

Enantiospecific enzymes responsible for the formation of phytoestrogens in flax

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Background and Aims: This research was designed to investigate the origin of stereochemical diversity of chiral phytoestrogens (responsible in preventing hormone dependent cancers) by cloning enantiospecific genes from *Linum usitatissimum*.

Methods: A cDNAs encoding a pinoresinol lariciresinol reductase (PLR) (PLRLu1) which is enantiospecific for the conversion of 8S, 8'S(-)-pinoresinol (SS-pinoresinol) via 8S, 8'S(-)-lariciresinol (SS-lariciresinol) to SS-(+)-secoisolariciresinol was cloned from the seeds *L. usitatissimum*. Another cDNA encoding a RR-pinoresinol- RR-lariciresinol reductase (PLRLu2) was cloned from the leaves of *L. usitatissimum* which converts only RR-pinoresinol to RR-secoisolariciresinol. After cloning and enzyme assay the conversion of enantiomers were analyzed by chiral column chromatography.

Results: In leaves and stems of *L. usitatissimum* accumulating the 8R, 8'R-enantiomers of lignans, only PLRLu2 was transcriptionally active. Both PLRLu1 and PLRLu2 transcripts were observed in seeds and contribute to the synthesis of SS- and RR secoisolariciresinol, respectively.

Conclusions: The enantiomeric composition of lignans in the organs of *L. usitatissimum* appears to be determined by the relative action of two PLRs with opposite enantiospecificities rather than by a single enzyme of low enantiospecificity.

Keywords: Pinoresinol lariciresinol reductase; *Linum usitatissimum*; Enantiospecificity; Lignin; Secoisolariciresinol; Transcript level