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GRDC

GROUNDCOVER SUPPLEMENT

CHICKPEAS DESIGNED FOR AUSTRALIA

Chickpeas capitalise on the convergence of science

By Dr Francis Ogbonnaya

Senior Manager, Genetic Technologies

There is a sweet point in research and development where the scientific outputs from human endeavour converge to create a step change for an industry. One of those points has been reached for Australian chickpeas.

With much research commitment, supported by GRDC and industry investment, chickpeas now play an important role in Australian farming systems. Used in rotations as a break crop for cereals, fixing soil nitrogen, managing diseases and improving yields of subsequent crops, their benefit is multitude.

As a result, Australian growers produced an average of 825,394 tonnes of chickpeas with an export value of $683 million per year over the last 10 years (2012–2022). This has seen Australia become the largest global exporter of chickpeas.

But the road to this sweet point for Australian chickpeas has not been easy.

Chickpeas arrived in Australia from India in the 1970s and were first supported by NSW Department of Primary Industries' research. Initially grown for stockfeed in drier areas, a shift in the Indian market led to a lucrative human consumption market. Breeding efforts focused on harvestability and phytophthora root rot resistance, drawing from global germplasm collections. The industry consolidated in the early 1990s despite challenges due to weather conditions, volatile markets and diseases. However, it was decimated in the late 1990s from Ascochyta blight, making recovery difficult even with rapid introduction of varieties from the International Center for Agricultural Research in the Dry Areas (ICARDA).

GRDC has been instrumental in rebuilding the industry working together with researchers across Australia.

Through extensive industry consultation and drawing on the successful approach of stepwise investments to grow the canola industry over several decades, the following constraints were identified:

- limited scale in the breeding program to meet the target opportunities;
- lack of genetic diversity for Australian breeders and researchers to draw on for valuable traits;
- chickpeas were not adapted to many Australian cropping regions – phenology, temperature (chilling tolerance at reproductive stages and susceptibility to heat shock events) and soil toxicities, including acid soils;
- lack of genetic resistance to important diseases – Ascochyta blight, root lesion nematode and phytophthora root rot;
- limited screening and indirect selection platforms to assist both pre-breeding researchers and breeding programs to accelerate genetic gain.

These priorities informed GRDC's strategic plan of interconnected research investments for chickpeas, which commenced in 2019. A number of these are showcased in this *GroundCover™ Supplement*:

- A multi-pulse species DNA chip has been developed by Agriculture Victoria Research (AVR) revolutionising pre-breeding and breeding efforts.
- Genetic resources have been accessed through investments with the University of California, Davis, and CSIRO.
- The genetic basis of phenological adaptation is being dissected by a team led by the University of Tasmania.
- Comprehensive resistance screening systems are routinely being used for Ascochyta blight by South Australian Research and Development Institute and AVR.
- Scalable and field-relevant screening systems have been deployed for acid soil tolerance by Murdoch University.
- Reliable methods for chilling tolerance screening are being used by WA Department of Primary Industries and Regional Development.
- Chickpea Breeding Australia is generating efficiencies in its breeding programs and capitalising on collaborative efforts of the above pre-breeding investments.

For Australian growers, this means science is converging to deliver more broadly adapted, robust chickpea varieties with improved yield and yield stability, as well as disease resistance to expand the region of production whilst providing a high-value pulse option in cropping rotations. □

More information: Dr Francis Ogbonnaya, francis.ogbonnaya@grdc.com.au

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WRITE TO: The Editor – *GroundCover™*, PO Box 5367, Kingston ACT 2604

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Infrastructure investment accelerates national chickpea development

Chickpea growers Australia-wide benefit from infrastructure developments at Tamworth, New South Wales

By Dr Sue Knights

Photo: CBA

New infrastructure for Chickpea Breeding Australia at the Tamworth Agricultural Institute is a joint investment by GRDC and the NSW Department of Primary Industries.

■ Tamworth has long been the hub for chickpea improvement, historically servicing New South Wales, but with the inception of Chickpea Breeding Australia (CBA) and an infrastructure investment injection, it stands to become the breeding powerhouse for Australian chickpeas.

A combined $10 million, five year investment from GRDC and the NSW Department of Primary Industries is helping. Commencing in 2021, the investment is delivering a new glasshouse, new polyhouses, storage facilities and capital items. These facilities are bringing efficiencies and boosting the scale of chickpea development.

Dr Kristy Hobson, CBA's senior chickpea breeder, says the new glasshouse is a step change to accelerate chickpea improvement.

"The 550-square-metre glasshouse is equipped with LED far-red lighting and sophisticated heating and cooling to assist rapid cycling 10,000 individuals through four generations per year," Dr Hobson says.

"This facility also assists us in synchronising flowering between domesticated and wild chickpeas, so we can cross multiple generations a year. Successful crossing percentages have increased from 30 to over 80 per cent regardless of the time of year."

Two polytunnels have been installed – one 460m² propagation tunnel with lighting, heating and cooling that can accommodate 5280 pots every four months, and one 340m² facility dedicated to Ascochyta blight screening fitted with misters and cooling.

"The 340m² facility is enabling us to screen 7752 individuals in a four-week period and accurately select more material at an earlier generation stage. This means we only take the best material to field-based Ascochyta blight screening; having this knowledge at an early generation ensures we don't waste resources on substandard material," Dr Hobson says.

New processing, cool-room and storage facilities, as well as freezers, grinders and a harvester, are improving efficiencies and increasing CBA program capacity.

"The new facilities have given us increased ability to collaborate with pre-breeders and researchers as we can accommodate more traits and germplasm."

ESTABLISHED AND EXPANDING REGIONS

CBA takes a methodical approach to breeding chickpeas adapted to Australian regions, with traits categorised as short-term and longer-term priorities. Its breeders work closely with pre-breeders, identifying appropriate germplasm with these traits for rapid inclusion in breeding pipelines.

"New breeding technologies have been adopted, such as genomics and high-throughput phenotyping as well as the latest in experimental design, much of this enabled by the new infrastructure."

CBA's efforts continue to focus on the established regions of northern NSW and central and southern Queensland where most of Australia's chickpeas are grown, but it is now well equipped to expand into regions of southern and western Australia.

"Improved Ascochyta blight resistance is the number-one priority and we are making good progress with increased resistance in pre-release lines. Fundamental to this progress has been GRDC's investment in the five-pronged program 'Towards effective genetic and sustainable management of Ascochyta blight of chickpea'.

"Phytophthora root rot resistance is important for chickpeas in the traditional areas and we have one desi chickpea line with improved resistance near release."

For expanding regions, CBA is making inroads for improved acid soil tolerance and chilling tolerance during flowering and podding, together with developing herbicide-tolerant varieties for improved weed management.

"CBA Captain(ϕ), released in 2020, continues to gain ground. It is an erect desi type with broad adaption to all regions of Australia, early to mid-flowering, moderate lodging resistance and has excellent harvestability.

"Collaboration with the research community and seed partners is critical to capturing the maximum value from new investments to deliver new chickpea varieties with improved yield and yield stability for Australian growers." □

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More information: Dr Kristy Hobson, kristy.hobson@dpi.nsw.gov.au

The Multispecies Pulse SNP bead chip array manufactured by Illumina Pty Ltd. Each array enables thousands of DNA markers to be simultaneously assayed on 96 samples. Photo: AVR

Multi-species DNA chip to boost chickpea improvement

Enabling technology and training are providing a step change for chickpea breeding

By Dr Sukhjiwan Kaur, Dr Gabriel Keeble-Gagnere, Dr Shimna Sudheesh, Debbie Wong, Dr Matthew Hayden

■ Agriculture Victoria Research (AVR), with investment from GRDC, has developed a cutting-edge, low-cost DNA genotyping platform for chickpeas, field peas, lentils and lupins.

The platform seamlessly connects research to breeding, heralding a significant efficiency boost and accelerating both pre-breeding research and breeding for the crops.

Data generated using this platform can be more easily combined with legacy, current and future datasets from GRDC projects and the public domain. The knowledge generated by combining datasets accelerates research outcomes and the translation of pre-breeding outputs into breeding programs.

The utility of the genotyping platform has been enhanced through Pretzel, a web-based tool developed by AVR that enables the data generated to be visualised and interrogated interactively in the context of other research and breeding information. This allows researchers and breeders without any specialised bioinformatic skills to combine data generated across projects, time and different technology platforms to objectively answer specific questions that are otherwise challenging to address.

The genotyping platform is delivered to the Australian pulse pre-breeding and breeding sectors through AVR and has been widely adopted, with more than 108,000 samples genotyped over the past two years.

UNIQUENESS OF CHIP DESIGN

The genotyping platform assays specific positions in the genome of each pulse crop known as Single Nucleotide Polymorphisms (SNPs). These positions reveal differences between individuals at the DNA sequence level. Across the four pulse crops, a total of 30,000 SNPs are assayed on the DNA chip.

The SNP content was carefully selected for two purposes.

The first set – called imputation SNP – was selected using a custom algorithm that chooses the minimum set of SNPs needed to unambiguously track the inheritance of DNA at the whole genome level during breeding. In other words, the imputation SNPs are selected to provide almost the same amount of information as whole genome sequence, where every DNA difference between individuals is known.

The selection of the imputation SNPs required a detailed understanding of the genetic variation that exists in globally diverse germplasm for each pulse crop. To generate this information, 1000+ accessions for each pulse crop were genotyped and 250 of the most genetically diverse accessions within each pulse crop group were whole genome sequenced. The accessions used included landraces, introgression lines, historical and released varieties and wild species, most of which were obtained from the Australian Grains Genebank.

The availability of whole genome sequence information for each pulse crop also made it possible to design assay probes for the selected SNPs that only work in the pulse crop for which they were designed. For example, an assay probe designed for chickpeas gives no signal in field peas, lentils and lupins. This design feature enables up to four DNA samples, one from each of the four pulse crops, to be jointly assayed to the same DNA array, effectively reducing the assay cost by up to fourfold.

