Wheat germplasm with improved yield performance under drought for Australian breeding programs

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Summary
This project has identified genomic regions, developed germplasm (alternative dwarfing genes, leaf-rolling, greater leaf area) and established screening methodologies (longer coleoptiles, leaf area, transpiration efficiency) targeted to improving water-use efficiency and yield under drought. Much of this germplasm has been released to breeding programs for use as parents in developing segregating populations. New germplasm, improved selection strategies and identification of genomic regions will aid in improving selection efficiencies for targeted traits in breeding programs aimed at improving yield under drought.

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Conclusions

Drought commonly limits productivity of Australian wheat crops. This project has confirmed and/or extended genetic variation for traits with potential for use in breeding for improved performance under drought, identified the nature of inheritance for some of these traits and underlying genetic control through quantitative trait loci (QTL), and released germplasm containing genes for traits not present within Australian breeding programs.

Conclusions arising from this work are summarised around each output.

Leaf-rolling

1. Genotypic differences in leaf-rolling expression were not associated with differences in soil water uptake or plant water status and appear to be associated with variation in the mechanical structure of the leaf lamina.
2. A new transversal curvature method provides a superior measure of leaf-rolling suitable for use in screening breeding populations.
3. Variation in leaf-rolling is highly heritable and under strong additive genetic control.
4. Preliminary evidence suggests that the leaf-rolling trait confers an advantage in increased grain size under drought.

Transpiration efficiency

1. Repeatable genotypic variation was observed for carbon isotope discrimination (CID) (a direct measure of transpiration efficiency) in the three National Wheat Molecular Marker Program (NWMMP) populations.
2. Genotypic variation in CID was under additive genetic control and of high narrow-sense heritability.
3. A number of QTL were identified for CID in each population. Many of these were repeatable across years and some repeatable across populations.
4. A number of the identified CID QTL are co-located with genomic regions for stomatal conductance and/or photosynthetic rate and highlight the potential for measures of stomatal conductance and/or canopy temperature and chlorophyll content in early-generation screening for CID.

Early vigour

1. Undertook diallel genetic studies to establish that coleoptile length and diameter were under strong additive genetic control.
2. Identified across different mapping populations a number of QTL for coleoptile length for use in marker-assisted selection (MAS).
3. Established leaf width was under additive genetic control and strongly correlated with leaf area.
4. Identified cycle 5 recurrent selection lines with leaf areas greater than barley and with root biomass approximately double that of current commercial wheats.
5. Developed and released high early vigour and alternative dwarfing gene, semi-dwarf wheats to national and Graingene Level 4, East Building | 4 National Circuit, Barton ACT 2600 | PO Box 5367, Kingston ACT 2604 | t. +61 6166 4500 | f. +61 2 6166 4599 | grdc@grdc.com.au | grdc.com.au
breeding programs.

Identification of QTL for coleoptile growth, CID and stomatal conductance represents the first reported genomic regions for these traits in wheat and shows considerable promise toward development of linked molecular markers for use in breeding improved performance under drought. However, availability of stomatal conductance and canopy temperature data could provide cost-effective means for rapid screening of segregating wheat populations for greater transpiration efficiency (TE).

The release of wheat germplasm containing alternative dwarfing genes with potential for greater coleoptile length has potential for overcoming constraints associated with existing Rht-B1b and Rht-D1b dwarfing genes while barley-like, high-vigour wheats show considerable promise as parental germplasm for wheats with improved water-use efficiency, weed-competitiveness, larger root systems/greater nitrogen uptake and potential for late sowing.

**Recommendations**

Recommendations arising from this work are contained in the Appendices (attached)

**Outcomes**

This project will have benefit to breeding programs and ultimately the wheat industry through selection for greater and more stable wheat yields under drought. The project was successful in providing germplasm and selection methods for enhancing development of wheat varieties with improved performance under drought via:

1. identification of new parental lines containing traits with potential for improved performance under drought;
2. development and release of germplasm with new traits for improved water-use efficiency;
3. selection methodologies aimed at recovering phenotypes with new traits;
4. identification of genomic regions associated with variation for water-use efficiency.

Together these benefits will assist in enhancing genetic gain for yield and quality under drought. For example, carbon isotope discrimination provides a direct measure of transpiration efficiency. CSIRO-developed drought-tolerant wheat varieties ‘Drysdale’ and ‘Rees’ were selected from a backcross-derived Hartog population on the basis of carbon isotope discrimination. However, carbon isotope discrimination is expensive, particularly in screening large segregating populations. In the current project, we identified carbon isotope discrimination to be:

1. highly heritable and so little affected by genotype/environment interaction;
2. under strong additive genetic control and so amenable to selection in early generations of a segregating population;
3. associated with genotypic variation in both stomatal conductance and photosynthetic capacity, enabling progeny to be screened indirectly for high water-use efficiency using both these component traits;
4. associated with a number of QTL repeatable across seasons and across separate, genetically-unrelated populations.

