Screening for resistance to chilling and Helicoverpa sp. in chickpea

Summary
Research at the Centre for Legumes in Mediterranean Agriculture (CLIMA) has addressed the common problem of flower and pod abortion at chilling temperature in chickpeas. The aim was to improve yield in new chickpea varieties, encourage chickpea production in cool dry areas, and therefore lead to a more sustainable cropping system. Collaboration between this project and the western module of the Australian Coordinated Chickpea Improvement Program (ACCIP) (Dr Tanveer Khan) has been a key to the release of two new chilling tolerant desi varieties, Sonali and Rupali. New tools have also been developed for screening and methods have been compiled as a technical guide that is targeted at chickpea breeders and researchers.

Report Disclaimer
This document has been prepared in good faith on the basis of information available at the date of publication without any independent verification. Grains Research & Development Corporation (GRDC) does not guarantee or warrant the accuracy, reliability, completeness or currency of the information in this publication nor its usefulness in achieving any purpose. Readers are responsible for assessing the relevance and accuracy of the content of this publication. GRDC will not be liable for any loss, damage, cost or expense incurred or arising by reason of any person using or relying on information in this publication. Products may be identified by proprietary or trade names to help readers identify particular types of products.
Conclusions

1. Pollen selection, through the inclusion of a cycle of low temperature stress at hybridisation in targeted crosses, is an effective means to increase tolerance to low temperature at flowering in chickpeas.
2. Chickpea varieties with improved tolerance to low temperature at flowering have a higher yield in cool dry environments such as that experienced in southern regions of Australia.
3. Early generations, segregating for tolerance to low temperature, are best screened in controlled environments through a combination of pollen tube measurements and assessment of pod set in pot grown plants.
4. Williams, 161km south-east of Perth (33°01’; 116°52’) is a reliable site for selection of early flowering, early podding lines, with average temperatures over 24 hours below 15°C extending from April to early November.
5. Merredin, 260km east of Perth (31°28’; 118°17’) is a useful location for screening of early podding at low temperature and assessment of the yield advantage over late podding lines and varieties. The site is prone to drought at the end of the season.
6. Molecular markers for chilling resistance were effective only for screening progeny of the original cross. The DNA based approach was not as reliable as pollen screening and controlled environment assays across the three different populations. Many plants, derived from more recent crosses, which were early podding at low temperatures in the field were not identified with molecular markers.
7. Sixty new segregating breeding lines, transferred to the ACCIP (Tanveer Khan), have chilling tolerance exceeding that of Sona and Heera. These lines contribute an important resource within the ACCIP, either directly for further selections in variety development, or as a source of elite parents in new crosses.
8. There is strong evidence that more than two genes are involved in chilling tolerance in chickpeas. The exact number was not determined in this study.
9. Some partial resistance to helicoverpa was achieved by further selections and development of breeding lines generated during this project. Selected lines may be suitable as parents to incorporate helicoverpa resistance into future varieties. The current material has not been selected for the local cool, dry environment.

Recommendations

1. Pollen selection for chilling tolerance should be incorporated into breeding programs for new desi and kabuli varieties for the southern regions of Australia.
2. Pollen selection for chilling tolerance should be included in crosses targeted for the cooler regions in central Queensland (QLD).
3. F2 generation segregating for the chilling tolerance trait should be screened in controlled environments.
4. F3 populations, identified as chilling tolerant, should be assessed for flowering and pod set at a site which experiences average temperature over 24 hours below 15°C (but not subject to regular frost) throughout September and October. For example, Williams (33°01’; 116°52’) in Western Australia (WA).
5. Elite F4 breeding lines should then be screened for ascochyta resistance and later generations trialled for yield at a cool, dry site such as Merredin (31°28’; 118°17’), WA.
6. It is strongly recommended that new breeding lines produced during this project be used in crosses to combine ascochyta resistance with the superior tolerance to chilling at flowering. At least one pollen selection cycle should be incorporated in the breeding schedule to ensure genes for chilling tolerance are introgressed quickly and not lost.

7. Breeding lines from the project, segregating for helicoverpa resistance, should be advanced and multiplied and later screened under field conditions where helicoverpa is prevalent (for example, northern India). If better resistance is identified, the best lines should then be used as parents in crosses targeted to combine helicoverpa resistance with chilling tolerance in the national breeding program.

8. Petri dish or cutting based screening methods for resistance to *Helicoverpa* spp. in chickpeas require further examination in order to establish that the results reflect the host plant resistance which occurs in the field.

9. Chickpea mapping will be required to identify the group of loci which may contribute to chilling tolerance in chickpeas. It is unlikely that a single molecular marker will be found to effectively select for the trait if it is under polygenic control.

