*, which resulted in the higher level of ABA and lower GA content to suppress seed germination. However, the expression levels of *GA2ox1, NCED6* and *NCED9* in imbibed seeds of *acd2-1* and *acd2-2* were higher than that in Col seed under HTs, whereas the expression of *GA3ox1, GA20ox1* and *CYP707A2* in the *acd2-1* and *acd2-2* was lower than that in Col seed, which is in agreement with the lower seed germination of *acd2-1/2* subjected to HT stress.

Furthermore, we also checked the effect of Put on the seed germination of *som* mutant, or the transgenic line overexpressing *SOM-GFP*. As the *som* mutant showed relatively higher seed germination, while *SOM-GFP* transgenic line showed lower seed germination under HT stress. Here we pretreated *som* or *SOM-GFP* line with Put and then checked their seed germination under HT stress. Unlike the wild-type Col in Put or Put inhibitor DMFA treatment promoted or suppressed its seed germination under HT stress, Put or DMFA treatment did not affect the seed germination of *som* mutant or *SOM-GFP* under HT, further supporting the opinion that Put treatment regulates seed germination through *SOM* under HT (Fig. 4B).

3.5 Genetic Analyses Reveal Put Acts Upstream of SOM to Regulate Seed Germination

Our above results indicated that Put depends on *SOM* to control seed germination. To understand the genetic relationship between *SOM* and *ADC2* during seed germination under HT, we crossed *som*, *SOM-GFP* line with *adc2-1* mutant and then checked their seed germination under HT. As shown in Fig. 5, we found that both of *som* and *adc2/som* double mutant showed higher seed germination under HT stress, whereas both of *SOM-GFP* and *SOM-GFP/adc2* line showed lower seed germination under HT, indicating that *SOM* was epistatic to *ADC2* to control seed germination under HT stress (Fig. 5A). Furthermore, pretreatment *adc2/som* double mutant seed with Put inhibitor also did not alter its seed germination under HT stress. Thus, these data further confirm the opinion that Put signal enhances seed germination through *SOM* under HT stress (Fig. 5A).

4 Discussions

Accumulated evidence suggests the important function of Put for plant's growth and development. There are two genes (*ADC1* and *ACD2*) encoding ADC in Arabidopsis. Both of them affect seed development, and the double *adc1/adc2* mutant is lethal [31,32,38]. It is reported that the expression of *ADC1* is constitutive and *ADC2* is inducible by environmental factors such as saline stress or iron deficiency, etc. [37,38]. Here we also found that ambient temperature did not obviously alter the expression of *ADC1*, but could quickly and strongly induce the expression of *ADC2*, which is in agreement with the previous study, suggesting the important function of *ADC2* for seed germination tolerance to ambient HT. Consistently, our results showed additional exogenous Put enhanced the seed germination. On the contrary, suppressing Put biosynthesis by a specific inhibitor aggravated the seed dormancy, confirming the positive function of Put seed germination under HT stress. We found that the *adc2-1* and *adc2-2* mutant showed a lower germination percentage compared with wild-type Col line under HT stress in particular. As the concentration of GA/ABA and its ratio determined the seed germination or dormancy status, here we also found that expression levels of *GA3ox1*, *GA20ox1* and *CYP707A2* associated with GA biosynthesis and ABA degradation in *adc2* mutants were obviously lower than that in Col under HT stress, whereas the genes of *GA2ox1, NCED6* and *NCDE9* associated with GA degradation or ABA biosynthesis in these lines were upregulated, suggesting that Put regulates seed germination under HT through altering GA and ABA metabolism.

