Australian Winter Cereals Pre-Breeding Alliance

Workshop on Pre-Breeding for Better Performance Under Drought

University House, ANU

5-6 September 2007

1. Background

This two-day workshop was organised by the Alliance, with the aim of developing a national strategy for improving the effectiveness of the national pre-breeding effort on the performance of water-limited crops. It provided an opportunity to bring together experts in various fields to:

- help identify traits that are likely to be useful over a large area
- outline ways for getting such traits well-phenotyped in realistic environments
- identify limitations to effective selection and ways of overcoming these
- facilitate the fruitful exchange of germplasm between breeders and pre-breeders

This is in keeping with the Alliance's goal of enhancing the speed of delivery of pre-breeding research outputs to Australian plant breeders.
The workshop was limited to about 30 participants (listed in Appendix 1) so that each participant would have ample opportunity to express his or her views.

The workshop was organised within three main themes (see Appendix 2 for the program):

- <u>defining the drought environment</u>, which aimed at broadly identifying areas of good prospect in relation to background agronomic, crop-physiological, climatologic and economic knowledge;
- <u>breeders' views</u> on what are and would be useful traits to target, and how the interactions between pre-breeding and breeding activities could be made more fruitful; and
- <u>pre-breeders' views</u> on what traits, candidate genes, and molecular markers would be worth developing, including current progress.

2. Defining the drought environment

Issues examined in relation to the drought environment included: its nature, the consequences it has on crop production, its implications for the livelihoods of farmers, and the prospects for producing cultivars that cope so well with drought that they will be widely adopted by farmers. These were posed as the following questions accompanied by background notes which were distributed to the participants in advance and which were addressed at the workshop. A synopsis of the background notes follows each of the questions.

<u>Severity</u>

How much pre-breeding activity should be directed at trying to alleviate the effects of severe droughts, and how much should be directed at getting the most out of the available water supply in moderate to good seasons?

To climatologists, insurers, or farmers, "drought" may mean an unusually long dry spell of weather, say, the driest decile of growing seasons. To field scientists it may mean a yield-limiting water supply, not necessarily severe. To lab scientists it may mean a watering regime that induces substantial water deficits in plants.

This question arises because farmers typically generate most of their average income in moderate to good (though typically still water-limited) seasons and do well to minimise losses during severe droughts. Its answer depends on analyses of economics and risk management, and the likelihood of the relevant R&D being effective in the field.

Within-season pattern

What is the frequency and severity of droughts at various stages of a crop's development? Are droughts at some stages more damaging, or are the problems they create more tractable, than at other stages?

From the point of view of coping with drought, winter cereal crops have 4 distinct phases, establishment, tillering, flowering and grain filling. Different traits are needed to deal with droughts at these different stages. Requirements may differ between agroecological regions.

Handling drought in the field: water as a limiting resource

From agronomic and crop-physiological experience in the field, what traits are most likely to improve water-limited yield?
Can this experience help unpack the large GxE interactions that occur when selecting for better yield in drought-prone environments, and thereby facilitate such selection?

During the last 20 years Australian farmers and agronomists have come to accept a now well-established benchmark performance for water-limited crops. This benchmark relates grain yield to the seasonal water supply, and is currently about 20-22 kg/ha of grain for every mm of water transpired.

This limit is often not attained in practice because of: poor distribution of the seasonal water supply; poor agronomic management; or stresses other than water, such as diseases, or other abiotic stresses such as frost.

There are many opportunities for pre-breeding that arise from viewing water as a limiting resource rather than as a stress.

Summary and Conclusions

The discussion on the drought environment broadly concluded that:

- because farmers get almost all of their income in moderate to good seasons and little or none during severe droughts, it is better to invest predominantly in ensuring that they can make the best of the moderate to good, albeit usually water-limited, seasons;
- further, because Australian breeders depend for income on end-point royalties, they have no incentive to invest in developing varieties that do comparatively well during severe droughts, when yields are necessarily low

- agronomic, physiological and biochemical evidence points to a current maximum biomass production of about 55 kg/ha per mm of water transpired by a wheat crop. This, coupled with a realistic maximum harvest index of about 0.40 means that there is an upper limit of grain yield of about 22 kg/ha per mm of transpired water.
- because of the robust relation between biomass production and water use, the main options for progress are in minimising losses of available water (such as direct evaporation from the soil) and by improving the pattern of water use during the season so that there is sufficient growth before flowering to set an appropriate number of seeds/m^2, and sufficient water available after flowering to enable the adequate filling of those seeds.
- improvement in the productive martialling of available water has, in recent years, been mainly through better practices on farm, though there have also been encouraging improvements in the water-limited yield of recently released cultivars.