Together, this information provides breeders with the tools to select for low carbon isotope discrimination either phenotypically through field evaluation or genotypically through linked molecular markers. Further, phenotypic evaluation for water use efficiency may be made directly on samples assayed for carbon isotope discrimination or indirectly via estimates of stomatal conductance and/or photosynthetic capacity. To this end, this project has identified that use of an infrared thermometer and a chlorophyll meter can provide very rapid estimates of stomatal conductance and photosynthetic capacity respectively.

This project has also developed germplasm with environmental benefits arising from selection for greater early vigour. Cycle 5 lines developed for greater early vigour through recurrent selection produced leaf areas double that of existing Australian wheat varieties and consistent in size with that of barley. These have potential for increased competitiveness with weeds, increased yield with late sowing and larger root systems with greater nitrogen uptake early in the season when nitrogen is leached below the roots into groundwater.

**Achievements/Benefits**

**Background**

Genetic improvement in water-use efficiency is critical to breeding wheat varieties with improved performance under rain-fed conditions. Drought resistance has been suggested for use by breeders but has been unsuccessful owing to yield trade-
offs when rainfall is plentiful and water non-limiting.

An understanding of the amount and timing of rainfall throughout the Australian wheat belt has identified improved establishment through a longer coleoptile, greater early vigour and increased transpiration efficiency as characteristics with potential for improving the water-use efficiency and yield of bread wheats.

By definition, greater transpiration efficiency increases the efficiency with which biomass is produced per unit of water transpired by the canopy. The release of the Hartog-derived, drought tolerant wheat varieties Drysdale\(^{(1)}\) and Rees\(^{(1)}\) were based on phenotypic selection for transpiration efficiency.

Longer coleoptiles enable growers to sow into moisture deep in the soil profile. As a result, sowing can occur earlier in the season to increase grain yield potential. Later sowing results in reduced anthesis biomass that reduces yield potential while later flowering delays grain-filling to when it is much warmer and drier. Studies have shown that delays in sowing reduce wheat grain yields by 0.1 t/ha for each week of delay in sowing after mid-May. Longer coleoptiles also allow for better seedling emergence and subsequent leaf area development under direct drill and/or stubble retention cropping systems. The shorter coleoptiles of current wheat varieties are unsuited to hard soils with direct-drilling and high stubble loads with stubble retention in conservation tillage. Also, better establishment through longer coleoptiles and reduction in water loss through greater early vigour reduces soil evaporation to improve water-use efficiency.

**Objectives**

While CSIRO has identified and assembled germplasm containing important traits from throughout the world, little is understood of their genetic control or how to select them efficiently in a commercial breeding program. Improved selection methods are critical given these traits are seemingly complex in inheritance. The need for adapted germplasm derived from these sources is also an issue, given the donors for many of these traits are poorly adapted to Australia.

Hence, specific objectives of this project were to:

1. Understand genetic control toward development of efficient and faster screening protocols for traits conferring greater water use efficiency in wheat.
2. Identify genomic regions associated with improved water use efficiency in wheat.
3. Develop germplasm containing traits with greater water use efficiency in wheat.

**Major achievements**

Major achievements of this project are summarised below. Further detail is provided in Appendices 1 to 3.

1. Leaf-rolling to maintain leaf area under drought.

   Leaf rolling genotypes use the same soil water (Appendix 1, Table 1.1) as non-rolling wheats and leaf-rolling occurs at the same leaf water potential (status) (App 1, Figure 1.1), indicating differences in leaf-rolling expression is related to mechanical structure of the leaf lamina.

   Leaf-rolling genotypes maintained photosynthetic leaf area longer to continue photosynthesis under water-limited conditions (App 1, Fig. 1.2).

   Under drought, leaves of leaf-rolling wheats are rolled like a needle by mid-morning to slow transpiration and water loss. However, these leaves unroll late in the day to continue assimilation late afternoon and early morning when aerial aridity (vapour pressure deficit) is lowest.

   A repeatable method was developed to improve the screening of leaf-rolling in large segregating populations (App 1, Fig 1.3).

   Two separate genetic studies identified the leaf-rolling trait to be predominantly under additive genetic control and of high narrow-sense heritability (App 1, Tables 1.4 and 1.5). This suggests that recovery of progenies carrying the leaf-rolling trait will be relatively simple in early generations of inbreeding and progeny-mean performance somewhat predictable according to parental differences in leaf-rolling.

   Bi-parental- and backcross-derived wheat populations were developed and phenotyped in two environments for identification of genomic regions associated with leaf-rolling. Range in leaf-rolling score among random progeny
encompassed scores for the rolling and non-rolling parents.