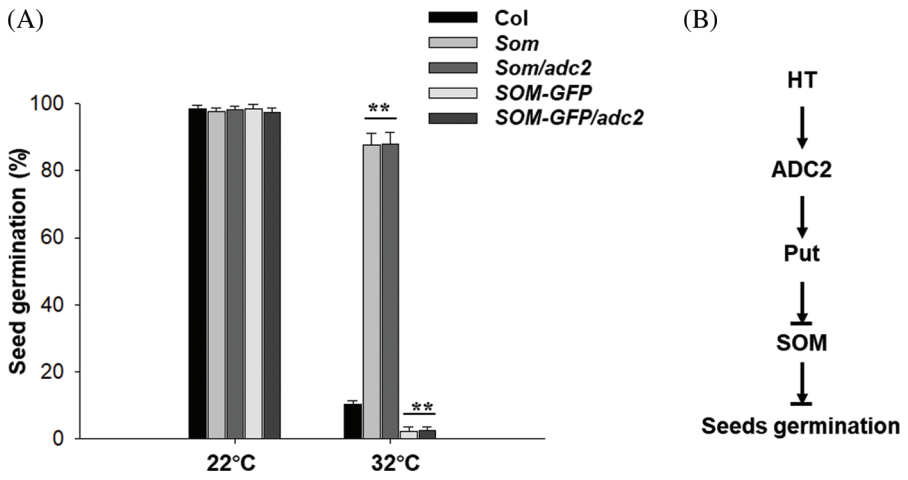

Figure 5: *ADC2* required *SOM* to regulate seed germination under HT. (A) Genetic analysis of the relationship of *ADC2* and *SOM* in controlling seed germination under HT. The Col, *som, SOM-GFP* and the crossed *adc2/som*, *SOM-GFP/adc2* seeds were incubated under 32°C for 3 days, and seed germination percentage was calculated. Quantitative analysis is the average of three repeats ±standard error. Asterisks indicate statistical differences between seed germination in col and other genetic materials under 32°C, as determined using student's *t*-test (**$P < 0.01$). (B) A proposed model to illustrate the novel functions of Put in enhancing seed germination tolerance to HT stress. In this model, we propose that HT induced the transient expression of *ADC2* to transiently increase Put content, however, long-term HT treatment did not sustain the high endogenous Put level. Additional exogenous Put suppressed the expression of *SOM* and subsequently altered GA/ABA metabolism to activate seed germination under HT stress

As *SOM* is the vital regulator to control phyB-dependent seed germination, it also participates into the seed germination regulation in response to HT stress [23,30,46]. *SOM* affects the genes associated with the GA and ABA metabolism, subsequently controls seed germination [23]. A series of transcriptional factors involved in GA/ABA signal transduction, including *ABI3*, *ABI5*, *DELLA*, and light-responsible transcriptional factor, such as *PIF1*, binds to the promoter region of *SOM* to activate its expression [22]. As a result, seed germination of *som* mutant shows insensitity to HT and far-red light irradiation, while overexpression *SOM* reduced seed germination after HT stress or red-light stimulation [23,30,46]. In this study, we found that Put also repressed the expression of *SOM*. Similar to the previous study, we found that Put treatment also altered the expression of genes related to GA/ABA metabolism, including upregulating *GA3ox1, GA20ox1* and *CYP707A2* for GA biosynthesis and ABA degradation, and down-regulating *GA2ox1, NCDE6 and NCED9* for GA degradation and ABA biosynthesis, consequently alleviated HT-induced seed dormancy. On the contrary, Put biosynthesis inhibitor treatment reversed the effect of Put on these genes' expression, thus increased seed dormancy. Genetic analysis also revealed that *ADC2* depended on *SOM* to regulate seed germination under HT, as the *adc2/som* and *som* mutant showed higher seed germination whereas both of *SOM-GFP/adc2* and *SOM-GFP* line displayed lower seed germination under HT stress, these data suggest that *SOM* is epistatic to *ADC2* for seed germination under HT.

In conclusion, we reported Put as the novel signal to enhance seed germination tolerance to HT stress. Based on our study, we proposed a model to illustrate the role of Put in regulating seed germination in response to HT stress. In Arabidopsis, *ADC2* is quickly induced by HT stress for Put biosynthesis, but such inducible effect is transient and induced Put is degraded during the continuous HT stress, therefore

additional Put treatment could enhance seed germination through suppressing the negative regulator *SOM*, therefore altering downstream GA/ABA metabolism, ultimately lessening the inhibitory effect of HT on seed germination. Together, our finding shows a new insight into the function of Put during plant response to heat stress, and proposes the potential application of Put as the plant growth regulator in promoting seed germination against ambient high temperature (Fig. 5B).

Author Contributions: The authors confirm contribution to the paper as follows: Ping Li and Xiangyang Hu designed the research and wrote the manuscript together. Shiyan Lu performed all the experiments. Yulan Hu and Yilin Chen obtained and identified the related materials. Yaru Yang and Yue Jin analyzed the data. All authors reviewed the results and approved the final version of the manuscript.

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Conflicts of Interest: The authors declare that they have no conflicts of interest to report regarding the present study.

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Supplement Information

Supplement Table 1: Primer sequences used for semi-quantitative RT-PCR

Gene name	Forward primer	Reverse primer
PP2A	TATCGGATGACGATTCTTCGTGCAG	GCTTGGTCGACTATCGGAATGAGAG
GA3ox1	CCGAAGGTTTCACCATCACT	CCCCAAAGGAATGCTACAGA
GA3ox2	TAGATCGCATCCCATTCACA	TGGATAACTGCTTGGGTTCC
GA2ox2	AATAACACGGCGGGTCTTCAAATCT	TCCTCGATCTCCTTGTATCGGCTAA
NCED6	ACCGGGTCGGATATAAATTGGGTTG	CCCGGGTTGGTTCTCCTGATTC
NCED9	AACCGCCGCTATGGTTTTAGACG	CCAGTCACCGGAAGGTTATGCAC
CYP707A2	ATGGGGTTGCCTTACATCGGAGA	TGGCTTGAACAAGTGAGCTTTGCT
SOM	ATGGATGTCGTTTGTACGGAACATCAA	TCAAGTCAAGAGATCATTGACCCATCC
ADC1	GTGACAGCGACGGAAAGATC	AGGTCCATCGCTCTGCAATA
ADC2	GAGGAAGCTGCGTCAATCTG	GTCAATCCCCAAACCACCAC