



Development of an interspecific hybrid protocol for *Lupinus*

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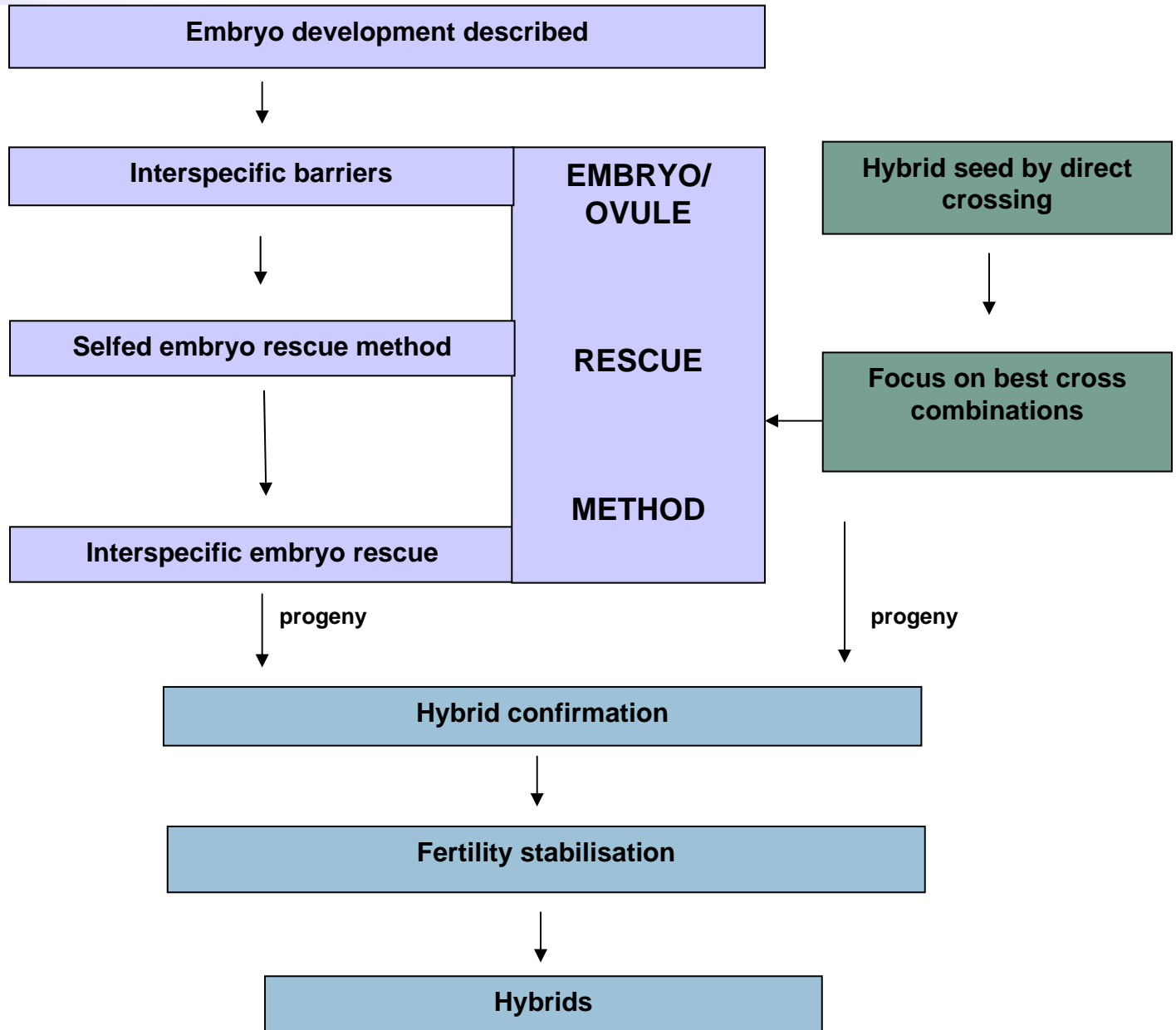
Future Farm Industries CRC, UWA

Department of Agriculture and Food WA







**Grains
Research &
Development
Corporation**





Transfer of traits among species?