The second type of SNP on the array – called knowledge-linked SNP – was selected to provide a strong connection between research and

breeding knowledge. These SNPs include trait-linked markers for each pulse crop that have been identified in national and international research and allow breeders and researchers to immediately know what trait alleles a genotyped accession possesses. The knowledge-linked SNPs also provide a direct connection to important germplasm and genomic resources that have been developed by the international research community.

For example, in chickpeas there are SNPs that provide a direct connection to a vast array of genomic resources developed by the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT).

The third set of knowledge-linked SNPs enable DNA introduced from wild species to be accurately tracked in domestic germplasm during breeding. Collectively, the knowledge-linked SNPs work to accelerate genetic gain for pulse crop improvement by enabling research outputs to be more seamlessly translated into breeding programs.

CHIP DEPLOYED WIDELY

The utility of the genotyping platform has been demonstrated across numerous GRDC pre-breeding investments including in chickpeas for improving Ascochyta blight resistance, reproductive-stage chilling tolerance and acid soil tolerance.

In the program for increasing Ascochyta blight resistance, the genotyping of more than 5500 diverse chickpea lines (including varieties, wild species, imported germplasm and breeding material) allowed the characterisation of existing genetic variation for resistance in breeding germplasm pools, as well as the identification of new sources of resistance.

The genotyping platform was also used to accelerate the crossing of new resistance sources into domesticated backgrounds to generate improved seed stocks for breeder evaluation.

In the chilling tolerance project, the genotyping platform was used to genotype a population of wild chickpea crosses that were known to carry novel sources of cold tolerance during the reproductive phase. The 1700 markers for tracking wild chickpea genomic segments in crosses with commercial lines allowed the identification of strong marker-trait associations and helped identify lines that can withstand cold temperatures during flowering.

Similarly, in the acid tolerance project the genotyping of more than 1500 lines (including wild crosses) led to the identification of germplasm with novel sources of tolerance, which are being crossed into elite backgrounds and will be ready for handover to breeders in the near future.

The genotyping platform has also been widely adopted by breeding programs, including Chickpea Breeding Australia, the national lentil and field pea breeding programs and Australian Grain Technologies (AGT) lupin breeding, to support the implementation of genomic selection, a technique used to accelerate the breeding of new improved varieties.

Breeders are using the ability to seamlessly link the genotype data generated to research knowledge to accelerate the adoption of pre-breeding outputs into their breeding programs.

PRETZEL TRAINING

A community of practice for Pretzel is being established and will be supported by extensive online training resources and tutorials. Researchers and breeders interested in taking part should contact gabriel.keeble-gagnere@agriculture.vic.gov.au. ☐

GRDC Code DAV1905-003RTX

More information: Dr Sukhjiwan Kaur, sukhjiwan.kaur@agriculture.vic.gov.au

A low-cost DNA chip for genotyping pulses has been developed by a team at Agriculture Victoria Research. From left to right (back row): Kirushanthy Kajendran, Dhriti Sharma, Sukhjiwan Kaur; front (left to right) Thanjua De Alwis, Shimna Sudheesh.

Photo: Adam Dimech

Expanding the climatic adaptation of chickpeas

Photo: Lia Olmedo-Pico

A collaborative approach is enabling the evaluation of chickpea phenology in different environments, with the focus on expanding production of the crop. Photographed here are Dr Fernanda Dreccer (CSIRO) and PhD student Alejandra Alfaro (University of Tasmania/CSIRO), who are assessing phenology in diverse chickpea lines at the field station in Gatton, Queensland.

Identification of phenology genes and acquisition of more diverse germplasm will expand the regions where chickpeas can be grown in Australia

By Dr Raul Ortega and Dr Jim Weller

Phenology, or the timing of key events in a plant's life cycle, is a crucial trait that breeders harness when improving crop adaptation and performance.

As climates become more unpredictable, phenology is becoming an increasingly important consideration in developing new varieties that are productive across a wider range of environments and climatic variables.

As such, understanding what controls phenology and being able to manipulate it more effectively through breeding are two important components in a far-sighted strategy for climate adaptation.

Compared with other crops, relatively little is known about the genes that control phenology in chickpeas. Discovering these genetic factors and how they intersect to determine phenology in different environments is one main goal of a GRDC-invested project underway at the University of Tasmania in partnership with the South Australian Research and Development Institute (SARDI) and CSIRO.

This knowledge will be an important asset in breeding programs for several reasons. It will provide breeders with new information and, when combined with genetic markers necessary to track specific gene variants, will enable selection for combinations of genes that confer diverse phenologies. It will also enable the development of genetically defined lines that help to assess the value of different phenologies for different locations and management options.

The Australian chickpea industry was founded on a relatively narrow subset of the genetic diversity available for the species globally. This means that breeders had limited access to the variation needed for the development of improved and diversified varieties.

To address this, the project is examining a much wider range of germplasm acquired from around the world that represents new diversity for phenology and related traits.

IDENTIFYING PHENOLOGY GENES

In many grain crops, there is good basic knowledge of genes that control flowering and progression through the reproductive phase. However, in chickpeas, only one major gene controlling flowering time has been identified so far – *early flowering locus 1* (*efl1*). This gene pushes phenology earlier, promotes pod filling and hastens maturity. It is important for short-season environments globally, where escaping terminal drought is a key problem.

In addition to *efl1*, the importance of at least five other genomic regions has been reported. To examine several of these in detail, researchers have generated populations from crosses between early and late lines. These populations have been grown in controlled-environment facilities at the University of Tasmania and assessed for variation in phenology, including the time of flowering, flower abortion and pod development.

The genetic analysis of these populations is underway and will allow researchers to better define several other major gene variants, including those that delay flowering. The researchers have already identified a second gene that provides a novel, and potentially contrasting, source of earliness to *efl1*.

One of the benefits of this detailed study is that, as different genetic means to achieving earlier or later phenology are identified, different genes are expected to have a different spectrum of effects on other traits such as growth habit, and also respond differently to environment. This will provide opportunities to expand the environmental regions of production for chickpeas.

The identification of specific genetic markers is another intended output from the project that can be used to follow the genes through the breeding process, and select specific combinations that may be desirable in a certain context. This is particularly important in situations where there may be alternative combinations with similar effects that need to be distinguished, or where it is useful to efficiently combine several gene variants with small effects.

These markers can be incorporated in emerging approaches in which breeders are increasingly utilising data from across the whole genome to guide their selections.

A FOCUS ON DIVERSITY

The project is also looking at phenology in relation to genetic diversity, carrying out a comparison between Australian and global germplasm. One broad aim of these activities is to identify greater variation that might not be represented in Australian breeding programs.

A second aim is to follow an alternative approach to unpick the genetic control of phenology through statistical association of trait differences with genes across the genome. This approach is complementary to the more-focused population/progeny approach described above, but has similar benefits with respect to generating markers.

To this end, the project, in collaboration with CSIRO, has assembled a panel of 400 lines from 46 different countries, which broadly captures the global variation in flowering time and genetic diversity and includes 28 accessions released by Australian breeding programs.

This panel is being multiplied for subsequent evaluation in the field and under controlled conditions of photoperiod and temperature, which will facilitate the identification of genomic regions regulating flowering in response to each of these components.

Importantly, the panel has also been genotyped using the multi-species pulse DNA chip developed for GRDC by Agriculture Victoria Research. Since this chip is used by pulse breeders and other pre-breeding researchers, it allows researchers to connect pre-breeding research outcomes with the chickpea breeding program, tracking the extent of relevant gene variants within the chickpea germplasm available in Australia.

TESTING PHENOLOGY GENES FROM GLASSHOUSE TO FIELD

In parallel with tracking down the identities of individual genes, it is also possible to test and validate their effects and interactions, and the project will address this in several ways. One way is to use previously generated populations and derived progenies that have been specifically selected to represent different combinations of gene variants.

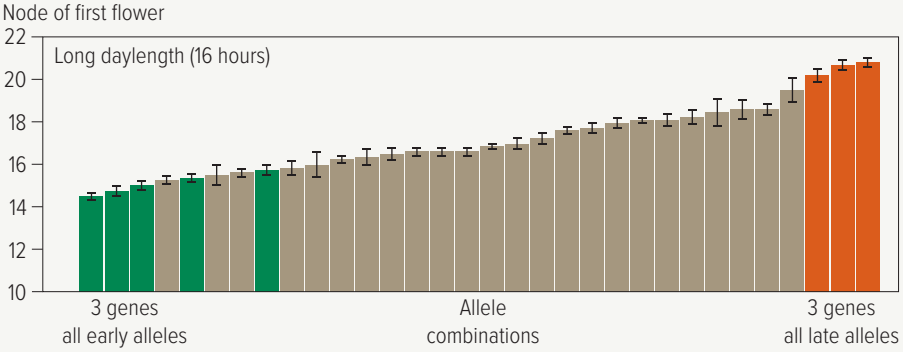
For example, the SARDI team has developed a set of closely related lines with different allelic combinations for *efl1* and two other flowering genes, and these are being assessed in both field and greenhouse. The results illustrate how variation in phenology can be obtained by combining alleles with distinct effects (Figure 1). Generation of similar sets of lines incorporating other genes is an ongoing activity within the project.

Ultimately, this material will be made available to pre-breeders, breeders and agronomists. It will enable testing of the adaptation of different phenology gene combinations across a wider range of environments, including different locations and sowing dates. ☐

GRDC Code UOT1909-002RTX

More information: Dr Jim Weller, jim.weller@utas.edu.au

Figure 1: Genes work together to affect flowering time. This graph shows when chickpea plants with different gene combinations start flowering. Green columns have early versions of three genes, orange columns have later versions and brown columns have a mix of the two sets of genes. Here they have been grown under long daylengths of 16 hours, resulting in a one-week difference, but under short daylengths the difference can be three weeks.



