ABSTRACT

Isoflavone synthase (IFS; CYP93C) plays a key role in the biosynthesis of the plant secondary metabolites, isoflavonoids. These phenolic compounds, which are well-known for their multiple biological effects, are produced mostly in leguminous plants (family Fabaceae). However, at least 225 of them have also been described in 59 other families, without any knowledge of orthologues to hitherto known *IFS* genes from legumes (with the single exception of sugar beet – *Beta vulgaris*, from the family Chenopodiaceae).

In view of these facts, this masters thesis has focused on two main objectives: (1) to identify isoflavone synthase genes in selected leguminous and non-leguminous plants exploiting the PCR strategy with degenerate and non-degenerate primers, and (2) to find a system for the verification of the correct function of these genes.

Our methodology for the identification of *IFS* orthologues was successfully demonstrated in the case of two examined legumes – *Phaseolus vulgaris* L. and *Pachyrhizus tuberosus* (Lam.) Spreng, in the genomic DNA of which the complete *IFS* sequences have been newly identified. To design a procedure for ascertaining the correct function of these genes and others once they have been completely described, a pilot study with IFS from *Pisum sativum* L. (CYP93C18; GenBank number AF532999) was conducted. *CYP93C18* was identified, cloned and introduced into the putative isoflavone pathway-free plant *Arabidopsis thaliana* using Gateway™ Technology. Its correct function was verified at four different levels by: PCR with *IFS*-specific primers (DNA), RT-PCR (RNA), Western blotting (proteins) and HPLC-MS (metabolites). In addition, CYP93C18::GFP fused proteins were transiently expressed in the leaves of *Nicotiana benthamiana*, and the localization of the GFP signal was observed on the endoplasmic reticulum using confocal microscopy, which is consistent with the predicted presence of a signal peptide in the IFS´s N-terminus, as well as with the model of IFS generated *in silico* on the basis of cytochromes P450 homology.