 <p>Narrow-leaved lupin</p>	 <p>Yellow lupin</p>	 <p>Albus lupin</p>	 <p>Pearl lupin</p>
<p>Well adapted & widely adopted Moderate resistance to Anthracnose Lower protein/oil $2n = 40$</p>	<p>Water logging & Brown spot tolerant Sulfur amino acids Acid-sandy soil High protein $2n = 52$</p>	<p>Tolerance to CMV, frost (some) & Simazine (Mod.) Calcareous soils Has cluster roots $n = 50$</p>	<p>High protein content High oil content Thin seed coat CMV resistance Frost susceptible $n = 48$</p>

Coromup

Pootalong

Andromeda

P26961

Developed by Mark Sweetingham and Jon Clements at DAFWA

Pre-fertilisation barriers

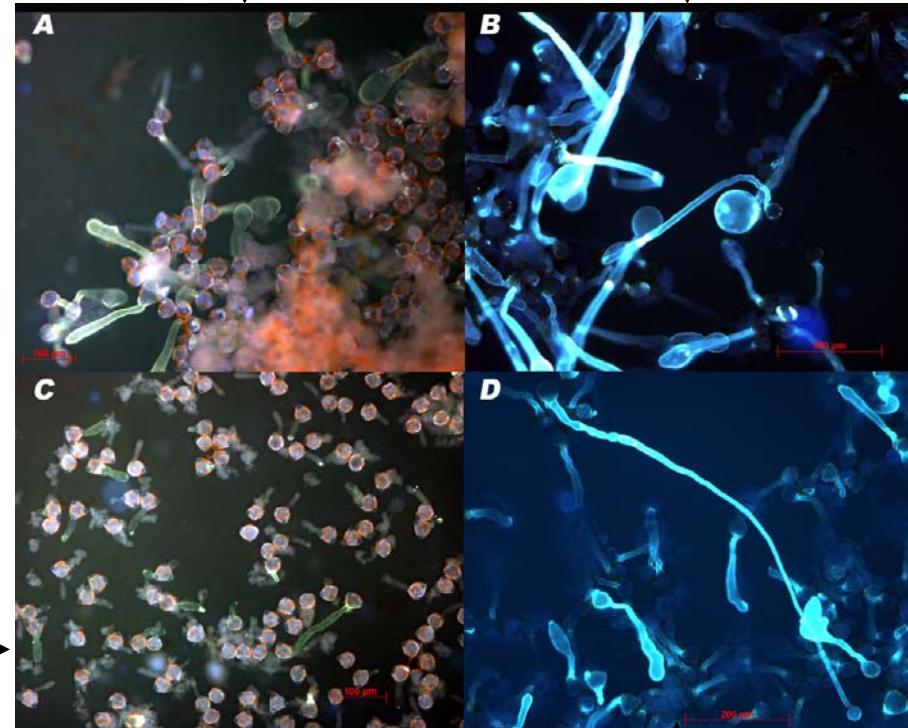
- Pollen germination *in vitro*
- Does pollen germinate on stigma?
- Do pollen tubes reach the ovules?

L. albus

- Short pollen tubes
- Lower viability (~70%)