Source: University of Tasmania

International profiling team tackles chickpea Ascochyta blight

Ascochyta blight is a continual threat to chickpea production in Australia, but a forensic approach is set to type both pathogen and chickpea genomes and improve the odds of success in the battle against the disease

By Dr Aladdin Hamwieh and Dr Lars Kamphuis

■ If you know your enemy and know yourself, you are much better prepared for any battle – and it is this approach that is being taken to better inform management of Ascochyta blight in chickpeas.

Internationally, studies on pathogen diversity have been conducted on a country-by-country basis, using different chickpea genotypes and without identifying specific resistance genes linked to particular pathotypes.

Unlike cereal pathogens, the interaction between chickpeas and Ascochyta blight (*Ascochyta rabiei*) suffers from a lack of known resistance genes or agreed differentials, as well as insufficient data on factors driving population changes in the pathogen.

To address this, a project led by the International Center for Agricultural Research in the Dry Areas (ICARDA) and the Centre for Crop Disease and Management (CCDM) at Curtin University aims to develop a set of chickpea lines with known markers for each resistant gene in the chickpea host plants and identify corresponding avirulence or virulence genes in the pathogen. This forms part of GRDC's five-pronged approach 'Towards effective genetic and sustainable management of Ascochyta blight of chickpea'. It involves creating segregating fungal populations of the pathogen to aid in the discovery of (a)virulence genes and determine the pathotype structure for the species.

Ultimately, this project will make novel Ascochyta blight-resistant genes accessible to Australian and overseas chickpea breeders, leading to improved Ascochyta blight resistance in future varieties.

By understanding the pathotype structure and (a)virulence genes discovered, chickpea growers worldwide will be empowered to enhance their production and profitability. This endeavour holds the promise of bolstering chickpea cultivation and ensuring food security not only in Australia but also on a global scale.

FUNGAL FINGERPRINTING

The CCDM team obtained 39 *A. rabiei* isolates from international collaborators and generated high-quality, chromosome-level genome assemblies for 13 of these isolates. Comparing these international isolate genomes with Australian ones revealed that Australian isolates have much less sequence diversity (Figure 1).

The low level of genomic diversity in Australian *A. rabiei* isolates is most likely due to the presence of only one mating type in Australia and therefore sexual recombination does not occur in Australian *A. rabiei* isolates.

A team led by Professor Rebecca Ford at Griffith University (part of GRDC's five-pronged Ascochyta blight investment) each year is screening *A. rabiei* isolates collected in Australia for their mating type to ensure the second mating type remains absent.

At Curtin University's biological containment facility, the team generated four fungal mapping populations to identify key genes in the pathogen's genome associated with causing disease. This required the development of a robust phenotyping process in the containment facility for Ascochyta blight resistance (Figure 2).

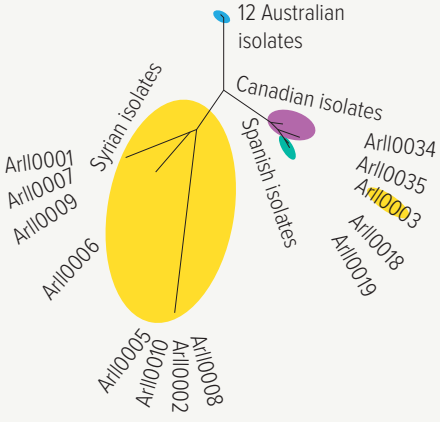
This method has been optimised to evaluate the response of the international isolates on 15 ICARDA differential chickpea lines as well as the Australian chickpea differential set used in other projects within the GRDC's Ascochyta blight five-pronged investment. The team has phenotyped the disease response of 83 offspring of the first fungal population so far.

GLOBAL CHICKPEA GERMPLASM GENERATION

A global consortium was formed by the project involving several countries (Australia, Tunisia, Turkey, Lebanon, Ethiopia, India and Morocco) to tackle Ascochyta blight in chickpeas. The scientists in these countries collected more than 150 isolates of the pathogen from different regions and tested their pathogenicity on various chickpea varieties under controlled conditions (Figure 3).

A range of responses was observed, with isolates showing low to high pathogenicity across all countries. The DNA of these isolates was sent to Australia for sequencing to gain a better understanding of the global diversity in the pathogen population. Additionally, the project created a collection of chickpea genotypes called the global Ascochyta blight

Figure 1: Whole genome relationship of 12 Australian and 13 international Ascochyta blight isolates sequenced to date. The 12 Australian isolates all cluster together and have a narrow genetic diversity when compared to the international isolates.



Source: Robert Lee, CCDM

resistant subset collection (GABRSC), which included various Ascochyta blight-resistant genes from different sources.

Seeds from this collection were shared among consortium members and tested in multiple locations in the participating countries for two years (2022 and 2023). The results revealed that certain genotypes (S0110227, S160454, S0110088 and S0110028) consistently showed resistance to Ascochyta blight irrespective of the country location and isolates used (Figure 4).

These chickpea genotypes were developed at ICARDA through the strategic combination of resistant parent plants over the past four decades, presenting a promising approach to creating sustainable resistance against Ascochyta blight in chickpeas. The GABRSC population is under quarantine with the Australian Grains Genebank and material will be disseminated to other projects within GRDC's Ascochyta blight five-pronged investment for evaluation with Australian isolates.

At ICARDA, more than 300 chickpea near-isogenic lines (NILs) were established. NILs are a valuable and fascinating tool in the field of genetics, designed to address complex traits such as Ascochyta blight disease in chickpeas. The chickpea NILs are a pair of lines that are genetically similar but differ in a small region of the genome. In our case, this region contains a single Ascochyta blight resistance gene in one line and not the other.

This unique structure allows researchers to isolate and study the effects of individual Ascochyta blight-resistant genes, making it easier to understand the genetic basis of characteristics of each resistant gene.

By comparing NILs to the original susceptible chickpea line ILL263, scientists can pinpoint the precise genes responsible for Ascochyta blight resistance, and develop markers to follow the different resistance genes in breeding programs. This will lead to significant advancements in chickpea crop improvement and ultimately contribute to global food security and sustainable agriculture. □

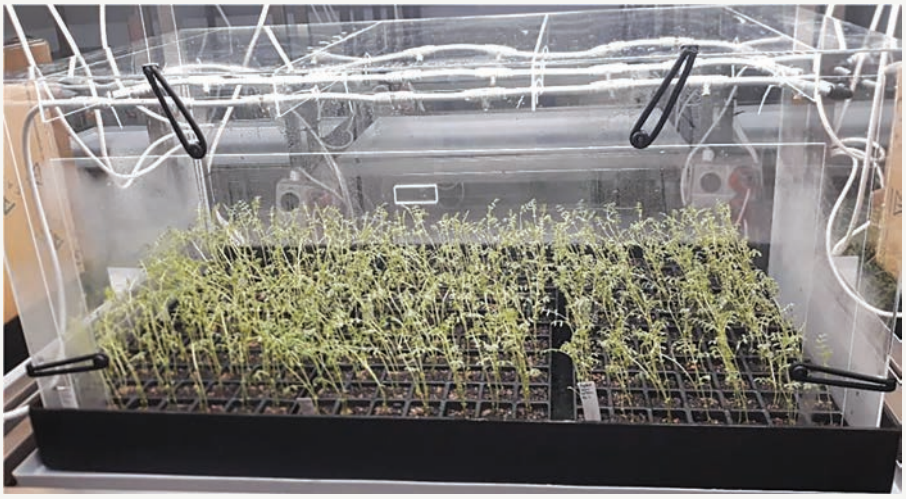
GRDC Code ICA2007-001RTX

More information:

Dr Aladdin Hamwieh, a.hamwieh@cgiar.org;

Dr Lars Kamphuis, lars.kamphuis@curtin.edu.au

Figure 2: Chickpea seedling Ascochyta blight assay in the misting hoods at Curtin University.



Source: Robert Lee, CCDM

Figure 3: Assessment of the pathogenicity of isolates collected from various locations. The evaluation was carried out in both a growth room and greenhouses to maintain controlled conditions and prevent contaminations between isolates.

Source: Aladdin Hamwieh, ICARDA, and Canan Can, Gaziantep University

Figure 4: Field evaluation of GABRSC performance during the 2022 growing season in a) Béja station (northwest of Tunisia) and b) Kafarshkhna station (West Lebanon). The experiments were laid out in an Alpha Lattice design, with two replications, and conducted under natural infection.

Source: Tawffiq Istanbuli, ICARDA; Mariem Buhadida, INRAT; and Canan Can, Gaziantep University

Holistic strategy to enhance chickpea blight resistance

Mass screening of chickpea lines is being combined with genomic resistance identification to improve the odds for chickpea's fight against Ascochyta blight

By Dr Judith Atieno, Dr Sukhjiwan Kaur, Sara Blake, Dr Ute Baumann, Marzena Krysinska-Kaczmarek, Dr Shahin Yazdifar, Dr Zibei Lin, Dr Josh Fanning, Dr Janine Croser

Enhanced, durable resistance to Ascochyta blight will aid more-reliable, lower-cost production for chickpeas across existing production areas while also promoting possibilities of expansion.

However, genetic resistance to chickpea Ascochyta blight globally appears to be limited, despite significant research efforts. It is the primary disease constraint for chickpeas in Australia.

The most-resistant variety (Genesis 090) is rated moderately susceptible with up to 64 per cent yield loss, with susceptible varieties such as PBA Striker(D) suffering up to 96 per cent loss.

Since 2020, GRDC has invested in a five-pronged program for Ascochyta blight with partners across Australia and internationally. Program 3 'Towards effective genetic and sustainable management of Asochyta blight in chickpea' is led by the South Australia Research and Development Institute (SARDI) by Dr Janine Croser and a multidisciplinary team incorporating researchers from SARDI, the University of Adelaide and Agriculture Victoria Research (AVR).

