Chickpea Improvement Through Genetic Analysis And Quantitative Trait Locus (QTL) Mapping Of Ascochyta Blight Resistance Using Wild *Cicer* Species

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Chickpea (*Cicer arietinum*)

- Important cool season pulse (~11 m ha; 700 kg/ha)
- Crucial source of protein
- In Australia, production is limited by ascochyta blight disease
- Two cultivated chickpea types: Kabuli and Desi
- Also many wild relatives. Only two species are crossable with chickpea: *Cicer reticulatum* *Cicer echinospermum*
Ascochyta blight (AB) disease

- Caused by *Ascochyta rabiei*
- Most damaging fungal disease of chickpea in Australia
- It attacks all aerial parts of the plant
- Yield losses from 10% to 100%
- Chemical control is difficult and expensive
Objective and research outline

The objective is to study resistance to ascochyta blight (AB) in chickpea and its wild relatives

This is being achieved successfully by:

- Developing segregating populations
- Developing a successful propagation method for precious hybrids
- Screening for disease in field and glasshouse
- Understanding the genetics of AB resistance
- QTL mapping of AB resistance & ‘domestication’ traits
Developing Populations

- **Cicer arietinum**
  - Desi type
    - ICC 3996 (R)
    - Lasseter (S)
    - 24B-Isoline (Multipinnate leaf)
  - Kabuli type
    - Almaz (R)
    - Kaniva (S)
    - Kimberly Large (Simple leaf)
    - IG 9337

- **Cicer reticulatum**
  - ILWC 118 (R)
  - ILWC 139 (S)
  - ILWC 184 (S)

- **Cicer echinospermum**
  - ILWC 181 (S)
  - ILWC 245 (R)
Propagation of chickpea using stem cuttings

- **Age of donor plant**
  - Pre-flowering
  - Post-flowering

- **Treatments**
  - Plant growth regulators (PGR, 0.5 mg/g IBA and 0.5 mg/g NAA)
  - Honey
  - Honey + PGR

- **Substrate for cutting**
  - Sand
  - Sand + Potting mix

- **Measurements**
  - Rooting rate
  - Survival rate
  - Root length
  - Root number

- **Result:**
  - **Highest success rate** was achieved:
    - Pre-flowering stage
    - PGR powder
    - Sand + potting mix substrate
    - Rooting rate from 87.5 to 100%

Vegetative propagation of interspecific hybrids
Ascochyta blight resistance - field trial

- Medina disease nursery
- Debris from previous year for inoculation
- Populations:
  - F₂ population
  - Diallel crosses
    - F₁ and F₂
- A 1-9 scale for disease assessment

1 = unaffected plant
9 = dead plant
The range of variation in Ascochyta Blight resistance in Kabuli ( ), Desi ( ),
*C. reticulatum* ( ) and *C. echinospermum* ( )

Danehloueipour *et al.* (2006) Genetic basis of ascochyta blight resistance in chickpea and wild
*Cicer* species. In '1st International Ascochyta Workshop on Grain Legumes', 2-6 July; Le Tronchet,
Brittany, France
### Inheritance of AB resistance in F$_2$ populations

**Medina field station, W.A., in 2005**

<table>
<thead>
<tr>
<th>Cross</th>
<th>Observed ratio in F$_2$</th>
<th>Fitted Model (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Two categories</td>
<td>Three categories</td>
</tr>
<tr>
<td>24 B-Isoline</td>
<td>18 R : 174 S</td>
<td>18R : 86 I : 88 S</td>
</tr>
<tr>
<td>Kimberley Large</td>
<td>6 R : 35 S</td>
<td>6R : 12 I : 23 S</td>
</tr>
<tr>
<td>Kimberley Large</td>
<td>25 R : 83 S</td>
<td>25 R : 46 I : 37 S</td>
</tr>
<tr>
<td>24 B-Isoline</td>
<td>60 R : 237</td>
<td>60 R : 157 I : 80 S</td>
</tr>
</tbody>
</table>

Relationships between \( W_r \) and \( V_r \) for AB resistance in \( F_2 \) half-diallel sets

- \( V_r \) = variance of all \( F_2 \) hybrids in each parental array
- \( W_r \) = Covariance between parental and \( F_2 \) progeny

Conclusions from inheritance studies

- Susceptibility dominant over resistance to AB resistance
- Recessive alleles in resistant parents ICC 3996 and Almaz
- High narrow sense heritability ($H^2 = 85\%$)
- No correlation between leaf types and AB resistance
- ICC 3996 (R) X ILWC 184 (S) best population for mapping studies
QTL Mapping

- **Plant Materials (Interspecific)**
  - *C. arietinum* - ICC 3996 (R, early flowering, semi erect growth habit)
  - *C. reticulatum* - ILWC 184 (S, late flowering, prostrate growth habit)

- **Generation**
  - $F_2$

- **Screening Method**
  - Clonal plants screened in controlled environment

- **Traits Measured:**
  - Ascochyta blight resistance
  - Days to flowering
  - Plant growth habit
Ascochyta blight resistance was assessed on 306 clonal plants produced by stem cuttings, while days to flowering and growth habit were measured on original F$_2$ plants.
Response to AB in a controlled environment

Data are the mean of eight clones per F$_2$ genotype
Phenotypic distribution of flowering time

Data from 306 F₂ hybrids
Phenotypic distribution of plant growth habit

Data from 306 F_2 hybrids
Segregation pattern in $F_2$ population
## Detection of QTL for AB resistance, flowering and plant habit

<table>
<thead>
<tr>
<th>QTL estimates</th>
<th>AB resistance</th>
<th>Flowering</th>
<th>Habit</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOD</td>
<td>3.56</td>
<td>19.7</td>
<td>19.7</td>
</tr>
<tr>
<td>Position</td>
<td>45.3</td>
<td>3.6</td>
<td>16.5</td>
</tr>
<tr>
<td>Proportion of explained variance (PEV)</td>
<td>22.7%</td>
<td>21.1%</td>
<td>4.9%</td>
</tr>
<tr>
<td>Total PEV</td>
<td>49%</td>
<td></td>
<td>90.2%</td>
</tr>
</tbody>
</table>

Danehlouejpour et al. (2007) QTL mapping of ascochyta blight resistance, days to flowering and plant growth habit in interspecific progenies derived from a cross between *Cicer arietinum* (chickpea) and *C. reticulatum*. *Theoretical and Applied Genetics*, to be Submitted
QTLs on linkage map of chickpea

Ascochyta blight resistance
Days to Flowering
Plant Growth habit
Conclusion

Ascochyta blight resistance:
- Three QTLs found in this study explained 49% of the phenotypic variation
- Developed a new method for disease screening
- Utilization of wild relatives to construct a genetic map
- Using developed plant materials in breeding programs

Days to flowering:
- Two major QTLs explained 90.2% of phenotypic variation
- Yield improvement through escape from late season drought
- A negative correlation between ascochyta blight resistance and days to flowering ($r=-0.22$, $P<0.001$, $n=306$)

Plant growth habit:
- A major single QTL explained 95.2% of phenotypic variation.
- Improve the efficiency of interspecific hybridisation
- A positive correlation between days to flowering and plant growth habit ($r=0.36$, $P<0.001$, $n=306$).
Publications from my PhD project

- Danehloueipour N, Nelson MN, Yan G, Clarke HJ, Siddique KHM (2007) QTL mapping of ascochyta blight resistance, days to flowering and plant growth habit in interspecific progenies derived from a cross between *Cicer arietinum* (chickpea) and *C. reticulatum*. *Theoretical and Applied Genetics*, to be Submitted


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