L. angustifolius

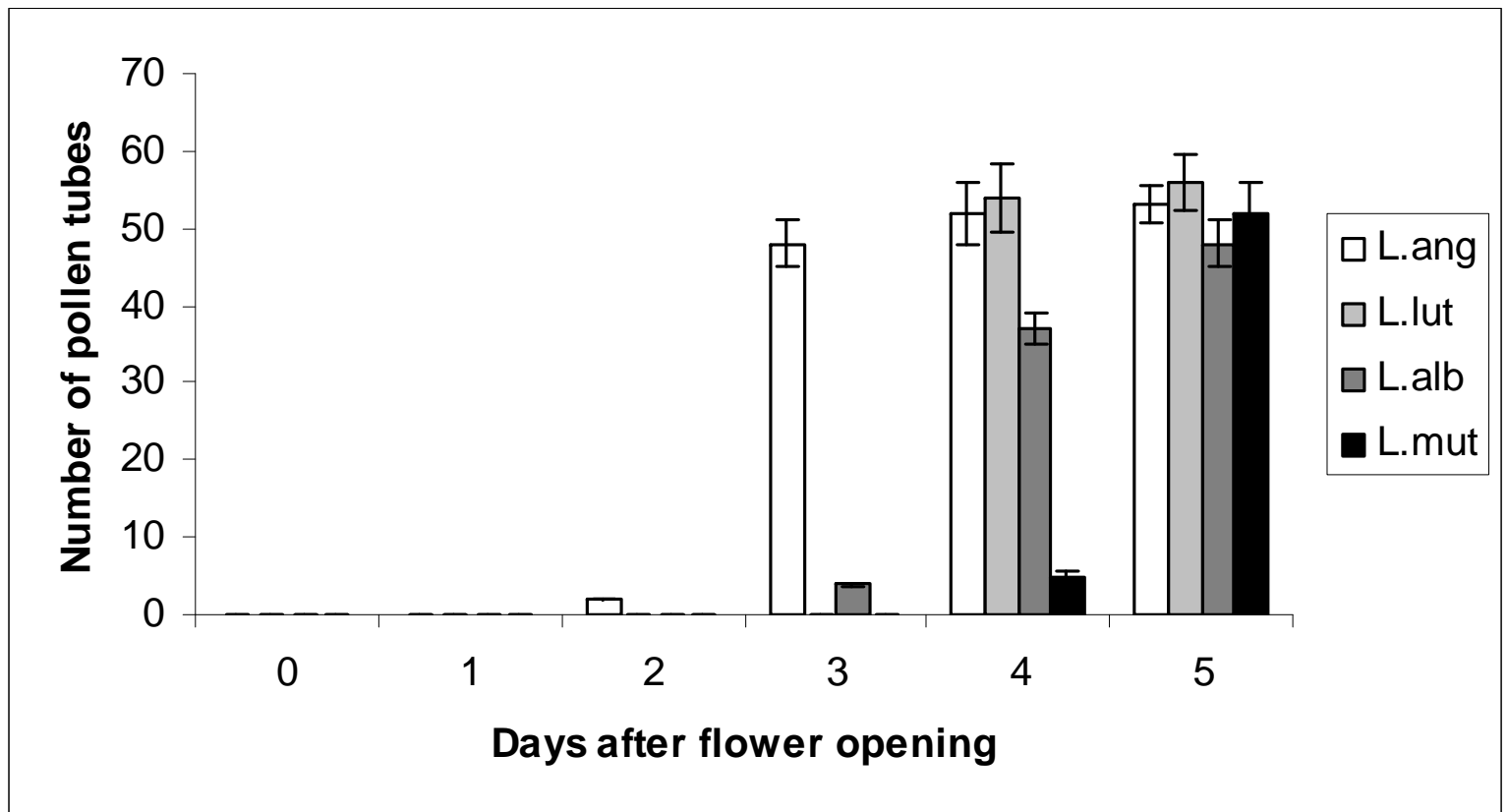
L. luteus



L. mutabilis



Pollen tube growth *in situ* for 'selves'



Pollen growth *in situ*



L. angustifolius

X



L. luteus



L. angustifolius x *L. luteus*

Embryo development 'selves'

■ *L. angustifolius* & *L. luteus* 'selves'