This partnership galvanises a holistic scientific approach to improve chickpea Ascochyta blight resistance. Field-relevant, pot-based phenotyping capabilities at SARDI assess both well-adapted and poorly adapted germplasm such as landrace and wild relatives that would struggle to grow under field conditions. Field disease nursery screening at AVR, led by Dr Joshua Fanning, provide scalable, high-throughput, grower-relevant disease response assessments.

The high correlation between the two phenotyping approaches means the data collected can be combined in the same analysis increasing statistical power to understand Ascochyta blight's genetic response. Similarly, trait dissection at SARDI and the University of Adelaide identifies functional genes that underlie Ascochyta blight disease resistance. Genomic prediction and speed breeding work led by Dr Sukhjiwan Kaur's team

Photo: Simon Michelmore, SARDI

Dr Judith Atieno (left) and Sara Blake on the hunt for improved genetic resistance for chickpeas against Ascochyta blight.

at AVR unravels the genetic basis of the disease response and identifies favourable alleles for resistance that are rapidly combined into seed stocks, ready for deployment in breeding. Underpinning these efforts is the multispecies pulse DNA chip, recently developed by Dr Kaur's team. This chip enables pre-breeding research outputs to be more seamlessly connected to breeding outcomes.

Ongoing breeding efforts for Ascochyta blight resistance in chickpea, under the mantle of Chickpea Breeding Australia (CBA), will now be fortified with outputs from these new pre-breeding efforts. Resistance levels are building as CBA pipeline material recorded no yield loss last season when exposed to Ascochyta blight infection. This effort will be constantly required as the pathogen is highly mutable, which means it keeps changing, creating an 'arms race' between researchers/breeders and the disease.

MASS SCREENING

The project has assessed Ascochyta blight resistance in 2465 different chickpea lines in the field and 1600 lines in a pot-based adult plant screening facility at SARDI.

These lines are as diverse as possible and include CBA breeding lines, international breeding program lines and wild relatives supplied by the International Center for Agricultural Research in the Dry Areas (ICARDA) Food Legume Improvement Program and the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), landrace lines (Vavilov) and wild relative (*Cicer reticulatum* and *C. echinospermum*) accessions. The international and wild relative germplasm seeds were supplied by Dr Sally Norton's team at the Australian Grains Genebank.

Disease assessment has been undertaken over three years and in two environments. The first, the SARDI 'Terraces', is a bird-net protected facility on the Waite Campus in Adelaide that provides a field-relevant, pot-based phenotyping platform in outdoor conditions. These pot-based methods enable adult plant assessment of poorly adapted germplasm.

Plants in the Terraces are assessed for resistance to Ascochyta blight based on four measures: per cent main stems broken, per cent stems with lesions, per cent side branches with disease and per cent leaves with disease to develop a disease index rating. This comprehensive rating provides the data required to help understand the biological mechanism of resistance.

The second environment is the Horsham field disease nursery, undertaken in partnership with Dr Josh Fanning and his team at AVR. Field assessment is based on per cent stem breakage. This project has demonstrated an excellent correlation between the Terrace and field environment, providing evidence of the field-relevance of the pot-based screening methodology. It has also shown the value and scalability of field-based screening for high-throughput disease screening.

By combining the data from these two approaches, 86 promising lines were identified. Seed from these lines has been harvested and provided to CBA to ensure breeding efforts can rapidly exploit novel resistance sources.

GENOMIC WORK

Concurrently, Dr Judith Atieno (SARDI) and Dr Ute Baumann (the University of Adelaide) have been working to identify genomic regions associated with resistance, using a method called Genome Wide Association Studies. This is a statistical method linking phenotyping information from SARDI with genotyping information from AVR.

This approach shows novel areas of the genome that are associated with resistance and has highlighted that several genes might need to be combined into a single genotype to develop durable resistance. The novel genomic areas have been targeted using sequence capture and this information will lead to a better understanding of the genetics of Ascochyta blight resistance.

To enhance the genetic analysis, SARDI has assembled the genome of Australian variety PBA HatTrick$^{\phi}$. The PBA HatTrick$^{\phi}$ genome and further information is now publicly accessible at hatchiblap.adelaide.edu.au.

In parallel, the AVR team focused on implementing a genomic selection approach to identify and combine, through breeding, both the major and minor genes that are responsible for Ascochyta blight resistance. This approach mitigates the risk of breakdown of the major disease resistance genes that occurs frequently due to the pathogen evolving over time.

Their research found that the newly identified large-effect resistance sources explained about one-third of the genetic variance for Ascochyta blight resistance, while the remaining two-thirds was explained by small-effect genes that are mostly already present in domesticated chickpea germplasm.

AVR has combined genomic selection with speed breeding approaches to develop seed stocks that are enriched with both major and minor gene resistance. The speed-breeding facility at AVR fast-tracks the development of fixed lines by reducing the generation cycle time of plants to eight to nine weeks, in comparison to more than six months in the field. A subset of these improved seed stocks is under validation in the Horsham nursery as well as SARDI Terraces. Once validated, these stocks will be delivered to CBA.

IN THE FUTURE

The pot-based phenotyping has identified wild relatives of domestic chickpeas with higher resistance to Asochyta compared to the cultivated species. Importantly, novel resistance has been found in the close relative *Cicer reticulatum*, which has been crossed at SARDI to domestic germplasm for incorporation in future breeding and research activities.

Utilising a genomic selection approach to further exploit current variation for Ascochyta blight resistance within cultivated germplasm will be important, as well as simultaneously and rapidly introducing newly identified major sources of resistance into new varieties. The molecular tools being developed by AVR will greatly assist breeders to achieve this.

The research teams will continue to seek opportunities to enhance the PBA HatTrick$^{\phi}$ genome assembly to take it to a level of accuracy and relevance for Australian breeding germplasm beyond what is currently available. □

GRDC Code UOA2005-011RTX

More information: Dr Janine Croser, 0448 990 281, janine.croser@sa.gov.au

A lateral irrigator can simulate conditions conducive for Ascochyta blight disease development regardless of the season at Agriculture Victoria's Horsham SmartFarm.

Chickpea blight – resistance phenotyping

Simulating conditions for Ascochyta blight disease screening has been challenging but has now been achieved with high throughput

Photo: Marty Shoo Photography

By Dr Sue Knights

■ High-capacity, cost-effective, accurate and reliable Ascochyta blight screening is now a reality for chickpeas. It is the result of combining years of accumulated disease knowledge with perfecting screening techniques.

Dr Joshua Fanning from Agriculture Victoria leads the development of the GRDC-invested screening program and says Ascochyta blight is the highest-priority disease of chickpeas nationally. The program is part of GRDC's five-pronged approach, 'Towards effective genetic and sustainable management of Ascochyta blight of chickpeas'.

"Ascochyta blight causes complete crop failure in the absence of suitable control. With limited genetic resistance available in commercial cultivars, growers are dependent on a restricted range of fungicides for disease control," Dr Fanning says.

"This imperative is driving the development of chickpea varieties with improved resistance, together with identifying new sources of resistance. To do this, a high-capacity, cost-effective, accurate and reliable means of phenotyping or screening chickpeas for Ascochyta blight was essential for the Australian industry.

"This project was developed to provide high-throughput phenotyping in paddock and controlled environments for breeders, pre-breeders and researchers both within GRDC's five-pronged Ascochyta blight initiative and more broadly."

METHOD DEVELOPMENT

Dr Fanning explains that there is a considerable amount of knowledge on Ascochyta blight that underpins the design of the phenotyping service.

"Over the years we have learnt about the epidemiology of the disease. We know that it has sexual and asexual reproductive capacity but only the asexual variant is present in Australia.

"Despite the reduced chance of genetic mutations of the disease due to the lack of sexual reproduction, there have still been several significant mutations resulting in the loss of varietal resistance in Australia."

The disease spreads via fungal spores that are carried from crop residues into new crops by wind or through seed transmission. Infection can occur at any stage of plant growth, provided conditions are favourable.

Moisture is essential for infection to occur and wet or foggy weather can exacerbate the disease as fungal spores are dispersed on to neighbouring plants by wind and rain splash.

Ascochyta can develop over a wide range of temperatures (5°C to 30°C), with leaf wetness key to disease development. Symptoms become visible in four to five days and fruiting bodies develop in fewer than 10 days. This allows rapid transmission.

"Horsham is an appropriate site for a large paddock-scale screening service as Victoria is home to Australia's more-aggressive Ascochyta blight isolates,

Photo: Marty Shoo Photography

Rain splash simulation by an automated overhead lateral irrigator for Ascochyta blight disease spread.

Photo: Agriculture Victoria

Dr Joshua Fanning assessing chickpea plants for Ascochyta blight in the phenotyping facility on Agriculture Victoria's SmartFarm in Horsham.

with both the major resistance breakdowns detected within Victoria," Dr Fanning says.

"The site is also ideally located to challenge chickpeas with the most relevant mix of stubble-derived isolates sourced from local growers."

Fundamental to the paddock-based screening method, Dr Fanning says, has been the simulation of suitable environmental conditions conducive to the disease's development in a reliable, repeatable way that can be used independently of seasonal conditions.

"Initially we started using infected stubble and a mister system to create rainfall drops and leaf wetness, which was automated. We started with a proof-of-concept approach over half a hectare, but we found it was cumbersome to sow plots in among a fixed set-up of pipes and misters.

"So, we moved to the use of a 57-metre automated lateral irrigator on the Agriculture Victoria SmartFarm and were then able to expand the area under irrigation to four hectares with a 25,000-plot capacity."

The site is irrigated at night or early morning to maximise the spread of disease and minimise the use of the water. The Horsham SmartFarm operators are installing a reverse osmosis plant to treat the town effluent and dams for storage and are looking at solar panels on the dams to operate pumps and reduce water evaporation.