3. The requirements of commercial breeding programs

Resistance to major diseases, acceptable grain quality, and greater productivity are the main targets of commercial breeding programs. Underlying the productivity are the well-established requirements of appropriate flowering time for the target environment and semi-dwarf habit. A new variety must have a clearly better combination of characters and must meet minimum acceptable standards for all important traits in its target production zone(s) or it will have little chance of being accepted in the market place. These issues were explored at the workshop by posing the following questions accompanied by background notes.

Novel traits

What properties must novel germplasm have to be attractive to commercial breeders?

Commercial breeders are heavily constrained in their activities and have limited time to experiment with any novel traits in a single breeding cycle, which might take 10 years from the initial cross until a new variety can be released. Yet, in the agricultural context, pre-breeding is doomed to be ineffective unless the novel germplasm it develops is adopted by commercial breeders.

Adoption will depend on how well a given trait can be screened for, whether or not it has been well-phenotyped in realistic environments, and whether or not it has been already incorporated into well-adapted germplasm.

New molecular markers

What are the best ways of aligning the development of molecular markers to the requirements of commercial breeders?

Many studies using mapping populations have identified markers linked to single major genes and to many QTLs in wheat associated with particular phenotypes. However, despite the many genotype/phenotype associations reported in the literature, few markers are being used routinely in breeding programs for drought. Using markers in breeding still requires that considerable hurdles be overcome in converting associations identified in mapping populations to markers useful to breeders.

An evident requirement is the development of strong linkages between laboratory and field scientists to ensure that the markers being developed are for traits that interest breeders and that are robust across different genetic backgrounds. The development of breeders' markers for QTLs that account for only a small part of the variation for an important trait with complex inheritance remains an important issue.

Summary and conclusions

The breeders made several strong and consistent points:

- They are not interested in specifically targeting severe droughts (see above)
- They are interested only in traits that have been proven to be useful in a given target area and that have no serious negative effects on their main selection targets, such as maturity, height, disease resistance, and grain quality
- They strongly prefer any promising traits to be incorporated into a good genetic background for the target area, especially if the selection tools for that trait are weak
- For routine selection of a given trait in a breeding program, they need selection tools for that trait that are cheap, simple, fast, and reliable.

4. Pre-breeding projects – the search for new and useful traits

The workshop sought to discuss what traits are likely to be the most promising, in terms of:

- their prospects of enabling plants to make more effective use of the water supply – by capturing more of that water for transpiration, by accumulating more dry matter for every mm of water transpired, or by increasing the harvest index.
- the scale (area, agroecological regions, seasonal rainfall deciles) over which they might be beneficial,
- their attractiveness to breeders, which depends in large part on the documented effectiveness of the trait, especially in the field, and on the ease with which it can be selected for and incorporated it into advanced breeding lines.
- the time it will take to be mature enough to be useful to breeders? (in 3, 5, or 10+ years)

Discussion on this theme was structured as follows:

From the point of view of agronomy and crop physiology, there are two broad classes of traits to discuss in relation to the water economy of a crop. These are:

- the competence of the roots (e.g. disease resistance, nutritional efficiency, tolerance of toxicities and difficult subsoils, effective deep roots), and
- the developmental control and carbon economy of the shoots (e.g. optimal flowering time, sensitivity of flowering to water stress, ability to establish well, ability to accumulate water-soluble carbohydrates for later transfer to the grain)

From the point of view of molecular biology/genetics, there are two main classes of approach, broadly aiming at trait and QTL/gene discovery:

- Candidate gene approach; functional analyses of known or novel drought-related or responsive genes (e.g. in transgenics, novel gene function through overexpression, increased desiccation tolerance) and
- the phenotypic and genetic analyses of drought tolerant germplasm using segregating mapping populations under controlled and field conditions in water-limited environments; (e.g. QTL, molecular marker and gene discovery of traits from target-environments)

<u>Summary and Conclusions</u>
The session on <u>pre-breeding</u> covered:

- promising physiological and morphological traits of both roots and shoots;
- a phenotypic and genetic analysis of a field-grown mapping population of progeny of a biparental cross between wheat cultivars that differed consistently in yield in water-limited environments;
- and a transgenic breeding program in wheat that involved several genes whose nature was commercial-in-confidence.

Several promising physiological and morphological traits are at an advanced stage of pre-breeding or have already been incorporated into cultivars. These include: coleoptile length, vegetative vigour, carbon isotope discrimination, depression of canopy temperature, copious water-soluble carbohydrates, and duration of green leaf area during grain-filling. It was recognised that different traits may be important at different stages of crop development. For example, long coleoptile and large grain size may be effective in improving establishment of the crop, whereas copious water-soluble carbohydrates stored in the stems may contribute to grain filling. This variety contributes substantially to the large interactions of genotype by environment experienced in breeding programs in drought-prone environments.

Some traits of long standing interest, for example osmotic adjustment, are widespread among current cultivars, though they seem not to have emerged in breeders' lines with large frequency. Others, such as boron tolerance, are now widespread. Likewise, resistance to root diseases, of crucial importance in ensuring that roots systems are competent to collect as much available water as possible, are also widespread; resistance to cereal cyst nematode is especially noteworthy.

It is notable that almost all of these traits discussed at the workshop are concerned with improving the development of the crop or its carbon economy, rather than with plant water stress per se. This observation reinforces the view, expressed several times at the workshop, that terms like "drought tolerance" have proven to be confusing and without consistent meaning in the context of pre-breeding.

No promising new traits emerged at the workshop, perhaps because so much thought and activity has already gone into pre-breeding for winter cereals, and the existing network is well-connected. A large amount of work concerned with pre-breeding for performance under drought concerns the expression, during severe water deficits, of genes having no well-articulated connection with the performance of water-limited crops in the field. Work of this nature, which does not espouse connections with commercial breeders, is perhaps best thought of as pre-pre-breeding. Such work may be largely outside the purview of the Pre-breeding Alliance, except for gene discovery in areas of known agronomic or crop-physiological significance.

There was agreement at the workshop that an appropriate balance should be struck between bottom-up gene discovery in relation to plant water stress, and top-down analysis, based on crop and plant behaviour, of where good opportunities lie. Internationally, the record of the former has been poor, with several thousand patents involved, and with only one in a thousand of these having made it through to published, and not wholly encouraging, field testing. Possible areas of interest in the top-down area were identified in the presentations and discussions. These include engineering more effective enzymes involved in photosynthesis and respiration; exploring ways of enabling wheat to fill its grain as fast as barley does; reducing the large amount of organic molecules that roots exude when growing in the field, thereby making up to 10% of net photosynthesis available for more productive use; and removing constraints on vegetative growth that often accompany mild water deficits and which may reflect overly conservative behaviour in a crop. This latter, which involves inhibitory signalling, may be amenable to gene silencing in synthetic pathways that generate inhibitory molecules.

The architecture of root systems is clearly important for capturing available water in the soil. It is however notoriously difficult to study. An association between vigour of roots and shoots suggests the potential to select for root growth using shoot vigour as a surrogate. Also, the use of large root boxes for exploring root growth through time and space offer some promise of, at least, selecting parents that might have more effective root architecture. Extrapolating from controlled environment to the field remains very difficult, though the use of molecular markers to characterise the distribution of roots in the field offers some hope of progress. Enabling roots to colonise and extract water from hostile subsoils remains a difficult problem, though boron tolerance has helped.