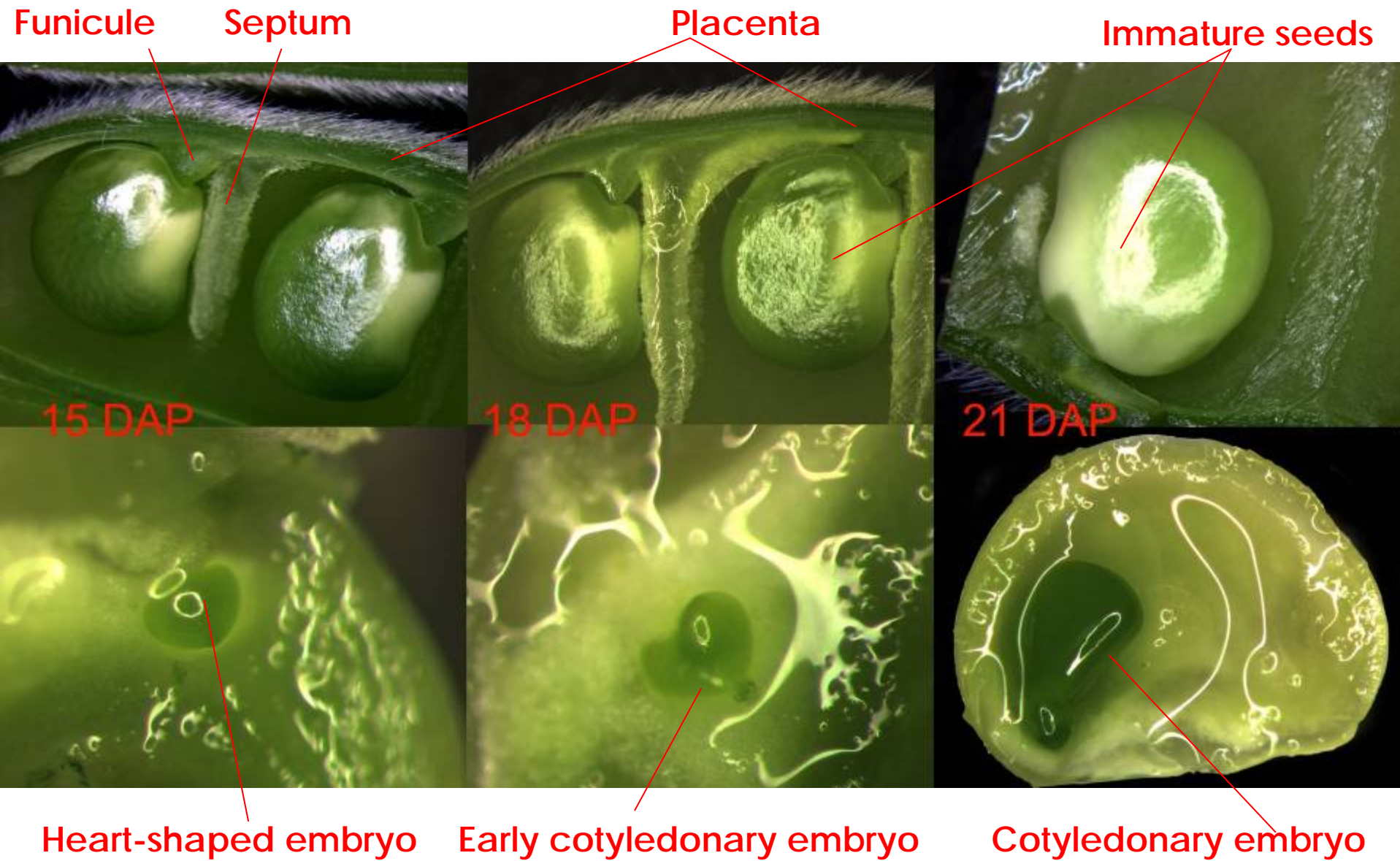
- Dissecting microscope
- Spurr's fixation for light mic & TEM

