

# Towards understanding of the biosynthesis and accumulation of lupin alkaloids

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Quinolizidine alkaloids (QA) are secondary metabolites distributed mainly in the genus *Lupinus*. QA accumulated in mature seeds of lupins are considered as antinutritional factors, due to their bitter taste and neurotoxic effects. Therefore, low QA content is one of the pivotal seed quality traits in lupins.

The biosynthesis and accumulation of lupin alkaloids is still only partially characterised at both biochemical and molecular level, with limited number of known genes and enzymes involved. At present, the narrow-leafed lupin (NLL; *Lupinus angustifolius* L.) has been the most extensively investigated in research targeted at unraveling alkaloid biosynthesis in lupins, as well as in selection of low-alkaloid cultivars through breeding efforts. Among several single recessive genes associated with reduced QA content, the *iucundus* allele has been incorporated in the majority of NLL cultivars. An improved molecular understanding of QA accumulation in lupins would greatly benefit the breeding efforts to produce cultivars optimised for the production of low QA content.

Within this study the comparative transcriptome analysis of leaf tissue, derived from NLL accessions with contrasting seed alkaloid content enabled the identification of candidate genes involved in QA biosynthesis pathway. Among a comprehensive landscape of differentially expressed genes APETALA2/ethylene response transcription, *RAP2-7* has been identified, which is likely a vital gene regulating the accumulation of alkaloids in this species. *RAP2-7* was found to co-segregate with a major locus that confers reduced QA content in seeds, *iucundus*, and was located within a region of major QTLs that affect alkaloid composition. The point mutation in *RAP2-7* gene sequence, credibly associated with alkaloid content, was further exploited to develop a co-dominant, gene-targeted marker useful in molecular breeding. Additionally, the expression profiles of QA-related genes, along with the alkaloid content, in various organs of sweet and bitter NLL accessions have been characterized, with special attention paid to the *RAP2-7*.

Moreover, to provide new insights into the diversity among lupins originating from the Old World, the alkaloid profiles in their seeds were explored, towards the evaluation of the potential use of these chromatographic fingerprints in species discrimination.