"We have worked with the statistics team from the University of Adelaide to further develop our methodology to determine the optimum number of replications and the spreader density of infected stubble to reduce variability as much as possible. This is generating highly accurate results."

During 2022, 2867 genotypes (8517 rows in a partially replicated design) were phenotyped in this facility or resistance to Ascochyta blight. These genotypes were for other programs in the Ascochyta investment led by the South Australian Research and Development Institute (SARDI) and for Chickpea Breeding Australia.

Dr Bikram Banerjee, in the Agriculture Victoria team, is now exploring the potential for drone imaging to speed up the assessment of disease level on plants and to relate the images captured to the use of molecular markers.

To complement the field program, in 2022 a new state-of-the-art glasshouse facility opened at the Horsham SmartFarm.

"This facility is used across all research programs at Agriculture Victoria, but is also used to screen chickpea material against specific Ascochyta blight isolates in a very controlled way," Dr Fanning says.

It is important to complement the field work with controlled environment screening so that isolates from other states can be safely screened in Victoria.

"Understanding the pathogen and how it causes disease is an important step in understanding why we lose resistance in chickpea varieties."

This part of the Ascochyta investment is led by Griffith University, with national collaborators.

To ensure all organisations in the five programs of the Ascochyta investment are screening material similarly, a diverse set of chickpea lines were screened by SARDI, Curtin University's Centre for Crop and Disease Management and Agriculture Victoria. This is known as 'ring testing' and it ensures that, nationwide, consistent and accurate results are provided. It also regularly verifies that the test methods being used across Australia are reliable and reproducible.

During 2022, a total of 10,500 chickpea pots were sown in the glasshouse to assess the pathogen aggressiveness of 194 Ascochyta blight isolates and the ring test.

Due for completion next year, this project will ensure that breeding and pre-breeding efforts to release varieties with improved resistance to Ascochyta blight will not be limited by the ability to determine the resistance accurately. □

GRDC Code DJP2007-001RTX

More information: Dr Joshua Fanning, joshua.fanning@agriculture.vic.gov.au

Global germplasm harnessed to deliver improved acid soil-tolerant chickpeas

By Dr Douglas Cook, Dr Amna Fayyaz, Dr Kiarash Jamshidi Goharrizi and Ali Said Yusuf

KEY POINTS

- A single major chickpea gene has been identified for seedling tolerance to aluminium in acidic soils
- Chickpea genetic resources, including aluminium-tolerant accessions, have been imported and are available to Australian researchers and breeders
- Genome-guided trait breeding is introducing the gene into adapted Australian chickpeas

Photo: Courtesy of Doug Cook

Dr Doug Cook (left) and Ali Said Yusuf from the University of California, Davis, are part of a team of international allies on the hunt for acid tolerance that will improve the adaptability of chickpeas to Australian soils.

About half of Australia's arable lands are acidified, which causes significant reductions in crop yields. This poses a challenge for the expansion of chickpea production, as pulse crops are particularly sensitive to soil acidity.

A majority of acid soils' negative effects on crop yields are attributable to trivalent aluminium (Al^{3+}), which is solubilised and becomes dominant in the soil solution as soil pH decreases. Al^{3+} is directly toxic to plants, reducing both root biomass and function while also reducing soil fertility by decreasing the availability of key nutrients such as phosphorus.

Importantly, low soil pH in agricultural systems can be the consequence of crop management practices, in particular overuse of inorganic nitrogen fertilisers. As inorganic nitrogen is oxidised (called 'nitrification') by soil microbes, protons are released to the soil, pH drops and aluminium becomes soluble and toxic.

Crop management practices – for example liming – can mitigate low soil pH. Developing crops that can tolerate low pH and aluminium is a complementary and cost-effective solution in soils that require extensive liming.

Although chickpeas and other pulses are especially sensitive to the effects of low soil pH and aluminium, paradoxically they might also be part of the solution. Through their capacity for biological nitrogen fixation, chickpeas return organic nitrogen to the soil, which is a form of nitrogen that is less readily nitrified. As a consequence, legumes enhance soil productivity and lower the risk of acidification compared with inorganic nitrogen fertilisers.

Crop wild relatives have frequently been the source of novel agricultural traits, but their use has been haphazard and intermittent. Previous investments by GRDC, in partnership with US government agencies, identified and conserved a diverse collection of the wild relatives of chickpeas.

Recently, in a collaborative project with scientists at the University of California, Davis (UC Davis), a single

gene that confers tolerance to low pH and aluminium has been discovered in a wild chickpea relative – a wild species, *Cicer reticulatum*. In parallel to trait discovery, the UC Davis scientists have developed genomic tools for more-rapid and precise trait breeding into elite Australian chickpea varieties.

FROM TRAIT IDENTIFICATION TO BREEDING AUSTRALIAN CULTIVARS

With GRDC investment, the UC Davis team developed a synthetic soil system in which aluminium concentration can be varied under conditions of low pH. This moderate-throughput greenhouse assay was used to quantify the response of thousands of individual plants, focusing on genetic populations of wild species crossed with cultivated species that were purpose-bred to identify wild traits of agronomic value.

Two distinct sources of aluminium tolerance were identified: one involving the crop's immediate wild progenitor species (*Cicer reticulatum*) and one in a more-distant wild relative (*Cicer echinospermum*). Although both species are cross-fertile with cultivated varieties, the genome of *C. reticulatum* has greater genetic compatibility and therefore the *C. reticulatum* trait has been the primary focus.

By combining quantitative data about aluminium tolerance with high-throughput genotyping data, a single region in the *C. reticulatum* genome was identified as the source of the trait. Through a series of increasingly detailed molecular tests, the UC Davis team narrowed the trait to a region containing approximately 0.03 per cent of the genome and 25 candidate genes.

As a prelude to more-detailed studies, the UC Davis team conducted field trials to compare a series of chickpea lines that either contained or lacked the wild aluminium tolerance gene. Tests were conducted in soils with a history of intensive fertiliser use that created low pH and aluminium toxicity.

Plants were sown into these aluminium-toxic soils, as well as into comparable control soils that were treated with calcium carbonate (lime) to eliminate acidity in agricultural fields. Excitingly, the researchers discovered that plants carrying the wild gene were not only uninhibited by acidic soils, but that their growth was stimulated in the acidic soils (Figure 1). By contrast, plants carrying the sensitive (cultivated) gene were significantly inhibited for growth in the presence of aluminium.

Armed with knowledge that the trait confers aluminium tolerance in agricultural fields, the UC Davis team is crossing the causal gene into the elite Australian chickpea variety CBA Captain$^{\Phi}$. Developed by breeders in the NSW Department of Primary Industries, CBA Captain$^{\Phi}$ has a suite of grower-preferred traits but is sensitive to aluminium. The goal is a modified CBA Captain$^{\Phi}$ variety that is tolerant to aluminium, which may be available for breeders by mid-2024.

To facilitate breeding, the UC Davis team used a new technology known as PacBio Revio to determine the complete genome sequence of CBA Captain$^{\Phi}$. Computational scientists at UC Davis then converted the genome data into a toolbox of genetic markers that will simultaneously select for the wild aluminium tolerance gene while retaining the CBA Captain$^{\Phi}$ identity throughout all other portions of the genome.

In the longer term, researchers envision that genome-assisted breeding efforts will be used to breed the wild aluminium tolerance trait into other chickpea varieties preferred by Australian chickpea growers. Moreover, the wild germplasm collection appears to contain other genes for aluminium tolerance, raising the prospect of developing successive generations of Australian chickpeas with increasingly greater tolerance to acid soils and aluminium.

DELIVERY TO AUSTRALIA

Hundreds of characterised chickpea genotypes, including aluminium-tolerant accessions, were imported through the Australian Grains Genebank and are now in the hands of Australian scientists including Professor Chengdao Li's team at Murdoch University.

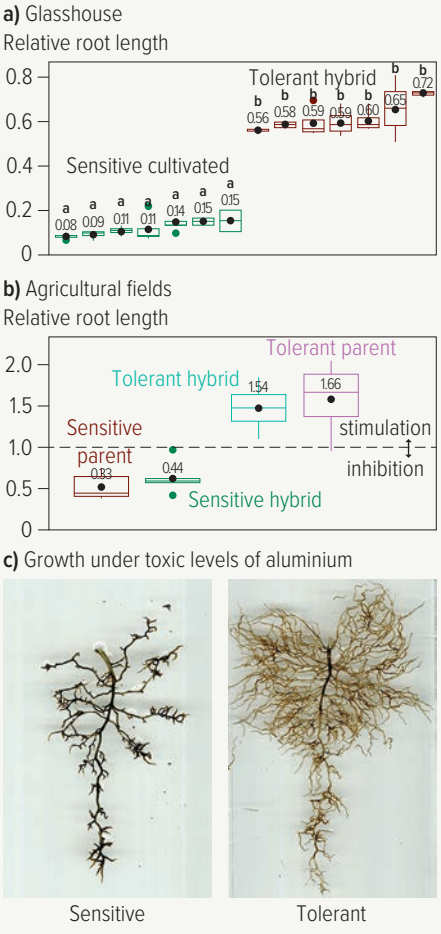
Full sequences of chickpea genomes and hundreds of validated molecular markers, including those linked to the aluminium tolerance trait, were deposited to GRDC databases and are available to Australian bio-informaticians and breeders. By 2024, a grower-preferred chickpea variety with the aluminium tolerance gene will be in Australia for local field trials and seed increase. □

Figure 1: Root growth tolerance to aluminium conferred by a single gene from wild chickpea.
a) glasshouse experiments comparing cultivated chickpea with a tolerant hybrid.

b) experiments in agricultural fields comparing plants with (tolerant) and without (sensitive) the wild aluminium tolerance gene.