■ General developmental stages

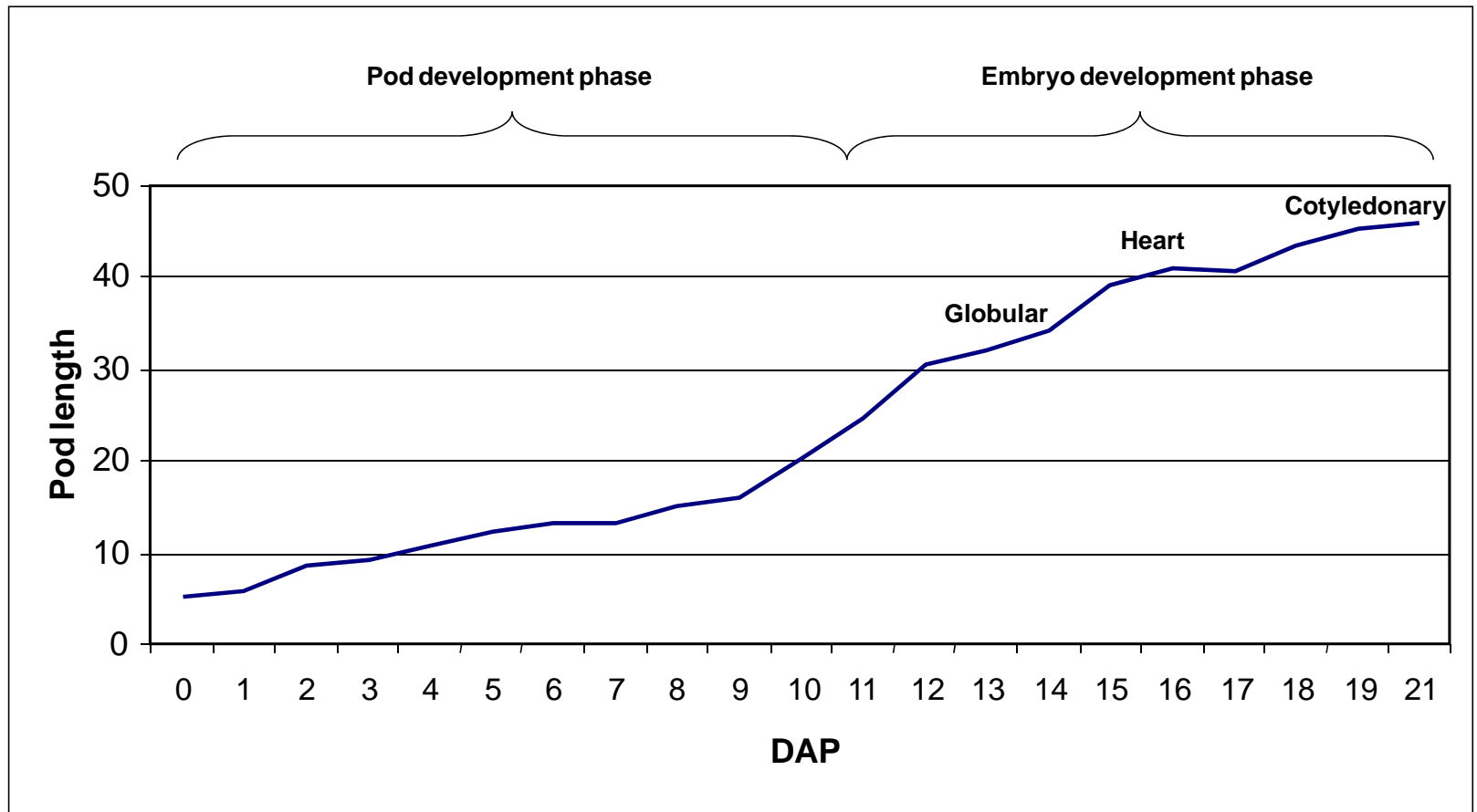
- 12 days globular
- 15 days heart ← Best stage for rescue
- 18 days early cotyledonary
- 21 days cotyledonary embryo



L. angustifolius 'selfs'



Embryo development 'selves'