Genetic approaches have included a systematic analysis of populations derived from parents that have been selected in breeding programs on the basis of differing performance in water-limited environments. Examples are doubled haploid or recombinant inbred lines derived from Seri x Babax, Excalibur x Kukri, and Berkut x Krichauff. These are being used to identify 'additive' genes for multiple and diverse traits each of which might provide an adaptive advantage under moisture stress but which are rarely if ever brought together in combination in a single variety. The aim is to have a better understanding of the genetic basis of these various contributory factors to enhance performance in dry conditions in elite varieties, as well as produce molecular markers that can facilitate rapid and efficient screening of early generations and the pyramiding of genes. However, success will depend on the analysis taking account of genotype by environment interactions, and therefore most of all depend on having reliable and accurate phenotyping of traits in the target environment.

The importance of appropriate phenotyping in realistic environments was touched on many times. The development of markers useful to breeders depends critically on it. The evident power of phenotyping in the field, using controlled drought treatments at various phenological stages of a crop such as CIMMYT has been doing at Obregon, attracted attention. Ideally it would be good to set up regional facilities in Australia. Such facilities could do much more than provide realistic environments. Providing that they are shared by breeders, agronomists, crop-physiologists, and pre-breeders, the ensuing interactions would greatly improve the effectiveness of pre-breeding by encouraging the development of agronomically significant traits that would be attractive to breeders. Breeders are understandably reluctant to put effort into inadequately tested traits in which they do not have full confidence.

The limitations of current genetic approaches were also discussed. Recognising that many of the key loci may show epistatic interactions rather than additive effects, there is a case to look at alternative breeding approaches. The aim would be to create breeding populations with high frequencies of alleles that when combined confer enhanced performance in water-limited environments. For example, experience in other species, such as tomato, suggests that new population structures that specifically address traits under complex epistatic control can lead to selection of lines with dramatically improved performance. For in-breeding winter cereals, such as wheat and barley, this would require novel population structures. These could be generated in a number of ways, including using large highly recombined populations, using recombinant chromosome substitution lines, or using complex inter-crosses where as many sources of drought tolerance as possible are crossed to one or more elite lines, then inter-crossed several times. Uniform (and relevant) phenotypic selection would be important for any of these approaches to succeed.

It is important to emphasise that novel traits and putative candidate genes for better performance under drought have little chance of being agriculturally useful unless they make some sense to agronomists and breeders, and unless pertinent screens can be invented for laboratory use, where initial explorations are usually done.

Finally, there was some general discussion about the wider community's high expectations of the promise of pre-breeding for better performance under drought. It seems to be not clear that there are major, well-understood, limits to the performance of water-limited crops which will be exceedingly hard to break. Most of the traits being discussed at the workshop aim not at exceeding the current limit, but at helping to ensure that a crop gets closer to its water-limited potential yield. Furthermore, even with seemingly simple traits (for example, coleoptile length), the development time is of the order of decades rather than years. Maintaining momentum across several funding cycles remains a challenge.

5. Recommendations

1. Because farmers get almost all of their income in moderate to good seasons and little or none during severe droughts, it is better to invest predominantly in ensuring that they can make the best of the moderate to good, albeit usually water-limited, seasons.

2. Given the wider community's high expectations of the promise of pre-breeding for better performance under drought, the message needs to be broadcast that there are major, well-understood, limits to the performance of water-limited crops which will be exceedingly hard to break.

3. Similarly, it is important to recognise that most traits discussed at the workshop, especially those in an advanced stage of development, aim not at exceeding the current limit to grain yield per mm of transpired water, but at helping to ensure that a crop uses more of the available water supply, and that it does so more effectively.

4. Given that the terms "drought tolerance" and "drought stress" have proven to be confusing and without agreed meaning in the context of pre-breeding for water-limited environments, their use should be avoided. Effort should be concentrated more on pursuing traits that enable crops to capture more of a limiting water supply and to use that water more effectively in developing grain yield.

5. To ensure uptake by breeders, promising traits should: have been well phenotyped in realistic environments; be incorporated into a good genetic background for the target area; be accompanied by selection tools that are cheap, simple, fast, and reliable; and have been shown to have no serious negative effects on breeders' main

selection targets, such as maturity, height, disease resistance, and grain quality.

