

Submission to the review of the Gene technology Scheme and Agreement.

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The major focus of this submission is the utilisation of gene technology as part of a multi-disciplinary strategy for crops to withstand the increasing stresses of climate change, which pose a major threat to agriculture in much of Australia.

The Gene Technology Scheme needs to support current and evolving gene technologies such as gene editing for inter-specific transfer of stress tolerant genes / QTLs from crop wild relatives (CWR) to domestic crops, and gene silencing, in order to widen the availability of genepools for plant breeding (Ebert and Schaflietner 2015, Walley and Moore 2015).

While Mendelian theory describes the basic genetic principles for heritability of major traits, further development is required for an understanding of genotype X environment interaction, and how this is integral to plant genetics. Mammals have evolved very sophisticated internal regulatory mechanisms for maintenance of relatively constant body processes that buffer against the effects of changeable weather conditions upon growth.

At this level the genetics of flowering plants and mammals diverge, with plants evolved to be highly responsive to environmental growing conditions. Varying between and within species, crops have respective optimal, maximum and minimum levels for responses to temperature, soil moisture and mineral nutrients. Crop genetic research needs to encompass both optimal growth conditions and the genetic variation required for adaptation to a wide spectrum of growth conditions.

Domestic crops evolved during the last 12,000 years with respective adaptations to benign crop environments. Climate change will push cropping into unprecedented stress environments of extreme high temperature spikes, frost and drought. These are expected to be more frequent and severe, as climate change rapidly becomes irreversible in the short to medium term this century. Grain crops are particularly vulnerable in the reproductive and seed set periods, and new sources of genetic variation will be required for crop adaption to a wider range of agricultural climates than previously experienced (Ortiz 2015).

The challenges from diseases and pests will also be affected by climate change, with an increased need for sources of resistances from both domestic and CWR genepools (Ebert and Schaflietner 2015).

New genetic variation must be identified to equip crops for climate change. Sources of stress tolerances are available as rare genes in extreme environments of some domestic gene pools but especially in CWR gene pools (Li Ling et al., 2012, Ebert and Schaflietner 2015). The latter tend to occur in harsher environments than for cropping.

The growing conditions for crops can be manipulated; by choice of sowing time in combination with appropriate choice of varietal maturity mechanisms; by choice of appropriate crop; by geographic change of location; and by modification of the crop soil and

moisture environments e.g. chemical fallow, no-till agriculture, wide crop furrows, weed control, and intensification of agriculture such as mixed cropping and relay cropping. A holistic combination of innovative genetics and cropping systems will be needed to address climate change (Redden et al., 2017).

There is little doubt that climate change is occurring and at an increasing rate. The polar ice sheets are melting especially in the arctic region, and the reduced sea ice results in more solar heat being absorbed by the ocean. Additionally melting of the upper soil thermo-frost layer releases methane which is a far more important pollutant than CO₂ for global warming. The result is self-reinforcing further global warming (Yadav et al., 2015).

Also, the continued growth in world population will inevitably accelerate the carbon footprint from humans, with further demand for manufactured goods, power and water, and urbanisation is becoming more dominant worldwide (Yadav et al., 2015).

New innovations in sources of power, electrification of transport, and efficiencies in power use such as no-till agriculture, north facing glass-fronted buildings with wide eaves in southern Australia for passive solar heating, may mitigate against global warming. However the planning, investment, implementation and public consensus required will take considerable time to become effective (e.g. Paris accord). Therefore over the next 30 years the worldwide situation for agriculture is expected to worsen, especially in Australia with much of the rain-fed cropping at risk of drought and heat stresses (Howden and Crimp 2011). This challenge needs to be addressed with both new genetics and innovative management to holistically equip our agriculture for more severe abiotic stresses, and to avoid dangers to the environment (Yadav et al., 2015).

A seven point genetic approach for sustainable crops in a changing Australian environment is next described under the following headings. Complementary agronomic measures as outlines above will not be discussed any further in this submission, but with the understanding that genetic measures are not presented as a stand-alone solutions.

1. Vulnerability of crops
2. Genetic diversity in domestic crop genepools
3. Wider genetic diversity for tolerances to abiotic stresses in CWR
4. Incorporation of stress tolerance from CWR to domestic gene pools and gene technology.
5. GMO regulation and approval.
6. Food safety considerations.
7. National genetic resources strategy to manage cropping under climate change.

Crop vulnerability.