Root length is expressed as the per cent growth of treated (aluminium stress) versus untreated (no aluminium stress) plants of the same genotype.

c) Under field conditions, tolerant plants have approximately 50 per cent growth stimulation in the presence of aluminium stress.



GRDC Code UMU1406-001RTX

More information: Professor Douglas Cook, drcook@ucdavis.edu

Firm foundations enable acid soil-tolerant chickpea development

Outputs from prior projects – together with the application of new technologies and knowledge – will expand the productive area for chickpeas

Photo: Evan Collis

Left to right: Sharon Westcott, Dr Darshan Sharma, Professor Chengdao Li and Dr Yong Jia are working to deliver improved acid-tolerant chickpeas to expand the areas in which the crop can be grown.

By Dr Sue Knights

Among all the commercially grown pulses, chickpeas are the most sensitive to acid soils. To further challenge this situation, there has been little variation in tolerance available in the breeding pool for the species. It is a problem that has hampered the adoption of chickpeas outside their traditional eastern Australian environment, says Professor Chengdao Li, director of the Western Crop Genetics Alliance at Murdoch University. Professor Li has been applying his knowledge accumulated from investigating the issue in barley to chickpeas.

"In Western Australia's grainbelt alone, more than 75 per cent of soils and subsoils are affected by acidity, which has been exacerbated with increased nitrogen input and climate change," Professor Li says.

Both aluminium and manganese cause damage to crops when grown on acid soils; in WA, aluminium is the dominant cause. Insoluble aluminium is abundant in most soils, but under acidic conditions (pH less than 4.5), some forms of aluminium are solubilised to release ions that are highly toxic to both roots and beneficial bacteria such as rhizobia.

Aluminium toxicity inhibits cell division and reduces root elongation of plants. Such effects on root growth not only impair nutrient acquisition by crops but also can exacerbate drought by restricting roots' access to subsoil water storage.

"Management practices such as liming reduce the effects of toxicity, but this is a costly and inefficient means to ameliorate subsoil aluminium toxicity in soils that may require extensive liming. An alternative would be to find a genetic solution through developing acid-tolerant crop varieties," Professor Li says.

"For chickpeas, we needed to start with developing an accurate and high-throughput hydroponic screening method and then methodically screen to look for variation – an analogous approach to what we had done for barley."

SOLID GROUNDWORK

A GRDC-invested pilot project led by Dr Darshan Sharma from the WA Department of Primary Industries and Regional Development (DPIRD) did much of the groundwork for chickpeas.

The project confirmed sources of inherent tolerance, bulked-up seed for future research, fine-tuned phenotyping methodology under glasshouse conditions, and developed genetic information that chickpea pre-breeders and breeders will use in developing varieties that would sustain yield potential of new chickpea varieties on acidic soils.

"The team developed a high-throughput hydroponic system and used it for the rapid screening of aluminium tolerance in 1243 chickpea lines," Professor Li says.

"Using 18 Australian chickpea cultivars as a reference, we found Australian chickpea cultivars to generally display susceptibility to aluminium toxicity."

The pilot project also drew upon the outputs of recently completed GRDC investments with Murdoch University, CSIRO, the University of Western Australia, Curtin University and the Australian Grains Genebank, which collected worldwide chickpea germplasm and developed genetic populations

"Of notable value have been the wild species from the pioneer work, supported by GRDC, by Professor Douglas Cook from University of California, Davis, including some of the interspecific derived lines he initially identified as reputed sources of acid soil tolerance, which our team has confirmed."

Although there are more than 40 chickpea species, only a select number are readily crossable and produce fertile offspring with domesticated chickpeas (*Cicer arietinum*).

Cicer reticulatum and *C. echinospermum* species are considered the closest progenitors that are readily crossable. Through screening, 13 wild *C. reticulatum* lines with better tolerance to soil acidity than the best-performing Australian line have already been found.

"Additionally, diagnostic molecular markers and specific candidate genes were identified for aluminium tolerance in chickpeas from this project," Professor Li says.

"Together with the effective hydroponic screening system, this work laid the foundations for genomic-assisted breeding, field validation and controlled-environment screening, and the potential to accelerate the transfer of tolerant genes to current elite chickpea varieties for improved aluminium tolerance and grain production."

The groundwork in previous GRDC investments has led to a five-year GRDC investment that started in 2023, 'Developing genetic tools to facilitate breeder use and deployment of high-value acid soils tolerant chickpea germplasm.'

Built on identification of acid soil-tolerant materials from the wild chickpea species, this project combines molecular markers, genomic prediction and rapid-cycling germplasm enhancement to transfer both major and minor genes into Australian elite varieties and evaluate their economic value in diverse Australian environments (western, southern and northern Australia).

BUILDING ON FOUNDATIONS

The new project is a collaboration between DPIRD, Murdoch University and Agriculture Victoria Research (AVR) and has several detailed goals over its five-year timeframe.

"We will establish a publicly accessible database to catalogue all phenotypic and genotypic data being generated from this project and other related projects and update it annually. This will be a valuable resource for chickpea improvement," Professor Li says.

The screening method will be further refined for both aluminium and manganese toxicity and validated in southern NSW, Victoria and Western Australia and be made available to breeders and pre-breeders.

"Advanced acid-tolerant chickpea lines will be evaluated under acid paddock conditions for compatibility with commercial rhizobial strains and improved strains near release."

Simultaneously, the AVR team, led by Dr Sukhjiwan Kaur (senior research scientist, genomic and predictive breeding), will focus on implementing a genomic selection-assisted speed breeding approach. This innovative strategy aims to identify and synergistically combine major and minor genes for acid tolerance.

The speed breeding facility at AVR expedites the development of fixed lines by significantly reducing the generation cycle time to just 50 to 60 days, a remarkable reduction when compared with the standard span of more than six months in a field environment. As the project advances, it will generate seed stocks that are enriched with both major and minor gene tolerances.

Field validation of these seed stocks will be carried out throughout the project and, once validated, these seed stocks will be delivered to chickpea breeders for deployment into breeding.

"These technologies are accelerating the rate at which we can deliver material to breeders and ultimately to growers."

The project builds upon GRDC-invested research by the University of California, Davis, developing crosses between aluminium-tolerant and sensitive chickpeas.

"Drawing from this collection imported through the Australian Grains Genebank, this year a collection of 510 chickpea genotypes covering both genotype and phenotype diversity was chosen for 2023 field trials to identify additional genetic factors that impact chickpea production in acid soils. They have been planted in Merredin, WA," Professor Li says.

"Field evaluation is very important as there can be complex interactions between aluminium, manganese and phosphorus that impact on chickpea growth that we cannot simulate in controlled environments.

"The investment by GRDC is also giving us the opportunity to investigate the potential of a chromosome block identified by Professor Rajeev Varshney in a chickpea line that reportedly confers abiotic stress tolerance such as tolerance to drought."

Professor Varshney identified this chromosomal region of chickpeas while working at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) in Hyderabad, India, before joining Murdoch University. He is now the director of Murdoch University's Centre for Crop and Food Innovation, director of the WA State Agricultural Biotechnology Centre, and international chair in agriculture and food security at Murdoch University's Food Futures Institute.

Professor Li says a similar approach will be used to backcross and speed breed these genes into elite material and evaluate them in paddock conditions.

"Collaboration and communication, and sharing knowledge, technology and resources are key to ensure that we can deliver improved chickpea lines to grow on acid soils and expand the area of production for chickpeas in Australia.

"We anticipate delivering knowledge, tools and germplasm carrying multiple diverse sources of tolerance to acid soils for deployment in chickpea breeding programs by 2027." □

GRDC Code DAW2205-004RTX, UMU1406-001RTX, UMU2303-003RTX

More information: Professor Chengdao Li, c.li@murdoch.edu.au

Photo: Evan Collis

Acid soil tolerance has been effectively transferred from wild chickpea species (right) to commercial cultivars (left).

Dr Yvette Hill investigating newly evolved rhizobia species.

Photo: Evan Collis

Adapted rhizobia to boost chickpea performance

Chickpeas can obtain a significant amount of nitrogen through a symbiotic relationship with rhizobia, but for maximum effect the rhizobia need to be adapted to the environment

By Dr Jason Terpolilli, Dr Yvette Hill and Dr Graham O'Hara

KEY POINTS

- A new group of rhizobia capable of nodulating chickpeas has evolved and is distinct from the commercial Group N strain
- There are diverse strains of this group across regions of Western Australia
- The new group provides a valuable resource for future inoculum development

■ Chickpeas are an introduced crop and the specific group of rhizobia that fix nitrogen with it are not naturally present in Australian soils. This means chickpeas need to be inoculated with the Group N inoculant rhizobia strain *Mesorhizobium ciceri* CC1192. This strain, originally sourced from Israel, has been used since 1977 as the inoculant for chickpeas across Australia.

Starting in the mid-1990s, work in New Zealand and Australia on introduced *Mesorhizobium* inoculant strains for pasture legumes showed this group of rhizobia carry symbiosis genes on a mobile piece of DNA that can transfer between bacteria in the soil.

Analysis of the chickpea inoculant strain by Murdoch University's Legume Rhizobium Sciences (LRS) centre showed that it similarly carries its symbiosis genes on a mobile region of DNA called an 'integrative and conjugative element', or ICE. The ICE carrying the symbiosis genes can move from CC1192 to a recipient strain that normally is unable to nodulate and fix nitrogen with chickpeas.

More-recent work has shown that

in Australian soils, strains receiving the symbiosis ICE appear to be groups of indigenous bacteria genetically similar to CC1192, but which lack their own symbiosis genes. When they pick up the symbiosis genes, they are converted into a symbiotic strain. The problem is that although these newly evolved strains have acquired the ability to nodulate chickpeas, they do not always fix nitrogen effectively.

It is not clear whether suboptimally effective novel strains are more competitive at nodulating chickpeas than CC1192. If they are, this could potentially reduce the efficacy of legume nitrogen fixation.