6. Investment decisions should be made in relation to realistic expectations about the lead time for a novel trait to be developed to the point at which it can be applied by breeders and incorporated into advanced breeding lines. Such lead times may be of the order of decades rather than years, unless the trait is evidently attractive and very easy and cheap to screen for (for example, visible duration of green leaf area during grain filling).

7. In order to reduce average lead times for the development of novel traits in a pre-breeding program, best-bet traits should be selected in close consultation with agronomists and breeders. Further, best-bet traits should be searched for by undertaking thorough comparative water balances of outstanding genotypes in the field – such studies remain rare.

8. Enabling roots to colonise and extract water from subsoils remains an important area for further research, especially given the recent discovery of the great value of such water when used during grain-filling.

9. An appropriate balance should be struck between bottom-up gene discovery in relation to plant water stress, and top-down analysis, of crop and plant behaviour, of where good opportunities lie. So far, very few traits have been successfully identified using a bottom-up approach. Possible new areas of research interest are suggested in this report.

10. Because the inheritance of water-limited productivity is complex and greatly influenced by environment, commonly used breeding strategies have not been very successful in raising this productivity. Alternative population structures and breeding schemes should be identified that capture a greater portion of additive and epistatic variation for water-limited productivity.

11. The importance of appropriate phenotyping in realistic environments must be recognised. Appropriate phenotyping facilities need to be developed in Australia, using controlled drought treatments in the field at various phenological stages. Such facilities could be shared by breeders, agronomists, crop-physiologists, and pre-breeders, and allow interactions that would greatly improve the effectiveness of pre-breeding.

John Passioura, (CSIRO Plant Industry, Canberra) 26 October 2007

Appendix 1: List of Participants

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Appendix 2: Workshop Program

Wednesday 5th

0900	coffee available
0930 – 0945	Jeremy Burdon, Chair AWCPA: *Welcome and Introduction*

The drought environment
Chair: John Passioura

0945 – 1030	Peter Hayman, SARDI. *The drought environment. 1. The impact of drought, whether mild or severe, on farm – best opportunities for improving farmers' livelihoods*
1030 – 1100	Coffee break
1100 – 1145	Victor Sadras, SARDI. *The drought environment .2a. Climate drivers of water use efficiency in Australia: implications for adaptive traits*
1145 – 1230	Richard Trethowan, U Sydney. *The drought environment. 2b. Identifying promising lines by imposing various drought treatments in the field*
1230 – 1330	Lunch
1330 – 1415	John Kirkegaard, CSIRO Canberra. *The drought environment. 3. Agronomy and crop physiology of water-limited crops – benchmarks and best opportunities for improving yield*

Requirements of commercial breeders
Chair: Richard Trethowan

1415 – 1530	*Current breeding activities addressing improved productivity under stress (H Braun, I Barclay, L. O'Brien)* *Needs, opportunities and limitations to producing more water efficient wheat*
1530 – 1600	Coffee break
1600 – 1645	*Breeder perspectives on traits to target* *Factors that limit/enhance trait adoption by plant breeders*
1645 – 1730	*Enhancing the link between pre-breeding and applied breeding (D Bonnett)* *- Keeping pre-breeding relevant* *- Logistics: improving the adoption of pre-breeding outputs*
1900	Dinner

Thursday 6 September

Pre-breeding projects – the search for new and useful traits
Chair: John Passioura

0830–0915	Jack Christopher, QDPI. *Traits relating to the competence of the roots*
0915–1000	Richard Richards, CSIRO PI. *Traits relating to the developmental control and carbon economy of the shoots*
1000-1030	Coffee break
1030-1115	Thorsten Schnurbusch, ACPFG. *Phenotypic and genetic analysis of drought tolerant germplasm*
1115–1200	Carl Ramage, MPBCRC *Candidate genes to increase drought tolerance*
1200 - 1230	Susanne von Caemmerer, ANU. *Prospects for increasing water-limited yield by engineering better Rubisco in the winter cereals*
1230 - 1330	Lunch

Wrap-up:

1330-1530	T*owards a national strategy for improving the effectiveness of the pre-breeding effort in water-limited winter cereals*