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Maize aldose reductase: A role in sugar-handling?

(submitted by Sylvia Morais de Sousa <<u>smsousa@cnpms.embrapa.br</u>>)

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Our initial interest in aldose reductase (AR) rose from its possible role in sorbitol metabolism by maize kernels. This is the only known enzyme in maize, other than sorbitol dehydrogenase, with capacity to synthesize or use the sorbitol so prominent in developing kernels. The reaction is reversible

(sorbitol+NADP<=>glucose+NADP[H]) and could allow sorbitol use by embryos. However, ARs can catalyze diverse reactions and may have multiple roles in sugar- and redox-handling. Aldo-keto reductases (AKRs) are widely distributed in nature and play numerous roles in metabolism. In this study, we present eight maize putative AKRs and characterize one of them, AKR4C13, due to its embryo specificity. To analyze in detail the expression of maize AKRs at the protein level we raised polyclonal antisera against the recombinant maize AR and used it in western blot assays. We also designed specific primers for each of the eight putative maize ARs to analyze their gene expression in different tissues and development times. The analysis of different maize tissues showed reaction with several polypeptides. The amount of each polypeptide also appeared to vary among tissues, consistent with potentially different roles for the AR-like polypeptides. Data on western blots were consistent with predicted molecular weights of the AR family members as well as their expression patterns. The AKR4C13 was embryo-specific, with a MW of 35,659 Da and was temporally correlated with seed maturation. Analysis of the recombinant AKR4C13 enzyme indicated that, in addition to DL-glyceraldehyde, reactions favored use of NADPH to reduce pentoses, but not D-glucose. Maize AKR4C13 was also able to oxidize sorbitol in the presence of NADP. One possibility is that the maize sorbitol pathway, and AR in vivo, has similarities to roles in humans, where their primary effect is that of balancing sugar pools and redox levels under high-sugar conditions.

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Monitoring Ds Transposition in Soybean Genome

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The maize two-component transposon system Ac/Ds has been used in many plant species as a means to generate insertional and activation tagged mutants. The long-term goal of this program is to develop a repository of transgenic soybean events harboring mapped Ds elements positioned approximately every cM, thereby creating a collection of Ds soybean events that will have utility for local mutagenesis. The usefulness of the system will be influenced by the ability of Ds to transpose when stacked with Ac. To investigate the transposition of Ds in the soybean genome we selected a set of five soybean events harboring gene or enhancer trap elements delineated by Ds termini. These events carry either one or two transgenic loci. To induce transposition we stacked the respective Ds events with an Ac cassette under control of either the constitutive 35S CaMV promoter or the reported meiosis specific promoter, DMC1, from Arabidopsis and generated 351 crosses. We found somatic transpositions in 144 F1 plants and 14 germinal transpositions in the progeny of 23 F1 plants analyzed so far. One specific transposition has a Ds delineated enhancer trap element re-inserted into the third intron of the ligand gated potassium ion channel (glyma 06g08110). In addition we have generated 461 events carrying an activation tag construct delineated by Ds termini and crossed selected events with the Ac carrying events to generate another 121 crosses. The data gathered from this study will allow us to test the influence of both level, and tissue specificity of Ac expression on transposition of Ds in the soybean genome.

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