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Article Addendum

Putrescine as a signal to modulate the indispensable ABA increase under cold stress

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Polyamines have been found to correlate frequently with biotic and abiotic insults, and their functional involvement in the plant responses to several stresses has been shown genetically with both gain and loss of function mutations. In spite of a large body of physiological and genetic data, the mode of action for polyamines at the molecular level still remains elusive. We have recently performed a detailed integrated analysis of polyamine metabolism under cold stress by means of metabolic studies, quantitative gene expression analyses, and gene inactivations, to characterize in more detail the role of polyamines in response to low temperature. Our data show a unique accumulation profile for putrescine compared to other polyamines, with a progressive increase upon cold stress treatment coincident with a similar transcriptional upregulation for the two arginine decarboxylase genes *ADC1* and *ADC2*. Loss of function mutants *adc1* and *adc2* display reduced freezing tolerance and alterations in ABA content and ABA-dependent signalling pathways under low temperature, compared to wild type plants. Phenotypical reverse complementation tests for both *adc* and ABA-defective mutants support our conclusion that putrescine modulates ABA biosynthesis at the transcriptional level in response to low temperature thus uncovering a novel mode of action for polyamines as regulators of hormone biosynthesis.

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Introduction

The intracellular accumulation of endogenous polyamines is well documented in plants subjected to environmental challenges and it has also been correlated with increased tolerance to abiotic stresses.[1] However, the details of molecular mechanisms by which polyamines act as cellular guard metabolites against stress remain ill-defined. The increased knowledge of metabolic pathways involved in polyamine biosynthesis and catabolism, and annotation of the gene sequences encoding enzymes involved in these processes allow an integrated analysis of these pathways in Arabidopsis.[2] We have recently used this conceptual approach in Arabidopsis plants challenged with low temperature conditions.[3]

Putrescine Requirement in the Plant Response to Low Temperature Conditions

The metabolic profile of polyamines confirmed previous metabolomic studies showing an accumulation of the diamine putrescine in response to conditions of low temperature.[4] However, our studies also revealed a thus far unexplained reduction of the high molecular weight polyamine spermine. The transcriptome of genes involved in polyamine biosynthesis was in agreement with previous data showing enhanced expression of arginine decarboxylase coding genes (*ADC1* and *ADC2*) with similar albeit not identical expression profiles.[5] The availability of T-DNA knock-out mutants (*adc1* and *adc2*) with reduced accumulation of putrescine upon cold stress compared to wild type, allowed functional test of putrescine accumulation in plants exposed to freezing stress. Freezing tolerance assays revealed that putrescine is required for plant survival under such limiting conditions. Both *ADC1* and *ADC2* were shown to be required for survival to freezing stress both in nonacclimated and cold-acclimated plants. Although the double *adc1-adc2* mutant is not viable, a plant completely depleted of putrescine would be expected to display a more dramatic phenotype of freezing sensitivity.

Putrescine Mediates the Cold-Induced ABA Response by Modulating its Biosynthesis at the Transcriptional Level

The immediate question that arose in the light of these data was

about the nature of the defects in the *adc* mutants that could explain their sensitivity to freezing tolerance. One central signalling pathway triggered by low temperature is the CBF-dependent pathway.[6] Several genes regulated by the CBF factors, the so-called CBF regulon, were monitored at the gene expression level for wild type and *adc* mutant plants challenged with cold stress. No significant alteration was observed, although lower transcript levels were detected for some genes in *adc* mutants compared to wild type plants. We tested, therefore, the cold induction of three CBF genes that are themselves cold-induced. While *CBF1* and *CBF2* behaved normally, *CBF3* showed slightly impaired induction in *adc* mutants compared to wild type. Another pathway involved in the plant response to low temperature is the ABA-dependent pathway, since ABA has been shown to mediate the transcriptional induction of some cold-responsive genes.[7] We therefore hypothesized that the ABA-signalling pathway operating under cold stress might be affected to some extent in the *adc* mutants with reduced putrescine content. To further investigate the relationship between putrescine and ABA signalling under cold stress, we studied the cold-induction of ABA-dependent genes such as *RD29B* and the key ABA biosynthetic gene *NCED3* that is also cold-inducible. In addition we also measured the ABA content at different time points of the low temperature exposure in both wild type and *adc* mutants. Our data indicated that, in contrast to wild type plants which display an endogenous maximum ABA content after 24 hours of cold treatment,[8] the *adc* mutant plants are unable to achieve a full ABA-dependent response. Thus, both the ABA content and the ABA-dependent gene induction are affected in the *adc* mutant plants, while both levels can be restored to wild type values upon exogenous putrescine application. Noteworthy, the supplementation of putrescine to the growth medium enhances the freezing tolerance even in the wild type plants. The reciprocal complementation tests of freezing tolerance for both ABA-defective mutant *aba 2–3* and putrescine defective mutants *adc1* and *adc2*, led to the conclusion that putrescine is required for a bona fide ABA response under low temperature conditions.

Conclusions

Our data point to a functional role for the diamine putrescine in freezing tolerance and cold acclimation that goes beyond a simple physico-chemical protective role. What remains to be elucidated is whether additional protective functions can be assigned to putrescine upon cold stress, and more importantly how putrescine modulates ABA synthesis at the molecular level. In this respect, it has been suggested that polyamines in mammals may participate in loops involving interaction with signal transduction pathways and activation/repression of proteins that control either cell death or cell growth.[9] Similar strategies could be used by plants.

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References

1. Alcazar R, Marco F, Cuevas JC, Patron M, Ferrando A, Carrasco P, et al. Involvement of polyamines in plant response to abiotic stress. Biotechnol Lett 2006; 28:1867-76.
2. Ferrando A, Carrasco P, Cuevas JC, Altabella T, Tiburcio AF. Integrated molecular analysis of the polyamine pathway in abiotic stress signalling. In: Amancio S, Stulen I, eds. *Nitrogen acquisition and assimilation in higher plants*. The Netherlands: Kluwer Academic Publisher 2005; 207-30.
3. Cuevas JC, López-Cobollo R, Alcázar R, Zarza R, Koncz C, Altabella T, et al. Putrescine is involved in Arabidopsis freezing tolerance and cold acclimation by regulating ABA levels in response to low temperature. Plant Physiol 2008; 148:1094-105.
4. Kaplan F, Kopka J, Haskell DW, Zhao W, Schiller KC, Gatzke N, et al. Exploring the Temperature-Stress Metabolome of Arabidopsis. Plant Physiol 2004; 36:4159-68.
5. Urano K, Yoshiba Y, Nanjo T, Igarashi I, Seki M, Sekiguchi F, et al. Characterization of Arabidopsis genes involved in biosynthesis of polyamines in abiotic stress responses and developmental stages. Plant Cell Environ 2003; 26:1917-26.
6. Van Buskirk HA, Thomashow MF. Arabidopsis transcription factors regulating cold acclimation. Physiol Plant 2006; 126:72-80.
7. Viswanathan C, Zhu JK. Molecular genetic analysis of cold-regulated gene transcription. Philos Trans R Soc Lond B Biol Sci 2002; 357:877-86.
8. Lang V, Mantyla E, Welin B, Sundberg B, Palva ET. Alterations in water status, endogenous abscisic acid content, and expression of Rab18 gene during the development of freezing tolerance in *Arabidopsis thaliana*. Plant Physiol 1994; 104:1341-9.
9. Pignatti C, Tantini B, Stefanelli C, Flamigni F. Signal transduction pathways linking polyamines to apoptosis. Amino Acids 2004; 27:359-65.