Combining agronomy and genomics to design future crops.

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Abstract
The history of agriculture shows that transformative impact can be achieved when new crop management strategies are combined with novel genetics. This paper outlines examples of how agronomy and genetics have been combined to enable progress in agriculture including recent highlights from Australia. Then, the potential to combine emerging genomics technologies with innovation in agronomy to deliver future "gene x management" breakthroughs is explored. The opportunity for crop pre-breeding strategies that combine emerging genomics technologies with research agronomy and crop breeding to deliver impactful outcomes is emphasised.

Keywords
Genome, selection, breeding, traits, farming systems.

Introduction
Farming systems drive the selection of crop genes. Genes for domestication traits, such as the non-shattering genes of cereals that allowed early farmers to harvest crops without losing grains, are archetypal examples (Simons et al. 2006). Another example is provided by genes for photoperiod insensitivity, which allow many crops to be grown across different latitudes (Turner et al. 2005). These genes were likely selected fortuitously during the early stages of agriculture when natural outcrossing or random mutation events gave farmers access to traits that improved the performance of local varieties.

Since the advent of modern breeding methods there have been successful efforts to harness global genetic diversity and to deliberately select for genes and associated traits that adapt crops to evolving farming systems. The "green revolution" semi-dwarf genes of cereals, for example, were selected to prevent lodging of plants in the high-yielding farming systems that were made possible by the use of industrial fertilisers (Evans, 1993). Another example is the tomato *SELFPRUNING (SP)* gene, which converts vine-like "truss" tomato into a compact bush with even fruit ripening (Pnueli et al. 1998). The *SP* gene enabled mechanised harvesting of field-grown tomatoes and was key contributor to the development of a new field crop industry (see Thompson and Blank, 2000). These examples demonstrate the transformative potential that comes from combining farming systems innovation with targeted genetic selection. In the examples outlined above, selection was based on trait phenotypes without any knowledge of underlying genetic mechanisms. This approach of targeted phenotypic selection is sometimes referred to as "physiological breeding".

Targeted selection of crops to suit evolving farming systems continues. A current example is the shift towards earlier sowing of wheat crops in some Australian growing regions (Hunt et al. 2019). This has been made possible by innovations in summer fallow management and by new technologies that allow deeper sowing. Benefits of earlier sowing include the ability of crops to take advantage of shifting rainfall patterns, with more summer rain versus less reliable autumn rainfall in some growing regions, and also the capacity of crops to establish high yield potential (Flohr et al. 2018). Earlier sowing requires wheats that have a long vegetative growth-phase to reduce the likelihood of precocious flowering before or during winter. This can be achieved through the selection of wheats that have a strong vernalization-requirement (the need for prolonged winter cold to stimulate flowering). The wheat genes for

vernalization requirement are known and this has allowed the use of genetic technologies to examine the relationship between specific gene variants, sowing dates and crop performance. For example, near-isogenic lines that contrast different vernalization gene variants in a common genetic background are available. These have been used to contrast and quantify the effect of genes that confer different vernalization-requirements on crop performance, alongside comparisons of elite varieties that differ for vernalization-requirement but also many other traits. This approach allowed definitive conclusions to be made about the overall value of genes for vernalization requirement for early sown wheats (Hunt et al. 2019). More importantly, diagnostic molecular markers are available for the genes controlling vernalization-requirement. These are useful for breeding wheats that are suited to early sowing because they can be used for parent choice and progeny selection. The first iterations of modern Australian wheats suited to earlier sowing are now reaching the market.

Increasing potential for genetic technologies

The intersect of agronomy and genetics can be considered as happening in waves, with the first wave being the opportunistic selection of natural variation and the second being deliberate phenotypic selection of natural diversity. Now, agriculture has transitioned to a third wave where genetic technologies and knowledge of gene function are starting to play an ever-increasing role in the selection of crops to suit farming systems. This is an ideal time to consider ways to connect emerging genetic technologies with research agronomy. Here the end-goal is to a deploy novel genetic solutions targeted to new farming systems opportunities. To achieve this, the primary driver has to be clearly defined opportunities for improved on-farm crop performance that are achievable through novel genetics. Assuming this first criterion is met, any genetic solution deployed has to also be compatible with other core traits for the target crop (no negative impact on other aspects of crop performance) and selection of novel genetics must be achievable if it is to be maintained within breeding populations, either through phenotyping or by using genetic markers. Regardless of the selection tool, a key question is, what is the architecture of the novel genetic solution? A simple trait that is controlled by one or two genes will be easier to deliver than a complex one that is controlled by many genes. That said, there is increasing potential to channel more complex genetics when the effort is justified.

For simple genetic traits, there are a range of tried and tested approaches to channel novel variation into target crops. These include the use of recurrent backcrossing to generate near-isogenic lines or using chemicals or radiation to induce mutations and thus generate new genetic variation for a trait in the target germplasm. Both these approaches have been used for many decades. Recent developments in molecular marker technology and genome-sequencing are facilitating these traditional approaches. For example, genome-sequencing can be used to detect mutations in target genes (Tsai et al. 2011). This is particularly useful in polyploid crops, where loss-of-function mutations typically exhibit no phenotype, since there are multiple copies of genes present (a phenomenon known as genome redundancy). Genome sequencing allows individual mutations in the different copies of a target gene to be identified and these can then be combined by crossing to generate a line that lacks all functional copies of the target gene (Tsai et al. 2011). This approach allows previously "hidden" variation that was only seen in diploid crops (e.g. rice) to be recapitulated in polyploids. Since many major crops are polyploid (e.g. wheat, oat, cotton, canola) this is an important advance.