Development of back- and top-cross-derived lines containing the leaf-rolling trait is near completion and will be released to national breeding programs under a material transfer agreement (MTA).

2. Genomic regions associated with variation in transpiration efficiency in wheat.

Three National Wheat Molecular Marker Program (NWMMP) populations were screened for transpiration efficiency (TE) across two to three years, revealing large genotypic variation for TE (App 2, Table 2.1). Further, there was little genotype x environment interaction for TE in any population. In turn, narrow-sense heritabilities were high (App 2, Table 2.1).

High narrow-sense heritabilities increased confidence in the range of genotypic means for TE (App 2, Fig 2.1). Distribution of genotypic means was approximately normal (gaussian), and given the high repeatability of progeny means, indicated genetic variation for TE was under polygenic control.

Range among progeny in each population varied but typically encompassed the two parents. Transgressive segregation in the Sunco/Tasman population indicated different genes for TE in either parent (App 2, Fig 2.1).

A number of significant quantitative trait loci (QTL) were identified for TE in the three populations (App 2, Table 2.2). Many of these were repeatable across years and across populations (eg. 1BL, 2DL, 3BS, 4AS, 4BS, 4DS, 5AL, 7AL, 7BS). This is the first report of QTL for TE in wheat.

Significant genotypic variation was observed for chlorophyll content, stomatal conductance and canopy temperature in the three populations (App 2, Table 2.3). Differences in stomatal conductance and canopy temperature were associated with TE in all three populations. Chlorophyll content strongly related to TE in the Sunco/Tasman population only (App 2, Table 2.4). Significant genotypic differences in the O^{18} oxygen isotope (O^{18}) were not well correlated with genetic differences in TE while the correlation for leaf and grain carbon isotope discrimination was weak. It was concluded that selection for greater TE on the basis of O^{18} or grain carbon isotope discrimination could be potentially misleading.

QTL were identified for stomatal conductance in all three populations (App 2, Fig 2.2). This is the first report of QTL for stomatal conductance and canopy temperature in any crop. Many of these QTL and QTL for chlorophyll content collocated with TE QTL providing evidence of TE trait dissection into its physiological components.

The small genetic effect of TE QTL will reduce their value for marker-aided selection. However, there is potential for use of stomatal-related traits (e.g. canopy temperature) in rapid screening of large segregating wheat populations.


A 12 x 12 full diallel was developed among short- and long-coleoptile parents for assessing genetic control of coleoptile length in wheat. Resulting F1 progeny were then assessed across four soil temperatures to reveal large genetic variance (range of 48 to 185 millimetres coleoptile length). The F1 variance was further partitioned to show large additive and little non-additive and maternal genetic control for coleoptile length and diameter (App 3, Table 3.1). Coleoptile length and diameter were themselves genetically uncorrelated, indicating the potential to develop long, thick coleoptiles for improved establishment. Little interaction with soil temperature suggested that parental effects (breeding value) were repeatable across temperatures, and so robust across environments. Small genotype x environment interaction resulted in high narrow-sense heritabilities for coleoptile traits.

A separate 12 x 12 full diallel was constructed using wheat parents identified to vary for early leaf area development. The F1 progeny were assessed in seedling trays and produced leaf-one widths ranging between 2.8 and 7.6 millimetres (mm). The Australian commercial controls averaged leaf-one widths of 4.1 mm. As for coleoptile length, most of the genetic variance was additive in origin (App 3, Table 3.2) indicating ease in selection for greater vigour during the early stages of inbreeding.

Large genotypic variation and a good repeatable screen enabled the identification of QTL for coleoptile length and diameter across a range of wheat populations. Repeatable QTL were identified on chromosomes 2B, 2D, 4A, 5D and 6B and on dwarfing genes Rht-B1b and Rht-D1b. This is the first report of QTL for coleoptile length and diameter for any winter cereal.

Access to high-vigour wheats from throughout the world provided an opportunity to combine genes from the different wheats through recurrent selection. This was maintained across five cycles resulting in progeny with leaf areas exceeding that...
of the cycle-one progeny, Vigour 18, and the barley control Beecher (App 3, Tables 3.3 and 3.4). Further, the leaf areas and biomass of selected lines was almost double that of Australian commercial wheats. Root biomass was reasonably correlated with leaf area (App 3, Fig 3.1). Selected high-vigour lines had almost double the root biomass of Australian checks (App 3, Table 3.4) and substantially exceeded that of elite International Maize and Wheat Improvement Centre (CIMMYT)-derived synthetic wheats.

Approximately 150 backcross- and top-cross-derived lines containing Rht4, Rht5, Rht8, Rht12 and Rht13 were sent to wheat breeding programs for use as parental germplasm in crossing. Information on linked molecular markers was included with this germplasm release.

