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CHARACTERIZATION OF A QUINOLIZIDINE ALKALOID O-TIGLOYLTRANSFERASE GENE IN WILD AND DOMESTICATED WHITE LUPIN *(Lupinus albus)*

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Alkaloids in lupins cause a bitter taste and are toxic. Wild white lupins have high levels of alkaloids (>1% w/w). Domesticated white lupin varieties have a very low content of alkaloids in seeds (<0.02%). Domesticated varieties were developed from mutants of wild white lupin, in which the mutations have disrupted the genes playing an important role in alkaloid synthesis [1-3]. Bitter genes from wild white lupins are a contamination threat to domesticated white lupin via cross pollination and spread through propagation. The *pauper* low-alkaloid gene results in a very low alkaloid level. Kiev-mutant, Start and Magna are pauper low-alkaloid varieties. The gene(s) for alkaloid synthesis have not been clearly identified, and the associated molecular background among wild white lupin, domesticated and contaminated domesticated plant materials is unknown.

So far only HMT/HLTase# cDNA has been cloned based on protein analysis [4,5]. which was suggested as encoding a quinolizidine alkaloid transferase regulating quinolizidine alkaloid biosynthesis. This gene has not yet been well characterized in different white lupin materials. We isolated the related sequence from the genome of a couple of different wild lupin accessions: P25758 (from Crete) and P27593 (from Azores). At the 3' end of this gene, an 880 base-pair exon from the stop code was identical in both P25758 and P27593, but the fragment near the 5' end of this gene differed between them (Figure 1, A). Such difference may also exist among varieties (Figure 1, B). Based on this we suggest that the domesticated varieties Kiev-mutant and Start may be originated from a P25758-type genetic background. On the other hand, the expression pattern as well as expression level didn't show difference between P25758 and Kiev-mutant (Figure 2). The sequence difference as well as the expression of this gene product doesn't show any correlation with bitterness (alkaloid content). This could be because the gene amplified here is not the one mutated to produce *pauper*, or that the *pauper* lesion is outside these amplified fragments. However, the sequence data of the two wild lupin materials may be useful in the identification of the contamination source of bitter seeds in commercial lupin seed lots. For example, the contaminated bitter seed samples analyzed in the current study may have originated from P27593 or Magna but not from Kiev-mutant, Start or P25758.

tigloyl-CoA:(-)-13alpha-hydroxymultiflorine/(+)-13alpha-hydroxylupanine O-tigloyltransferase

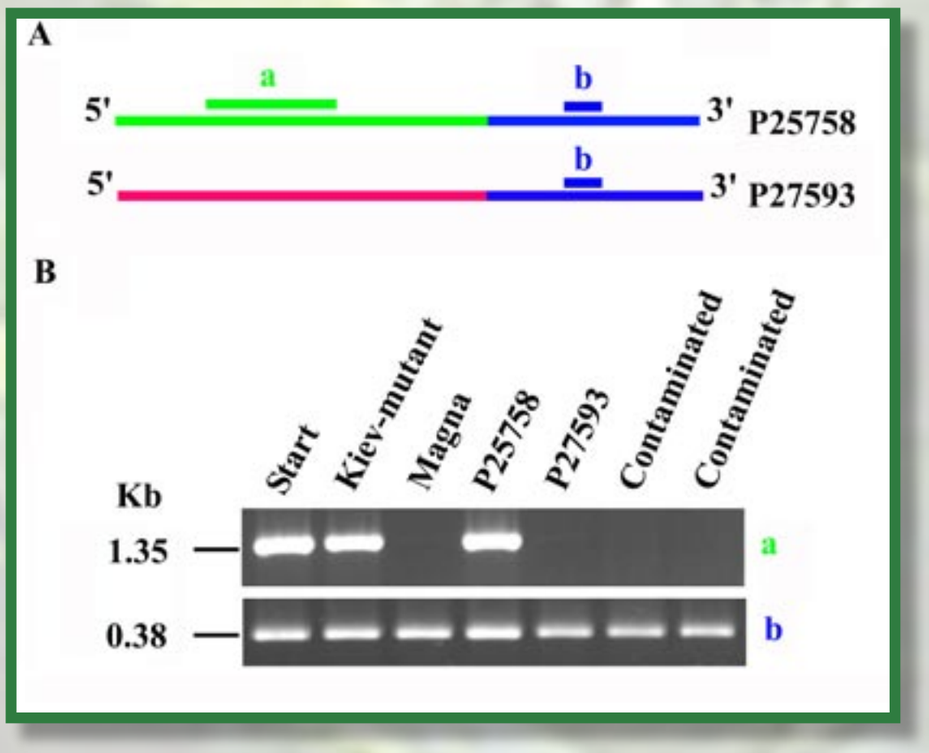

Figure 1. HMT/HLTase gene sequence in different white lupin materials. A. Primers were designed from different part (a and b) of this gene in two wild white lupins. Identical sequences were indicated by the same colour. B. PCR product b presented in all the materials analyzed. PCR product a specific to P25758 was present in Kiev-mutant and Start, but not in Magna and contaminated seeds from commercial crops.

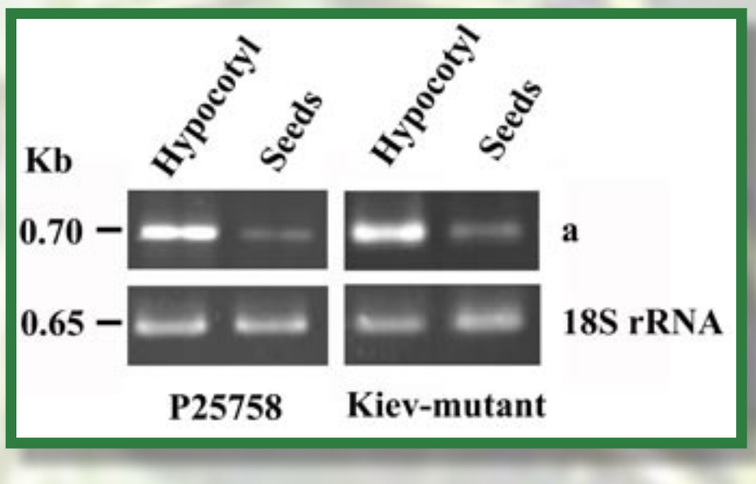

Figure 2. RT PCR product of HMT/HLTase gene fragment a (see Figure 1.) in different tissue of P25758 and Kiev-mutant. 18S rRNA was used as internal standard. The brightness of the products represents their expressing level. Whilst the expression pattern of the analyzed product was similar in wild and domesticated white lupin, namely, expressed more in hypocotyls than in seeds, the expression level of this product in seeds didn't show significant difference between wild white lupin P25758 and variety Kiev-mutant.

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