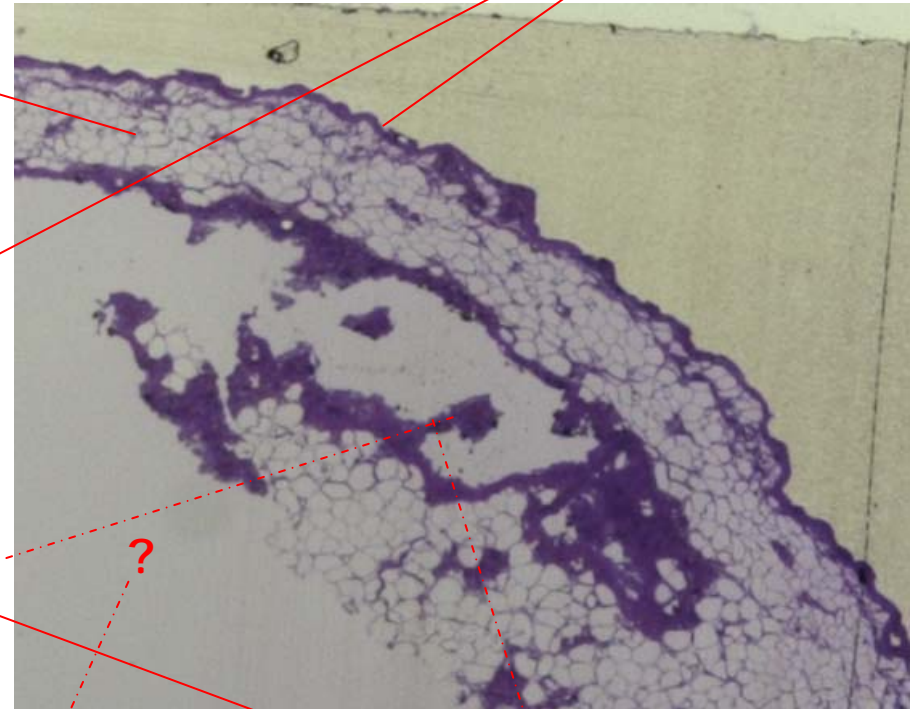
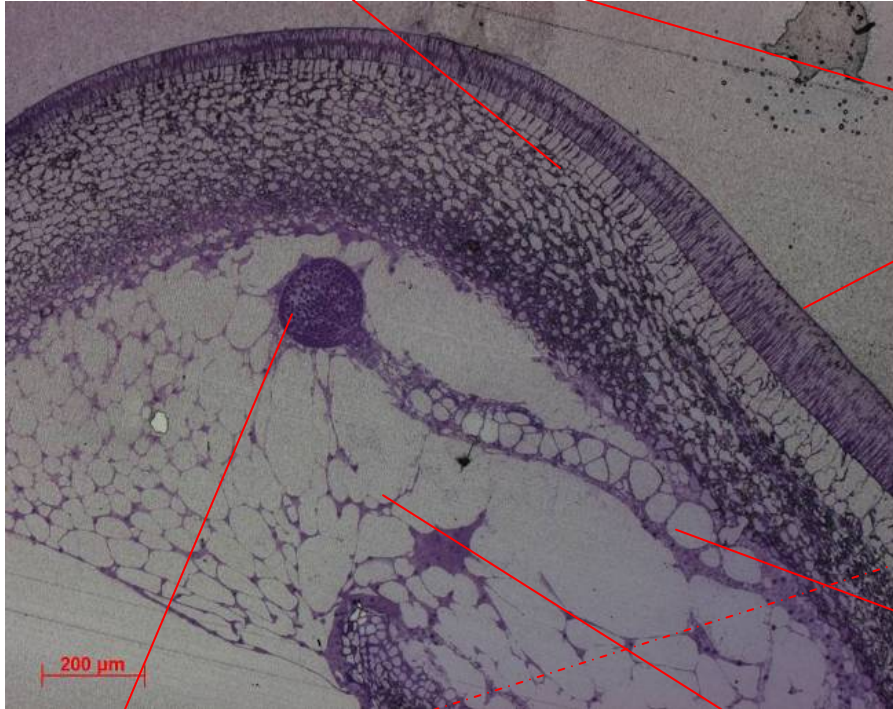


L. angustifolius

Sections through immature seeds

Integument

Seed coat



Globular embryo

Endosperm

Suspensor cells

L. angustifolius 'self' 12 DAFO

L. angustifolius x *L. luteus* 40 DAP

Hand crossing

- Crossing in the field
- Reciprocal crosses
- Main stem only
- Delayed pollination
 - *L. albus* & *L. mutabilis*
- Pod scored at 21 days after crossing
- Seeds collected if mature
- Embryo rescue attempted



L. angustifolius – pod scoring



Needs
rescue
True
hybrid?

