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 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 to other polyamines, with a progressive increase upon cold stress. The availability of T-DNA knock-out mutants (adc1 and adc2) with similar albeit not identical expression profiles. The immediate question that arose in the light of these data was the 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.

Key words: ABA, cold acclimation, freezing tolerance, polyamine, putrescine, arginine decarboxylase, gene expression
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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