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Gene silencing and overexpression studies in concurrence with promoter specific elicitations reveal the central role of WsCYP85A1 in biosynthesis of triterpenoids: Withania somnifera (L.) Dunal

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Withania somnifera (Ashwagandha) synthesizes triterpenoids (including brassinosteroids, phytosterols and withanolides) that are produced via an intricate isoprenoid pathway whose biosynthetic and regulatory mechanism is yet elusive. Their pharmacological examinations make them as significant bioactive molecules demanding their higher production. Studies reveal that P450s mono-oxygenizes are the main players in their biosynthesis and assist in functionalizing molecule core structures including withanolides. Present study entails the isolation and functional characterization of cytochrome P450 (CYP85A1), a key enzyme of brassinosteroids biosynthetic pathway from W. somnifera. The full length WsCYP85A1 having open reading frame of 1367 bp encodes for 462 amino acid residues. Further, in vitro enzymatic activity assay using 6-deoxocastasterone as substrate confirmed its oxidative functionality. Validation of product formed was done using LC-PDA-MS analysis by displaying peak at m/z value of 487[M+Na]+ and 506[M+ACN]+. In planta transient overexpression of WsCYP85A1 in W. somnifera significantly enhanced its transcript levels resulting in an increased in castasterone, stigmasterol and withanolides contents. By contrast, their contents were reduced in plants wherein the expression of WsCYP85A1 gene was suppressed using artificial micro-RNA mediated silencing. Taken together, these non-complementary approaches suggest unambiguous information regarding the regulatory role of WsCYP85A1 in context to withanolides and stigmasterol biosynthesis. Furthermore, promoter analysis of WsCYP85A1 resulted in the identification of several potential cis-regulatory elements. Methyl jasmonate was found to be the potent inducer of expression levels of WsCYP85A1 with concomitant increased castasterone levels as compared to abscisic acid and cold treatment. Overall, these empirical findings suggest that functional characterization of WsCYP85A1 may plausibly be helpful to unravel the mechanism of brassinosteroids biosynthesis and could also pave way for metabolic engineering.

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