JUMPING RHIZOBIUM GENES

To get a handle on how widespread symbiosis ICE transfer is and its impact on nitrogen fixation, work at LRS has examined the genetic diversity and effectiveness of chickpea rhizobia isolated from soils sampled from farms across all of Western Australia's agro-ecological zones.

Using a rhizobia-specific genome sequencing pipeline developed at LRS, this has shown that 28 per cent of strains isolated were not the inoculant strain. The distribution of CC1192 versus novel ICE recipients isolated varied considerably across the zones. The Ord River irrigation area and Western Australian central agro-ecological zones had 81 per cent and 51 per cent novel strains, with decreasing proportions through the eastern, northern and sandplain zones at 22, 12 and 4 per cent of the strains isolated respectively.

Additionally, the diversity of the ICE recipients collected from across the state that are capable of nodulating chickpeas is considerable, with more than 31 different novel strains detected based on molecular fingerprints. All the novel strains have the CC1192 symbiosis genes, meaning they have evolved recently by acquiring these genes from the inoculant strain.

The study so far has found some novel strains are suboptimally effective, with work to understand their prevalence and impact on nitrogen fixation ongoing. Current work is also extending this survey to chickpea growing areas in NSW and Queensland to better understand symbiosis ICE transfer in areas of sustained and intensive chickpea production.

Photo: Evan Collis

Selecting nodules to assess nodule occupancy by rhizobia and nodule morphology.

ADAPTED TO AUSTRALIAN SOILS

Several novel strains have been identified from the WA study that fix nitrogen on par with CC1192, but with potential for greater tolerance to acid soils and desiccation than the inoculant strain.

These strains are being evaluated through a national program that tests their performance throughout chickpea regions across the country, in comparison to the current commercial inoculant strain. If they prove superior to CC1192, they could ultimately become a new inoculant for chickpeas, but this will take up to 10 years depending on future research outcomes and extensive field trials and inoculum stability tests.

Importantly, although the novel strains isolated from Australian soils are genetically very diverse, their symbiosis genes are not – they are in fact all derived from CC1192.

This means the likelihood of finding a strain that is more effective than CC1192 from isolations made in Australia is very low. CC1192 has been the commercial inoculant for chickpeas in Australia for 45 years, yet this strain was originally selected from a very small cohort of five strains.

In contrast, modern strain selection programs for other agricultural legumes have delivered new elite inoculant strains after screening many hundreds, if not thousands, of potential strains. Therefore, more rhizobia germplasm needs to be sourced from international locations with long histories of chickpea cultivation and similar climatic and soil conditions to Australia.

These collections will have a much better chance of identifying symbiotically diverse chickpea rhizobia that could well provide Australian growers with a more-effective and robust inoculant strain for this grain legume. □

GRDC Code UMU1901-002RTX, UMU1810-001RTX

More information:

Dr Yvette Hill, y.hill@murdoch.edu.au;

Dr Jason Terpililli, j.terpolilli@murdoch.edu.au

Wild relatives recruited to improve chickpea chilling tolerance

Increasing chickpea production in Australia requires enhancing chilling tolerance in the species through the exploration of greater trait diversity in its wild relatives

By Dr Jens Berger

Established production regions for chickpeas are in the relatively warm areas of northern New South Wales and southern Queensland. To expand production of the pulse to cooler southern and western regions, improved chilling tolerance at flowering is required. Incorporating this trait could also stabilise yields in northern regions experiencing radiation frosts.

Chilling is a significant stress on

Dr Abdulkadir Aydogan from the Turkish Ministry of Agriculture cold screening chickpea lines for Australia in Turkey.

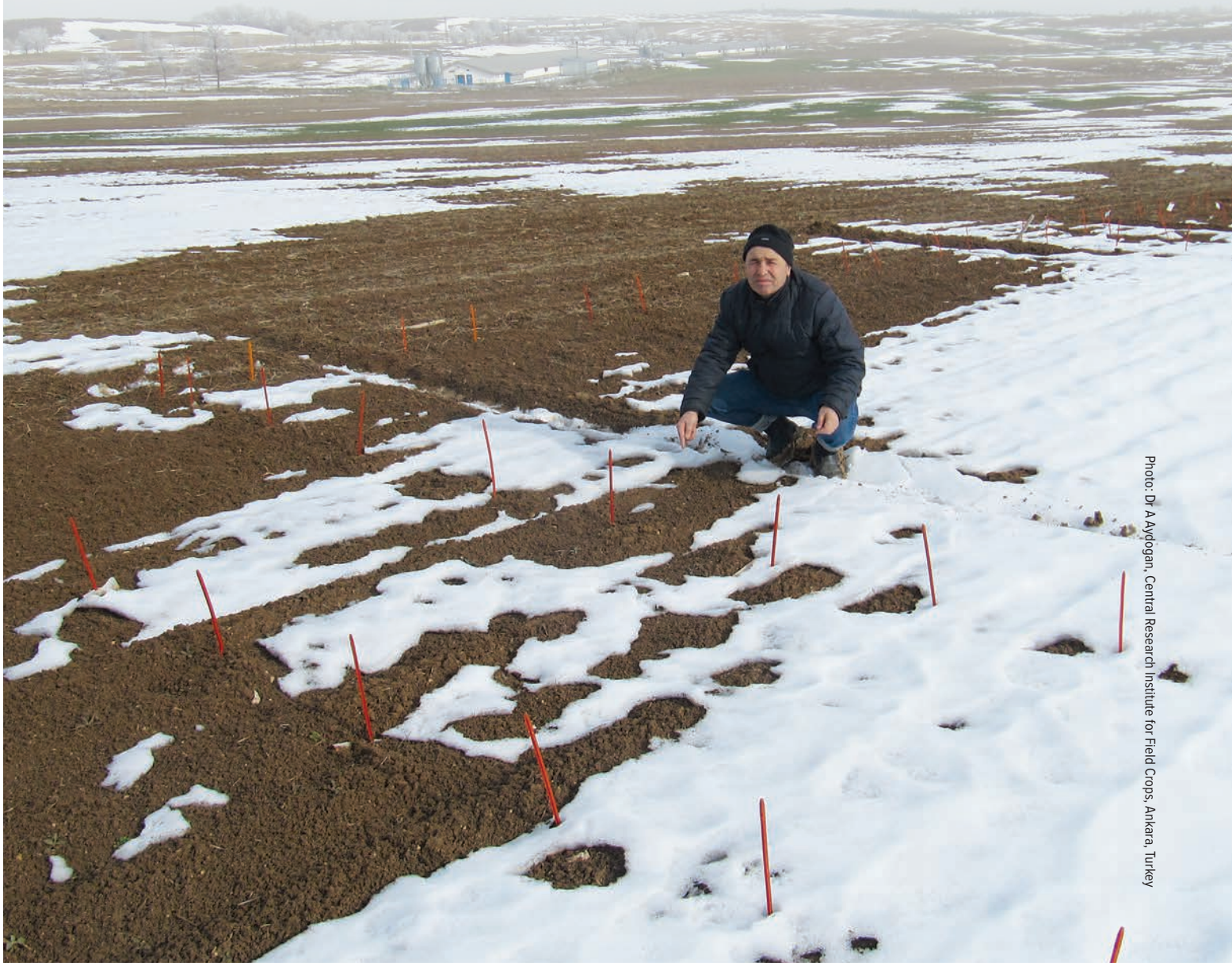


Photo: Dr A Aydogan, Central Research Institute for Field Crops, Ankara, Turkey

chickpeas because at flowering it disrupts fertilisation and pod set. It delays the reproductive phase and exposes plants to greater heat and drought stress later in the season, which decreases yield while increasing risk.

THE CHALLENGE

Low genetic diversity constrains chickpea improvement by limiting variability for traits of interest. This is particularly the case for reproductive chilling tolerance, which delays pod set and exposes the crop to terminal drought throughout Australia, reducing yield and yield stability.

This reflects domesticated chickpeas' unique evolutionary history whereby the crop escaped winter stress in both space and time, moving from West to South Asia (Indian subcontinent) in the Bronze Age and returning as a spring-sown crop.

In contrast, its close wild relatives are true winter annuals that germinate with the autumn opening rains in low-temperature habitats in south-eastern Turkey and have greater than 100-fold genetic diversity compared with domesticated chickpeas. While this combination suggests great potential for improving domestic chilling tolerance, until recently there were very few wild accessions to work with.

This changed with recent collection efforts supported by GRDC, the Turkish Ministry of Agriculture and Forestry, a range of Turkish universities and the University of California, Davis, with CSIRO in South-East Anatolia returning to Australia with hundreds of wild accessions that can be crossed with domestic chickpeas.

This collection includes 229 *Cicer echinospermum* and 484 *Cicer reticulatum* accessions, the latter two species being wild relatives of the domesticated chickpea (*Cicer arietinum*). Notably, the team also discovered a *Cicer* species new to science, now known as *Cicer turcicum*, which appears to have very high heat tolerance and resistance to bruchids.

The wild collection has subsequently been screened for low-temperature flowering, podding and pod production rate in both Turkey and Australia and identified tolerant lines that have been crossed into CBA Captain(Φ).

These populations are under development and have not yet been screened for low-temperature tolerance. However, researchers have been able to screen a large wild by domestic diversity panel created earlier by Curtin University, which crossed the most diverse *C. echinospermum* and *C. reticulatum* collected in 2013 with PBA HatTrick(Φ).

Reproductive chilling tolerance screening is carried out in the field in cool locations at Mount Barker, Western Australia, and Wagga Wagga, NSW. The trials are hand-sown early using vernalised seed and artificial lighting to prompt early flowering when temperatures are low.

The results are promising. The data demonstrates that wild *Cicer* is more cold-tolerant than chickpeas, with much more stable phenology, higher growth rates, earlier pod set and higher rates of low-temperature pod production.

