



Transgenic Wheat Has Increased Polyamines

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Importance of arginine decarboxylase in plants

Crop productivity in modern agriculture relies heavily on abundant supplies of water for irrigation. Although drought and salt tolerance genes are present in wheat, breeding for stress tolerance is time- and labour-intensive and complicated by the multigenic nature of stress tolerance and the complexity of wheat genetics. The polyamine biosynthetic pathway in higher plants is a useful model in which to examine the components that affect the levels of intermediates and end products in the pathway. By introducing appropriate transgenes into plants and measuring the effects of transgene products on end product accumulation, we may begin to understand how individual components of the pathway contribute towards their concerted regulation¹.

The polyamines spermidine and spermine, and their precursor putrescine, are ubiquitous in all living organisms and are involved in many diverse physiological, developmental, and biochemical processes. Pyridoxal phosphate (PLP)-dependent ornithine decarboxylase (ODC) is the initial enzyme in the pathway committed to polyamine synthesis. In plants and some bacteria, putrescine can also be synthesized from arginine via arginine decarboxylase (ADC) through the intermediate agmatine. Putrescine is further converted into spermidine and spermine by spermidine synthase (SPDS) and spermine synthase (SPMS), respectively. These enzymes add aminopropyl groups generated from *S*-adenosylmethionine (SAM) by SAM decarboxylase (SAMDC). In plants, the two alternative pathways appear to have specific roles in growth and development. While ODC appears to be implicated in the regulation of the cell cycle in actively dividing cells and meristematic zones, ADC is the primary enzyme for putrescine synthesis in non-dividing elongating cells, secondary metabolic processes, and in cells under various stresses².

Arginine decarboxylase is a low copy number nuclear gene that lacks introns in the sequences described to date. Several *ADC* cDNA clones have been isolated and characterized from various species. Whereas in some plants a single gene encodes ADC, in the *Brassicaceae* family at least two paralogues exist in all members studied to date except for the basal genus *Aethionema*. In *Arabidopsis*, protein sequences derived from *ADC1* (U58851) and *ADC2* (AF009647) genes show 80% homology; however the activities of these enzymes differ. Much of the difference between ADC1 and ADC2 protein amino acid sequences is at the N-terminus, suggesting that the subcellular location of the two proteins might be different. Polyclonal antibodies raised against tobacco *ADC* (AF321137; 99% homology to the tobacco *ADC2* AF127241) detect ADC protein in all plant organs analyzed: flowers, seeds, stems, leaves, and roots; however, depending on the tissue, the protein is localized in two different subcellular compartments, the nucleus and the chloroplasts. These results suggest that the intracellular location of ADC in plants might account for different roles for the enzyme in different locations. Thus the notion was advanced that chloroplastic ADC might be involved in photosynthesis, whereas the nuclear form may play a role in cellular signalling³.

Engineering the polyamine biosynthetic pathway in wheat

Wheat (*Triticum aestivum* L.) is a staple crop for about 35% of the human population. Its great adaptability to varied climatic conditions makes it one of the most widely cultivated crops, with a short growing season and a good yield per unit area. Breeders have produced disease-resistant, drought-tolerant (to a certain degree) and high-yielding varieties using conventional methodology. However, wheat productivity has declined as a result of deteriorating soil conditions, the quality and quantity of available water for irrigation, and general environmental degradation. Consequently, increases in productivity have fallen below the rate of population growth.

One of the major targets for wheat improvement is drought tolerance. Our group is interested specifically in elucidating the role of polyamines in abiotic stress tolerance in cereal crops. We generated transgenic wheat plants expressing an oat (*Avena sativa* L.) *ADC* cDNA driven by the maize 1 ubiquitin (*Ubi*) promoter and first intron⁴. These plants accumulate up to 2-fold putrescine, spermidine, and spermine in leaves. The two-fold increase in the three polyamines measured in leaves of primary transformants is maintained in the T1 generation, thus confirming the heritable nature of polyamine accumulation in transgenic wheat plants expressing the transgene.