Temperature is the major environmental factor for annual crops, with periods of hot day stresses in relation to growth stage and maximum and minimum temperatures having the greatest effect on growth and production (Hatfield and Preuger 2015). Optimal growth temperatures for crops vary by species – higher for soybean than for *Phaseolus* bean and

higher for maize than for wheat (Hatfield and Preuger 2011). Pollination during the reproductive growth stage is the most vulnerable to high temperature, with failure of maize pollen above 35°C, and greatly reduced grain yield. In rice the daytime maximum temperature is 33 °C with yield falling to zero above 40°C. Genetic variation does exist for these responses, though limited. The minimum temperature during pollination is important also, as night time minimums have risen faster than day time maximums with global warming. Reduced rice yields in the tropics show an inverse trend with a rise in minimum night temperatures (Lafarge et al., 2011).

Diversity in the domestic gene pool for tolerance of abiotic stresses.

Diverse varieties of cowpea from the African Sahel were screened by Hall (2011) in a desert environment with temperatures above 40°C at flowering. A small number of varieties managed to set seed and were later shown to have reproductive heat tolerance. However the adaptation range of most crops does not extend into high temperature environments, but exploration of the diversity in domestic crops can sometimes provide important tolerances of heat stress. Li Ling et al., (2012) identified sites in China where pea landraces were collected that had a climatic history of heat, frost or drought stresses during the reproductive stage, to prioritise landraces for investigation of abiotic stress tolerances.

A shorter season crops such as pearl millet is better adapted than sorghum to the heat and drought stresses of the Sahel. Hence the choice of crop may become significant as climate change stresses become more severe (Redden et al. 2011)

The current trend of climate change and of within growing season temperature spikes are unprecedented since crop domestication (Redden et al., 2014), and CWR with a much longer evolution and wider dispersal into harsher environments could provide the required tolerances to combined heat and drought stresses. These may only be partly offset by a positive growth response to increased CO₂ levels with climate change (Redden et al, 2011).

Crop wild relatives and sources of tolerance to abiotic stresses.

Crop wild relatives are the progenitors of domestic crops and have evolved to persist without cultivation, with their ranges extending into hostile environments with heat and drought stresses and sometimes frost stresses too (Redden 2015). Only a minute portion of CWR diversity with mutations favourable for domestication was selected for use as crops, often as a single event with little further flow of genes from CWR, to result in a genetic bottleneck for crop diversity (Ortiz 2015). Domestic tomato has only 5% of the diversity found in tomato CWR (Ebert and Schaflietner 2015). Thus CWR with a much more ancient evolutionary history than for crops, wider diversity and adaptation ranging into high stress environments (Lawn 2015, Ortiz 2015), extend the genetic diversity available for crops with novel stress tolerance genes, and novel sources of disease and pest resistances.

Examples of the contribution of CWR to crops are given by Porceddu and Damania 2015. Wheat is a polyploid comprising the genomes of three different *Aegilops* (*Triticum*) species. The respective tolerances of *Aegilops* CWR show high temperature tolerance at anthesis, and for yield expression via both higher grain number per spike and increased seed weight

(Redden et al., 2014). In recent decades wheat has been reconstituted as synthetics directly from these relatives. Varieties released from synthetics may have increased grain yield, tolerance of water logging, improved drought tolerance with deeper roots, novel sources of disease resistances, and increased protein content (Trethown and Mahmood 2011, Ortiz 2015). Rice wild relatives have been sources of resistance to grassy stunt and other viruses, bacterial diseases, male sterility, drought avoidance, novel proteins, and pest resistances. Maize CWR are diverse with both annual and perennial species, including flood prone habitats, sources of resistance to the weed “striga”, and of tolerances to aluminium and to drought. Unusually a progenitor wild relative often grows in maize fields in central – south America, thus maize has 75% of the diversity in CWR whereas this is only 30-40% for wheat and rice (Porceddu and Damania 2015). Potato has 199 CWR which have been important sources of resistance to viral, bacterial and fungal diseases, as well as for frost/cold tolerance. Similarly CWR have provided new genetic variation to grain legume crops such as chickpea and lentils for improved grain yield, cold tolerance and disease resistances, though there is scope for much more exploitation of these CWR (Porceddu and Damania 2015).

Incorporation of stress tolerance from CWR to domestic gene pools and gene technology.

There are many complications to consider for introgression of traits from CWR into domestic crops.

Expressions of disease resistance in CWR may be the same in both CWR and when introgressed into the domestic gene pools of crops, if controlled by 1-2 major genes, often with little modification by other domestic background genes. However for multi-genic traits controlled by quantitative trait loci (QTL) from CWR the trait expression can be greatly influenced when introgressed by interaction with the genetic background of the crop (Walley and Moore 2015, Baute et al. 2015). Thus QTLs from CWR are best evaluated for stress tolerance expressions after introgression into the domestic gene pool. There are many examples where QTLs unexpressed in CWR have conferred improved yield potential with introgression into the domestic gene pool (Ortiz 2015, Ebert and Schaflietner 2015).

