Genomic research to improve the value of narrow-leafed lupin (*Lupinus angustifolius* L.) grain: quinolizidine alkaloids

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Narrow-leafed lupin

Recently domesticated crop (1960s - )
Cultivars have limited genetic and adaptive diversity
Wild lupin used to increase genetic diversity

**Quinolizidine alkaloids**

Toxic, bitter secondary metabolites

Synthesised in aerial tissues and transported via the phloem to seeds

Provides insect resistance

*iucundus* mutation reduces alkaloids from ‘bitter’ wild lupin to ‘sweet’ cultivars
Alkaloids reduce the value of lupin grain

Grain must remain below 0.02% for food and feed purposes, which is sometimes exceeded

Large impact of genotype and environment on grain alkaloid content
- Wide variation in response of lupin grain alkaloid content
- Lupin crops grown over 357 sites in WA showed 26% probability of crops exceeding the alkaloid threshold, but in an unpredictable way

Data: Jens Berger
Project aims

Identify candidate genes involved in lupin alkaloid biosynthesis, transport and regulation

- Using transcriptomic and genomic data

Identify the gene responsible for *iucundus* low-alkaloid phenotype

Measure alkaloid production (gene expression and alkaloid levels)

- In lupin tissues
- Under stress (drought, increased temperature, aphid predation)
Sites of alkaloid biosynthesis in lupin tissues
Alkaloid biosynthetic gene expression

Lysine/ornithine decarboxylase (L/ODC)

- **Relative expression**
- **Root**
- **Stem**
- **Leaf**
- **Flower**
- **Seed**

Copper amine oxidase-like

- **Relative expression**
- **Sweet cultivar**
- **Bitter wild lupin**
**iucundus ‘low alkaloid’ gene**

Recessive mutation difficult to track in breeding programs

Located to a 26 gene region

  Using lupin genome assembly and flanking markers

This region harbors regulatory genes and those possibly involved in alkaloid biosynthesis

Comparing gene sequences between 12 lupin cultivars and 30 wild lupin varieties to identify candidate genes
Candidate *iucundus* gene

Premature STOP codon in all cultivars, absent in most wild varieties
Changes in alkaloid production in lupin grain under drought and temperature stress
Grain alkaloid content in lupin cultivars

Drought and increased temperature can increase grain alkaloid content, is dependent on genotype
Currently measuring alkaloid levels in leaves
Changes in alkaloid production in lupin grain under aphid predation
**Susceptible lupin**

- Control
- Aphid Infested

**Resistant lupin**

- Control
- Aphid Infested

- **% grain alkaloid content**
  - 0.08
  - 0.07
  - 0.06
  - 0.05
  - 0.04
  - 0.03
  - 0.02
  - 0.01
  - 0

- **Species**
  - lupanine
  - 13-hydroxylupanine
  - a-isolupanine
  - angustifoline

- **Photographs**
  - Plants showing aphid infestation.
Identification of candidate seed alkaloid transporters
Identification of metabolite transporters for ‘transport engineering’

Control accumulation of metabolites in a tissue-specific manner without compromising biosynthesis and plant defence

Alkaloid transporter gene families in other plant species
- Multidrug and toxic compound extrusion (MATE)
- Purine uptake permease (PUP)
- ATP-binding cassette (ABC)

Aim: Identify transporters involved in import of lupin alkaloids into grain to look for non-functional genes in mutagenized population

Shoot cells ➔ Phloem ➔ Seed cells
Identification of lupin seed transporters using transcriptome data

To identify genes with transporter protein annotation more highly expressed in bitter lupin seed

Differential expression analyses carried out

Between bitter vs. sweet lupin (all tissues)
  • 22 transporter genes upregulated

Between tissues (seed vs. flower, root, stem, leaf)
  • Sweet lupin – 23 transporter genes upregulated
  • Bitter lupin – 13 transporter genes upregulated

Candidate list of transporters involved in uptake of alkaloids into grain (9)
Candidate alkaloid transporter gene expression

**Relative expression**

- **Sweet lupin**
- **Bitter lupin**

**Seed development stage** (days after anthesis)

- 4-8
- 9-12
- 13-16
- 17-20
- 21-26
- 27-32
- 33-38

**Root**
**Stem**
**Leaf**
**Flower**

* Significant difference
Future prospects

Understand how growth conditions affect alkaloid production
  e.g. Drought, increased temperature and presence of aphids

Need to better understand genetic variation for alkaloid production
  Is increased grain alkaloid content due to increased production or transport?
  How are crosses with wild lupin increasing alkaloid levels?

Breeding approach: ‘bitter sweet’ phenotype
  Identify individuals with non-functional seed alkaloid transporters
  Non-GM, rapidly taken up by industry