

CHARACTERIZATION OF *MEDICAGO TRUNCATULA* LHA GENE AS MULTIFUNCTIONAL OXIDASE INVOLVED IN THE BIOSYNTHESIS OF HAEMOLYTIC SAPONIN

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Saponins are a group of glycosidic compounds present in several plant species whose aglycone moieties are formed by triterpenoid or steroidal skeletons. In spite of their importance as antimicrobial compounds and their possible benefits for human health, knowledge of the genetic control of saponin biosynthesis is still lagging behind. Here we report on the identification of a cytochrome P450 gene (LHA) involved in saponin synthesis in *M. truncatula* using a combined approach of genetics and biochemistry. Genetic loss-of-function analysis and complementation proved that LHA is responsible for an early step in the saponin pathway, as the mutants reported were unable to produce haemolytic saponins (*lha*) but only synthesized soyasaponins. In vitro enzymatic activity assay indicates that LHA catalyzes oxidation of β -amyrin and erythrodiol at C-28 position, to yield oleanolic acid. Transcriptome changes in the mutant lacking haemolytic activity showed a modulation in the main steps of triterpenic saponin biosynthetic pathway: squalene cyclization, β -amyrin oxidation and glycosylation.

This report provides a first evidence for a key oxidative step in haemolytic saponin biosynthesis.