CWR have evolved for survival in the wild, hence may have traits undesirable for agriculture, such a shattering of grain from brittle flowering heads, seed dormancy to enable germination to be spread over many weather events and seasons, and various seed dispersal features. These traits can be genetically linked to the QTLs being introgressed to result in unwanted linkage drag of undesirable genes from CWR when sourcing desirable traits (Walley and Moore 2015). The QTL transfer can be isolated from linkage drag through repeated backcrossing to the chosen crop variety, along with selection for the desired trait and against the unwanted linked traits.

In future the genetic diversity of domestic and CWR collections will in principle be explored, and mapped at the genomic level, especially as the cost of genome sequencing continues to reduce (Baute et al. 2015). Breeders need to be able to choose which accessions to evaluate

and utilise for tolerance of abiotic stresses. Current resequencing of genomes of crop varieties and CWR could be extended to whole genebank collections, identifying rare genes and novel QTLs from CWR and leading to efficient use of CWR. The size of CWR collections can then be planned to maximise genetic diversity, target collection gaps – in combination with eco-geographic data on collection sites, and reduce duplication (Accessions are often renamed in each collection they are transferred to, and unless full genebank passport data is also transferred it is unknown whether different genebank sources of accessions are similar). Genomic technologies provide the marker density required for identification of novel rare alleles, and help to resolve taxonomic relationships (Baute et al. 2015). The high marker density obtained from high throughput sequencing can help detect the allelic combinations per accession, and outlying rare alleles resulting from local adaptation to stress environments, enabling CWR accessions to be targeted for crop improvement. Multi-parent crosses provide increased recombination events for mapping target traits, and enable genomic selection to facilitate breeding for complex traits. Complications include the genome size per species, and the frequency of repetitive sequences which in wheat make up 80% of the genome (Baute et al. 2015).

Cost effective sharing of large volumes of sequence data between genebanks and research organisations, will require open and transparent disclosure of procedures and methods, software settings, quality filtering, contamination removal and metrics for software quality (Baute et al. 2015). Thus data standardisation will be important to reach the goal of complete genotyping of diverse collections, combined with sharing of passport data and use of eco-geographic climatic information (Li Ling et al. 2012).

The taxonomic classification of CWR into sub-species, and genera related to crops does not always reflect their crossability relationships and separation into primary, secondary and tertiary gene pools of crops (Salisbury and Barbetti 2011, Walley and Moore 2015). Crossing barriers are also complicated by differences in ploidy levels. The success in recovering embryos may be very low, dependent upon the choice of parents, direction of crossing (domestic X CWR and/or vice versa), crossing techniques and associated treatments, and upon embryo rescue with excision from the maternal cytoplasm into a culture medium. Viability then may be further improved with further backcrossing to the domestic parent (Walley and Moore 2015). Differences between genomes of polyploid parents can be another complication, for example amongst the diploid and allotetraploid *Brassica* sp. (Salisbury and Barbetti 2011).

Evolutionary distance between CWR and crops may not align with geographic distance. *Vigna* CWR in Australia are still cross-compatible with domestic mung bean from Asia, and their habitats include semi-desert hostile environments in northern Australia (Lawn 2015), a potential source of tolerances to drought and heat stresses. There are more than 20 rice (*Oryza sativa*) CWR species and one progenitor genome also occurs in northern Australia, and is still cross compatible after millions of years of geographic separation (Nevo and Henry 2015). They also suggest that greater diversity may be found in populations in more stressed environments.

The dilemma in screening CWR to identify potential accessions as sources of abiotic stress tolerance, is whether to screen CWR for these traits directly or after backcrossing with selection for the trait. Expression of a trait from CWR can be influenced by epistatic interactions with the background genotype of the domestic parent (Walley and Moore 2015). In some cases a CWR phenotypic trait such as greater root growth can be directly associated with tolerance of drought stress in chickpea (Imtiaz et al., 2011), and selected for in backcrosses. However a number of QTLs may be jointly conferring stress tolerance, hence the need to genetically map these expressions and identify markers [e.g. with single nucleotide polymorphisms (SNPs)] associated with the major QTLs for the desired traits. Mapping populations developed as recombinant inbred lines (RILs) are fixed and then allow screening of many CWR accessions for the most promising parents (Walley and Moore 2015).