Benefits to industry

Achievements arising from this work have the potential to bring substantial benefits to the Australian Grains Industry.

1. Access to new germplasm with variation substantially exceeding that currently available in any breeding program (e.g. alternative dwarfing genes for increasing coleoptile length, greater early vigour, larger root systems). Longer-coleoptile wheats, for example, will allow growers to sow deeper and with higher stubble loads than is possible with current wheats.
2. Understanding of genetic control and development of efficient screening protocols to enhance genetic gain in breeding programs targeting improved adaptation to drought.
3. Identification of genomic regions associated with variation in coleoptile length and TE in wheat. These should assist in marker-assisted selection (MAS) for these traits, although the high repeatabilities established in this project suggest good genetic gain for either trait through direct standard phenotypic selection. Indirect selection for TE using canopy temperature should provide a useful cost-effective means of selection for greater TE in large wheat-breeding populations.

Other research

This research has achieved outcomes for improved performance under drought not previously reported in wheat or any other winter cereal. The mapping-QTL outcomes for transpiration efficiency and dissection into stomatal conductance/canopy temperature and photosynthetic capacity are particularly exciting and are leading to:

1. a better understanding of how water-use efficiency improves adaptation to drought
2. selection strategies for development of germplasm with improved performance under water-limited conditions.

New research arising out of this project includes:

1. Validation and implementation of genomic regions associated with greater coleoptile length in wheat.
3. Establishing the potential for grain carbon isotope discrimination (CID) in understanding contribution of pre- versus post-anthesis assimilation to grain yield under drought.
4. The potential for combining genes for greater transpiration efficiency and high soluble reserves to increase grain yields and kernel size under drought.
5. Further backcrossing of recurrent selection-derived, early-vigour wheats in development of wheats with improved weed competitiveness and greater root growth (for better access to water and nitrogen).
6. The favourable transmission of light associated with the leaf-rolling trait should increase grain yields and grain protein for wheats grown in higher-yielding environments. Leaf-rolling thus shows promise for the high rainfall and irrigated zones.
7. Development of short-season wheats in conservation tillage cropping systems. PhD research by Dr Rahul Amin showed late sowing (mid-August) of current wheat varieties decreased wheat yields by up to 50% in central and northern NSW. Dr Amin also showed that high-vigour wheats developed by CSIRO Plant Industry intercepted more light and accumulated biomass more rapidly to increase grain yield than Australian cultivars.
8. Development of ‘barley-like’ wheats can give growers greater flexibility:
   1. with late season breaks; and
2. Where knockdown herbicides are applied in late July, early August to control herbicide-resistant weeds.

Growers have shown considerable interest in barley-like wheats that can be harvested within 120 days of sowing. Given that much of the success of H45 was attributed to its high early vigour and earlier flowering, there is real opportunity in development of more vigorous wheats, particularly where growers are moving to wider row spacings and retaining stubble. The recurrent-selection developed wheats are as vigorous as the most vigorous barleys tested and so offer potential in development of shorter-season wheats.

9. The availability of rapid and repeatable screens for a range of traits associated with improved performance under drought should enable the assessment and confirmation of the physiological basis for improved performance reported for the International Maize and Wheat Improvement Centre (CIMMYT) -developed synthetic wheats. Current research (Dreccar et al., 2006) suggests that yield advantages in the synthetics are not repeatable across environments (gxe > g by three-fold). Hence, utility of the synthetics for new genetic variation requires an understanding of factors contributing to specific adaptation for targeting by breeding programs.

**Intellectual property summary**

New germplasm containing alternative dwarfing genes was released to wheat breeding programs. These are backcross- and top-cross-derived lines with potential for use as parental lines in crossing. Leaf-rolling and high early vigour germplasm will be available to breeding programs under a material transfer agreement (MTA).

There is some potential for commercial release in these lines. Any commercialisation would be conducted under arrangements mutually agreeable to CSIRO, GRDC and the wheat breeding entity.

IP held in the form of new varieties would be protected under PBR and held by the above three parties. These parties would license a seed-selling agency to trade in such varieties. The seed-selling agency would not have any IP ownership.

**Additional information**

In total, 10 research papers were published in international journals arising directly or indirectly from this research, with another four papers in preparation. The quality of this work was recognised with invitations to write several book chapters and present at several conferences. Four industry/GRDC articles were also published and information was disseminated at GRDC Updates (Bendigo, Wagga, Adelaide and Temora) and at grower field days (Condobolin, Griffith and Gundibindyal).

Additional information is provided as attachments to this project

- Attachment 1 – Leaf rolling as a useful trait for performance under drought
- Attachment 2 – Genetic analysis of carbon isotope discrimination in wheat
- Attachment 3 – Genetic analysis of seedling vigour