Whereas an increase in polyamine content is rare in rice leaves, such increases are more common in seeds. For example spermidine and spermine levels are significantly increased in seeds of transgenic rice plants expressing *Ubi:DsSAMDC*⁵. Multiple independent transgenic wheat lines expressing *Ubi:AsADC* accumulate up to 7-fold putrescine, 1.5-fold spermidine, and two-fold spermine in seeds, compared to wild type plants. These levels are maintained and even enhanced in progeny.

Results from our earlier studies in rice and also our current investigation in wheat demonstrate that less metabolically active



tissue, such as seeds, accumulate higher levels of polyamines. Our results are in line with experiments in which metabolites such as pre-vitamin A and pharmaceutical antibodies accumulate at high levels in seeds of rice, wheat, and pea⁶. It is not surprising that higher levels of accumulation occur in seeds, because these storage organs are dormant or certainly less metabolically active compared to vegetative tissue. The above examples show that this behavior is not limited to small molecular weight metabolites. Rather it is more general, extending to the accumulation of recombinant proteins, which in extreme cases can form paracrystalline structures in the endosperm⁶.

The wheat genome contains at least two arginine decarboxylase paralogs

Genomic characterization of the transgenic wheat plants indicated high homology between the oat and wheat *ADC*. Digestion with several enzymes and hybridization with a cloned partial wheat *ADC* probe confirmed the presence of at least two *ADC* genes in the wheat genome. Only two *ADC* genes have been cloned from monocotyledonous plants to date—the first from oat (X56802) and more recently a second gene from rice (BAA84799). Chattopadhyay et al.⁷, upon digestion of rice and oat genomic DNA with a specific enzyme and subsequent probing with a 498-*OsADC* probe, observed two distinct molecular species in both rice and oat genomic DNA blots. An ancestral *ADC* gene appears to have been duplicated early in the origin of the *Brassicaceae* family, thus yielding two paralogs. These two different *ADC1* and *ADC2* genes have been described in *Arabidopsis*. Although the two genes share 80% homology in their amino acid sequence, they exhibit different expression patterns: *ADC1* is expressed constitutively, whereas *ADC2* is mainly expressed in cauline leaves and siliques and is induced by different abiotic stresses. Two *ADC* paralogous genes have been characterized also in *Pringlea antiscorbutica*, *Nicotiana tabacum*, and *Malus sylvestris* L (crabapple).

Using the gene-specific probe for *TaADC*, we detect two transcripts of ca. 2.6 and 3.1 kb in wheat. The transcripts are expressed in leaves and roots, with the 2.6 kb species exhibiting higher steady-state accumulation. No significant changes are measured in levels of steady-state *TaADC* (long or short mRNA species) or in *TaSAMDC* levels in transgenic plants expressing the *AsADC* gene. It is therefore apparent that the heterologous transgene operates independently of its wheat homologs, and consequently any increase in polyamine content is due to expression of the transgene alone. Some evidence exists for post-transcriptional and post-translational regulation of *ADC* in plants. *TaADC* might generate two different forms of the transcript through alternative splicing. Alternative processing of a transcript is common in animal cells, where expression from a single gene may produce protein variants that differ in function, tissue specificity, or sub-cellular localization. There are also reports of alternative splicing in plants. For example Zhang et al.⁸ described the cloning of three cDNAs from apple (*MdSPDS-1*, *-2a* and *-2b*) encoding SPDS. *MdSPDS-2a* and *MdSPDS-2b* originate from *SPDS2* by alternative splicing and are differentially regulated in a tissue- and developmentally-specific manner.

In conclusion, we generated and characterized a transgenic wheat population expressing *AsADC*. In the course of this characterization, we detected the presence of multiple *ADC* homologs in wheat, coinciding with gene duplication in other plant species with complex genomes. This transgenic wheat population will be useful in studies to elucidate further the role of polyamines in drought tolerance and also to ascertain differences and similarities between rice and wheat in their general response to abiotic stresses. Such studies are important because they will allow us to determine whether polyamines are general response mediators that enhance tolerance of plants to abiotic stress.

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