At Mount Barker in 2021, average low-temperature (<13.4°C) pod production rates were almost three times greater in wild *Cicer* than domestic chickpeas, while the wild cross domestic hybrids were intermediate between the two (Figure 1).

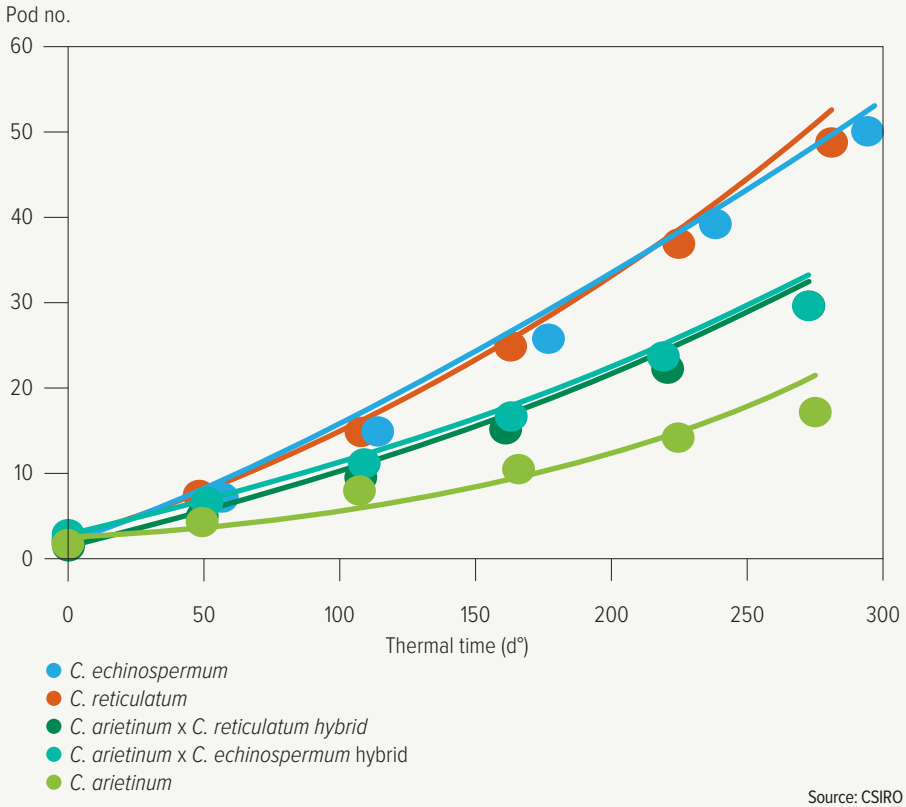
Importantly, Figure 1 shows that it is possible to cross this tolerance into chickpeas, even in a population designed for maximising genetic diversity rather than cold tolerance.

Indeed, some of the wild cross domestic hybrids have low-temperature pod production rates as high as the most-tolerant wild *Cicer* accessions.

These results are very consistent across years; the same species performance was observed at Mount Barker in 2022 and stable resistance was identified among wild and wild cross domestic hybrid accessions.

Work is ongoing to better understand both the temperatures responsible for chilling stress and wild and domestic responses to these, as well as their underlying genomic basis. The work so far suggests that day temperatures are likely to be more important than night temperatures, which will have significant implications for where chickpeas can be grown. □

Figure 1: Mean responses of wild, wild cross domestic and domestic species of chickpea to chilling temperature in the field at Mount Barker, Western Australia in 2021.



Source: CSIRO

GRDC Code CSP2102-006RTX

More information: Dr Jens Berger, jens.berger@csiro.au

Chilling tolerance phenotyping for chickpeas

By Dr Amanuel Bekuma and Dr Brenton Leske

KEY POINTS

- A reliable phenotypic method to screen chickpeas for chilling tolerance has been developed
- Chilling-tolerant chickpea lines have been identified that flower and set viable pods during the chilling period when daily mean temperature is less than 13°C
- Information about the promising lines has been shared with Chickpea Breeding Australia

Chickpeas, compared with other grain legume species, are highly vulnerable to cool or chilling temperatures, leading to substantial fluctuations in their yields. Winter conditions impede seedling growth, while mean daily temperatures below 15°C result in flower and pod abortion. Consequently, chilling tolerance has become a priority trait for Australian chickpea growers and breeding programs.

However, improving chilling tolerance is complex with two main hurdles impacting progress: limited genetic variation within elite germplasm; and the absence of reliable screening or phenotyping methods.

These issues are now being addressed, the first thanks to a prior GRDC investment with CSIRO, which identified chilling tolerance sources in wild chickpea species originally from Turkey. Progress was made possible through trials conducted in Turkey and Australia, culminating in the crossing of this genetic material with commercial lines and the development of elite chickpea populations.

To overcome the second hurdle, GRDC has invested in a one-year project to develop a reliable and efficient field-based method of phenotyping for use in pre-breeding and commercial applications with the Western Australian Department of Primary Industries and Regional Development (DPIRD). It aims to complement and strengthen phenotyping efforts at CSIRO.

By achieving these objectives, more-accurate identification and selection of chilling tolerance traits during pre-breeding activities can be realised, increasing the likelihood of these traits being adopted and selected through commercial breeding programs. Eventually, commercial chickpea varieties with these traits will be released to Australian growers.

Photo: Amanuel Bekuma

The chickpea chilling tolerance team, from left to right: Brenton Leske, Michelle Boyd, Nathan Height, Chaiyya Cooper, Ghazwan Al-Yaseri and Amanuel Bekuma. Missing in the image is Bob Shackles.

METHOD DEVELOPMENT

Chickpeas are particularly susceptible to chilling temperatures during flowering. At this critical stage, when either the pollen and/or the ovary are exposed to chilling temperatures, successful fertilisation is inhibited, leading to embryo abortion and compromised pod set.

In cases where fertilisation does occur, the embryo is still vulnerable to chilling temperatures, causing it to abort and leaving the pods empty and non-viable. These are all factors that determine pod viability, with a viable pod being one with all grain filled.

As pod viability is considered one of the most-important traits for chilling tolerance in chickpeas, a dependable field phenotyping methodology known as 'pod marking' based on this trait has been developed.

The chickpea chilling tolerance field trial site at Dale, Western Australia.

Photo: Amanuel Bekuma

This is a simple method that involves the use of a paintbrush and a water-based, UV-resistant paint. Pods that set during the chilling period are dot-marked and, after four weeks of grain filling, they are harvested. This approach allows for the assessment of seed-bearing pods that were formed exclusively during the chilling period and excludes pods that formed later outside this period. The chilling period is characterised by an average screen temperature below 13°C.

FINDINGS

Using the phenotyping method, chickpea lines have been identified that flowered and set viable pods in chilling conditions from a set of 378 chickpea lines (that included breeding lines and commercial varieties).

The viability of pods varied significantly among different lines, ranging from 21 per cent to 97 per cent. Among the commercial lines there were no significant differences in pod viability. PBA Striker$^{\phi}$ had the highest pod viability at 89 per cent, followed by PBA Drummond$^{\phi}$ at 88 per cent and CBA Captain$^{\phi}$ at 73 per cent.

Interestingly, there were 29 lines that exhibited slightly higher pod viability than PBA Striker$^{\phi}$. These were crosses between domesticated chickpea and wild species.

The wild chickpea species *Cicer echinospermum* and crosses between this species and commercial chickpeas (*C. arietinum*) were the top four lines with high pod viability. Generally, the *Cicer arietinum*/*echinospermum* hybrids displayed higher pod viability compared with a cross to another wild species, *Cicer reticulatum*.

The results indicate that chickpea hybrid 937-1-218 had the highest number of viable pods with a total of 121, followed by a cold-tolerant wild parent (Bari3_100) with 86 viable pods and Kayat_077 with 76 viable pods. Bari3_100 and Kayat_077 were both collected from the province of Mardin, Turkey. PBA Striker$^{\phi}$ remains the highest among the commercial genotypes, with 68 viable pods.

937-1-218 is a hybrid between a cold-tolerant wild parent, Gunas_100, collected from the province of Diyarbakir in Turkey, and the domesticated parent Kyabra$^{\phi}$, which is commercially available in Australia. The hybrid produces twice as many pods as the cultivar PBA Striker$^{\phi}$ and 70 per cent of the set pods on the hybrid plant are still viable, which is a relatively high number compared with PBA Striker$^{\phi}$. Overall, it appears that 937-1-218 has some desirable traits, including high pod viability and increased pod production, which could make it a valuable candidate for a future crossing program.

Further investigation into the specific chilling-tolerant traits present in these wild species and their subsequent crosses would be worthwhile.

By selecting and promoting lines with a greater proportion and number of viable pods, breeders and growers can enhance the overall resilience and productivity of crops in challenging environments.

A new GRDC investment will build on this recently concluded project. The new project will combine the field phenotyping approach and development of diagnostic molecular markers for chilling tolerance.

The project will also aim to develop high-throughput techniques to accelerate chilling tolerance screening in chickpeas. Collaborative expertise from DPIRD, CSIRO and Agriculture Victoria will be harnessed to speed up the delivery of chilling-tolerant germplasm to breeders and ultimately new varieties to growers. □

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More information: Dr Amanuel Bekuma, amanuel.bekuma@dpird.wa.gov.au; Dr Brenton Leske, brenton.leske@dpird.wa.gov.au

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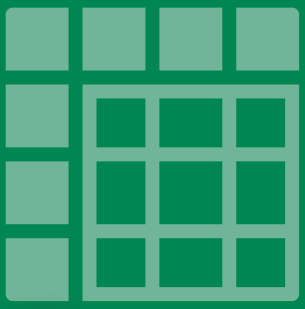

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P Level 4, 4 National Circuit, Barton ACT 2600 | PO Box 5367, Kingston ACT 2604
T 02 6166 4500 **F** 02 6166 4599 **E** grdc@grdc.com.au