Advances in genomics especially sequencing technologies (New Generation Sequencing), and targeting of gene insertion [using Clustered Regularly Inter-Spaced Palindromic repeats (CRISP-Cpf1, CRISP-Cas), Zn finger nucleases and Transcription Activator Like Effective Nucleases (TALEN)], 'provide new tools to characterise CWR sources of genetic variation for use in adaptation of agriculture to climate change' (Nevo and Henry 2015). Marker assisted backcrossing can then be used to recover a QTL target region in the relatively isogenic background of the target variety (Walley and Moore 2015). This enhances the speed and practicality of exploiting CWR traits in crop breeding for adaptation to climate change. Thus CWR can now provide access to vastly more diverse and useful gene pools than within those for domestic crops.

GMO regulation and approval.

There are now many genetic transformation procedures in crops that do not fit the original definition of a genetically Modified Organism (GMO) as being the inter-specific transfer of DNA. Gene silencing of a susceptibility expression may involve the knockout of a single base pair nucleotide, a gene activator, and of RNA expressions, while gene editing can target genomic regions or introduce sequence changes that mirror a CWR allele (Walley and Moore 2015). Also it is increasingly possible to synthesise DNA in the laboratory as an alternative to inter-specific transfer of an allele.

The GMO legislation is now outdated. Advances in biotechnology widen the range of options for genetic manipulation. The pace of change in this science in this science is accelerating, in step with increasing understanding of gene regulation and tissue specific expression. The between species boundaries within the *Panicaceae* grass and the *Brassicaceae* families are becoming blurred with chromosomal and part chromosomal substitutions between species (Salisbury and Barbetti 2011, Trethowan and Mahmood 2011).

A revised approach is needed for the Gene Technology Scheme. Perhaps the very restrictive rules about the location and buffer area required for approved GMO testing in the field, plus keeping of the test location clear of plant growth in the following year, also need to be reconsidered.

Food safety considerations.

GMO in various forms exist now in a wide range of food and pastures and fibre crops. They have been credited in substantially reducing the number of synthetic chemical spray events for pest and disease control, saving in energy and in expenses for weed control to also enable conservation of soil moisture, and many aspects of crop improvement from gains in yield to improved tolerance of biotic and abiotic stresses.

The majority of maize and soy bean crops in North America have been GMO for the past three decades, and converted into many forms of processed food and derived food ingredients without presenting any health problems. There appears to be no substance to concerns about health from consuming food from GMO crops.

Undoubtedly some people will continue prefer to eat only GM free foods. Realistically this is an aesthetic preference, as GM foods may longer be seriously considered to be a health risk.

National genetic resources strategy to manage cropping under climate change.

Climate change presents an unprecedented challenge to agriculture in Australia and the world. A national plan is required to mobilise all available genetic strategies and facilitating agronomic practices, and this includes the use of GMO technology for crop improvement in abiotic and biotic stress tolerances and in food quality improvement.

It is time for all states to remove remaining moratoria on use of GM crops, so that a unified national approach can be implemented. National leadership is needed for organisations, both public and commercial, to share data with information standards on genome sequencing and identification of markers at least after an agreed period of commercial implementation / patenting.

To lead a national strategy for agriculture to combat climate change, a holistic plan is needed. This must present options available to farmers and incorporate farmers' own options. Agronomic considerations with increased cropping intensity could include innovations such as mixed and relay cropping: wide row spacing for tall crops with early maturity inter-sown with shorter later crops either at the same time or later as a relay crop, with the objective of spreading the peak growth periods of the two crops to achieve complementarity. Other options may include rotation with inoculated pulse crops, thereby utilising nitrogen fixation to provide nitrogen benefits both directly to legume crops and in crop residues to subsequent non-legume cereals, or oilseed crops.

The strategy also requires a sound understanding of farming systems, inclusion of livestock, and tailoring of agronomic / genetic options to individual farmer's situation with equipment, crop choices and farming risk, and economic management. Thus a national strategy needs to incorporate a sound knowledge of integrating disciplines, and above all be a team effort with farmers.

With limits to Australia's research infrastructure, public and private, there is a need to facilitate collaboration amongst institutions, public, private and semi-private/public. Public institutions need to deal and be seen to deal equally with other institutions, have joint

strategy meetings with all stakeholders, and joint ownership in developing and promoting the national agenda for agriculture and climate change. This is more than an aspirational recommendation for an agricultural strategy, but increasingly as climate change becomes irreversible in the short-medium term (to 2050), crises will best rural communities and food security by commodity may become an issue within Australia and worldwide.

Action is needed now for the capacity building and infrastructure development to address climate change on a national basis.

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