Gene-editing technologies offer an even more powerful approach to generate novel traits. For example, CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats, CRISPR Associated Protein 9) technology allows DNA modification enzymes to be targeted directly to genes, to trigger sequence changes that can abolish or enhance gene activity (see Sukegawa et al. 2021). To apply gene-editing the target crop must be transformable, because gene-editing enzymes are delivered as a transgene. That being said there are intensive efforts to develop transformation free gene editing for crops. Another important consideration is that there must be sufficient knowledge of the trait-of-interest to identify appropriate target gene sequences for editing. Advances in genome-sequencing have meant

that access to gene sequences is not limiting but sufficient understanding of gene function remains an important consideration. There are key aspects of plant biology, such as phenology or plant height, where genetic pathways are well understood. Additionally, the functions of genes in these major pathways is often conserved, so knowledge can be translated to many crop species including those that have not been the focus of intensive research. So, these types of traits are ideal targets for gene editing. Gene editing has been applied to crops such as soybean to generate novel variation (Cai et al. 2018) and has also been used to accelerate domestication of wild or near-wild crops by rapidly introducing domestication traits (Lemmon et al. 2018, Li et al. 2018). Gene-editing can also target multiple copies of a gene in a single editing event and so is highly effective approach to generate novel genetic variation in polyploid crops (Abe et al. 2019). One important consideration is that some forms of gene-editing are currently classed as genetically modified organisms (GMOs) in some countries, which can give rise to significant regulatory burdens and market restrictions. Notably, simple gene edits (classed Site Directed Nuclease-1, SDN-1) are not considered as GMOs in Australia and this type of gene editing is highly effective at altering gene function.

Advances in genomics and allied technologies also offer new ways to introduce complex traits into elite crop varieties. One strategy is to simultaneously target multiple regions for selection through genomic prediction, which used genome-wide marker information to predict phenotypes of individuals. Genomic prediction can be used to screen larger populations than might be achieved by direct phenotyping and thereby enrich a population for a target trait. This approach is increasingly being used to target complex traits in plant breeding, including yield and other complex traits, such as drought tolerance (Cooper et al. 2014). Genomic prediction relies on knowledge of marker-trait relationships. There are a number of ways to build this knowledge using genetic populations, including Genome-Wide Association Studies (GWAS), Nested Association Mapping (NAM) and Multi-Parent Advanced Inter Cross (MAGIC) populations (see Adamski et al. 2020). The resulting knowledge of marker-trait relationships are then incorporated into models or algorithms that predict phenotype based on marker data. Machine-learning methods, such as Random Forest, are proving useful in this respect, being able to rapidly generate predictive algorithms with no prior knowledge and able to detect and incorporate genetic interactions (Charmet et al. 2020). Similarly, methods such as Random Forest can resolve gene-environment interactions to allow cross-environment trait prediction, though this can be a challenge for crops that grow in variable climates (e.g. rainfed wheat), requiring trial data from multiple sites and seasons.

The marker-trait associations used to predict phenotype can also be used to identify targets for gene editing. It is reasonable to expect that as gene-editing technologies advance it will be possible to edit many genes simultaneously, offering the potential to engineer increasingly complex traits in the near future. Taken together, increasing potential to use genomics to rapidly engineer simple or complex genetic traits offers many potential solutions to challenges faced in farming systems. There is great potential to fine-tune the performance of existing crops to suit shifting management practices or to repurpose crops for entirely new niches or end uses. The capability to rapidly improve orphan crops (those that have not been a focus for modern breeding) and even to accelerate domestication of wild plants might also allow the uptake of new crops.

Future integration of genome-biology and agronomy

To achieve the full potential of crop genomics there needs to be a cohesive model for innovation that links research agronomy, emerging genetic technologies and also crop breeding. There are examples of where this type of innovation model already operates, particularly with phenology research. Examples include section of early-sown wheat (see above) and dual-purpose canola (Paridaen and Kirkegaard, 2015). In both instances, innovation opportunities have been defined by agronomists working closely with growers and this was the catalyst for focussed genetics research. These projects also have clarity in terms of the intersect between research and breeding. The priority for genetics in these projects is to develop deeper understanding of variation accessible in breeding populations and to develop markers

that allow breeders to effectively harness that variation alongside other core target traits. More specifically, there have been deliberate efforts to deliver knowledge that allows breeders to interpret phenology using the same in-house marker platforms that are being applied to genomic selection. While these projects successfully connect agronomy and genetics, we suggest that there is scope for more adventurous combined innovation efforts to harness innovations in crop management together with emerging gene technologies, particularly genomic prediction and gene editing. The focus should be on identifying farming systems opportunities or gaps that can be addressed via novel genetics. Of particular interest is where emerging farming systems approaches or technologies create niches for new or modified crops, protected cropping for example.

Conclusion

Advances in gene-technology will allow new genetic solutions to be rapidly developed to meet the needs of farming systems. Existing crops can be redesigned for new purposes or adapted to new niches. Traits identified in one crop can rapidly be translated in others by leveraging conservation of gene-sequences and gene function. There is even potential to rapidly domesticate new crops. For this potential to be achieved there needs to be an innovation pipeline that seamlessly integrates agronomy, genetics and crop breeding.

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