

## Safeners, Tools for Artificial Manipulation of Herbicide Selectivity: A Zea mays Case Study

**Authors :** Sara Franco Ortega, Alina Goldberg Cavalleri, Nawaporn Onkokesung, Richard Dale, Melissa Brazier-Hicks, Robert Edwards

**Abstract :** Safeners are agrochemicals that enhance the selective chemical control of wild grasses by increasing the ability of the crop to metabolise the herbicide. Although these compounds are widely used, their mode of action is not well understood. It is known that safeners enhance the metabolism of herbicides, by up-regulating the associated detoxification system we have termed the xenome. The xenome proteins involved in herbicide metabolism have been previously divided into four different phases, with cytochrome P450s (CYPs) playing a key role in phase I metabolism by catalysing hydroxylation and dealkylation reactions. Subsequently, glutathione S-transferases (GSTs) and UDP-glucosyltransferases lead to the formation of Phase II conjugates prior to their transport into the vacuole by ABCs transporters (Phase III). Maize (*Zea mays*), was been treated with different safeners to explore the selective induction of xenome proteins, with a special interest in the regulation of the CYP superfamily. Transcriptome analysis enabled the identification of key safener-inducible CYPs that were then functionally assessed to determine their role in herbicide detoxification. In order to do that, CYP's were codon optimised, synthesised and inserted into the yeast expression vector pYES3 using in-fusion cloning. CYP's expressed as recombinant proteins in a strain of yeast engineered to contain the P450 co-enzyme (cytochrome P450 reductase) from *Arabidopsis*. Microsomes were extracted and treated with herbicides of different chemical classes in the presence of the cofactor NADPH. The reaction products were then analysed by LCMS to identify any herbicide metabolites. The results of these studies will be presented with the key CYPs identified in maize used as the starting point to find orthologs in other crops and weeds to better understand their roles in herbicide selectivity and safening.

**Keywords :** CYPs, herbicide detoxification, LCMS, RNA-Seq, safeners

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