Develops
into
fertile
seed

Shade house 2006

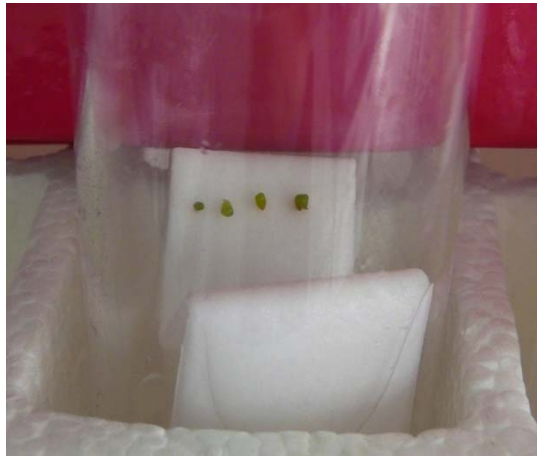
Seeds (% / flower)



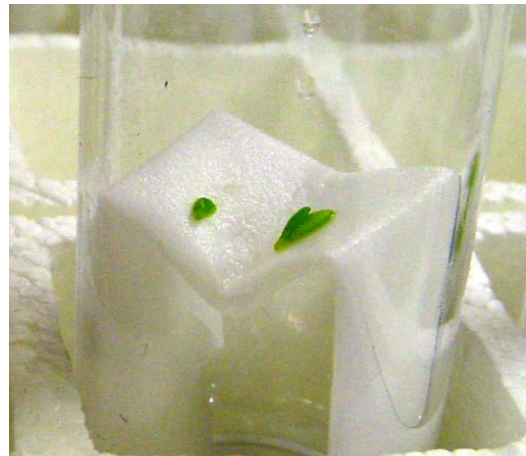
Species	<i>L.angust</i>	<i>L.luteus</i>	<i>L.mutab</i>	<i>L.albus</i>	Total
<i>L.angust</i>	-	6	6	6	6
<i>L.luteus</i>	2	-	2	0	2
<i>L.mutab</i>	0	0	-	5	1
<i>L.albus</i>	1	0	2	-	2
Total	1	5	3	4	3

Crossing effort in 2007 focussed on these 4 combinations

Embryo rescue: *L. angustifolius* selfs



Globular (?) embryo



heart & cotyledonary embryo



immature seed rescue



Embryo derived plantlet



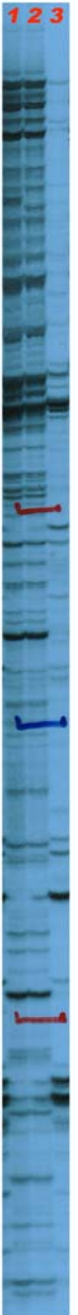
embryo derived shoots

Testing hybridity...

Evaluating RGAP

- DNA fingerprinting method
- Pair of primers designed for conserved regions of plant disease resistance genes.
- Can determine species and some cultivars
- All 'hybrids' had maternal profile

Primer Name	SEQUENCE (5' – 3')	Tm (by Sigma)
P loop-F	GGI GGI GTI GGI AAR ACN AC	53.2
GLPL-R	GAG IGC IAG IGG IAR ICC	51.5



Interspecific hybridisation - summary



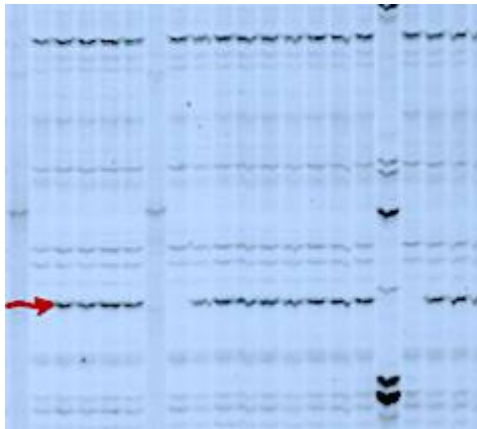
**Crossing in glasshouse
& field conditions**



***L. angustifolius*
Pollen tube formation**



***L. angustifolius* x *L. luteus*
Putative hybrid (?) pod**



**DNA analysis to
test hybrids**



**Transfer
to glasshouse**



**Embryo rescue of embryos
& immature seeds**

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Application of plant growth regulators at crossing & 2DAP