**Outcomes**

**Economic Outcomes**

Collaboration between this project and the western module of the ACCIP (Dr Tanveer Khan) has been a key to the release of two new desi varieties, Sonali and Rupali, in September 2004. The improved chickpea varieties are expected to have a significant positive impact on the chickpea industry, particularly in the short season environments of the northern and central grainbelt of WA. The new varieties also perform well in the drier areas of South Australia (SA), Victoria (VIC) and southern QLD. Sonali and Rupali flower 10-15 days earlier and start podding at a lower temperature than Sona (average temperature 10-12°C over 24 hours, compared with 14°C). Both varieties, therefore, mature earlier than Sona and all other varieties in Australia. Sonali yielded 31% more than Sona in 17 WA trials in 2000-2003. Rupali yielded 5% more than Sona and is of interest to industry because of its attractive light coloured seed. Both varieties have partial resistance to ascochyta blight (scores of Sonali 4.3-6.5 and Rupali 5-6 compared to Howzat 6.3-9 and Sona 6.8-8). Sonali is also resistant to fusarium wilt.

Improvements in the new varieties are a result of faster, more effective breeding for tolerance to low temperature at flowering and to ascochyta resistance.

Further segregating germplasm is currently being developed in the same manner. The expected outcome has not changed since the project was initiated.

**Environmental Outcomes**

It is predicted that new varieties with partial resistance to ascochyta will lead to a reduction in fungicide sprays. It is also expected that the early podding variety will reduce pod borer infestation and require less pesticide application. Adoption of chilling tolerant varieties will encourage chickpea production and therefore lead to a more sustainable cropping system.

**Social Outcomes**

Improved chickpea varieties with better tolerance to chilling stress and disease resistance will assist the chickpea industry in WA.

**Achievements/Benefits**

**Background:**

The abortion of flowers and pods at chilling temperature is a significant limitation to yield in chickpeas, particularly in the cool, dry regions of southern Australia. Stress during reproduction in chickpeas has not received much attention, and trials at the International Centre for Agricultural Research in Dry Areas (ICARDA) in Syria has only focused on tolerance to freezing temperatures during the vegetative stage of growth in chickpeas. In recent years, the expansion of chickpea production into new regions, and the desire to stabilise yield have directed research at the International Crops Research Institute for the Semi-Arid Tropics in India (ICRISAT) and CLIMA towards breeding for tolerance to chilling temperature at the reproductive stage. Yield and quality in chickpeas are also reduced by Heliothine moths, of which *Helicoverpa punctigera* and *H. armigera* are now major pests in the Australian farming system. The need for adequate control in chickpeas, by up to four sprays per season in the worst affected areas, is now a significant cost to growers. It is evident that early podding chickpeas will improve yield, particularly in the cool dry regions of southern Australia. Combined with better resistance to *Helicoverpa* spp., early podding will also be a significant advantage in avoiding severe budworm damage later in the season at the time that the moths are most active.
In the past few years, CLIMA has taken some novel approaches to yield improvement in chickpeas with successful results. Project UWA354 continued this work as part of the ACCIP, providing practical information for breeders and researchers on screening methods, as well as developing new segregating breeding lines for the western module of the national program.

At the start of this project, it was evident at CLIMA that floral abortion and abscission during reproductive growth were associated with abnormal pollen development at chilling temperatures and to retarded growth of pollen tubes before fertilisation. A fast track breeding schedule incorporating a new pollen selection technique was initiated as a result. This technique relies on the competitive ability of the most chilling resistant pollen to fertilise the eggs when hybridised under a cold stress. Such hybridisations allowed selective introgression of genes for chilling resistance into susceptible varieties. Despite the success of this accelerated breeding program, lack of an efficient method for screening the large number of new lines was a major limitation for chickpea improvement. Traditionally, new lines are selfed and grown to maturity in the field to identify elite plants. This is costly in terms of time and facilities. An additional problem was the dramatic effect of small changes to environmental conditions in the field, such as light, moisture, humidity, etc. on flower and pod abortion when plants are already stressed at a critical temperature, making experiments difficult to interpret and impossible to repeat. It was evident that an efficient and reliable method for selection of elite lines was essential for the identification and transfer of genes for chilling resistance. Recent research at CLIMA investigated the use of Polymerase Chain Reaction (PCR) based analysis for the identification of molecular markers in chickpeas. Progeny from CLIMA crosses provided variable populations for marker development. At the start of this project, a number of bands, based on Amplified Fragment Length Polymorphisms (AFLPs), were identified as being linked to chilling resistance in chickpeas. The application of the DNA based method to screening was tested during this project, as well as a comparison made between field, controlled environment and laboratory based screening of the new segregating chilling tolerant breeding lines. At the same time, new hybrid lines with chilling tolerance were selected at the second and third generations, providing new germplasm with higher yield in cool environments for the western module of the ACCIP. Better resistance to *Helicoverpa* spp. was also achieved by improving current screening methods. The main focus of the project was on chilling tolerance, as requested by GRDC. A better understanding of the inheritance of these traits, and improved screening techniques, also contributes to enhanced efficiency of the ACCIP, and the release of chickpea varieties expressing these characteristics.

