

FLAVONOID METABOLISM IN *MEDICAGO TRUNCATULA* MUTANTS

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Barrel medic, anthocyanin biosynthesis, gene expression, metabolism

Barrel medic (*Medicago truncatula* Gaertn.) is a self-fertile, annual, and diploid plant that has been selected as a model legume. Flavonoids are plant antioxidants synthesized by the phenylpropanoid metabolic pathway and their benefits for plant, human and animal health are known. In this work six mutants of barrel medic affected in flavonoids biosynthesis have been characterized. These mutants, obtained by chemical (tilling) or physical (fast-neutron radiation) mutagenesis, showed either an altered pattern or an absence of pigmentation in leaves and flowers compared to wild-type plants. A strong reduction of the total amount of anthocyanins present into mutant leaves was also found. At metabolite level, the amount of flavones measured with LC-MS was also affected by the mutations. The tricetin-3GluAc was the most accumulated product in mutant leaves compared to apigenin-3GluAc in wild-type. We analysed the expression of structural genes and selected transcriptional factors (*Myb*, *Myc* and *MADS*-box genes, WD40 protein) involved in flavonoids biosynthesis by RT-PCR and qPCR and we measured altered expression profiles in the mutant compared to wild-type leaves. For instance, when the amount of anthocyanins was very low, the glutathione S-transferase (GST) expression was strongly reduced; one mutant showed a complete suppression of the UDP-glucose:anthocyanin 5-O-glucosyltransferase (5GT) expression (the last enzyme of anthocyanin biosynthesis). We observed a correlation between the amount of anthocyanins and the expression of PAPI, a specific *myb* gene related to anthocyanin synthesis.