UNDERSTANDING LYSINE METABOLISM IN CEREAL CROPS

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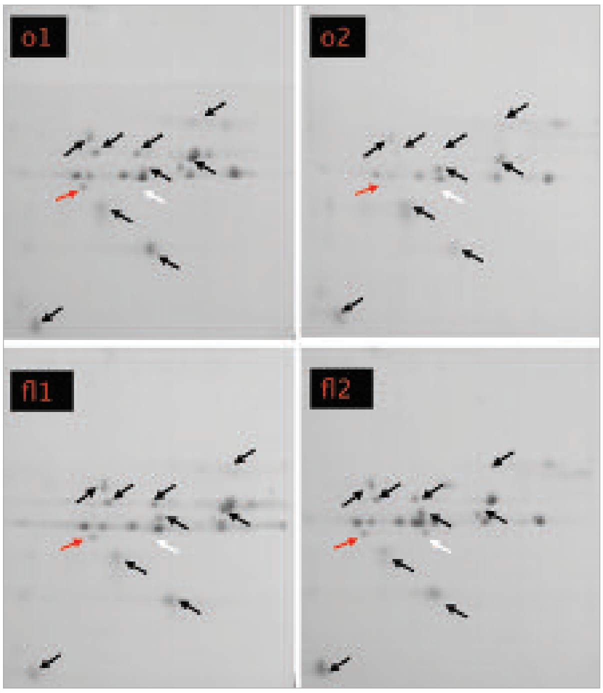

2D-PAGE of Zein Storage Proteins:
Red and white arrows indicate examples of a drastically reduced expression of a protein (in the o2 maize mutant) and a newly expressed protein (in the fl2 maize mutant), respectively. Azevedo et al. 2003. European Journal of Biochemistry. ***270****: 4898-4908*

Hunger and malnutrition are among the most devastating problems affecting a large part of the world's population. Nearly 30% of people in the developing world are suffering from one or more forms of malnutrition. In fact, one billion people worldwide are affected by nutritional deficiencies, 800 million people are chronically undernourished, more than 40% of women in the developing world are underweight and/or anemic, and one third of the world's children are affected by delayed growth and development caused by malnutrition. The tragic consequence of malnutrition includes disability, stunted mental and physical growth and death, affecting all age groups. Protein quality is based on their origin (animal or vegetable), amino acid composition (particularly their relative content of essential amino acids) and their digestibility. Therefore, high quality proteins are those that are easily digested and contain the essential amino acids in quantities that correspond to human requirements. Amino acid and protein requirements are metabolic demands of the organism. The aspartate metabolic pathway is extremely important since aspartate is the precursor of four essential amino acids: lysine, threonine, methionine and isoleucine. The challenge of producing crops with a high-lysine concentration in the seeds appeared to be in sight a few years ago, however, apart from the quality protein maize lines currently commercially available, the release of high-lysine cereal crops is still in its first steps. We are left with the question: Is the production of high-lysine crops still a challenge? The main goal of this project is to better understand the regulation of lysine metabolism in cereal crops. Key enzymes involved in the regulation of the aspartate pathway, the biosynthesis and the accumulation of seed storage proteins of distinct cereal crops were the main focus of our attention. In parallel, we are also investigating nitrogen use efficiency and amino acids metabolism.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

We have been studying naturally occurring maize (opaque and floury) and sorghum high-lysine lines and quality protein maize (QPM) lines obtained by Brazilian Agricultural Research Corporation (EMBRAPA). The maize storage proteins, albumins, globulins, zeins and glutelins of the maize endosperms from *opaque1*, *opaque2*, *floury1* and *floury2* mutants and their wild-type counterpart, *Oh43+*, were analyzed by two dimensional gel electrophoresis (2D-PAGE, 8-18% gradient gel), which allowed us to detect several specific changes for each mutant. In addition, enzymes involved in lysine, threonine and methionine biosynthesis, and two enzymes involved in lysine degradation, were analyzed indicating that the high-lysine levels in the mutants cannot be solely explained by one particular event, but are due to a combination of modified enzyme feedback regulation and differential distribution of the storage proteins. We were also able to perform relative quantification of gene expression for some of the enzymes in some of the genotypes. For instance, in developing seeds, a *LL-DAP aminotransferase* (*LL-DAP-AT*) gene was induced in both QPM lines (designated L161o and L161q) at 14 days after pollination and an enhanced expression was observed at 20 days after pollination in L161o. At 24 days after pollination, L161q exhibited up-regulation for both, *LL-DAP-AT* and *threonine synthase* (*TS*) genes, but *LL-DAP-AT* was three times more expressed than *TS*. In collaboration with EMBRAPA, we have been able to undertake a new strategy in which we have introduced a high-lysine containing protein encoding gene (zeolin) into maize and expressed it specifically in the seed endosperm by using a *γ-kafirin* promoter. Preliminary PCR results of transgenic plants have indicated several positive transformed plants. SDS-PAGE biochemical characterization has indicated that protein is properly expressed in the endosperm affecting the overall protein concentration (70-160% increase in zeins). Further genetic and biochemical characterization will focus on the inheritance of gene, lysine levels in the seeds and agronomical performance of the putative high-lysine maize lines.

MAIN PUBLICATIONS

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