**Project Achievements:**

1) Pollen selected varieties, Sonaliand Rupali, released in 2004

Two new desi chickpea varieties, Sonali and Rupali, were released in September 2004 in WA bred by novel technologies at CLIMA and the Department of Agriculture WA (DAWA) by Drs Tanveer Khan and Heather Clarke. This is a major achievement for the GRDC funded chickpea research at CLIMA which was initiated in response to the needs of chickpea growers in Australia. Sonali and Rupali are adapted to a range of environments but are particularly suited to the short season environments of the northern and central grainbelt of WA. Both varieties flower 10-15 days earlier than Sona and podding starts when the average temperature over 24 hours is 10-12°C compared to 14°C for Sona. Both varieties, therefore, mature earlier than Sona and all current varieties in Australia. Sonali yielded 31% more than Sona in 17 WA trials in 2000-2003. Rupali yields 5% greater than Sona and is of interest to the industry because of its attractive light coloured seed. Both varieties have partial resistance to ascochyta blight (scores of Sonali 4.3-6.5 and Rupali 5-6 compared to Howzat 6.3-9 and Sona 6.8-8). Sonali is also resistant to fusarium wilt.

There was an attempt to combine pollen selection and a molecular marker for chilling tolerance which ultimately could be used in any breeding program. This was achieved only within the initial segregating population and two markers were used, in combination with controlled environment screening, in the early development of Sonali and Rupali. The project was less successful in the transfer of these markers to new germplasm arising from the project. Fortunately, as a result of pollen selection, the improvement in tolerance to chilling at flowering in chickpeas has been significant and the advantage in the local environment is clearly reflected in early pod set and greater yields in new breeding lines. Therefore, more recent pollen selected lines have been advanced for variety development as a result of controlled environment screening and conventional selections in field trials carried out in collaboration between CLIMA and DAWA, rather than through molecular marker selection. The large improvement in yield, despite set backs caused by the additional need to incorporate ascochyta resistance in the chilling tolerant breeding lines, has been a major achievement for the project.

2) Manual for breeders describing methodology for pollen selection and screening of early generation breeding lines for resistance to chilling at flowering

Technical guidelines for new screening methods and how to incorporate pollen selection for chilling tolerance into a chickpea breeding program have been compiled at CLIMA (see Attachment 1). Options for breeders and researchers to screen
pollen in the laboratory, individual plants in controlled environment growth rooms and populations of segregating material in the field are presented. The development of an AFLP marker for chilling tolerance is detailed from DNA extraction to and its application in the breeding program. Its limitations are clearly described. Scientific papers and reviews to support methods have also been published internationally.

3) A further sixty hybrid lines selected for better tolerance to chilling at flowering
The primary aim of the project, to identify superior chilling tolerant lines among recombinant progeny recently developed at CLIMA, has been fulfilled. Sixty hybrid lines were selected with tolerance to low temperature at flowering equal to, or better than, the new desi varieties. F4 seed from the sixty lines were passed to Dr Tanveer Khan in March 2003. The material was planted in replicated plots at Merredin for yield assessment and seed multiplication as part of the western module of the ACCIP in 2003. Elite lines selected from Merredin are being trialled at replicated sites and a proportion of seed is being screened at the ascochyta disease nursery in 2004. Selected chilling tolerant lines will also be transferred for trials in central QLD in 2004 where early flowering and chilling tolerance are desirable but ascochyta is less prevalent.

4) Bulked F4 seed segregating for chilling tolerance screened for ascochyta resistance in 2003
In addition to elite breeding lines (above), bulked F4 seed segregating for chilling tolerance are also being screened for ascochyta resistance in 2003. This material was transferred to Dr Tanveer Khan in March 2003 and was planted at the disease nursery at Merredin. Individual plants showing resistance were selected for further trials and for use as parents in further crosses.

5) Segregation of H. punctigera (budworm) identified among recombinant breeding lines (F3)
Broad variation in resistance to H. punctigera was recorded among a segregating F3 population of a cross between Annigeri (susceptible) and WAD79 (partial resistance, imported from ICRISAT). Controlled environment screening of 50 F3 individuals identified 14 with resistance estimated to be equal or greater than WAD79. No absolute resistance to the pest was detected, but slower weight gain and higher rate of mortality was apparent on the selected lines. It was also clear that the trait is still segregating in this population. This material was transferred to Dr Tanveer Khan for further development as (a) bulked F3 populations segregating for helicoverpa resistance and (b) 14 selected F4 lines identified with partial resistance to H. punctigera in controlled environment screening at CLIMA. A summary of this work is given in Attachment 2.

6) Controlled environment screening identifies genotypes which perform best in the field in cool dry environments
Assessment of breeding lines in controlled environment facilities at CLIMA provided a reliable method to identify chilling resistant genotypes among segregating F2 and F3 populations in the early stages of the breeding program. A combination of pollen tube measurements and pod set data from pot grown plants was most effective. Most of these selections outperformed other lines and varieties in the field, at a site with low night temperatures at flowering and pod set (range: 15-23 °C day/ 0-7°C night). This approach was identified as the most reliable method for selecting early generation hybrids to advance to field trials. Pollen selection for chilling resistance and early screening of hybrid lines in controlled environments is now being adopted by chickpea breeders. Dr Tanveer Khan proposes to include this approach in crosses targeting higher yield through chilling tolerance at flowering in new varieties for southern regions of Australia. Breeders in Horsham (Victoria) and Queensland may also include pollen selection in their chickpea breeding programs.

7) International communication
The research has been well received internationally through presentations at the 4th European Conference on Grain Legumes (Poland 2001), the International Chickpea Conference (India 2003), and the 12th Australian Plant Breeders Conference (Perth 2002). Scientific papers have been published in international journals based on results from the project.

Benefits
The project falls within the research priorities of GRDC programs 1 and 2, with the common objective of a wider range of pulses suited to different environments, higher yielding varieties to increase profitability and enhanced pest and disease resistance in pulses. The most significant benefit to the chickpea industry has been the development of two new desi chickpea varieties, Sonali and Rupali, released in 2004. These lines have been developed by Dr Tanveer Khan and Dr Heather Clarke as a collaboration between DAWA and CLIMA. The new varieties are chilling tolerant at flowering with partial resistance to ascochyta blight and are particularly well adapted to the cool, dry environments. The project also benefits the industry with better techniques for breeding, as well as new early generation germplasm feeding into the national chickpea breeding program. In general, the project achieved outcomes of improved screening techniques and assessment of breeding methodologies to the stage where they will be routinely applied in a breeding program. The new
breeding technologies enable chickpea breeders to respond faster to growers' needs with direct benefit to the industry. Segregating germplasm at early generation has added significantly to material for variety development in WA benefiting the ACCIP. The project has extensive support from the industry and is strongly supported by Mr Ted Knights, national coordinator ACCIP, and Dr Tanveer Khan, Pulse Breeder DAWA.

**Other research**

Early generation germplasm arising from the project will be further developed as part of the Western module of the ACCIP. Plans are also in place to incorporate pollen selection and screening for chilling tolerance into chickpea crosses in WA.

Interspecific hybridisation between chickpeas and wild relatives is now being investigated as a means to access new genes for traits such as ascochytta resistance, chilling tolerance and resistance to *Helicoverpa* spp. Protocols for rescue of hybrid embryos from wide crosses among the gene pool are being developed through internet collaboration with Canada and India (UWA00036). FI plants derived from the crosses will be tested for hybridity with molecular markers during the project. A postgraduate study in CLIMA will also examine cytogenetics among annual species in the genus *Cicer*.

AFLP markers are currently being applied at CLIMA to characterise the world collection of annual wild *Cicer* species (PDF38). Knowledge of relationships between the species and the extent of diversity among the collection is being used to identify key parents for interspecific hybridisation. DNA profiles will be a useful tool for management of the collection and for targeting new collections of *Cicer* to maximise diversity and increase variation in available germplasm.

There is a strong possibility that the pollen selection method could improve yield in other crops which might benefit from better tolerance to low temperature in certain environments. There is also an opportunity to apply the technique during breeding for heat stress in chickpeas. Funding will be sought to investigate this potential.

**Intellectual property summary**

Chickpea breeding lines used in the study of chilling resistance were generated at CLIMA. Accessions from overseas, used as parents, were imported under a Material Transfer Agreement between CLIMA and donor institutions. CLIMA and its partners will share in the Plant Breeder's Rights (PBR) of new varieties resulting from this project, according to the terms and conditions set out in the agreement.

**Additional information**